

TRAINING MANUAL FOR FOOD SAFETY REGULATORS

**Vol IV – ENSURING FOOD SAFETY & STANDARDS THROUGH FOOD
SAMPLING & ANALYSIS**

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THE TRAINING MANUAL FOR FOOD SAFETY REGULATORS WHO ARE INVOLVED IN IMPLEMENTING FOOD SAFETY AND STANDARDS ACT 2006 ACROSS THE COUNTRY

FOOD SAFETY & STANDARDS AUTHORITY OF INDIA
(MINISTRY OF HEALTH & FAMILY WELFARE)
FDA BHAVAN, KOTLA ROAD,
NEW DELHI – 110 002
Website: www.fssai.gov.in

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Sampling involves the selection of a certain portion, number of container and product units from a particular lot of the same food. It must be as representative as possible of the whole consignment or from lot.

Objective:

Samples are usually collected from a lot of food for random surveillance, collection of data for a specific purpose, or monitoring/and to determine whether the food is unsatisfactory for any reason. The samples may be collected from factory premises, or from the market. Food Safety officer while taking samples of food for analysis under clause (c) of sub-section (1) of section 47 of the Act or an authorized officer taking sample of imported article of food for analysis under sub-section (5) of section 47 of the Act shall, follow the procedure specified herein for taking samples and sending them for analysis.

Notice: When a sample of food is taken for analysis, the person taking the sample shall give notice in writing then and there of his intention to have the sample so analysed to the person from whom he has taken the sample and simultaneously, by appropriate means also to the person whose name, address and other particulars have been disclosed under rule 3.6 of FSS Rules.

Sample Collection

It is important to clearly define the population that is to be sampled. The population may vary in size from a production lot, a day's production to the contents of a warehouse. Extrapolating information obtained from a sample of a production lot to the population of the lot can be done accurately, but conclusions cannot be drawn from data describing larger populations, such as the whole warehouse.

Populations may be finite, such as the size of a lot, or infinite such as in the number of temperature observations made of a lot over time. For finite populations, sampling provides an estimate of lot quality while for infinite populations it provides information about a process. Regardless of the population type, that is, finite or infinite, the data obtained from sampling are compared to a range of acceptable values to ensure the population sampled is within specifications.

Importance of Sample Collection

The reliability of analytical data thus obtained depends on several factors, sampling being the major factor. Current analytical methods require only few grams of food sample to analyze. Thus, it is necessary that a sample be as representative of the population as possible.

There are three basic activities involved in analysis of food products:

- 1) Collection of representative sample;
- 2) Sample preparation;
- 3) Analysis using appropriate methods and instruments.

These activities, although independent in nature, yet can have decisive influence on each other. Furthermore, each of these activities has their own potential sources of variations that contribute to the uncertainty level associated with any analytical result. Thus, care must be taken to identify the sources of variation and minimize or avoid them while accomplishing any activity, on the part of the laboratories, it is therefore necessary to develop a plan for the proper performance of each activity, and then establish quality standards and written procedures in compliance with the standards. Many times, the activity of sampling falls outside the purview of laboratory's mandate or control. This is especially true in commercial testing laboratories where the "first contact" is the arrival of samples. To improve the overall quality of analytical process, a laboratory must do all it can to receive appropriate, applicable, defensible samples. The development of appropriate plans will depend upon an understanding of the problems involved in each activity, and then the application of reasonable judgments in seeking solutions.

A sample should represent a population as adequately as possible. To ensure proper sampling, the analysts need to be consulted time to time concerning proper sample size, suitable containers for sampling or the use of appropriate preservatives to prevent any spoilage or transformation in a sample before analysis, one common cause of lack of precision or lab-to-lab variation in analytical results for a particular population can be traced back to erroneous sampling. When significant difference in results occurs among laboratories which have supposedly analyzed the same sample, a serious conflict may arise questioning the competence and credibility of the laboratories. Many of these situations can be avoided if sample delivered to the laboratory represents the composition of the parent lot.

There are at least two ways to measure a given lot of goods: one, that we often assume to be the "proper" way, is to find its "true value", by which we mean its average value. The other way, often discovered accidentally as a result of "poor" sampling, is to measure its variability.

Types of Sampling

a. Bulk sampling

It involves the selection of a sample from a lot of material that does not consist of discrete, identifiable or constant units. Sampling may be performed in static or dynamic situations. Bulk sampling poses special problems requiring certain decisions to be made: the number of increments to be taken, the size of the increments, from where in the pile or stream they should be drawn, the sampling device to be used, and how to reduce the increments taken to a reasonable size of sample for delivery in the laboratory.

b. Acceptance sampling

It differs from the bulk sampling and involves the application of predetermined plan to decide whether a lot of goods meet defined criteria for acceptance. The risks of accepting "bad" or rejecting "good" lots are stated in conjunction with one or more

parameters. Statistical plans can be designed to regulate the probabilities of rejecting good lots or accepting bad lots.

Precautions during sampling

The condition of the sample received for examination is of primary importance. If samples are improperly collected and mishandled or are not representative of the sampled lot, the laboratory results will be meaningless. Because interpretations about a large consignment of food are based on a relatively small sample of the lot, established sampling procedures must be applied uniformly. A representative sample is essential when pathogens or toxins are sparsely distributed within the food.

The number of units that comprise a representative sample from a designated lot of a food product must be statistically significant. The composition and nature of each lot affects the homogeneity and uniformity of the total sample mass. The proper statistical sampling procedure, according to whether the food is solid, semisolid, viscous, or liquid, must be determined by the collector at the time of sampling.

Whenever possible, samples must be submitted to the laboratory in the original unopened containers. If products are in bulk or in containers too large for submission to the laboratory, representative portions must be transferred to sterile containers under aseptic conditions. There must be no compromise in the use of sterile sampling equipment and the use of aseptic technique.

Clean, dry, leak-proof, wide-mouthed and sterile containers of a size suitable for sample of the product must be used. Containers such as plastic jars or metal cans that are leak-proof may be hermetically sealed. Whenever possible, avoid glass containers, which may break and contaminate the food product. For dry materials, use sterile metal boxes, cans, bags, or packets with suitable closures. Sterile plastic bags (for dry, unfrozen materials only) or plastic bottles are useful containers for line samples. Take care not to overfill bags or permit puncture by wire closure. Identify each sample unit (defined later) with a properly marked strip of masking tape. Do not use a felt pen on plastic because the ink might penetrate the container. Whenever possible, obtain at least 100 g for each sample unit. Submit open and closed controls of sterile containers with the sample.

Dry or canned foods that are not perishable and are collected at ambient temperatures need not be refrigerated. Transport frozen or refrigerated products in approved insulated containers of rigid construction so that they will arrive at the laboratory unchanged. Collect frozen samples in pre-chilled containers. Do not freeze refrigerated products. Unless otherwise specified, refrigerated samples should not be analyzed more than 36 h after collection.

Sampling plan:

Samples should be collected following a particular plan or procedure. The FSO/ Authorized Officer should also bear in mind the capability of the laboratory to carry out analyses, particularly if the samples are perishable. The following criteria should be considered in formulating a sampling plan:

- type of food product
- the size of food articles to be sampled (production units, cans, packages, etc.)
- the nature of the defect: bacterial contamination, chemical toxin or residue, insufficient heat exposure, etc.
- the degree of hazard to human health
- the potential for fraud
- acceptance and rejection criteria: absence of pathogens, adulteration, tolerance limits, faulty seams, compositional standards, net contents
- and degree of confidence required so that the test result is valid.

Objective sampling must be conducted on a random basis. Each unit should have an equal chance of being picked as a sample. The best way to assure this is by using random number tables or picking a sample according to a predetermined time from a production chain.

In dealing with selective or biased sampling, random sampling is unnecessary or even undesirable. When lots are contaminated, adulterated, defective or in violation in some visible manner (e.g., swollen canned goods, or flour containing live insects), the FSO/ Authorized Officer, after examination of the lot, should select the units which will most clearly demonstrate the violation. For this selective procedure, he should choose a sufficient number of units to demonstrate what he found in the lot, along with a few normal units, and report the number or proportion of such defective units present in the entire lot or in the number of units examined.

Where no specific instructions are given, the general rule is to collect samples from the square root, plus one, of the number of units in the lot. Ordinarily, with respect to large consignments in factories, on wharfs or in warehouses, not less than 12 or more than 36 units should be collected, and each unit should come from a different container. For example, if the consignment consists of cartons containing 48 cans of food product, one can would be collected from each carton. If there are less than 12 containers, all containers should be sampled equally.

With regard to bulk containers, e.g., barrels, up to 2 kg should be taken from each, depending on the product and the analysis likely to be required. Each subsample should be packaged separately, and every effort must be made to ensure that the food product in the original containers is not contaminated or otherwise made unfit or unsatisfactory for use as food. The container must be properly and securely closed after the subsample has been taken.

Products manufactured under non-homogeneous conditions (e.g., canned fruits and vegetables) are subject to random sampling. There may be differences in the processing of the various portions of the lot, or the units may vary in composition, and evidence of insanitary manufacturing conditions may be found in different amounts in various packages in the lot. In order to obtain data for average composition, a representative cross-section sample of the lot must be collected and examined.

Sampling tools and containers:

Whenever possible, a sample should consist of an adequate number of intact retail containers of a product. This is not always possible, because food products are often transported, stored or sold in bulk containers such as barrels, bags, trucks, etc. Samples collected from bulk packages or unpackaged foods sold at retail must be placed in suitable containers for storage and handling to be presented for laboratory analysis.

Sampling tools:

The tools available to FSO/ Authorized Officer range from common tools for general purposes to special tools to be used in specific situations and for specific examinations of particular food products. All sampling equipment must be clean and dry when used to collect a sample. Common tools such as pliers, spoon, screwdriver and knife are useful for opening shipping containers, cutting bags and scooping out food products. A special opener is available for opening cardboard shipping cartons with the least damage to the carton. Dippers of suitable size can be used to collect samples of liquids such as milk. The dippers should be of smooth metal construction, preferably of stainless steel, to facilitate sterilization. A dry borer tube may be used for flour, dried milk and dried milk products. The product will usually have to be pushed out of the trier with a spatula. This trier should not be used for bacteriological sampling. A suitable stainless steel or aluminium spoon may also be used. A special probe is needed for sampling railway wagons or lorry loads of dried grains such as wheat and maize. When the closed probe is in the grain, it is opened over a large cloth to collect the grain. A conical-shaped metal probe often referred to as a "bag thief", is used for sampling bags of grain, coffee beans and spice. The probes are stored in leather or cloth sheaths when not in use. Special probes or triers are used for butter and cheese. The trier is stuck into the product, then turned to cut out a cylindrical plug. For sampling cheese only, a sealing compound composed of paraffin, beeswax and white petroleum jelly will be needed to reseal the sampled areas. A boot or flour trier is used for sampling elevator boots (bottoms) in a large flour mill or bakery. The trier is stuck into the static material in the equipment, then lifted out for examination. Presterilized, disposable plastic spoons and pipettes, for aseptic sampling for bacteriological analysis, are necessary. Rubber or latex surgical gloves may also be used to permit handling without adding bacteria to the sample. A metal screen and a collecting pan are used for checking bulk grains for insects, rodent faeces, and other foreign material.

Isopropyl alcohol or ethyl alcohol should always be carried by the FSO/ Authorized Officer in a plastic bottle, for disinfecting sampling tools.

Sample containers:

In general, for liquids the FSO/ Authorized Officer should use clean, dry containers of appropriate waterproof and greaseproof material, including glass, stainless metal, and suitable plastic material which can be sterilized by heat if necessary. The containers must have a secure closure of rubber or plastic stoppers, or a screw-cap of metal or plastic, coated with an insoluble, non-absorbent and greaseproof material. The containers and closures must be such as not to influence the odour, flavour, pH, or composition of the sampled products. For solids or semi-solids, clean, dry, wide-mouth, cylindrical receptacles of suitable waterproof, greaseproof material should be used. These containers may also need to be sterilized. All containers must have air-tight closures. Suitable plastic bags may also be used. Plastic bags or containers should not be used for pesticides samples. For butter, suitable wide-mouth jars should be used. The butter must not be allowed to come into contact with paper or any water or fat-absorbing surface.

Sample Collection Techniques:

Product Information

Before collecting any sample, the FSO/ Authorized Officer must observe the lot from which the sample is to be collected, and record relevant observations. Information obtained should include the following as appropriate;

- (a) name of the food;
- (b) lot size;
- (c) type of packing;
- (d) container size or sizes;
- (e) product code or control numbers;
- (f) number of consignments;
- (g) labelling information;
- (h) condition of the lot, i.e., broken packages, evidence of rodent or insect infestation, debris, etc.;
- (i) general condition of the area or building in which the lot is stored.

If the subsamples for packaged food are drawn from boxes or crates, the sample units should be marked with numbers. Corresponding numbers should be written inconspicuously on the boxes or crates, together with the FSO/ Authorized Officer's initials and the date. The boxes or crates are thus identified, as is the entire lot, so that they can be recognized later if they are re-sampled.

Sampled Unit (Lot):

Every effort should be made to restore the lot from which the sample is collected to its original condition. Boxes or crates should be refilled, re-glued and re-stacked. Sacks or bags which have been opened should be refilled and closed. These operations require some physical effort, but it is essential to leave the stock in good saleable condition. Refilled units must not be contaminated.

It may not be possible to restore some lots completely to their original condition, as the sampling operation may do some damage that the FSO/ Authorized Officer cannot avoid or correct. This problem should be discussed with the owner of the goods before sampling begins, so that a satisfactory arrangement can be reached.

Whenever possible, samples should be collected from previously unopened boxes or crates, unopened retail packages, and unopened bulk containers such as sealed barrels, etc. The FSO/ Authorized Officer may, however, often find that the bulk containers from which he wished to collect the sample have already been opened by the dealer. Often samples may have to be collected from bulk containers which have been inadequately covered. When this happens, the existing condition should be described in detail, keeping in mind the effect that opening could have had on the composition of the product.

Sample integrity:

Because of the large variety of food products which may be sampled, it is impossible to provide specific guidelines for each product. However, the FSO/ Authorized Officer must always be aware of the perishability of the sample and that, for analytical significance, the sample must reach the laboratory in a condition similar to that at the time of sampling.

In taking official samples, many food control authorities prescribe the use of special tamper-proof containers or sealing with wax and a seal with the FSO/ Authorized Officer's designated identification number. It is usually a good precaution to have the owner of the goods sign for the owner's portion of the sample.

The FSO/ Authorized Officer is responsible for collecting, holding, sealing, storing and delivering the sample in a manner that will prevent it from being changed after sampling. Whoever receives the sample at the laboratory has the same responsibility from that time on. It is very important that the FSO/ Authorized Officer be able to document sample integrity from time of collection to delivery to the analyst, particularly when enforcement action is being considered.

Field examination:

Field examination deals with observable conditions, the most frequently encountered being rodent and insect-contaminated foods. For insect-infested or rodent-damaged lots, the FSO/ Authorized Officer should report the number and location of live and dead insects, and amount of rodent excreta, hair or other filth discovered inside the containers as well as on their exterior surface. He should give measurements of areas of rodent-urine or chemical stains on each container and the extent of penetration. Findings of the unit-by-unit examination can be correlated with any photographs and with the physical subdivisions collected.

Where the field examination is carefully described and documented, the samples collected from obviously mishandled lots may be reduced to carefully selected exhibits. The field examination and the report of findings will serve as evidence in place of laboratory analysis.

When a lot is visibly or obviously adulterated, it is desirable to list recent deliveries from the lot to other customers so that follow-up may be made to prevent consumption of this material. When other shipments are to nearby areas, the FSO/ Authorized Officer can make an examination and take appropriate action. When deliveries have been made to areas too distant for his examination, he must communicate with the local FSO/ Authorized Officers for examination and action.

When rodent infestation is observed, the FSO/ Authorized Officer should select the portions actually sampled to reflect the violated nature of the lot, i.e., in respect of which the lot fails to meet legal requirements. Suspected urine stains can be examined with an ultra-violet lamp. This examination should be conducted in as near total darkness as possible.

Samples of rodent contamination may be collected in the following manner:

- (a) cutting standard material, such as bags, along the stained area, leaving about 2 cm of bagging material along each side. The same method can be used for cutting the material around a gnawed hole. If the material is multi-layer paper bags, all layers should be kept together;
- (b) placing the cuttings between two pieces of white paper and folding or rolling them, or leaving them flat and placing the entire sample in a plastic or paper bag. This will help hold the evidence in place and prevent possible loss of hairs or parasites;
- (c) submitting a minimal amount of product from under the stained or gnawed areas, preferably sampling the clumped product only;
- (d) identifying the various pieces of evidence with the FSO/ Authorized Officer's initials, the date and an exhibit number. On the report submitted with the sample, the exact location and circumstances of each exhibit should be clearly noted.

Direct contact with rodent-urine contaminated materials should be avoided as much as possible. The moist urine of chronically infected rodents can contain *Leptospira* organisms that may be transmitted to man, contact with bruised skin or mucous membranes being the suspected mode of transmission.

The FSO/ Authorized Officer should collect samples from lots suspected of dry chemical contamination in much the same manner. After collecting a sample of the contents from immediately beneath the suspected area, he should collect residues from the

surface of the bag or container. When infiltration of loosely woven bags is suspected or observed, the bag should be shaken or tumbled over a large sheet of clean paper to collect the sifting as a sample.

In addition to these samples, exhibits should include diagrams and photographs to demonstrate the violative conditions reported. The FSO/ Authorized Officer should prepare diagrams of the top and side views of stacked lots, and indicate which contaminated bags were sampled and photographed.

Sample collection For analysis of Pesticide Residues:

Submission of food samples to the laboratory for pesticide analysis presents a special set of problems which must be considered. For pesticide analysis of fresh produce, agricultural products, and milk or other animal products, a selective sample should be collected to substantiate inspection or other evidence of suspected misuse of a specific pesticide for the particular crop, grower, or growing area. The stability of the pesticide residue must be considered, as transport and storage could permit the residue to diminish, giving results which do not reflect conditions at the time of sampling. In other instances, general objective samples should be collected from lots for which there is no such evidence or suspicion. To retard decomposition if a product sample is normally held or transported under refrigeration in commercial practice, the sample collected must be held in cold storage for a minimum time or preserved with appropriate chemicals until ready to be delivered to the laboratory. Samples of perishable products must be handled and analysed as promptly as possible.

Screening of Dry Products:

When examining for filth by screening cereals and pulses, etc., packed in large containers, the FSO/ Authorized Officer should use a portable screen, set up at an angle. He should examine 5 to 10 kg, briefly observe the screening from each bag macroscopically, and subjectively report findings as to live or dead insects, rodent excreta pellets, or other obvious filth. Screenings should be submitted as a separate unit or exhibit.

Sampling for Microbiological Examination of Foods

Generally, Regulatory bodies of Governments have employed three methods to control microbiological hazards. These are, creating awareness by education and training; inspection of facilities and food processing units and microbiological testing. Microbiological testing is through the application of microbiological criteria for foods. Microbiological criteria is defined as "a statement of the criterion of acceptance to be applied to a lot, based on examination of a required number of sample units by defined analytical methods" (International Commission on Microbiological Specifications for Foods :ICMSF, 1986). Central to the assessment of the role of microbiological criteria are the concepts of probability and sampling involved in the definition of a sampling plan. In particular, the choice of sampling plans should take into account: risks to public health associated with the hazard; the susceptibility of the target group of consumers and the heterogeneity of distribution of microorganisms where variables sampling plans are employed. The distribution of microorganisms in food normally follows either a Poisson (random) or more usually a negative binomial (contagious) distribution that is approximated by a log-normal distribution.

Sample of the lot should be representative. It should reflect, as far as is possible, the composition of the lot from which it is drawn. In drawing a representative sample it is important to avoid bias by sampling at random, where the sample units are drawn using

random numbers. Every sampling plan has a certain amount of risk by which a good lot is rejected or a bad lot is accepted. In other words, this can be classed as a Consumers' or Producers' risk. The producers risk describes the probability that an acceptable lot if offered will be falsely rejected. The consumers risk describes the probability that a bad lot when offered will be falsely accepted, that is a lot whose actual microbial content is substandard as specified in the plan, even though the determined values indicate acceptable quality. Drawing greater numbers of smaller samples provides greater protection than drawing the same total weight of sample in fewer sample units. In the concept of "multiple samples" where a number of samples are drawn, there is an increased chance of detecting contamination due to uneven distribution of contamination.

A sampling plan includes the sampling procedure and the decision criteria to be applied to a food lot, based on examination of a prescribed number of analytical sample units by defined methods. The main advantage of using sampling plans is that they are statistically based and provide a uniform basis for acceptance of a lot against defined criteria.

Sampling plans should include the following:

- Microbe or groups of microbes of interest
- The number of samples to be tested (n) chosen independently or randomly
- Test method (eg: Most Probable number, Standard Plate Count)
- Level of contamination considered to be
 - Acceptable (cfu/g < m)
 - Marginally acceptable ($m < \text{cfu/g} < M$)
 - Critical (M , cfu/g)
- The number of samples which fall into each class (of acceptable/marginal/unacceptable) if the lot is to be accepted.

There are two widely accepted types of sampling plans as defined by the (ICMSF). Two-class plan is used essentially for pathogens and/or where a presence/absence test is to be performed, whereas a Three-class plan is frequently used to examine for hygiene indicators where enumeration of microbes in a unit-volume or mass is possible.

Two-class sampling plan:

n = number of units making up the sample

m = threshold below which all results are considered satisfactory

c = number of units in the sample allowed to exceed m (i.e. presence of an organism or counts above a preset limit denoted by m)

Probability is the number of times the outcome did occur among the trials conducted. It is independent of distribution of contamination in the lot. It is usually a test for the presence (+) or absence (-) of an organism or whether they are above (+) or below (-) a preset concentration. It is defined by two numbers

E.g. FAO/WHO standards for Ice cream, for Salmonella, $n=10$, $c=0$, $m=0$.

Three-class sampling plan

n = number of units making up the sample

m = threshold below which all results are considered satisfactory

M =acceptability threshold, the result is considered unsatisfactory if one or more units give values = or $> M$

c = number of units in the sample that can fall between m and M .

The Three-class attribute plans is similar to the two-class plans except for the introduction of a new term M . A count above M for any sample unit is unacceptable and the quality of the product can be divided into three attribute classes:

- counts $< m$ (acceptable)
- $m < \text{counts} < M$ (marginal)
- counts $> M$ (unacceptable)

When determining m and M , the points to be considered are:

- The level of the test organism that is acceptable and attainable in the food is m . This is reflected by what is attainable by GMP or is the hazardous level in 2 class sampling plans.
- Choice of the value of M is based on whether it poses a health hazard, is a spoilage-shelf life index or is a general indicator of sanitation

Choice of n and c varies with the desired stringency, which is determined by the level of hazard. For high stringency n is high and c is low. Choice of n is usually a compromise between what is an ideal probability of acceptance and the work load the laboratory can handle as sampling plans should be administratively and economically feasible.

Eg: FAO/WHO standards for Ice cream, for Aerobic Mesophilic bacteria: $n=5$, $c=2$, $m=10^2$, $M=10^3$.

Stringency is the probability that a lot of food of defined microbial quality will be rejected by a prescribed sampling plan compared to other sampling plans. Three-class plans are more effective at reducing the consumers risk while the Two-class plans are more effective at reducing the producers risk. The sampling plan is usually independent of lot size if the lot is large compared to the sample size eg. multiplying the sample number by 4 will only halve the likelihood of making wrong decisions.

Variable plan: is one which employs statistics of a sample, such as the mean and standard deviation, rather than segregation of samples into attribute classes. Probability assumes normal distribution of contamination in the lot and should be checked before application of variable **sampling** plans.

The lot is rejected if $\bar{x} + ks > C$, Where,

- \bar{x} is the sample mean,
- s is the standard deviation
- k is the plan stringency value obtained from appropriate tables for a given number of sample units,
- n , and the desired probability of rejecting the lot if defective product is present, and
- C is the limit value, which is selected as a specification for safety.

Routine versus Investigational sampling:

Circumstances of unusual hazard in routine sampling will require a more intensive “investigational” examination. Circumstances include, conditions of severe risk (eg. critical pathogen like *C. botulinum*); failure of a series of lots to pass routine inspection; to confirm that a potential problem exists or provide information on possible sources of the problem *etc.*

In choosing a sampling plan the following must be borne in mind.

- The required stringency of plan. This should be based on the degree of hazard to the consumer from pathogenic or spoilage microbes. This is a function of types and numbers of microbes, which may spoil the product, indicate possibility of contamination by pathogens; Cause mild illness but don't multiply rapidly; or Cause severe illness
- The degree of hazard due to storage conditions must be considered. The options are, an increase in bacterial numbers; no change in bacterial numbers; or decrease in bacterial numbers.
- Other factors include the Food-borne disease record of this particular food type, the probable past history of processing, the expectation of subsequent spoilage due to storage conditions or due to the possible exposure to hazardous pathogens.

The statistical performance characteristics or operating characteristics curve should be provided in the sampling plan. Performance characteristics provide specific information to estimate the probability of accepting a non-conforming lot. The sampling method should be defined in the sampling plan. The time between taking the field samples and analysis should be as short as reasonably possible, and during transport to the laboratory the conditions (e.g. temperature) should not allow increase or decrease of the numbers of the target organism, so that the results reflect - within the limitations given by the sampling plan - the microbiological conditions of the lot.

Precautions during sampling:

The condition of the sample received for examination are of primary importance. If samples are improperly collected and mishandled or are not representative of the sampled lot, the laboratory results will be meaningless. Because interpretations about a large consignment of food are based on a relatively small sample of the lot, established sampling procedures must be applied uniformly. A representative sample is essential when pathogens or toxins are sparsely distributed within the food.

The number of units that comprise a representative sample from a designated lot of a food product must be statistically significant. The composition and nature of each lot affects the homogeneity and uniformity of the total sample mass. The proper statistical sampling procedure, according to whether the food is solid, semisolid, viscous, or liquid, must be determined by the collector at the time of sampling.

Whenever possible samples must be submitted to the laboratory in the original unopened containers. If products are in bulk or in containers too large for submission to the laboratory, representative portions must be transferred to sterile containers under aseptic conditions. There must be no compromise in the use of sterile sampling equipment and the use of aseptic technique.

Containers that are clean, dry, leak-proof, wide-mouthed, sterile, and of a size suitable for samples of the product must be used. Containers such as plastic jars or metal cans that are leak-proof may be hermetically sealed. Whenever possible, avoid glass containers, which may break and contaminate the food product. For dry materials, use sterile metal boxes, cans, bags, or packets with suitable closures. Sterile plastic bags (for dry, unfrozen materials only) or plastic bottles are useful containers for line samples. Take care not to overfill bags or permit puncture by wire closure. Identify each sample unit (defined later) with a properly marked strip of masking tape. Do not use a felt pen on plastic because the ink might penetrate the container. Whenever possible, obtain at least 100 g for each sample unit. Submit open and closed controls of sterile containers with the sample.

Dry or canned foods that are not perishable and are collected at ambient temperatures need not be refrigerated. Transport frozen or refrigerated products in approved insulated containers of rigid construction so that they will arrive at the laboratory unchanged. Collect frozen samples in pre-chilled containers. Do not freeze refrigerated products. Unless otherwise specified, refrigerated samples should not be analyzed more than 36 h after collection.

Quantity of Food Samples to be collected for Analysis:

Under the provision of Rule No. 13 (FSSAI), the quantity of sample of food to be sent to the Public Analyst / Director for analysis shall be as specified in the table below:

Article of food	Approximate quantity to be supplied
1. Milk	500 ml.
2. Sterilized Milk/UHT Milk	250 ml.
3. Malai/Dahi	200 gms.
4. Yoghurt/Sweetened Dahi	300 gms.
5. Chhana/Paneer/Khoya/Shrikhand	250 gms.
6. Cheese/Cheese spread	200 gms.
7. Evaporated Milk/Condensed Milk	200 gms.
8. Ice-Cream/Softy/Kulfi/Ice Candy/Ice lolly	300 gms.
9. Milk Powder/Skimmed Milk Powder	250 gms.
10. Infant Food/Weaning Food	500 gms.
11. Malt Food/Malted Milk Food	300 gms.
12. Butter/Butter Oil/Ghee/Margarine/Cream/ Bakery Shortening	200 gms.
13. Vanaspati, Edible Oils/Fats	250 gms.
14. Carbonated Water	600 ml.
15. Baking Powder	100 gms
16. Arrow root/Sago	250 gms
17. Corn flakes/Macaroni Products/ Corn Flour/ Custard Powder	200 gms.
18. Spices, Condiments and Mixed Masala(Whole)	200 gms.
19. Spices, Condiments and Mixed Masala (Powder)	250 gms.
20. Nutmeg/Mace	150 gms.
21. Asafoetida	100 gms.
22. Compounded Asafoetida	150 gms.
23. Saffron	20 gm.
24. Gur/jaggery, Icing Sugar, Honey, Synthetic Syrup, Bura	250 gms.
25. Cane Sugar/Cube sugar/Refined Sugar/Dextrose, Misri/Dried Glucose Syrup.,	200 gms
26. Artificial Sweetener	100 gms.
27. Fruit Juice/Fruit Drink/Fruit Squash	400 ml
28. Tomato Sauce/Ketch up/Tomato Paste, Jam/ Jelly/ Marmalade/Tomato Puree/Vegetable Sauce	300 gms.
29. Non Fruit Jellies	200 gms.
30. Pickles and Chutneys	250 gms.

31. Oilseeds/Nuts/Dry Fruits	250 gms.
32. Tea/Roasted Coffee/Roasted Chicory	200 gms.
33. Instant Tea/Instant Coffee/Instant Coffee Chichory Mixture	100 gms.
34. Sugar Confectionery/Chewing Gum/Bubble Gum	200 gms.
35. Chocolates	200 gms.
36. Edible Salt	200 gms.
37. Iodised Salt/ Iron Fortified Salt	200 gms.
38. Food Grains and Pulses (Whole and Split)	500 gms.
39. Atta/Maida/Suji/Besan/Other Milled Product/ Paushtik and Fortified Atta/Maida	500 gms.
40. Biscuits and Rusks	200 gms.
41. Bread/Cakes/Pastries	250 gms.
42. Gelatin	150 gms.
43. Catechu	150gms.
44. Vinegar/Synthetic Vinegar	300 gms.
45. Food colour	25 gms.
46. Food colour preparation (Solid/Liquid)	25 gms Solid/100 ml liquid
47. Natural Mineral water/Packaged Drinking water.	4000 ml in three minimum original sealed packs.
48. Silver Leafs	1 gm
49. Prepared Food	500 gms.
50. Proprietary Food, (Non Standardised Foods)	300 gms.
51. Canned Foods .	6 sealed cans
52. Food not specified	300 gms.

Note: - Foods sold in packaged condition (Sealed container/package) shall be sent for analysis in its original condition without opening the package as far as practicable, to constitute approximate quantity alongwith original label. In case of bulk packages wherever preservatives to be added, as per the requirement under these rules, the sample shall be taken after opening sealed container or package and the contents of the original label shall also be sent alongwith the sample for analysis. However, such samples shall not be fit for microbiological analysis.

Rule 14. Contents of one or more similar sealed containers having identical labels to constitute the quantity of food sample:-

Where food is sold or stocked for sale or for distribution in sealed containers having identical label declaration, the contents of one or more of such containers as may be required to satisfy the quantity prescribed in shall be treated to be a part of the sample.

Rule 15. Quantity of samples of food packaging material to be sent to the public analyst :-

The quantity of sample of food packaging material to be sent to Public Analyst / Director for analysis shall be as specified below:-

<i>Name of food packaging material</i>	<i>Approximate quantity / surface area to be supplied</i>
Food packaging material of plastic origin	8 x 100 x 9 sq. cm surface area.

Rule 16. Quantity of sample to be sent considered as sufficient :-

Notwithstanding anything contained in Rule 13 and rule 15, the quantity of sample sent for analysis shall be considered as sufficient unless the public analyst or the Director reports to the contrary.

Payment of cost: Where a Food Safety Officer or the purchaser takes a sample of **an** article of food for analysis or an authorized officer takes a sample of imported article of food for analysis, he shall pay, the cost of such sample, to the person from whom the sample is taken calculated at the rate at which the article is sold to the public.

Provided that in case where the sample of article of food is imported, the cost of sample as well as the cost of testing is to be borne by the importer.

Sample Preparation, Sealing/Packing, Identification and Dispatch

Preparation

Samples to be taken in clean bottles, jars or other suitable containers: Sample of article of food (whether imported or domestic) for the purpose of analysis shall be taken in clean dry bottles or jars or in other suitable containers which shall be closed sufficiently tight to prevent leakage, evaporation or to avoid entrance of moisture in case of dry substance and shall be carefully sealed.

The person taking the sample shall divide the sample in four parts and mark and seal or fasten up each part in such a manner as its nature permits and take the signature or thumb impression of the person from whom the sample has been taken.

PROVIDED that where such person refuses to sign or put his thumb impression, the Food Safety officer or Authorized officer taking the sample shall call upon one or more witnesses and take his signature or thumb impression, in lieu of the signature or thumb impression of such person.

PROVIDED FURTHER that in case the paper slip containing the signature of the Designated Officer is of such a size that it does not cover completely from the bottom to the top of the container, the Food Safety Officer shall affix additional sheet/s of paper to the slip containing the signature of the Designated Officer so as to cover the container completely and the Food Safety Officer shall affix his signature on each of the joints for the purpose of identification.

PROVIDED FURTHER that where the sample is taken by a purchaser or an authorized officer, the paper slip need not contain the signature of the Designated Officer.

Bottles or containers to be labeled and addressed: All bottles or jars or other containers containing the samples for analysis shall be properly labeled and the parcel shall be properly addressed. The label on any sample of food sent for analysis shall bear

- i. Code number of the sample
- ii. Name of the sender with his official designation
- iii. Date and place of collection
- iv. Nature of articles being sent for analysis

v. Nature and quantity of preservative, if any, added to the sample

PROVIDED that in the case of a sample which has been taken from Agmark sealed container, the label shall bear the following additional information

- (i) Grade
- (ii) Agmark label No./Batch No.
- (iii) Name of packing station.

After a sample is collected, the FSO/ Authorized Officer must mark and seal it so that its integrity and identity are properly maintained. The sample must be prepared, handled and dispatched to prevent breakage or spoilage, and to ensure that the sample examined by the laboratory is the same as that collected and documented by the FSO/ Authorized Officer.

IDENTIFICATION

In order to enable the FSO/ Authorized Officer to identify his sample subsequently, he should mark each sampling unit with a number, date of collection and his initials. This identification mark can be made on the labels of retail packages, on the coded end of tin cans or of jars, or on the label attached to a special sample container. It is important that the identifying mark be permanent, and ingenuity may be required to develop a satisfactory way of identifying some units. Plastic or wax-paper containers present a special problem, since they will not take ink permanently. Felt-tip pens, using phenol-base inks, are suitable for writing on many surfaces.

Every subsample must be marked with a separate "subsample number". Where significant, this number must be correlated with the manufacturing control code number. When multiple subsamples are taken from the same case, bale, box, etc. in the lot, a combination of letter and a number may be used. For example, if two tins are taken from each case in a lot, the cans may be marked with subsample numbers 1a, 1b, 2a, 2b, etc. to identify units a and b from case No.1, units a and b from case No.2 etc.

PACKING AND SEALING THE SAMPLES

In order to maintain integrity, packages containing exhibits should be secured or sealed to prove their authenticity, i.e., to ensure that they could not have been tampered with or changed from the time they left the FSO/ Authorized Officer's hands until they were received at the laboratory. The seal can be a self-adhering, specially printed paper sticker with space for the date, the FSO/ Authorized Officer's signature, Other adhesive substances, such as sealing-wax or paraffin, that can be imprinted with a special, distinctive design or hologram, may also be used.

i.The stopper shall first be securely fastened so as to prevent leakage of the contents in transit

ii.The bottle, jar or other container shall then be completely wrapped in fairly strong thick paper. The ends of the paper shall be neatly folded in and affixed by means of gum or other adhesive.

iii.A paper slip of the size that goes round completely from the bottom to top of the container, bearing the signature of the Designated Officer and code number of the sample, shall be pasted on the wrapper, the signature or thumb impression of the person from whom the sample has been taken, shall be affixed in such a manner that the paper slip and the wrapper both carry a part of this signature or the thumb impression

iv. The paper cover shall be further secured by means of strong twine or thread both above and across the bottle, jar or other container and the twine or thread shall then be fastened on the paper cover by means of sealing wax on which there shall be distinct and clear impression of the seal of the sender, of which one shall be at the top of the packet, one at the bottom and the other two on the body of the packet. The knots of the twine or thread shall be covered by means of sealing wax bearing the impression of the seal of the sender. **The outer covering of the packet shall also be marked with the code number of the sample.**

DISPATCH OF SAMPLE

After the sample is prepared and sealed, it may be necessary to dispatch it to an authorized examining laboratory. The sample must be handled and packaged in such a manner that subsequent handling will not change its identity or cast doubt on its integrity. All samples packaged for dispatch must be secured with shock-absorbing materials to protect them from damage en route.

Samples of frozen foods to be sent overnight may be packed in insulated cartons containing dry ice that will last for that length of time. To prove that the sample did not thaw in transit, a jar containing chips of ice may be included in the package. If thawing did not take place, the chips will be intact on arrival of the sample parcel. Liquid samples requiring chilling must not be frozen, but should be maintained chilled during dispatch.

Since abnormal metal containers such as hard swells may explode in transit, they should be wrapped heavily in paper and cushioning material for dispatch, and submitted promptly.

If special precautions in handling or storing samples are needed, the FSO/ Authorized Officer should ensure that persons who will be handling the samples are informed. If the sample is to be kept frozen, this should be clearly shown on the package. If the sample is fragile, the package should be so marked.

When frozen, perishable or dangerous items are sent, the receiving laboratory should be notified of the dispatch by telephone or telegram, with the following information: sample number, name of product, number of samples in the parcel, name or carrier, waybill number, estimated time of arrival, and any other pertinent remarks. The collection of such samples can also be scheduled so that they arrive at the laboratory on a pre-arranged day of the week. This will enable the laboratory to perform any analyses, especially microbiological, during regular working hours. Dispatch may be difficult, depending on road conditions, availability of air connections, season of the year and so forth. These factors must be carefully considered so that analytical results have relevance to the food which was sampled.

DOCUMENTATION -

There are specific formats of various procedural formalities which have to be completed by respective officers. This enables clarity of the process being carried out.

- Seizure of articles of food by the Food Safety Officer and matters connected therewith (as per Part 3.3 ;Food Safety & Standards Rule,2009)

Rule 3.3.1.: Form of receipt for article of food seized by a Food Safety officer– For every article of food seized under clause (b) of sub-section 1 of Section 38 of the Act, a receipt in **Form II** shall be given by the Food Safety Officer to the person from whom the article of food was seized.

Rule 3.3.2 Form of order/bond not to dispose of the stock–

Where the Food Safety Officer keeps any article of food in the safe custody of the vendor under clause (c) of sub-section (1) of Section 38

Article

1. He shall, after sealing such article of food, make an order to the vendor in **Form III (as mentioned below)** and the vendor shall comply with such an order, and
2. He may require the vendor to execute a bond in **Form IV. (as mentioned below)**

FORM III

[Refer rule 3.3.2.(1)]

(to keep any article of food in safe custody of the vender)

To

(Name and address of the vendor)

.....
.....
.....

Whereas *.....intended for food which is in your possession appears to me to be adulterated/misbranded:

Now therefore under clause (c) of sub-section (1) of section 38 of the Food Safety and Standards Act, 2006 (34 of 2006), I hereby direct you to keep in your safe custody the said sealed stock subject to such orders as may be issued subsequently in relation thereto.

Food Safety Officer

Area.....

Place:

Date:

**Here give the name of article of food.*

FORM IV
[Refer rule 3.3.2.(2)]
(Vendor to execute bond)

SURETY BOND

Know all men by these presents that we (i)son of.....resident ofand (ii) son ofresident of.....proprietors/partners of Messrshereinafter called the Vendor(s) and (iii).....son ofresident ofand (iv).....son ofresident of.....hereinafter called the surety/sureties are held and firmly borne upto the President of India/Governor of.....hereinafter called the government in the sum ofrupees to be paid to the government, for which payment will and truly to be made.

We firmly bind ourselves jointly and severally by these presents.

Signed thisday ofwhereas Shri.....Food Safety Officer has seized.....(here, insert the description of materials together with number/quantity and total price hereinafter referred to as the said article) from(specify the place);

An whereas on the request of the Vendor(s) the government agreed to keep the said article in the safe custody of the Vendor(s) executing a bond in the terms hereinafter contained and supported by surety/ two sureties which the Vendor(s) has/have agreed to do.....Now the condition of the above written obligation is such that if in the event of the Vendor(s) failure to produce intact the said article before such court or Authority and on such dates(s) as may be specified by the said Food Safety Officer from time to time the Vendor(s) and /or the surety/sureties forthwith pay to the government on demand and without a demur sum ofrupees the said bond will be void and of no effect. Otherwise the same shall be and remain in full force and virtue.

These presents further witness as follows:

(i) The liability of the surety/sureties hereunder shall not be impaired or discharged by reason of time being granted by or any forbearance, act or omission of the government whether with or without the knowledge or consent of the sureties or either of them in respect of or in relation to all or any of the obligations or conditions to be performed or discharged by the Vendor(s). Nor shall it be necessary for the government to sue the Vendor(s) before suing the sureties or either of them for the amount due, hereunder.

(ii) This Bond is given under the Food Safety and Standards Act,2006 for the performance of an Act in which the public are interested.

(iii) The government shall bear the stamp duty payable on these presents.

In witness whereof these presents have been signed by the Vendor(s) and the surety/sureties the day hereinabove mentioned and by Shri.....on behalf of the President of India on the date appearing below against his signature.

Witnesses:

1..... (Signature)
(Name and address).....

2..... (Signature)
(Name and address).....

Signature..... (Vendor).....

Signature..... (Vendor).....

Signature..... (Surety).....

Signature..... (Surety).....

for and on behalf of the President
of India / governor of
Signature.....
(Designation).....

Form of Notice: The Notice to be given by the Food Safety Officer or an authorized officer or the purchaser to the person from whom he has taken the sample and to the person, if any, whose name and address and other particulars have been disclosed **under** rule 3.6 **of** these Rules, shall be in **Form No. V**.

FORM V
(Refer rule 3.4.1. (3))

To

.....
.....

Dear Sir/s/ Madam:

I have this day taken from premises ofsituate at.....
.....samples of food specified below to have the same analysed
by the Food Analyst for _____.

Details of food:

Code number:

Place:

Sd/-) Food Safety Officer

Date:

Address:

Manner of dispatching containers of samples: The containers of the samples shall be dispatched in the following manner namely

a) the sealed container of one part of the sample for analysis and a memorandum in **Form VI** shall be sent in a sealed packet to the Food Analyst not later than the immediate succeeding working day by any suitable means.

FORM VI
(Refer rule 3.4.3 (7))

Memorandum to Food Analyst

From:

.....

..... Date: _____

To

Food Analyst

.....

.....

MEMORANDUM

(Refer rule (v)a of 3.4.1(8))

1. The sample described below is sent herewith for analysis under ___ of ___ of section ___ of Food Safety and Standards Act, 2006

- (i) Code Number
- (ii) Date and place of collection
- (iii) Nature of articles submitted for analysis
- (iv) Nature and quantity of preservative, if any, added to the sample.

2. A copy of this memo and specimen impression, of the seal used to seal the packet of sample are being sent separately by post/courier/hand delivery (strike out whichever is not applicable)

(Sd/) Food Analyst
Address:

b) the sealed container of the second and third parts of the sample and two copies of memorandum in Form VII shall be sent to the Designated Officer immediately but not later than the succeeding working day by any suitable means and

c) the sealed container of the remaining fourth part of the sample and a copy of memorandum in **Form V** shall be sent to an accredited laboratory, if so requested by the food business operator, under intimation to the Designated Officer.

PROVIDED that in the case of a sample which has been taken from Agmark sealed container, the label shall bear the following additional information

- (i) Grade
- (ii) Agmark label No./Batch No.
- (iii) Name of packing station.

Memorandum and impression of seal to be sent separately:

The Food Safety Officer shall send to the Food Analyst to whom the sealed container of first part of the sample was sent, a copy of the memorandum and specimen impression of the seal used to seal the packet and the same shall be sent not later than the immediate succeeding working day, by any suitable means.

Addition of preservatives to samples

The Food Safety Officer or the authorized officer, while taking sample for the purpose of analysis under the provisions of the Act may add to the sample, a preservative as may be prescribed from time to time in the rules or in the regulations for the purpose of maintaining it in a condition suitable for analysis.

COMMODITY SPECIFIC SAMPLING PROCEDURES

(As per ISO/BIS specifications)

A major priority of any specific food sampling programme should be that of providing protection of food from microbiological contamination, chemical hazards and natural toxicants.

1. Food products such as meat, poultry, fish, milk and dairy products, processed eggs, improperly processed low-acid canned foods, foods prepared and sold at the street level and in open markets are major items of concern from the microbiological safety point of view. Other products such as groundnuts, pulses and cereal grains can become contaminated with fungal toxins under certain growing and storage conditions.
2. Products such as flour, pulses and baked goods, while not normally involved in food-borne disease outbreaks of microbial origin, are prone to serious insect and rodent infestation. While contamination by rodents and insects may be more of an aesthetic than true health hazard, generalizations about this subject should be avoided. Rodents and insects have been shown to be vectors in food-borne disease and thus vigilance in controlling their invasions into food products is essential. Furthermore, contamination of food by rodents and insects results in major food losses and, from this perspective alone, adequate control measures are essential.
3. Certain other foods are targets for adulteration. For example, a relatively inexpensive oil may be substituted for a more expensive oil and sold fraudulently as the more expensive product. Similarly, colours are often used fraudulently to increase the appeal of certain foods. All too often, adulteration crosses the economic fraud area when toxic or inedible adulterants are used and human illness and chemical poisoning result.
4. Storage and handling of foods in jute, canvas or other porous bags or sacks are another area requiring close surveillance. Spills of hazardous chemicals on such bags can become a major source of food-borne illness with significant lethal potential for those consuming the contaminated food product.
5. Food control also includes responsibilities for prevention of economic fraud. Inspection activities should, therefore, include review of labelling, check of net contents, identity and compositional aspects of food standards, and false and misleading food advertising. These activities should be integrated within the food sampling programme.

1. Sampling of Milk and Milk Products.
(As per IS: 11546-1999, ISO: 707-1997)

2. **ITEMS:** Milk, Evaporated Milk, Sweetened Condensed Milk, Milk Concentrates, Edible Ices, Semi-Processed Ices, other Frozen Milk Products, Dried Milk and Dried Milk Products, Butter and related products, Butterfat and related products, Cheese.

3. **SAMPLING** – This standard gives guidance on methods of sampling milk and milk products for microbiological, chemical, physical and sensory analysis except for sampling of ex-farm milk from individual animals and sampling of milk within quality payment schemes.

4. **General Arrangements**

- The following instructions are not necessarily applicable for routine sampling.
- The parties concerned or their representatives shall be given the opportunity to be present when sampling is performed.
- Whenever special requirements are given for the sampling and/or arise from a specific analysis to be performed, these requirements shall be followed.

4.1 Sampling personnel

Sampling shall be performed by an authorized person, properly trained in the appropriate technique. That person shall be free from infectious disease. Sampling for microbiological examination shall always be undertaken by a person experienced in the technique of sampling for microbiological purposes.

4.2 Sealing and Labelling of Samples

Samples shall be sealed (in the case of a legal requirement or an agreement between the parties concerned) and a label attached, reproducing integrally the identification of the product, the nature of the product and, at least, the identification number, name and the signature (or initials) of the person responsible for taking the samples. If necessary, additional information may be included, such as the purpose of sampling, the mass or volume of the sample, the unit from which the sample was taken and the condition of the product storage conditions at the moment of sampling.

4.3 Replicate Samples

Samples shall be taken in duplicate, or in plural in the case of a legal requirement or an agreement between the parties concerned. It is recommended that additional sets of samples to be taken and retained for arbitration purposes, if agreed between the interested parties.

4.4 Preparation of a Sampling Report

- a) Sample shall be accompanied by a report, signed or initialled by the authorized sampling personnel and countersigned- as far as necessary or agreed by the parties concerned- by witness present. The report shall give the following particulars:
- b) The place, date and time of sampling (mentioning the time of sampling is only require when agreed by the parties concerned);
- c) The names and designation of sampling personnel and of any witness;
- d) The precise method of sampling, if this differs from the instructions given in this international standard;
- e) The nature and number of units constituting the consignment, together with their batch code markings, where available;
- f) The identification number and any four markings of the batch from which the samples were taken;
- g) The number of samples duly identified as to the batches from which they were taken;

- h) If possible, the name and address of the producer or trader or of the person responsible for packing the product.

When appropriate, the report shall also include any relevant conditions or circumstances (for example, the condition of the product containers and the surroundings, the temperature and humidity of the atmosphere, the age of the product, method of sterilization of the sampling equipment, whether a preservative substance has been added to the samples), and any special information relating to the product being sampled, for example difficulty in achieving homogeneity of the product.

5. Apparatus

5.1 Sampling equipment

5.1.1 General

Sampling equipment shall be made of stainless steel, or other suitable material of adequate strength, which does not bring about a change in the sample which could affect the results of subsequent examination. All surfaces shall be smooth and free from cervices. All the corners shall be rounded. The equipment shall be dry prior to use.

5.1.2 Sampling for microbiological examination

Sampling equipment shall be clean and sterilized prior to use. Disposable plastic equipment shall be sterile. If solder is used in the manufacture of the equipment, it shall be capable of withstanding a temperature of 180°C. If possible, sterilization shall be performed by one of the two following methods:

Method A: Exposure to hot air at 170°C to 175°C for not less than two hours.

Method B: Exposure to steam at 121° C + 1°C for not less than 20 minutes in an autoclave.

After sterilization by method A or Method B, Sampling equipment shall be stored under sterile conditions prior to use.

If, in a particular situation, sterilization by Method A or B is impossible, the following alternative methods, which shall be regarded as secondary methods only, can be used, provided that the sampling equipment is used immediately after sterilization:

Method C: Exposure to a suitable flame so that all working surfaces of the sampling equipment come into contact with the flame;

Method D: Immersion in at least 70% (V/V) ethanol solution;

Method E: Ignition with 96% (V/V) ethanol.

CAUTION 96% ethanol is hygroscopic and may change its concentration over a period of time.

Method F: Exposure to a sufficient dose of gamma radiation.

After sterilization by method C, D or E, sampling equipment shall be cooled under sterile conditions or, in the case of method D, be rinsed with the ethanol solution before sampling.

5.1.3 Sampling for chemical and physical analysis and sensory examination

Sampling equipment shall be clean and dry and shall not influence the properties, such as odour, flavour or consistency or the composition of the product. In some cases sterile equipment is required to avoid microbial contamination of the product.

5.2 Sample Containers

- Sample containers and closers shall be of materials and construction which adequately protect the sample and which do not bring about the change in the sample which could affect the results of subsequent analysis or examinations. Materials which are appropriate include glass, some metals (e.g. stainless steel) and some plastics (e.g. poly propylene). The containers should preferably be opaque. If necessary, transparent filled containers shall be stored in a dark place. Containers and closures shall be dry, clean and either sterile or suitable for sterilization one of the methods described in 5.1.2.
- The shape and capacity of the containers shall be appropriate to the particular requirements of the product to be sampled (sterile and non sterile) and suitable plastic bags, with appropriate methods of closure, may also be used.
- Containers other than plastic bags shall be securely closed either by means of a suitable stopper or by means of a screw –cap of metal or plastic material, having, if necessary, a liquid- tight plastic liner which is insoluble, non- absorbent and grease proof, and which will not influence the composition, properties or the odour and flavour of the sample.
- If stoppers are used, they shall be made from, or covered with, non- absorbent, odourless and flavourless material.
- Containers for samples for microbiological examination shall not be closed with cork stoppers or caps with cork seals, even if provided with liners. Containers for solid, semi-solid or viscous products shall be wide- mouthed
- In the case of small retail containers, these are considered as sample containers: the sample shall consist of the contents of one or more intact, un-opened containers.
- Requirements for insulated containers for the transport of old, frozen or quick-frozen are samples are given in annex B.

6. Sampling technique

- Sampling shall be carried out in such a way as to obtain representative samples of the product.
- If samples for microbiological, chemical and physical analysis and sensory examinations are taken separately, samples for microbiological examinations shall be taken first using aseptic technique and containers 5.1.2.
- Care shall be taken to assure that when taken samples for sensory examinations the flavour of the samples is not adversely affected by sterilization of the sampling equipment or sampling cocks, e.g. flaming with ethanol.
- The precise method of sampling and the mass or volume of product to be taken varies with the nature of the product and the purpose for which samples are required. If product contains coarse particles, it may be necessary to increase the minimum sample size. The sample containers shall be closed immediately after sampling.
- For small retail containers, the sample consists of one or more un- opened containers. If necessary, a further sample should be taken for temperature control during transportation to the testing laboratory

7. Preservation of the samples

Preservatives shall normally not be added to samples intended for microbiological or sensory examination.

Preservatives may be added to some milk products, provided that

- ✓ An instruction to do so is issued by the testing laboratory,
- ✓ The preservative is of a nature that does not interfere with subsequent analysis, and testing of texture and flavour shall not be performed,
- ✓ The nature and quantity of preservative are stated in the sampling report and, preferably, indicated on the label.

8. Storage and transport of samples

Storage and dispatch of the samples shall be such that the state of the sample at the time of sampling is not adversely affected to any considerable extent. During transport, where necessary, precautions should be taken to prevent exposure to off-odours, direct sunlight and other adverse conditions.

If cooling is necessary, the minimum requirements to meet are the temperature ranges which are either legally requested or specified by the manufacturer. The storage temperature after sampling should be attained as quickly as possible. The time and temperature shall be considered in combination and not independently. Storage temperatures are given in Table 1.

TABLE-1 SAMPLE PRESERVATION, STORAGE TEMPERATURE AND MINIMUM SAMPLE SIZE

Sampling according to Clause	Product	Preservation Permitted For Samples intended For Chemical and Physical Analysis	Temperature ¹⁾ Before and During Transport (°C)	Minimum Sample Size
9	Non –sterilized milk and liquid milk	Yes	0-4	100 ml or g
9	Sterilized milk, UHT milk and sterilized liquid milk products in unopened containers	No	Ambient, max. 30	100 ml or g
9	Sterilized milk, UHT milk and sterilized liquid milk products after sampling from the production line or from one or more original packs	Yes	0-4	100 ml or g
10	Evaporated milk, Sweetened Condensed milk and Milk Concentrates	No	Ambient, max. 30	100 g
11	Semi- Solid and solid milk products except Butter and Cheese	No	0-4	100 g
12	Edible Ices and Semi- Finished Ice Products	No	-18 or lower	100 g
13	Dried milk and Dried milk Products	No	Ambient, max. 30	100 g
14	Butter and Butter Products	No	0-4(In The Dark)	50 g
15	Butter Fat(Butter Oil and similar products)	No	0-4 (In The Dark)	50 g
16	Fresh Cheese	No	0-8	100 g
16	Processed Cheese		Ambient, max. 30	100 g
16	Other Cheeses		4-8	100 g
<p>1) The temperature mentioned in the table is meant as general guidelines. For specific analysis purposes, other temperatures may be more appropriate. It may be, under certain practical conditions, not always easy or even impossible to maintain the “ideal” or desirable temperatures specified in this table. This therefore recommended to use suitable containers in all cases where it is necessary (see also annex B) and to monitor and record temperatures in a suitable way.</p> <p>2) A large sample size may be necessary according to the test required and the type of product.</p>				

Samples shall be dispatched to the testing laboratory immediately after sampling. The time for dispatch of the samples to the testing laboratory shall be as short as possible, preferably within 24 h. If requested, samples shall be dispatched as instructed by the testing laboratory.

9. MILK AND LIQUID MILK PRODUCTS

9.1 APPLICABILITY

The instructions given in this clause are applicable to raw and heat-treated milk (except raw milk from individual animals and raw milk taken within quality payment schemes), whole, partly skimmed and skimmed milk, flavoured milk, cream, fermented milk, butter milk, liquid whey and similar products.

9.2 APPARATUS

Sampling equipment shall correspond to that given in clause (2).

9.2.1 Apparatus for Manual Mixing

Agitators for mixing liquids in bulk shall have a surface sufficient to produce adequate disturbance of the products. In view of the different shapes and sizes of containers, no specific designs of agitators can be recommended for all purposes, but they shall be designed in such a way as to avoid damage of the inner surface of the container during mixing.

9.2.1.1 Apparatus for Manual Agitation in Small Vessels

For mixing liquids in small vessels (e.g. in buckets and cans) a stirrer (plunger) of the design and dimensions as shown in figure A.1 is suitable. The length shall be adjusted to the depth of the vessel.

9.2.1.2 Apparatus for Manual Mixing In Large Vessel

A stirrer (plunger) of the design and the dimensions as shown in figure A.2 is suitable for use for larger vessels (e.g. road and farm tanks).

9.2.2 Apparatus for Mechanical Agitation

9.2.2.1 Built in Agitators

The product to be mixed in tank or vessel determines the technical characteristics and construction of built –in agitator.

Various types of agitators are used but no attempt has been made to describe any of them in this international standard.

9.2.2.2 Removable Agitators

They are usually provided with a propeller and are introduced into transport, road and rail tanks through the manhole. Best stirring results are achieved at a depth corresponding to 0, 7 of the filling height. It is recommended that the stirrer be inclined 5° to 20° as this allows vertical mixing of the liquor liquid as well as horizontal movement.

9.2.3 Apparatus for Taking Samples

9.2.3.1 Apparatus for Sampling

A dipper of the shape and size as shown in figure A.3 is suitable to be used for sampling. The tapered form of the cup permits nesting of the dippers.

9.2.3.2 Sample Containers

The capacity of the sample containers shall be such that they are almost completely filled by the samples and allow proper mixing of the contents before testing, but avoid churning during transport.

9.2.3.3 Thermally Insulated Transport Containers

See annex B.

9.3 SAMPLING

Thoroughly mix all liquids, by inverting stirring, by pouring to and from one product container to another of the same volume, until sufficient homogeneity is obtained. The equipment described in (9.2.1 and 9.2.2) may be used.

Take the sample immediately after mixing. The size of the sample shall not be less than 100 ml.

9.3.1 Sampling for microbiological examination

Take samples for microbiological examination always first using aseptic techniques, wherever possible, from the same product containers as those taken for chemical and physical analysis and for sensory examination. Sterilized sampling equipment and sample containers as described in 5.1.2. Proceed as described in 9.3.2.

9.3.2 Sampling for chemical and physical analysis and sensory examination

In certain cases sampling equipment and sample containers shall be sterile for chemical and physical analysis and sensory examination.

9.3.2.1 Small vessels, Milk buckets and Cans

Thoroughly mix the milk, for example by transfer, stirring or plunging (plunger).

9.3.2.2 Milk Tanks or Vats

Mechanically agitate the milk for at least 5 minutes, until sufficient homogeneity is obtained. If the tank is equipped with a periodical, time programmed agitation system, sampling may be carried out after only a short duration of agitation (1-2 minutes). In those instances where the propeller of the agitator is close to the surface of the milk, do not use the agitator since this is likely to lead to the formation of foam.

9.3.2.3. Weighing bowl

It is essential for the milk to be adequately mixed in the weighing bowl if a representative sample is to be obtained. The degree of mixing achieved when milk is tipped into the weighing bowl varies and does not allow proper sampling it is essential to supplement this by additional agitation. The amount of additional mixing shall be determined by experiment. When the volume of milk to be sampled exceeds the capacity of the weighing bowl, a sample representative of the whole consignment shall be obtained.

9.3.2.4 Large vessels, storage, rail and road tanks

In each case, thoroughly mix the milk by an appropriate method before sampling, for example mechanical agitation, stirring with clean compressed air without foaming or by plunging (plunger). When compressed air is used, any adverse influence on the product to be mixed must be avoided.

The extent of mixing depends on the period of time over which the milk has been a rest.

In those instances where the propeller of the agitator is close to the surface of the milk, do not use the agitator since this is likely to lead to the formation of foam.

Mixing using a plunger or a removable agitator to be used in road, rail tanks or vessels of similar size shall be performed as follows.

- a) When samples are taken within 30 min after filling the container, mix the milk for at least 5 min by plunging or stirring with an agitator; when the milk has been stored in the tank for a long period of time mixing shall be extended to at least 15 min;
- b) When the tank is completely filled as is normally the case with transport, road and rail tanks, proper mixing of milk showing pronounced creaming phenomena can only be achieved by mechanical agitation.

In a large vessel the bottom discharge outlet or a sampling cock installed at another place, there may be, at the discharge outlet, a small quantity of milk which is not representative of the whole contents even after mixing. Accordingly the samples preferably be taken through the manhole. If samples are taken from the discharge outlet valve or the sampling cork, discharge sufficient milk to ensure that the samples are representative of the whole.

The efficiency of the method of mixing applied in any particular circumstances shall be demonstrated as being adequate for the purposes of the analysis envisaged; the criterion of mixing efficiency is the repeatability of analytical results from samples taken either from the different parts of the whole, or from the tank at intervals during discharge.

9.3.2.5 Containers of different design

Special equipment will be required from taking samples from shallow containers.

9.3.2.6 Sub divided quantities

Unless a part of the bulk is to be tested individually, take a representative quantity from each container after mixing the contents and state the quantity and the container relative to the sample in the sampling report, as described in 4.4.

9.3.2.7 Sampling from closed systems

For taking samples from these systems (e.g. UHT plants, aseptic techniques), in particular for microbiological analysis, the working instructions for the installed sampling equipment shall be observed.

9.3.2.8 Retail containers

The content of an intact and unopened container constitutes the sample.

9.3.3 Applicability to products other than milk

9.3.3.1 Butter milk, fermented milk and flavoured milk

Choose a suitable method from those described for milk and take a sample before fat or other solid matter has had time to separate. If the latter has occurred, proceed to take a representative sample from a homogenous product as described in c.1.

9.3.3.2 Cream

When using a plunger or a mechanical agitator for mixing cream, thoroughly mix the cream at the bottom of the container with the upper layers.

To avoid foaming, whipping or churning of the cream, do not raise the disc of the plunger above the surface of the cream during plunging. The equipment described in b.1 (see figures A.1 and A.2) may be used. When mechanical agitators are used, avoid the incorporation of air.

9.3.3.3 Whey

Choose a suitable method from those described for milk.

9.4 PRESERVATION, STORAGE AND DISPATCH OF SAMPLES

See clauses 4 and 5.

10 EVAPORATED MILK, SWEETENED CONDENSED MILK AND MILK CONCENTRATES

10.1 APPLICABILITY

The instructions given in this clause are applicable to evaporated milk, sweetened condensed milk and milk concentrates and similar products.

10.2 SAMPLING EQUIPMENT

See.5.1

10.2.1. Mixers

See (9.2.1 and 9.2.2).

10.2.2. Stirrers

Stirrers, broad- bladed, of sufficient length to reach the bottom of the product container and having one edge shaped to the contour of the container (see figure A.4).

10.2.3 Dippers

See (9.2.3).

10.2.4 Rods

Rods, about 1 m long, of diameter about 35 mm.

10.2.5 Containers

Containers, for sub sampling, of capacity 5 litres, wide- mouthed.

10.2.6. Spoons or spatula

Broad- bladed.

10.2.7 Sample containers

See 5.2.

The capacity of the sample containers shall be such that they are almost completely filled by the sample and allow proper mixing of the contents before testing.

10.3 SAMPLING EVAPORATED MILK

Take the sample immediately after mixing. The sample size shall not be less than 100 g.

10.3.1 Sampling for microbiological examination

Always take the samples for microbiological examination first using aseptic techniques and, whenever possible, from the same product containers as those taken for chemical and physical analysis and for sensory examination. Sterilize the sampling equipment and sample containers as described in 5.1.2.

Proceed as described in 10.3.2, however using aseptic techniques.

10.3.2 Sampling for chemical and physical analysis and for sensory examination

In certain cases, the sampling equipment and sample containers shall be sterile for chemical and physical analysis and sensory examination.

10.3.2.1 Large vessels (e.g. 2 kg and 4 kg)

Thoroughly mix the evaporated milk by plunging or stirring using a manual stirrer, by mechanical agitation, or by pouring from one container to another, until sufficient homogeneity is obtained.

However, in most cases, sufficient distribution of fat is only obtained if the containers have been left standing in warm water at about 45°C for 30 min before shaking. If it proves difficult to obtain sufficient homogeneity, take samples from different portions of the product container to obtain a representative total sample of not less than 100 g. State on the label and in the sampling report if the sample is a mixture of subsamples (see 4.4).

10.3.2.2 Large vessels (containers) of 500 kg and more and road tanks

Mixing is, in principle, performed in the same manner as described for milk (9.3.2.4). The intensity of mixing is dependent on the degree of concentration.

10.3.2.3 Retail containers

The content of an unopened container constitutes the sample. Take one or more containers to provide a sample size of not less than 100 g.

If a sample is taken from retail containers, preheat it before as described in (10.3.2).

10.4 SAMPLING SWEETENED CONDENSED MILK AND MILK CONCENTRATES

Take the sample immediately after mixing. The sample size shall not be less than 100 g.

10.4.1 General

The sampling of bulk containers may be a matter of extreme difficulty, particularly when the product is not homogenous and is highly viscous.

Problems of sampling may arise through the presence of large crystals of sucrose or lactose, through precipitation of various salts which may occur throughout the body of the product or adhere to the walls or through the presence of lumpy matter. Such conditions will become apparent when a sampling rod is introduced into the product container (see 10.2.4) and is withdrawn after exploring as large the volume of the container as possible. Provided that the size of sugar crystals is not larger than 6 µm, difficulties in sampling should not be experienced from this cause. Since sweetened condensed milk is frequently stored at atmospheric

temperature, it is recommended that the contents of bulk are brought to a temperature of 25°C + 5°C. Crystallized concentrate in storage tanks cannot be sampled representatively unless the tank is designed for and equipped with a power-driven agitator.

When the product is not homogenous and particularly when the crystals are not evenly distributed state this fact in the sampling report (see 4.4). Perform sampling immediately after mixing.

10.4.2 Sampling for microbiological examination

Take the sample for microbiological examination always first using aseptic techniques, whenever possible, from the same product container as those taken for chemical and physical analysis and for sensory examination.

Sterilize the sampling equipment and containers as described in 5.1.2.

10.4.2.1 Bulk containers

Thoroughly clean, sterilize and rinse with cold sterile water the outside end of the product container, or of the drum, if it is an end-opening type (bung hole), before opening the container or removing the end cover (bung). For sterilization, the surface can be flamed using alcohol, repeatedly if required (see 5.1.2).

Proceed as described in 10.4.1. However, using aseptic techniques.

In the case of condensed milk which flows readily and is of uniform consistency, turn drums with bung holes. Take the sample while the product is draining. Bung holes with screw-caps are difficult to disinfect, so particular care shall be taken here. When the product has become viscous, remove the surface layer to a depth of 2 cm to 3 cm by means of a sterile spoon and then take the sample from the drum.

When surface samples are taken, sampling shall be performed according to special instructions corresponding to the particular purpose.

State the type of bulk container in the sampling report (see 4.4).

10.4.3 Sampling for chemical and physical analysis and for sensory examination

In certain cases, the sampling equipment and sample containers shall be sterile for chemical and physical analysis and sensory examination.

10.4.3. Open-ended containers (drums with cover)

Thoroughly clean and dry one end of the container before opening to prevent foreign matter falling into the container during the opening process. Mix the contents by means of a stirrer (see figure A.4). Scrape the blade round the sides and the bottom of the container to remove any adhering product.

Thoroughly mix the contents by a combination of rotary and vertical movements, with the stirrer inclined diagonally, taking care to avoid the incorporation of air in the sample. Withdraw the stirrer and transfer the condensed milk adhering to it into a container (5 litres) by means of a spatula or spoon. Repeat the mixing and withdrawal until 2 litres to 3 litres have been collected. Mix the volume until homogenous and take the sample.

10.4.3.2 Enclosed containers (drums) with outlet (bungs) at one end or at the side

For reasons described in 10.4.1, sampling through the outlet (bung hole) is suitable only with condensed milk which flows readily and is of uniform consistency. Mix the contents by inserting a rod through the bung hole and agitate and stir, as far as possible in all directions.

Withdraw the rod and proceed as described in 10.4.3.1 (sampling with a stirrer).

10.4.3.3 Bulk container of capacity 500 litres with manhole

The procedure is, in principle, the same as for milk (see 9.3.2.5).

10.4.3.4 Retail containers

The content of an intact, unopened container constitutes the sample. Take one or more containers to provide a total sample size of not less than 100 g.

10.5 PRESERVATION, STORAGE AND DISPATCH OF SAMPLES

See clauses 7 and 8.

11. SEMI-SOLID AND SOLID MILK PRODUCTS EXCEPT BUTTER AND CHEESE

11.1 APPLICABILITY

The instructions given in this clause are applicable to puddings, desserts and milk products of the fermented or not fermented, semi-solid or foamed type, with or without the addition of stabilizers, binding agents, fruits, nuts or other ingredients, as well as other products, the semi-solid or solid texture being the common property.

11.2 SAMPLING EQUIPMENT

See 5.1.

11.2.1 Apparatus for mixing

See 9.2.1.

11.2.2 Apparatus for sampling

See 9.2.3.1.

11.2.3 Sample containers

See 5.2.

The capacity of the sample containers shall be such that they are almost completely filled by the sample and allow proper mixing of the contents before testing.

11.3 SAMPLING

The sampling of these varying products from large containers may be a matter of extreme difficulty, particularly when the product is highly viscous or if it contains constituents which may contribute to a particular extent to in homogeneity. Mixing shall, therefore, be adjusted to the particular requirements of the product. If possible, preference should be given to lots of retail containers. In special cases the instructions given in 11.3.2.1 and 11.3.2.2 shall be adjusted to the specific properties of the product.

Take immediately after mixing. The sample size shall not be less than 100 g.

11.3.1 Sampling for microbiological examination

Always take the samples for microbiological examination first using aseptic techniques and, whenever possible, from the same product containers as those taken for chemical and physical analysis and for sensory examination.

Sterilize the sampling equipment and containers as described in 5.1.2.

Proceed as described in 11.3.2, however using aseptic techniques.

11.3.2 Sampling for chemical and physical analysis and for sensory examination

In certain cases, the sampling equipment and sample containers shall be sterile for chemical and physical analysis and for sensory examination. The product type and subsequent examination required are the decisive factors for the sampling technique to be employed.

11.3.2.1 Containers or tanks

Mix the product by plunging or stirring by mechanical agitation until sufficient homogeneity is ensured. Mix gently to avoid foaming, whipping, whey-separation and disruption of lumpy ingredients (see also 9.2.1).

If it proves difficult to obtain sufficient homogeneity, take samples from different portions of the product container to obtain a representative total sample of not less than 100 g. State on the label and in the sampling report if the sample is a mixture of subsamples (see 4.4).

11.3.2.2 Retail containers

The content of an intact, unopened container constitutes the sample. Take one or more containers to obtain a total sample of not less than 100 g. Large containers from which portions are taken for sale or consumption shall be taken as a whole.

11.4 PRESERVATION, STORAGE AND DISPATCH OF SAMPLES

See clauses 7 and 8.

During transit, precautions shall be taken to prevent exposure to vibration.

12 EDIBLE ICES, SEMI-PROCESSED (SEMI-FINISHED) ICES AND OTHER FROZEN MILK PRODUCTS

12.1 APPLICABILITY

The instructions given in this clause are applicable to edible ices, semi-processed ices and other frozen products.

12.2 SAMPLING EQUIPMENT

See 5.1.

12.2.1 Borers

Borers, of sufficient length to reach the bottom of the product container.

12.2.2 Spoon, knife or spatula, or ice scoop

12.2.3 Sample containers

See 5.2.

The sample containers shall be placed in an appropriate thermally insulated transport container (see 9.2.3.3) which has been suitably refrigerated (e.g. with solid carbon dioxide) for not less than 30 min before use.

12.3 SAMPLING

Sampling from containers from which portions are to be taken can best be performed at product temperatures between -12°C and -18°C. If the consistency of the product is too firm for sampling, the whole container constitutes the sample.

The size of the sample shall not be less than 100 g.

12.3.1 Sampling for microbiological examination

Always take samples for microbiological examination first using aseptic techniques and, whenever possible, from the same product containers as those taken for chemical and physical analysis and for sensory examination. Sterilize the sampling equipment and containers as described in 5.1.2.

Use the sterilized spoon, knife or spatula (12.2.2) to remove the surface layer of the product in the centre of the container from the sampling area to a depth of at least 10 mm. Take the sample of adequate size with a sterilized instrument from the area removed. If required obtain a “surface sample” by uniformly scraping the product surface to be tested using a sterilized spoon or spatula to a minimum depth.

When the microbiological condition of the product as presented to the customer is to be examined, the retailer’s vending operations normally used for dispensing shall be applied for the purpose of sampling.

Transfer the sample as quickly as possible into the sterile sample container which shall be closed immediately. Place the container immediately in pre-cooled transport containers (12.2.3).

Proceed as described in 12.3.2, however using aseptic techniques.

12.3.2 Sampling for chemical and physical analysis and for sensory examination

In certain cases, the sampling equipment and sample containers shall be sterile for chemical and physical analysis and for sensory examination.

Transfer the sample immediately after sampling into the pre-cooled transport container.

Take only original packages for physical analysis.

12.3.2.1 Retail containers

Retail containers include small packages, ice lollipops, multi-layered ices and marbled ices.

Collect and dispatch the samples in their original containers, keeping the samples deep frozen until analysed.

12.3.2.2 **Soft Ice**

Soft ice is ice which is sold directly from the freezer. When the condition of the product as presented to the retail customer is to be examined, the retailer's vending operations normally used for dispensing shall be applied for the purpose of sampling.

When information is required regarding the condition of the product in the freezer, take the sample directly from the freezer. To this end, thoroughly clean and disinfect the outlet first, as described in 5.1.2.

Let out a sufficient amount of the product. Fill the requisite number of sample containers in succession from the freezer while it is continuing to operate.

12.3.2.3 **Semi-processed ices**

Sampling of semi-processed ices (as e.g. concentrates and powders for the production of edible ices) is performed as described in clauses 9 to 13.

12.4 PRESERVATION, STORAGE AND DISPATCH OF SAMPLES

See clauses 7 and 8.

The storage and transport temperature may vary according to the purposes of the product and the analysis envisaged. The temperature should be -18°C or in certain cases even lower.

13 DRIED MILK AND DRIED MILK PRODUCTS

13.1 APPLICABILITY

The instructions given in this clause are applicable to products, as, for example, milk powder with different fat contents, dried whey, milk protein products and their derived products, co-precipitates and other powders with high milk protein contents. The method described is also applicable to lactose in powder form.

The sampling method described is not suitable for powders in large bulk containers (silo). In such containers a number of small samples shall be taken during loading or unloading of the container to allow access to the entire consignment (batch). Special attention shall be paid to exclude the influence of atmospheric moisture.

13.2 SAMPLING EQUIPMENT

See 5.1.

13.2.1 Borers

Borers, of sufficient length to reach the bottom of the product container. The borer shall be made entirely of polished stainless steel.

13.2.2 Scoop, spoon or broad-bladed spatula

13.2.3 Sample containers

See 5.2.

The capacity of the sample containers shall be such that they are three-quarters filled by the sample and allow proper mixing of the contents by shaking before testing.

13.3 SAMPLING

Precautions shall be taken to ensure there is no uptake of atmospheric moisture by the contents of the product container during sampling for microbiological examination or in the period prior to sampling for chemical and physical analysis and for sensory examination. The product container shall be securely reclosed after sampling.

The size of the sample shall not be less than 100 g.

13.3.1 Sampling for microbiological examination

Always take samples for microbiological examination first using aseptic techniques and, whenever possible, from the same product containers as those taken for chemical and physical analysis and for sensory examination.

Sterilize the sampling equipment and containers as described in 5.1.2 using Method A or Method B. Pre-sterilized (disposable) equipment may also be used.

Use the sterilized spoon or spatula (13.2.2) to remove the surface layer of the product from the sampling area. Take the sample with a sterilized borer, if possible, from near the centre of the container using the technique described in 13.3.2. Transfer the sample as quickly as possible into a sterilized sample container, which shall be closed immediately, taking aseptic precautions. If there is the likelihood of dispute concerning the microbiological condition of the top layer of powders in the product container, first take a special sample from this layer.

13.3.2 Sampling for chemical and physical analysis and for sensory examination

In certain cases, the sampling equipment and sample containers shall be sterile for chemical and physical analysis and for sensory examination.

Pass the clean dry borer (13.2.1) through the product, if necessary with the container laid on its side, with the slit oriented downwards and an even rate of penetration being used. When the borer reaches the bottom of the container, rotate it through 180°, withdraw it and discharge the contents into the sample container.

According to the purpose of testing envisaged, a sample may also be taken with a scoop.

Immediately close the sample container once sampling is completed.

13.3.3 Retail containers

The content of an intact and unopened container constitutes the sample. Take one or more containers to obtain a sample of not less than 100 g.

13.4 PRESERVATION, STORAGE AND DISPATCH OF SAMPLES

See clauses 7 and 8.

14 BUTTER AND RELATED PRODUCTS

14.1 APPLICABILITY

The instructions given in this clause are applicable to butter, butter with additives and half fat butter, and similar products.

14.2 SAMPLING EQUIPMENT

See 5.1.

14.2.1 Butter triers

Butter triers, of sufficient length to pass diagonally to the bottom of the product container, and of dimensions suited for the purpose envisaged (see figure A.7).

14.2.2 Spatula

Spatula, broad-bladed.

14.2.3 Knife

Knife, of sufficient size.

14.2.4 Sample containers (see 5.2).

The capacity of the sample containers shall be adequate for the size of the sample.

The use of opaque sample containers is recommended. If required for the tests to be performed, wrap the container or core in aluminium foil. Use carton boxes for samples of 2 kg.

In some cases it is essential that the sample containers be completely filled or provided with inert gas and have an airtight closure, e.g. when fat indices are to be determined.

14.2.5 Sample containers, for sensory examination (see 5.2).

Suitable containers include carton boxes, which can be adequately closed and which are provided on the inside with a sufficiently large piece of aluminium foil or plastic-coated parchment paper.

The capacity of the boxes shall be such that they are almost completely filled by the sample.

14.3 SAMPLING

The size of the sample shall not be less than 50 g.

14.3.1 Sampling for microbiological examination

Always take samples for microbiological examination first using aseptic techniques and, whenever possible, from the same product container as those taken for chemical and physical analysis and for sensory examination.

Sterilize the sampling equipment and sample containers as described in 5.1.2.

Use the spatula (14.2.2) to remove the surface layer of the product from the sampling area to a depth of not less than 5 mm. Proceed as described in 14.3.2

using aseptic techniques. Use a sterilized trier each time for taking a core of the product.

For microbiological examination of the surface, sampling shall be performed according to special instructions depending on the purpose envisaged.

14.3.2 Sampling for chemical, physical analysis and for sensory examination

For a number of sensory examinations and especially for physical analyses, take a sample of 2 kg.

In certain cases, the sampling equipment and sample containers shall be sterile for chemical, physical analysis and for sensory examination.

14.3.2.1 Retail containers (with a content of 1 kg or less).

The contents of intact and unopened containers constituting the sample. Take one or more containers to obtain a sample of not less than 50 g.

14.3.2.2 Products in bulk or packets (with content of more than 1 kg).

Pass the butter trier of suitable size from the edge diagonally through the product, ensuring that the trier does not penetrate the bottom surface. Rotate the trier through a half turn and withdraw it with the core.

Discard the upper 25 mm of the core.

Remove the rest of the core by the means of a spatula from the trier and transfer it either directly or after wrapping in aluminium foil to the container. The temperature of the butter, sampling room and the butter trier should be about the same. Sampling of butter stored under deep freezing conditions requires special care and experience.

14.3.2.3 Large containers (for sample sizes of more than 2 kg).

For sampling from a large containers or sample sizes of more than 2 kg, cut with a knife a block of the product which will fit into the sample box, wrap the block in aluminium foil and place in it the box. Avoid deformation of the product during cutting and wrapping.

14.4 PRESERVATION, STORAGE AND DISPATCH OF THE SAMPLES

See clauses 7 and 8.

15. BUTTERFAT (BUTTER OIL) AND RELATED PRODUCTS

15.1 APPLICABILITY

The instructions given in this clause are applicable to anhydrous milk fat, butterfat, butter oil and similar products.

15.2 SAMPLING EQUIPMENT

See 5.1.

15.2.1 Butter triers

Butter triers, of sufficient length to pass diagonally to the bottom of the product containers and of dimensions suited for the purpose envisaged (see figure A.7).

15.2.2 Spatula

Spatula, broad-bladed.

15.2.3 Agitator (plunger), describe in 9.2.1.

15.2.4 Dipper

Dipper, of capacity 25 ml to 100 ml.

15.2.5 Sample containers (see 5.2).

The capacity of the sample containers shall be such that they are almost completely filled by the sample and allow proper mixing of the content before testing.

In some cases it is essential that the sample containers shall be completely filled or provided with inert gas and have an airtight closure; e.g. when fat indices are to be determined.

15.3 SAMPLING

The size of the sample shall not be less than 50 g.

15.3.1 Sampling for microbiological examination

Always take samples for microbiological examination first using aseptic techniques and, whenever possible, from the same product containers as those taken for chemical and physical analysis and for sensory examination.

15.3.2.1 Retail containers (with a content of 1 kg or less).

The contents of an intact and unopened container constitute the sample. Take one or more containers to obtain a sample of 200 g.

15.3.2.2 Product in bulk

15.3.2.2.1 Liquid products

Thoroughly mix the product by plunging or by mechanical agitation until sufficient homogeneity is obtained.

15.3.2.2.2 Solid products

Take a sample as described in 14.3

15.4 PRESERVATION, STORAGE AND DISPATCH OF SAMPLES

See clauses 7 and 8.

16. CHEESE

16.1 APPLICABILITY

The instructions given in this clause are applicable to cheese, in particular hard, extra hard cheese, semi-hard, semi-soft, soft cheese, fresh cheese, acid curd cheese, cheese in brine, pre-packed cheese, processed cheese, processed cheese preparations, flavoured processed cheese and cheese products.

16.2 SAMPLING EQUIPMENT AND CHEMICALS (see 5.1)

16.2.1 Cheese triers

Cheese triers, of shape and size appropriate to the cheese to be sampled (see figure A.6).

16.2.2 Knife

Knife, with a pointed blade and a smooth surface.

16.2.3 Spatula

16.2.4 Cutting wire

Cutting wire, of sufficient size and strength.

16.2.5 Sealing compounds

Sealing compounds, for example a mixture of paraffin, wax and beeswax, prepared by heating, which shall be in compliance with the food law of the specific country.

16.2.6 Ethanol, undenatured, 70% (V/V)

16.2.7 Sample containers (see 5.2)

16.3 SAMPLING

The size of the sample shall not be less than 100 g.

16.3.1 General

Immediately after sampling, place the samples (cores, slices, sectors, entire small cheese, etc.) in a sample container of suitable size and shape. The sample may be cut into pieces for insertion into the container, but it shall not be compressed or ground. Storage of cheese samples tightly wrapped in aluminium foil inside or even outside a sample container is especially suitable to prevent moulding of the cheese surface.

Unless otherwise specified, whatever the method of sampling used, the sample shall include any surface layer of the cheese (such as mould and rind).

If it is necessary to examine the surface layer (for example to examine surface flora), special sampling instructions shall be observed according to the purpose envisaged.

Take the heterogeneity of the product into account when collecting samples.

16.3.2 **Sampling of cheese other than fresh cheese and cheese sold in brine, oil etc.**

Always take samples for microbiological examination first using aseptic techniques and, whenever possible, from the same cheese as those taken for chemical and physical analysis and for sensory examination.

Sampling is performed, depending upon the shape, mass and type, by taking an entire cheese, packed or pre-packed portions or a sector, slices or cores.

16.3.2.1 Sampling by taking an entire cheese, or cheese in pre-packs

This method is normally used for small cheeses, small portions of cheese or pre-packed cheeses.

Take a sufficient number of packets or portions to obtain a sample of not less than 100 g. Place the sample in the original packaging in the sample container (plastic bags, etc.).

16.3.2.2 Sampling by taking sectors, slices or cores

Remove any other wrapping from the cheese; inner wrapping, such as for example wax or plastic film, is not removed.

16.3.2.2.1 Sampling by cutting sectors or slices

Cut by sample by means of a knife of sufficient size or a cutting wire. The sectors or slices shall be of sufficient thickness.

16.3.2.2.2 Sampling by taking cores

16.3.2.2.2.1 Sampling for microbiological examination

Always take samples for microbiological examination first using aseptic techniques and, whenever possible, from the same product (core) as those taken for chemical and physical analysis and for sensory examination.

The amount of sample taken for the surface samples may be smaller than 100 g. The sampling equipment and containers shall be sterile as described in 5.1.2.

Disinfect the cheese surface around the sampling site with undenatured ethanol (16.2.6). For taking cores; take a short core of a larger diameter first. To do this, insert a trier into the cheese to a depth of 25 mm. Rotate the trier through one complete turn and withdraw it with the cor. Keep this core and use it later to close the core. Keep this core and use it later to close the core hole.

If the core is required as a surface sample, immediately place it in a sample container. Then insert a smaller trier of sufficient length through the inner surface of the cheese exposed by the core hole. Rotate the trier through one complete turn and withdraw it with the core.

With the aid of a knife, transfer the core to the sample container. Repeat this procedure until a sample of not less than 100 g is obtained. Then close the core hole by means of the first outer core; if the latter is required as a surface sample, seal the core hole with a suitable sealing compound (see 16.2.5).

16.3.2.2.2.2 Sampling for chemical and physical analysis and for sensory examination

Take a core by inserting a Trier of sufficient length into the cheese Rotate the trier through one complete turn and withdraw it with the core. Close the core hole with

the outer plug (approximately 10 mm to 20 mm) and, if the outer plug is required as a surface sample, seal the core hole with a suitable sealing compound as described in 16.2.5.

Wrap the cores in aluminium foil before placing them in the sample container if analysis is not performed immediately after sampling.

16.3.3 **Sampling of fresh cheese**

For sampling of fresh cheese the containers shall be intact and unopened. The containers shall not be opened until immediately before analysis.

Take a sufficient number of sample containers to obtain a sample of not less than 100 g. Containers from which portions are taken shall be taken as a whole.

16.3.4. **Sampling of cheese sold in brine, oil, etc.**

Sample cheese by taking fragments of at least 100 g each (without brine, oil etc.)

During storage in brine in particular, the composition of the cheese will change, depending on time and temperature. The testing laboratory shall specify whether the sample shall include brine, oil etc. Normally brine, oil etc. are included. Whenever possible, the original ratio of cheese and liquid shall be maintained and the latter shall completely cover the cheese. If brine is included, take a sufficient amount of brine so that the cheese is completely covered. If brine is included, take sufficient amount of brine so that the cheese is completely covered. If brine is included, take a sufficient amount of brine so that the cheese is completely covered. If brine is not included, the cheese or cheese fragments shall be dried with filter paper and placed in the sample container.

The testing laboratory ca specifies the temperature at which the sample has to be stored or dispatched.

Indicate in the sampling report whether the sample has been taken with or without brine, oil, etc.

16.4 PRESERVATION, STORAGE AND DISPATCH OF SAMPLES

See clauses 7 and 8.

ANNEX A

(Normative)

Sampling equipments and shapes of samples

A.1 Sampling Equipment

A.1.1 Agitators (plungers)

See figures A.1 and A.2.

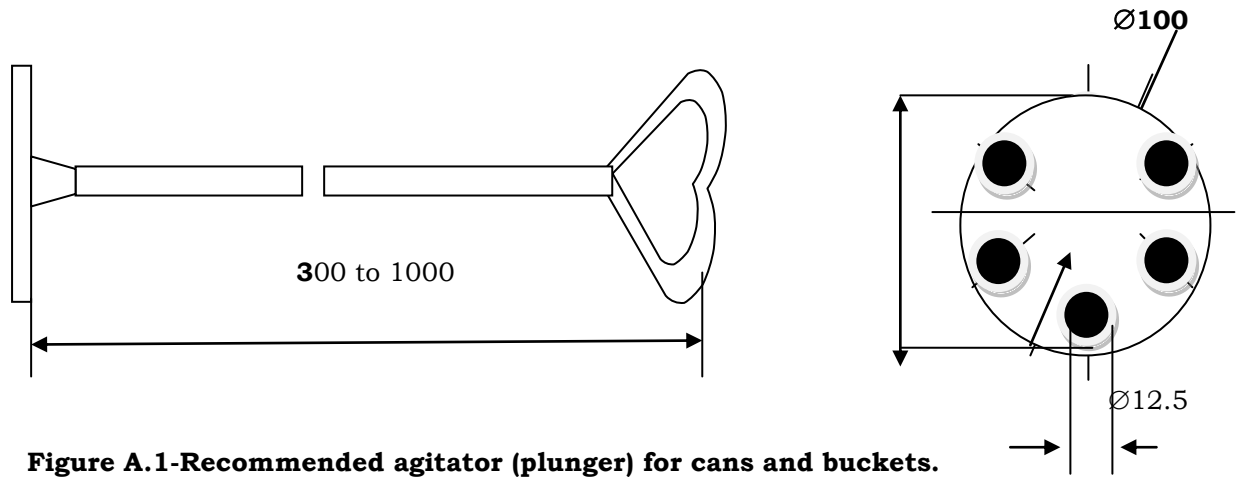


Figure A.1-Recommended agitator (plunger) for cans and buckets.

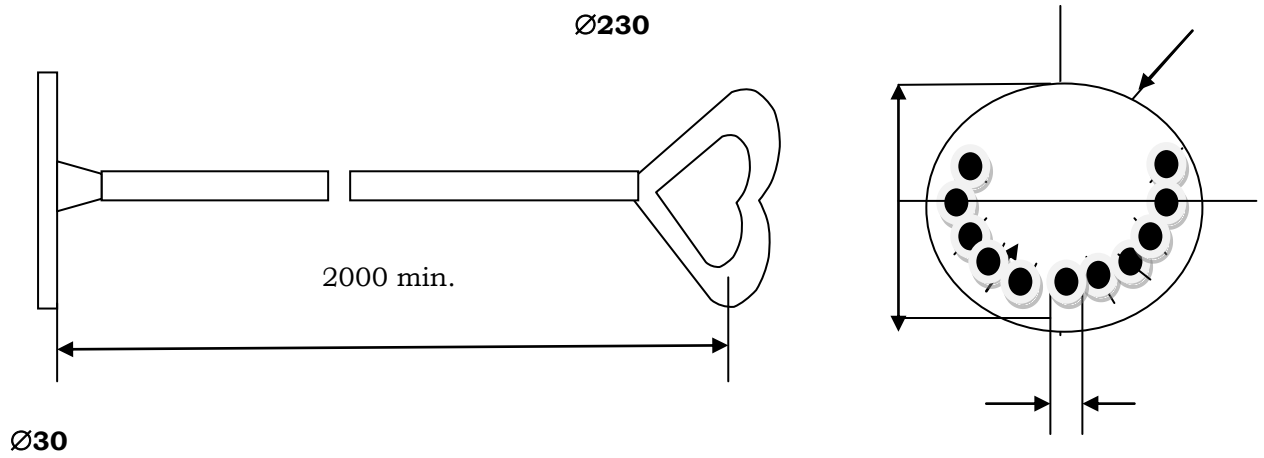


Figure A.2-Suitable agitator (plunger) for road, rail and farm tanks.

A.1.2 Dippers

See figure A.3

A.1.3 Stirrers

See figure A.4.

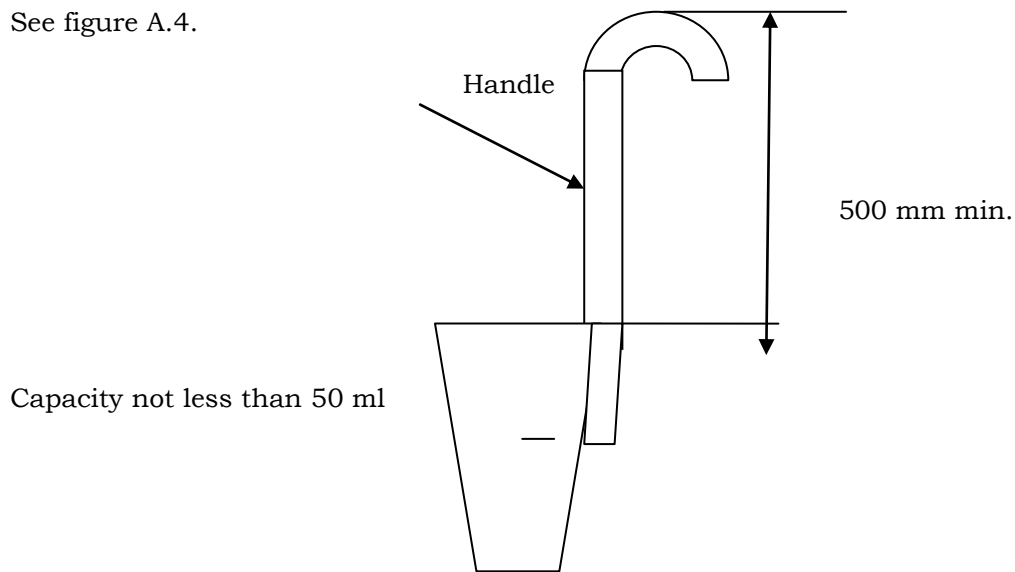


Figure A.3- Suitable dipper for liquids.

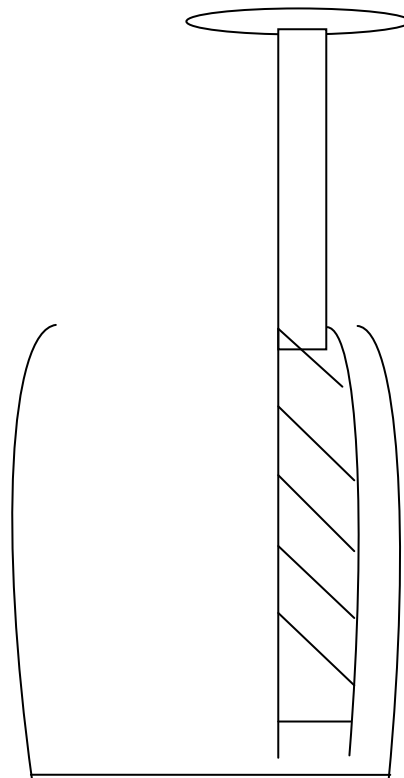


Fig A.4 Suitable stirrers for mixing sweetened condensed milk in barrels.

1. Sampling of Cream. (as per IS:3509-1966)

2. Items: Cream and Malai.

Note: *MALAI* is that portion of milk which is rich in milk fat and which rise to the surface of the heated milk on standing.

3. Sampling

3.1 Sampling shall be carried out by an experienced person as it is essential that sample should be representative of the bulk which may comprise of a large number of packages. Sampling, therefore requires most careful attention of the detail if the subsequent analysis is to be of value. As a guide to the selection of sample, useful information may be found in documents and certificates which normally accompany the consignment and usually include classification markings. However, it is recommended that the method given in these documents and certificates should be adhered to wherever practicable.

3.2 Selection of units for sampling.

3.2.1. Lot- All the units in a single consignment belonging to the same batch manufacturing shall be grouped together to constitute a lot. If a consignment consists of different batches of manufacture, the batches shall be marked separately and the group of units in each batch shall constitute separate lots.

3.2.2. Selection of units from the lot- These units shall be selected at random from a lot. To ensure the randomness of selection, a random number table as agreed between the purchaser and the supplier shall be used. In case such a table is not available, the following procedure shall be adopted.

Starting from any unit, count them as 1, 2, 3, ... up to r and so on, in one order, where r is equal to the integral part of N/n , N being the number of units in the lot and n the number of units to be selected. Every r^{th} unit thus counted shall be withdrawn to give the required number of units in the sample.

3.2.3 Bulk Units-

3.2.3.1 Units known to be uniform- If cream is supplied in bulk units like churns, and is known to be of uniform quality, the number of units to be selected for sampling shall be as follows:

<i>Number of units in the lot</i>	<i>number of units to be selected</i>
N	n
1	1
2 to 9	2
10 to 49	3
50 to 99	4
100 to 199	5
200 to 999	<i>5 for the first 200 plus one for each additional 200 units, or fraction thereof</i>
1000 and over	<i>9 for the first 1000 plus one for each additional 1000 units, or fraction thereof.</i>

3.2.3.2 Units with wide variation- While sampling small units, like bottles and small-tins, incidence of sampling may be varied according to the circumstances. Sampling may also vary according to the information, if any, of the division of the consignment into manufacturing batches. The testing laboratory may be consulted regarding the number and the method of selection of units.

3.2.4 Small units

3.2.4.1 Units with wide variation- While sampling small units, like bottles and small tins, incidence of sampling may be varied according to the circumstances. Sampling may also vary according to the information, if any, of the division of the consignment into manufacturing batches. The testing laboratory may be consulted regarding the number and the method of selection of units.

3.2.4.2 Units expected to be uniform- For consignment, or parts of consignment expected to be of uniform quality, the following minimum numbers of units shall be selected at random from separate crates, cases or packages:

<i>Number of units in the lot</i>	<i>Number of units to be selected</i>
<i>N</i>	<i>n</i>
<i>1 to 100</i>	<i>1</i>
<i>101 to 1000</i>	<i>2</i>
<i>1001 to 10000</i>	<i>4</i>
<i>Over to 10000</i>	<i>4 for the first 10000 plus one for each additional 2500 units, or fraction thereof.</i>

3.2.5 The samples shall consist of the unopened retail units selected.

3.3 Sample containers:

3.3.1 Wide mouth jars and bottles of 50 and 100 ml capacities and following approximate dimensions in millimetres may be used:

<i>Nominal Capacity</i>	<i>height</i>	<i>Width</i>	<i>Width of the mouth</i>
50	60	50	30
100	70	60	45

3.3.2 The jar and bottle shall be closed by means of a screw cap lined with butter paper or a glass stopper. For chemical analysis, bottles may be closed with rubber stoppers lined with butter paper if organoleptic tests are not to be made.

3.3.3 Jars, bottles, caps and toppers shall be suitable for sterilization.

3.3.4 Sample containers shall be perfectly clean and dry, and shall not impart any foreign odour or flavour.

3.4 Sampling Appliances

3.4.1 Material- Plungers, dippers and tubes used for sampling cream shall be of stainless steel, or other suitable material of adequate strength and stout construction to prevent distortion in use. They shall, however be sufficiently light in weight or be operated to be able to move them rapidly through the cream. If solder is used in the manufacture of the sampling appliances, it shall withstand sterilizing temperature of 180 deg C. All surfaces shall be smooth and free from crevices, and all corners shall be rounded.

3.4.2 Plungers- The plunger shall be of sufficient area to produce adequate disturbance in the unit. A form of plunger recommended as being suitable for mixing cream has been shown in Fig 1. It consists of a disc of 150 mm diameter, perforated with six holes each of

12.5 mm diameter, and a pitch cycle to 100 mm diameter, the disc being fixed centrally to a metal rod the other end of which forms a loop handle. The length of the rod, including the handle, should be approximately one meter.

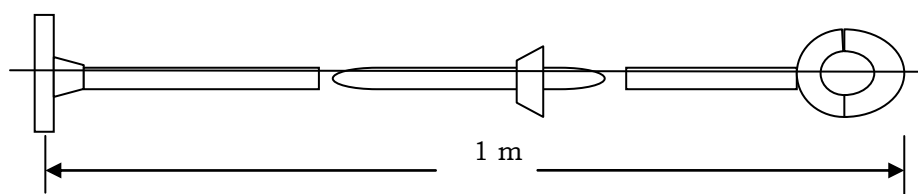


Fig 1. Plunger.

3.4.3 Dippers- Sampling dippers shall be fitted with a solid handle at least 150-mm long and it shall have a capacity of not less than 80 ml. A form of sampling dipper recommended as being suitable is shown in fig 2

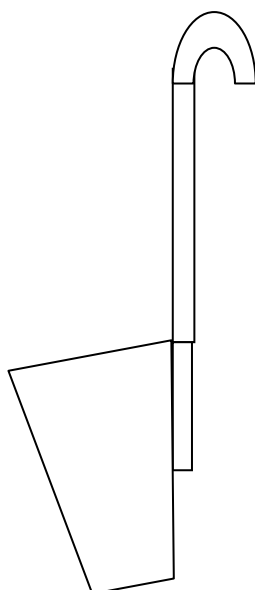


Fig 2. Sampling Dipper.

3.4.4 Sampling Tube- The sampling tube shall consist of a straight seamless metal tube about 600 mm long of 6 mm inside diameter and about 1.6 mm thickness.

3.4.5 All sampling equipment shall be perfectly clean and dry and shall not impart any foreign flavour or odour.

3.5 Mixing of cream in containers

3.5.1 When cream is thin and in small containers, it shall be mixed either by six transfers or by plunging not less than ten times. The position of the plunger shall be moved from place to place to ensure that the whole of the cream at the bottom of the vessel has been thoroughly agitated and mixed with the upper layer. To avoid whipping and churning, the disc of the plunger shall not be brought above the surface of the cream.

3.5.2 When cream is thick or in bulk containers, it shall be mixed by plunging described in 3.5.1.

3.5.3 When the cream is sour the material shall be warmed so as to attain a temperature 30°C and 40 °C and, while cooling it to room temperature, the container shaken gently or the contents stirred. Keep the contents covered as much as possible.

3.5.4 In all cases the sample shall be taken immediately after mixing.

3.6 Preparation of composite samples.

3.6.1 Taking equal amount of cream from each of the containers selected (3.2.3 or 3.2.4), collect about 100 g of the material which shall be mixed and divided into three parts. One of these samples shall be for the purchaser, one for the vendor and the third for the referee.

3.6.2 The normal size of the final sample 3.6.1 shall be 100 g, but may be less if the examining laboratory so instructs.

3.7 Sampling for bacteriological analysis

3.7.1 All sampling equipment, including plunges, dippers, sampling tubes, bottles and stoppers, shall be sterile and the samples shall be collected under aseptic condition. The equipment shall be sterilized by one of the following methods:

- a. Heating in a hot air for not less than 2 hours at 160°C; or
- b. Autoclaving for not less than 15 minutes at 120°C.

Note- For field conditions. Equipment may be sanitized by immersion for at least 5 min in boiling water.

Note 2- Rubber stoppers shall be sterilized in an autoclave as in (b). Treatment by immersion in boiling water for 10 minutes would be satisfactory if they are used immediately.

3.7.2 The cream in the container shall be mixed well as described in 3.5. Immediately after mixing, a sample of about 300 g shall be drawn with a sterilized or sanitized plunger. Transfer directly to a sterilized or sanitized wide-mouthed sample container. Use spoon to assist in removing the cream from the plunger so as not to allow the product to come in contact with the exposed lip of the sample container. The sample shall be divided into three equal parts and placed in three sterilized or sanitized wide-mouthed sample containers. One sample shall be for the purchaser, another for the supplier and third for the reference. Replace the closure of the containers and refrigerate the samples.

3.8 Preservation of the sample

3.8.1 When required for chemical analysis, a suitable preservative may be added if instructed by the examining laboratory. The nature and quantity of such preservative shall be indicated on the label of the container. The preservative shall not interfere with the examination. If the examining laboratory is expected, the sample shall be cooled and held at a temperature of 0°C to 5°C.

3.8.2 No preservative shall be added to any sample required for bacteriological or organoleptic examination. The sample shall be cooled and maintained strictly within the range of 0° and 5°C.

3.8.3 All samples shall be protected from light and heat, and kept in a cool place.

3.9 Transport and storage of sample

3.9.1 Sample shall be sent as quickly as possible to the examining laboratory and shall be protected from light and contaminating odours. The samples shall be cooled in ice and placed in an insulated container capable of maintaining the temperature between 0 °C and 5°C. Exposure of samples to temperature below freezing point shall be avoided.

3.9.2 Bacteriological examination shall be undertaken within 24 hours of the time of sampling.

3.10 Preparation of sample for chemical and Bacteriological Analysis.

3.10.1 The preparation of the sample depends upon its physical condition. If, at room temperature, the cream is thin to pour easily, mix by repeated inversion of the container; if it is too thick, stir gently, taking care that the top and bottom layers get well mixed.

3.10.2 It may not be possible to mix by gentle stirring, if the cream is very thick and the fat is partially separated or if on stirring the cream becomes thick or the fat separates. Under these circumstances, warm the sample to a temperature between 30° and 40°C and, while cooling it to room temperature, shake the container gently or stir the contents at intervals. Keep the container covered as much as possible to avoid loss of moisture by evaporation.

3.10.3 In the preparation of the sample for bacteriological analysis, suitable aseptic precautions shall be taken to prevent contamination of the sample.

Sampling of Ghee (Butterfat)

(As per IS:3508-1966, (Reaffirmed 2003))

ITEMS: Ghee

SAMPLING:

1. Sampling shall be carried out by an experienced person. It cannot be too strongly emphasized that correct sampling is a difficult problem and one which requires the most careful attention to details if the subsequent analysis is to be of value. A sample which is representative of the bulk is essential and is particularly difficult to obtain from a consignment consisting of a large number of packages. However, as a guide to a selection of samples, useful information may be found in documents and certificates normally accompanying the consignments and usually include classification markings. It is recommended that the method given should be adhered to wherever applicable. Particular circumstances may render some modifications of the recommended method necessary.
- 2. Scale of Sampling**
 - (a) *Lot*- All the containers in a single consignment belonging to the same batch of manufacture shall be grouped together to constitute a lot. If a consignment is declared to consist of different batches of manufacture, the batches shall be marked separately and the group of containers in each batch shall constitute separate lots.
 - (b) The number of containers to be selected for sampling shall depend upon the lot size and shall be in accordance with Table 1.

TABLE 1 NUMBER OF CONTAINERS TO BE SELECTED FOR SAMPLING

Number of Containers In the Lot <i>N</i>	Number of Containers To Be Selected <i>n</i>
1	1
2 to 40	2
41 to 110	3
111 to 300	5
301 to 600	7
601 and above	10

- (c) These containers shall be selected at random from the lot. To ensure the randomness of selection, a random number table as agreed to between the purchaser and the supplier, shall be used. In case such a table is not available, the following procedure shall be adopted.

Starting from any container, count them as 1, 2, 3,upto r and so on, in one order, where r is equal to the integral part of N/n , N being the total number of containers in the lot and n the number of containers to be selected (see Table 1). Every r th container thus counted shall be withdrawn to give required number of containers in the sample.

- (d) If there is a possibility of wide variation among the different units, for example, in the consignment of ghee from an individual producer, every unit shall be sampled.

3. Sample Containers

- (a) Wide mouth jar and bottles and in containers of 50, 100 and 200/250 ml capacities and of following approximate dimensions are convenient to use as sample containers:

Nominal capacity 50 ml	Height 60 x Width 48 x Width of the mouth 60 mm
Nominal capacity 100 ml	Height 70 x Width 60 x Width of the mouth 44 mm
Nominal capacity 200/250 ml	Height 97 x Width 70 x Width of the mouth 60 mm

- (b) The jars shall be closed by means of a screw cap lined with butter paper. Bottles shall be glass-stoppered. Tin containers shall be closed with the press-on type of lids. For chemicals analysis, bottles may also be closed with rubber stoppers lined with butter paper if organoleptic tests are not to be made.

4. Sampling Appliances

- (a) The sampling instrument shall be such that it is possible to sample the contents throughout the whole depth of the containers by it.
- (b) *Sampling Concentric Tubes*- A convenient sampling instrument consists of two concentric tubes closely fitted into each other throughout their entire length, so that one tube may be rotated within the other. A longitudinal opening of about one-third the circumference is cut in both tubes. In one position the tube is open and admits the ghee; by turning the inner tube it becomes a sealed container.

The inner tube may be 19 to 38 mm in diameter and undivided in its length. The two tubes are provided with V- shaped ports at their lower ends, so placed that ghee contained in the instrument can be drained through them when the longitudinal openings are open.

The instrument should be inserted closed; it is then opened to admit ghee and finally closed and withdrawn.

- (c) *Sampling Plain Tube*- The sampling tube may be used when ghee is fluid and is known to be quite uniform. It consists of a metal or thick-walled glass tube which may vary from 20 to 40 mm in diameter and should be 375 to 750 mm long. The upper and lower ends are conical narrowed down to about 6 to 12 mm. At the upper end there are two rings to assist handling.

To take an individual sample the apparatus is first closed at the top with the thumb, or stopper, and lowered until the desired depth is reached; it is opened for a short time to admit the ghee and finally closed and withdrawn.

- (d) The sampling appliances shall be made preferably of stainless steel. The surface of the instruments shall be polished.
- (e) All sampling equipment shall be perfectly clean and dry and shall not impart any foreign odour or flavour. Sampling instruments may be cleaned with hot soapy water or other detergent, care being taken to wash away the last traces with

scalding hot water. If a source of steam is available the instruments may be given a final cleaning in a jet of steam.

5. Sampling Technique

- (a) Sampling shall be carried out in such a manner as to protect the sample, the sampling instruments and the containers in which the samples are placed from adventitious contamination, such as rain and dust.
- (b) Material adhering to the outside of the sampling instruments shall be removed before the contents are discharged.
- (c) A sample shall be drawn from each container to be sampled with the sampling instrument which is inserted through a convenient opening in such a manner as to sample the entire depth of the contents.
- (d) All samples from the same consignment shall be put into a clean and dry receptacle, preferably of stainless steel. The contents of the receptacle shall be thoroughly mixed and the required sample drawn into a clean and dry sample container.
- (e) The sample container shall be closed, leaving sufficient air space at the top for expansion. On the other hand this space shall not be too large, as air exerts detrimental action.
- (f) All samples shall be protected from light and heat, and kept in a cool place.

6. **Preparation of Composite Sample**- Taking equal amount of ghee from each of the containers selected {2. (b)}, collect at least 300 g of the material as described in (5.) which shall be mixed and divided into three equal parts. Each part shall be transferred to a separate sample container. One of these composite samples shall be for the purchaser, one for the vendor and the third for the referee. Store the containers at a cool and dark place.

7. **Transportation and Storage of Samples**- Samples should be sent as quickly as possible to the examining laboratory, and should be protected from light and contaminating odour. The sample shall be kept in a cool and dark place.

8. Preparation of Sample for Score Card and Analysis

(a) *Sample for Score Card of Ghee*- Testing shall be carried out soon after opening the container. In case of large containers, soon after opening, the contents shall be thoroughly mixed and about 200 g shall be transferred to a glass bottle with a well-fitting stopper. The sample shall not be heated before the score card is prepared.

(b) *Sample for the Determination of Moisture and General Analysis*

- i. Mix the sample in the container in which it is received until homogenous. Carry out this operation in a cool place, away from direct sunlight, and complete it in shortest possible time. In the event of any separation taking place in between, that is, mixing and commencement of the analysis for moisture, remix the sample. Use this for the determination of moisture.
- ii. After the determination of moisture place the bottle in a water-bath at a temperature not higher than 50°C till completely melted. Filter through a dried, fluted open-texture 15 cm filter paper (for example, Whatman No. 4) with the help of a hot water funnel, directly into the receiving bottle. Continue the filtration until it is complete, or not more than 3 or 4 ml of ghee grains. The filtered ghee should be bright and clear.

Sampling of Butter

(As per IS:3507-1966, (Reaffirmed 2003))

ITEM: Butter.

SAMPLING:

Sampling requires the most careful attention to details if the subsequent analysis is to be of value. It is, therefore, essential that a truly representative sample is drawn. But this is difficult task and it becomes more difficult when a consignment consists of a large number of packages. However, for the purpose usual information given in the documents and certificates accompanying the consignment may be utilized to serve as a guide, and the method given in this standard should be adhered to wherever practicable. If the modification is desirable, the laboratory should be consulted regarding the selection of sample.

1. General Requirements

- a) Samples shall be drawn by an experienced person in a protected place not exposed to damp air, bright light, dust or soot. The material shall preferably be at a temperature between 0 to 15°C at the time of drawing the sample.
- b) The sampling instruments shall be clean and dry, and shall not impart any foreign odour or flavour.
- c) Samples shall be placed in clean, odourless and dry glass containers.
- d) Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers from adventitious contamination.
- e) While drawing the sample for bacteriological examination, all equipment and containers shall be sterile and the samples shall be collected under aseptic conditions. Equipment shall be sterilized either by heating in a hot air oven for not less than 2 hours at 160°C, or by autoclaving for not less than 15 minutes at 120°C.
- f) Each container shall be sealed air-tight after filling and marked with full details of sampling, batch or code number, name and address of manufacturer, and other important particulars of the consignment.
- g) Samples shall be sent to the examining laboratory as quickly as possible and shall be protected from light and contaminating odours. The samples shall be stored suitably at a temperature between 0 to 5°C. No preservative shall be added to the butter at the time of sampling.
- h) Bacteriological examination shall be undertaken within 24 hours of the time of sampling.

2. Sampling Appliances

- a) Bitter triers shall be used for drawing the sample. A butter trier shall have at least 30-mm diameter and sufficient length to pass diagonally to the base of the container. The butter triers may conform to the dimensions given in fig 1.
The details of construction of the trier may be as given below:
 - The blade and stem of the trier should be made of stainless steel of appropriate hardness. The grip may be of stainless steel or any other suitable material which would withstand repeated sterilization.
 - The blade and stem may be in one piece and the transition from stem to blade shall be smooth.
 - The stem may be circular in cross-section and run-parallel to the blade.
 - The blade may be tapering to the point. The degree of tapering may be less in the case of blades of triers intended for structural examination.
 - The grooves of the blade may have sufficient depth and the edges of the blade may be sufficiently sharp so as to facilitate the sampling of hard butter.
 - The surface of the blade should be well polished.

- Shape, material and the finish of the trier should permit the trier to be easily cleaned and sterilized.
- b) Spatulas and knives may be used for removing portions of samples from the triers and should be made of stainless steel.

3. Sample Containers

- a) Wide-mouth jars and bottles of 50 and 100 ml capacity shall be used as sample containers. The approximate dimensions of the containers may be as given in Table 1.

TABLE 1 DIMENSIONS OF SAMPLE CONTAINERS

S. No.	Nominal Capacity	Height	Width	Width of the mouth
	(ml)	(mm)	(mm)	(mm)
i)	50	60	50	30
ii)	100	70	60	45
iii)	200-250	100	70	60

- b) The jar or bottle may be closed by means of a screw cap lined with butter paper or a glass stopper. Sample containers for chemical analysis other than organoleptic tests may also be closed with rubber stoppers lined with butter paper.
- c) Jars, bottles, bottle caps, and stoppers should be suitable for sterilization.
- d) For bacteriological examination of butter, only glass-stoppered bottles shall be used.

4. Scale of Sampling

- a) *Lot*- all the units in a single consignment belonging to the same batch of manufacture shall be grouped together to constitute a lot. If a consignment is declared to consist of different batches of manufacture, the batches shall be marked separately and the group of units in each batch shall constitute separate lots.
- b) If the butter is supplied in bulk units like cakes or boxes, the number of units to be selected for sampling shall depend on the size of the lot and shall be in accordance with Table 2.

TABLE 2 NUMBER OF BULK UNITS TO BE SELECTED FOR SAMPLING

Number of Bulk Units In The Lot <i>N</i>	Number of Units to Be Selected <i>n</i>
1	1
2 to 9	2
10 to 49	3
50 to 99	4
100 to 199	5
Over 200	5 for the first 200 and 1 each for 200 Additional units for fraction thereof

- c) If the lot is of small units like packets or tins having same batch number, the number of units selected for sampling shall be in accordance with Table 3.

TABLE 3 NUMBER OF SMALL UNITS TO BE SELECTED FOR SAMPLING

Number of Small Units In the Lot <i>N</i>	Number of Units to Be Selected <i>n</i>
1 to 100	1
101 to 1000	2

1001 to 10 000	4
Over 10 000	4 for the first 10 000 and for 2500 Additional units or fraction thereof

- d) The units to be selected for sampling shall be selected at random from the lot. To ensure randomness of selection, a random number table as agreed to between the purchaser and the vendor shall be used. In case such a table is not available, the following procedure may be adopted:

Starting from any unit in the lot, count them as 1, 2, 3,, up to r and so on, in one order, where r is equal to the integral part of N/n , where N is the total number of units in the lot and n is the number of units to be selected. Every r th unit thus counted shall be withdrawn to give the required number of the units in the sample.

5. Sampling Technique

For Chemical Analysis

➤ *Hard and semi- hard butter kept under cold storage*

- From churns-* Four cores shall be drawn with the help of a trier at equal distances. At least two should be near the centre of the churn.
- From trollies-* Four cores (one each from the two ends and the other two from the sides) shall be drawn with the help of a trier.
- From boxes-* Three cores shall be drawn by inserting a trier vertically through the block. One core would be at the centre and the other two near diagonally opposite corners of the open end.
- From casks-* Three cores shall be drawn by inserting a trier at three points equidistant from the circumference of one end of the block and directed through the centre of the block.
- From small packets-* The samples shall consist of the unopened packets. After taking the sample for bacteriological test, the rest shall be used for chemical analysis.

➤ *Pasty butter kept under warm conditions*

- When the product is in small quantities, remove a sample from the deeper layers of the product at the centre of the block and two other points roughly equidistant from the central point, located 2 to 3 cm away from the ends. A suitable, clean, dry spoon, spatula or a trier should be used.
- When the product is in the form of large heaps or blocks, select three points, one at the centre, the second about 2 to 3 cm away from the bottom and the third at an equal distance from the centre on the opposite sides. At each point, draw from the deep layers three cores, roughly equidistant on the circumference. A suitable, clean, dry spoon, spatula or a trier should be used.

➤ *Preparation of composite sample*

Taking equal amount of butter from each of the containers selected in {4. (b), (c)}, collect about 300 g or more of the material which shall be mixed and divided into three equal parts. Each part shall be transferred to a separate sample container. One of these composite samples shall be for the purchaser, one for the vendor and the third for the referee.

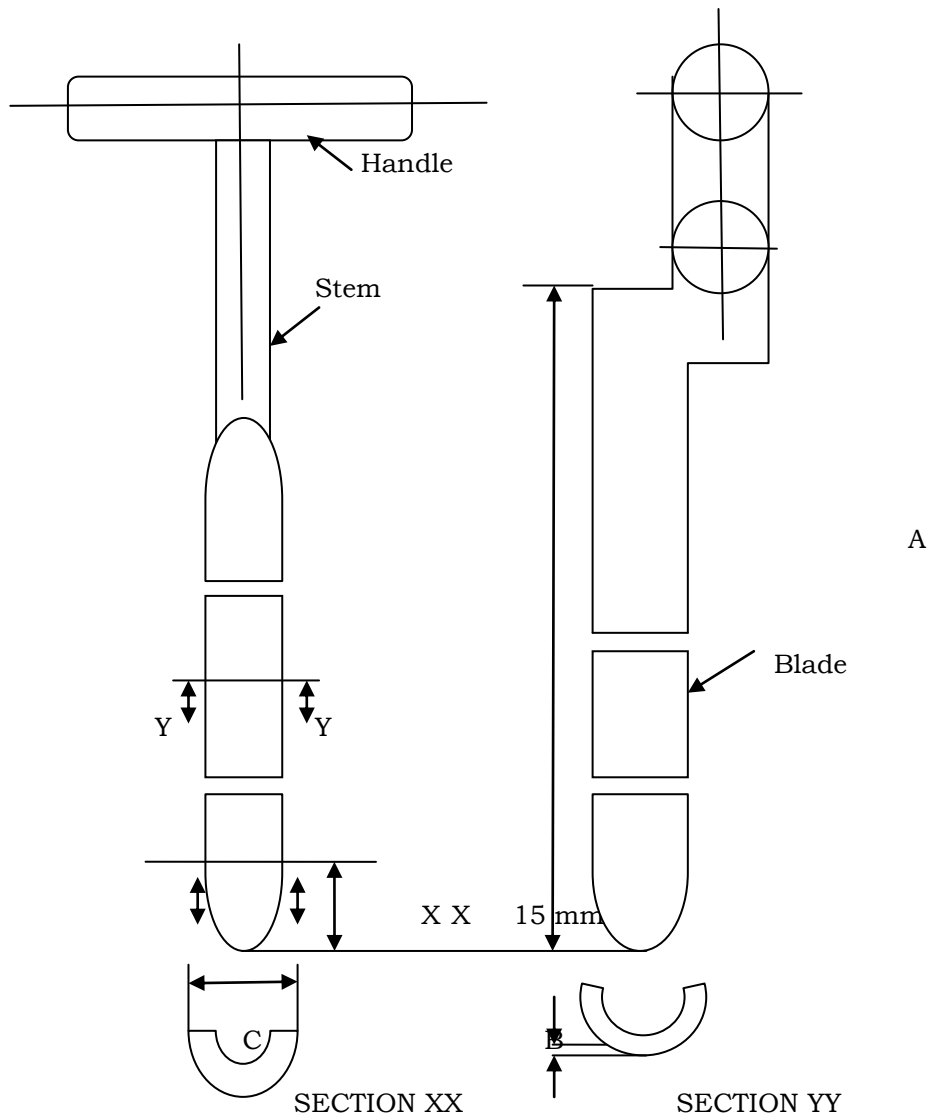
For Bacteriological Examination

➤ *Hard and semi- hard butter kept under cold storage*

- From chunks or from butter trollies-* With a sterile or sanitized spatula or trier take a small amount of butter from not less than four different locations in the churn so that the total amount of butter is not less than 300 g. Transfer directly to a sterile or sanitized glass- stoppered bottle. Use another sterile or sanitized spatula or spoon to assist in removing the butter from the sampling instrument so as not to

allow the product to come in contact with the exposed lip of the glass- stoppered bottle.

- (b) *From boxes and bulk packages-* With a sterile or sanitized trier bore diagonally through the container (tub or box) and remove at least two plugs with a minimum total weight of 300 g. If desired, the surface butter may be removed from the top of the plug. With the end of a sterile or sanitized spatula or spoon transfer the product to a sterile or sanitized glass-stoppered bottle, so as not to allow the product to come in contact with the exposed lip of the glass- stoppered bottle.
 - (c) *From small retail packets-* Since there is difference in surface areas of 100, 250 and 500 g packs, remove samples from packet butter with sterile or sanitized trier in such a manner as to ensure uniformity in surface area per sample. Take 7.5 to 10 cm slice from the end of each packet and transfer it (including surface portion) with the aid of a sterile or sanitized spatula or spoon to a glass- stoppered bottle which has previously been sterilized or sanitized.
 - (d) *Preparation of the sample-* The sample (a, b or c) shall be divided into three equal parts and placed in three sterile or sanitized glass- stoppered bottles. One sample shall be for the purchaser, another for the supplier, and the third for the referee. Close the glass- stoppered bottle and refrigerate the sample.
- *Pasty butter kept under warm conditions*
- (a) When the product is in small quantities, remove a sample from the deeper layers of the product at the centre of the block and two other points roughly equidistant from the central point located 2 to 3 cm away from the ends, so that the total amount of butter is not less than 300 g. A sterile or sanitized spatula or trier should be used for drawing samples.
 - (b) When the product is in the form of large heaps or blocks, select three points, one at the centre, the second about 2 to 3 cm from the bottom and the third at an equal distance from the centre on the opposite side. At each point remove a sample so that the total amount of butter is not less than 300 g. A sterile or sanitized spatula or trier should be used for drawing samples.
 - (c) *Preparation of the sample-* Transfer directly the sample {(a) or (b)} into a sterile or sanitized glass- stoppered bottle. Use another sterile or sanitized spatula or spoon to assist in the removing the butter from the sampling instrument so as not to allow the product to come in contact with the exposed lip of the glass- stoppered bottle. The sample shall be divided three equal parts and placed in three sterile or sanitized glass- stoppered bottles. One sample shall be for the purchaser, another for the supplier and the third for the referee. Close the glass- stoppered bottles and refrigerate the sample.
- *Preparation of sample for chemical analysis*
- (a) *Sample for analysis of butter-* Warm the sample in an air- tight container with the lid screwed down tightly or with the glass stopper, in an oven or water- bath not exceeding 39°C until by frequent vigorous shakings a homogenous fluid emulsion (free from unsoftened pieces) is obtained at the lowest possible temperature.
 - (b) *Sample for analysis of butterfat-* heat a portion of emulsified butter in a beaker to a temperature of 50- 60°C until the fat separates. Filter the fat layer through a dried filter paper into a dry vessel at a temperature above the solidification point of the fat, using a hot-water funnel, if necessary. Re- filter the filtrate under the same conditions, until it is clear and free from water. Liquefy the fat completely and mix before taking samples for analysis.
- NOTE-* Exposure to light and air of the butter sample or the butterfat obtained from it shall be as short as possible and analysis shall be carried out without delay.



Particulars	Principal Dimensions		
	Long	Medium	Short
A*	540	265	125
B	1.8	1.5	0.5
C	17	16	11.6
*Tolerance for A + 10 percent			

All Dimensions in millimetres.

Fig 1. Butter Trier.

1. Methods for sampling oilseeds and oil bearing fruits.

(as per IS:4115-1967 (Reaffirmed 1994, 2006))

2. Items: oilseeds and oil bearing fruits.

- 2.1 **Small oil seeds:** Sesame seeds, mustard, poppy, rape, linseed, hemp, cotton seed and other oilseeds in the same size-range.
- 2.2 **Medium oil-seeds:** Castor, palm kernel, groundnut, mahua, soybean and other oilseeds in the same size rang.
- 2.3 **Large oilseeds:** Copra and other oilseeds in the same size range.

3. Sampling Equipment: For drawing representative samples of oilseeds, suitable sampling instruments shall be needed.

- a. Slotted-Tube Sampler: The slotted tube sampler is suitable for drawing samples from bulk and from bags of oilseeds. It is suited for small and medium sized oilseeds. It consists of two metallic tubes having several oval slots, fitted one inside and the other, and free to rotate. After inserting the sampler into the oilseeds, the inner tube is rotated so that the slots or the tubes co inside and an amount of oilseeds are deposited into the inner tube. By rotating it further, all slots of the inner tube are closed and he sampler is withdrawn from the oilseeds.
- b. Parkhi type sampler: The Parkhi tube sampler is used for drawing representative sample from bagged oilseeds. It is useful from drawing samples from all type of oilseeds except from bagged oilseeds. When a Parkhi type sampler is used it is inserted in the diagonal position and he sample is collected at the other end by gently turning.
- c. Thermo Sampler: The thermo- sampler is a device by which a sample it taken from the bulk storage with simultaneous recording of temperature at different depths. This device is used in oilseeds storage godowns where bulk storage is practiced. With the exception of copra, this type of sampler can be use for oilseeds for all sizes.
- d. Other samplers:

The deep bin-probe can be used to a depth of 1.5 m in sampling from heaps or other bulk s of oilseeds. It is inserted at an angle in the closed position and is opened when the desired depth is reached. After allowing about half a minute to collect the ample the tube is closed and pulled out.

Pelican type sampler: Scoop and sack type sampler can be used when he oilseeds are in motion and he increments are require to be pick up at regular intervals depending upon the rate of flow.

All samples shall be kept in suitable containers so as to preserve, as far as possible, all the charactestics till the time of their use in testing. They shall also carry labels with full particulars and identification.

4. Procedure: Formation of lots and sub-lots

a. **Lots:** The consignment shall be broken into lots such that each lot consists of oilseeds belonging to the same species, variety, type, grade, source, and the year of production a far as possible.

b. **Sub Lots:** The object of dividing a lot into a suitable number of sub-lots is only to facilitate the drawing of representative gross samples rather than to indicate its physical division. However, if the lot admits of a distinct qualitative division, each sub-lot may be formed only on quantitative basis. Depending on the size and uniformity of the lot the number of sub-lots may be 2 or more, preferably in accordance with Table 1 or Table 2. In case of un-bagged materials sub-lots may be identified by demarcation lines on the surface.

Table 1. Number of sub-lots for oilseeds in bags.

Number of bags in a lot	Minimum number of sub-lots
Up to 300	2
301 to 1000	3
1001 to 3000	4
3001 and over	5

4.0 Sampling from wagons during loading or unloading.

- 4.1** For the purpose of sampling all the wagons in a lot shall be divided into a suitable number of sub-lots of approximately equal weight in accordance with the requirements of table 1 or table 2 as applicable.
- 4.2** One gross sample shall be drawn from each of the sub-lots so that there are as many gross samples as the number of sub-lots into which a lot is divided.
- 4.3** In order to get a representative gross sample, the oilseeds shall be sampled, as far as possible, in steady motion during loading or unloading. As a first step a minimum of 25% of wagons shall be selected at random from the sun lot.
- 4.4** In the case of bagged oilseeds, the number of bags to be selected from each sub-lot shall be accordance with table 3.

Table 2 Number of sub lots for oilseeds in bulk	
Weight of oilseeds in a lot	Minimum number of sub-lots
Up to 30	2
31 to 100	3
101 to 300	4
Over 300	5

Table 3 Scale of sampling for bagged oilseeds		
Number of bags in the lot	Number of bags to be sampled for	
	Small oilseeds	Medium and large oilseeds
Up to 50	5	8
51 to 100	8	13
101 to 150	1	20
151 to 300	20	32
301 and above	32	50

- 4.5** The bag shall be evenly distribute over the selected wagon with a view to determining the necessary number of bags that shall be collected from each of the selected wagons of the sub-lot. The requisite number of bag shall be collected at regular intervals at the time of loading or un-loading. From each of the selected bags one increment shall be taken with the help of suitable sampling instruments.
- 4.6** In the case of unbagged oilseeds a minimum of 50 increments shall be collected from each sub-lot. These increments shall be uniformly spread over the selected wagons, and shall be drawn at regular intervals at the time of loading or unloading.

4.7 All the increment from the same sub-lot shall be thoroughly mixed and blended to constitute a gross sample to represent a sub-lot. The minimum size of the gross sample shall be at least 2 kg for small oilseeds, 6 kg for medium oilseeds and 10 kg for large oilseeds. If the gross sample as obtained above is less than this minimum quantity, additional increments shall be drawn from the sub-lots so as to make up the required quantity. The manner of drawing these increments shall be as those described above.

5. Sampling from ships during loading or unloading

5.1 The entire quantity of oilseeds in a ship shall be divided into a suitable number of sub-lots of approximately equal weights in accordance with Table 1 or Table 2.

5.2 One gross sample shall be drawn from each of the sub-lots so that there will be as many gross samples as the number of the sub-lots into which the lot has been divided.

5.3 Sampling of oilseeds from ships shall be carried out as far as practicable when the material is in motion. The requisite number of bags or increments shall be drawn during loading of the ship in accordance with point no 4.4 and 4.6.

5.4 The same treatment as explained in sub point 4.7 shall be given to the increments selected in 5.1 and 5.2.

6. Sampling from stockpiles or warehouses

6.1 For the purpose of sampling, the quantity of oilseeds in a stockpile or warehouse shall be divided into a suitable number of sub-lots of approximately equal weight as specified in table 1 and table 2.

6.2 One gross sample shall be drawn from each of the sub-lots so that there will be as many gross samples as the number of sub-lots into which the lot has been divided.

6.3 As far as practicable the oilseeds shall be sampled when the material is in motion that is, while making or breaking stockpile or while storing or removing oilseeds from the warehouse. The number of bags or increments to be selected and the method of collecting them shall be in accordance with 4.4 and 4.6.

6.4 In case of bagged oilseeds in stationary stockpiles, the number of bags to be selected from a sub-lot shall be as specified in table 3. These shall be chosen randomly from different layers of the stockpile. For easy accessibility of all the bags in a sub-lot, it may be more practicable to restrict the height of the stock pile to 1.5 m or so. From each of the bags so chosen, an increment shall be collected with the help of suitable sampling instruments.

Table 3. Scale of Sampling for bagged oilseeds		
Number of bags in the lot	Number of bags to be sampled for.	
	Small oilseeds	Medium and large oilseeds
Up to 50	5	8
51 to 100	8	13
101 to 150	13	20
151 to 300	20	32
301 and above	32	50

- 6.5** In the case of un-bagged oilseeds in stationary stockpiles, the sub-lots should be indicated by suitably marking the line of demarcation on the surface of a lot. The surface of each of the sub-lot shall be levelled and from the various parts randomly chosen from the surface, a minimum of increments shall be drawn with the help of suitable sampling instruments. As in the earlier case it would be advisable to have stockpiles of less than 1.5 m high so that the sampling instruments can probe deep enough to obtain a representative sample.
- 6.6** The same treatment as explained in 4.7 shall be given to the increments selected in 5.4 shall be given to the increments selected in 6.4 and 6.5. The manner of drawing these increments shall be the same as those described in 4.4 and 4.6 if the material is in motion or as in 6.4 or 6.5 if the material is stationary.

Sampling of oils and fats

Items: Vegetable oils and fats

Equipments

- a) Sampling bottle or can (for large vessels and tanks)
- b) Sampling tubes – closed type sampling tubes-divided or undivided; for sampling of liquids and semi-liquids that may not be homogeneous, open type sampling tubes for sampling of homogeneous liquids.
- c) Sampling scoops: for vegetable fats and hydrogenated vegetable oils.
- d) Sample containers: Clean dry containers, preferably of glass or tin-plate. Glass bottles of 500 ml capacity are recommended for liquid oils and glass jars for solid vegetable oils or fats.

Procedure

- e) Oils in Bulk in Storage Tanks and Tank Wagons: IS: 548 (Part I)-1964, 3.5.1
- f) Oils in Barrels, Casks, Drums and Tierces
 - i. Liquid or semi-solid oils - Roll the container to mix the contents and insert the sampling tube slowly through the bung hole or any other convenient opening. If possible, the sample should be drawn from end to end. As soon as the tube is fully inserted, close the upper constriction with the thumb or a stopper, withdraw the tube and transfer the sample into a clean dry container. Take several portions in this manner from this and other packages. Mix thoroughly. The sample containers should be almost but not completely filled. The sample containers shall be sealed with sealing wax in such a manner that it is not possible to remove the contents and the label without breaking the imprint of the seal.
 - g) Preparation of test sample: Normally, all the samples drawn as described above shall be put into a clean dry receptacle, such as a bucket or tub, and the contents of this receptacle shall be thoroughly mixed and at least four uniform samples (test samples) shall be drawn there from. One test sample shall be sent to the purchaser and one to the supplier.

Each sample container after filling shall be sealed and marked with full details of sampling, the number of packages sampled, the date of sampling and other particulars of the consignment.

Size of test sample: The minimum size for each test sample shall be 0.5 kg.

Special instructions: Rubber stopper should not be used to close the containers. In case of glass containers, glass stoppers or new good quality velvet corks should be use and, in the case of tine containers, tin caps should be suitably soldered on the top, avoiding contamination of fat with acid flux. Use of resin as a flux is recommended. Tinfoil or grease-proof paper may be wrapped round the corks to prevent contact of sample with them, and it is recommended that this be done in the case of refined or deodorized edible oils. In case of oils with high acid value, neither metal containers nor tinfoil is recommended.

Lot sampling

- h) Lot – all the containers in a single consignment of one type and grade of material drawn from a single batch of manufacturer shall constitute the lot. If a consignment is declared to consist of different batches of manufacture, the batches shall be marked separately and the groups of containers in each batch shall constitute separate lots. Should the consignment not be uniform in quality, the parts of the consignment which appear to be similar may be collected together and each quality treated as a separate lot.
- i) Gross sample – the general procedure for taking a gross sample is tho draw a number of portions from the bulk quantity or a number of portion s form all or several packages, and mix them thoroughly. Representative portions of the gross sample shall be transferred to air-tight containers to suitable size for the test samples
 - i. Gross sample from bulk quantities – shall be drawn in quantities of not less than 2 kg per 2000 kg or less.
 - ii. Gross sample from small packages – when sampling form drums, barrels, etc, the packages from which the samples are drawn shall be selected at random from the lot. The following schedule is recommended for the number of packages to be sampled:

Number of packages in the Lot	Number of packages to be Sampled
1 to 4	Each package
5 to 100	At least 20 percent with a minimum of 4 packages
More than 100	At least 10 percent with a minimum of 20 packages

1. Sampling of Cereals, pulses and milled products.

(as per IS:14818-2000, ISO:13690-1999)

2. Items: Cereals, pulses and milled products.

3. Equipments:

General:

Many different types of instruments are available. Those given in annex B and their dimensions are included, therefore, solely as a guide. Annex C is included to help in the selection of suitable sampling instruments it is known that use of various types of equipment can give rise to differing samples from the same lot.

Where possible, the type of equipment to be used and the procedures for its use shall be determined by agreement between the parties concerned. Pneumatic samplers should not be used for milled products. All instruments used shall be suitable for the product being sampled.

Instruments for general usage:

- I. Sampling from bulk:** Use appropriate apparatus for obtaining increments from static bulk (example, hand- held spears, mechanical or air-assisted apparatus).
- II. Sampling from bags:** Use sack type spears.
- III. Mixing and dividing:** Use shovels and dividing apparatus or automatic random dividing apparatus.

4. Procedure:

4.1 Location and time of sampling

The location and time of sampling shall be determined by the agreement between the parties concerned.

4.2 Method of taking samples:

General: Unless otherwise specified in the contract, consignments shall be considered in lots of a maximum of 500t or such part thereof as constitute a single consignment.

4.2.1 Sampling from bags:

Unless otherwise specified in the contract or unless the practice at the port or elsewhere requires otherwise, increments shall be taken from different part of a bag (for eg. Top, middle, bottom) by means of a sack/ bag spear from the number of bags specified in table 1.

Table 1 – Number of bags to be sampled.

Number of bags in consignment	Number of bags to be sampled
Up to 10	Each bag
10 to 100	10, taken at random
More than 100	Square root (approx.) of total number, taken according to a suitable sampling scale ^a .
^a See Annex A.	

When using mechanical sampler, increments shall be taken from a minimum of three different sampling points.

4.3.3 If the type of wagon, vessel or commodity does not allow samples to be taken in this manner, or if there is a separate agreement between the buyer and seller, the grains shall be sampled during discharge of the wagon/vessel.

4.4 Sampling from silos, bins or warehouses

4.4.1 Increments shall be taken throughout the whole depth of the lot. Suitable instruments must be used to achieve this requirement. If the depth of the lot does not

permit use of this method, sampling should be carried out from the flowing cereal in accordance with ISO 6644.

4.4.2 The grain should be sampled using a grid system, for example similar to that used for rail/road wagons, barges or ships (4.3.2).

4.4.3 Sufficient increments should be taken to satisfy the requirements given in (4.4.4).

4.4.4 The number of increments to be taken shall be determined as follows.

Take the square root of the tonnage in the static bulk. Divide by two and round up to the next whole number. This is the minimum number of increments that is to be obtained. If circumstances dictate that more increments are required to obtain fair average samples of the static bulk. For examples see table 2.

Table 2. Number of increments for bulk grain of more than 500 t

Tonnage	Square root	Number of increments
500	22,4	12
1000	31,6	16
2000	44,7	23
4000	63,2	32
6000	77,4	39
8000	89,4	45
10000	100	50

Annexure A:

Sampling Scheme for consignment of more than 100 bags.

The consignment shall be dividing into (n-1) groups containing n or (n-1) bags: the remaining bags constitute a group.

Examples

- a. A consignment comprising 200 bags.

The square root of 200=14, 142, therefore n=14:

---makeup 14 group of 14 bags (i.e. total of 196 bags);

---Draw up a list from 1 to 14; cross out one number, for e.g. 7;

---Sample the seventh bag from each group of 14 bags;

---the remaining group (i.e. 4) is smaller than 14 bags, so sample one bag from this group at random.

A total of 15 bags have thereof been selected.

- b. A consignment comprising 2000 bags

The square root of 2000=44,721, therefore n=45:

---make up 44 groups of 45 bags (i.e. total of 1980 bags);

---draw up a list from 1 to 45;

---cross out one number, for example 20;

---sample the 20th bag from each group of 45 bags;

---the remaining group (i.e. 20) is smaller than 45 bags, so sample one bag from this group at random.

A total of 45 bags ha therefore been selected.

4.2.2. Pre packed units are usually transported in outer cases or carton containing a convenient number of units. The procedure applicable to bags (described in 4.2.1) shall be used to determine the appropriate number of outer cases or cartons to be sampled. If the total number of outer cases or cartons in the consignment does not exceed 1000, only one pre packed unit hall be taken from each of the outer cases taken for sampling.

4.2.2.1. Care shall be taken to ensure that a pre- packed unit is taken in a random manner from the entire contents of the outer case or carton for sampling.

The selection of pre-packed units occupying the same corresponding position in a number of outer cases or cartons shall be avoided.

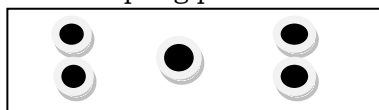
The pre-packed units taken in this manner shall be considered as increments.

4.3 Sampling from rail or road wagons, Lorries, barges or ships.

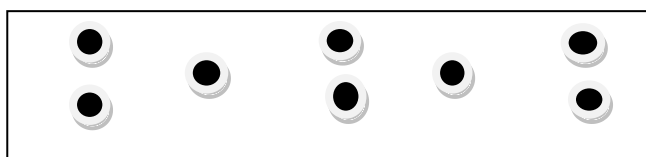
4.3.1 Unless otherwise specified in the contract, each laden wagon, lorry, barge or ship shall be sampled.

4.3.2 Increments shall be taken throughout the whole depth of the lot. Suggested patterns are as follows

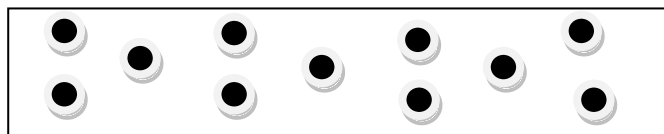
a. Up to 15 t: 5 sampling points.



b. From 15 t to 30 t: 8 sampling points



c. from 30 t to 500 t: minimum of 11 sampling points.



d. Above 500 t: see Table 2.

Annexure B

Note: The type of equipment to be used and the procedures for its use should be determined by agreement between the parties concerned.

B.1 Instruments for sampling cereals:

B.1.1 Instruments for sampling from static bulk, tote bins and rigid containers.

B.1.1.1 Concentric hand spears

- Open handle: single and multi aperture.
- Closed handle with compartments: multi-aperture.
- Open handle sequentially opening slots: multi-aperture.

B.1.1.2 Gravity spears with extension rods and T handles.

- Gravity spear: concentric type.
- Gravity spear cup type

Minimum bore size item B.1.1.1 and B.1.1.2: 20 mm diameter.

B.1.1.3 Mechanical Samplers

There are three main types:

- Gravity sampler.
- Suction (sometimes called "vacuum") sampler
- Air-assisted sampler.

Minimum aperture size grain: 120 mm X 20 mm.

Minimum aperture size: 25 mm.

B.1.2 Instruments for sampling from sacks and bags including bulk bags

B.1.2.1 Dynamic sack spears.

Minimum bore: 17 mm diameter, aperture 40 mm X 15 mm.

B.1.2.2 Walking stick type

Concentric tubes, minimum bore 20 mm diameter:

- a. open handle: single and multi-aperture;
- b. With compartments: single and multi aperture.

B.1.2.3 Conical sampler

B.1.2.4 gravity spears

These have extension rods and T handlers for open-topped bags.

B.1.2.5 Screw augers.

These are usually small and portable and electrically powered.

B.2 Instruments for sampling pulses

B.2.1 Instruments for sampling from a static bulk.

These are as for cereals (B.1.1)

B.2.2 Instruments for sampling from sack and bags.

These are as for cereals (B.1.2), but bore and aperture dimensions should be appropriate to the size of the pulses to be sampled.

B.3 Instruments for sampling milled products, excluding pelleted materials.

B.3.1 Instruments for sampling from static bulk

These are as for cereals (B.1.1) with the exception of mechanical samples.

For mechanical samplers, only two types are suitable for milled products:

- a) Electric mechanical screw auger
- b) Gravity mechanical sampler.

In general, air – assisted samplers are excluded for this use.

B.3.2 Instruments for sampling from sacks and bags

These are as for cereals (B.1.2)

B.4 Instruments for division of samples

These are manufactured from materials which will not contaminate the samples.

B.4.1 quartering irons

B.4.2 Multiple slot (riffle or blade type)

- i. Small laboratory dividers for ground samples:
Min. 12 slots 12, 7 mm chutes.
- ii. Medium-size dividers for cereal grain samples:
Min. 18 slots 12, 7 mm chutes.
- iii. Dividers for large pulses:
Min. 18 slots 25 mm chutes.

B.4.3 Conical dividers (Boerner type).

B.4.4 Centrifugal (rotary) divider

One to eight samples may be obtained simultaneously.

These instruments should not be used for division of pulses because damage to samples may occur

B.5 Mechanical instruments for sampling from static bulk.

B.5.1 Electric screw auger

B.5.2 Gravity sampler.

Annexure C: Guide to appropriate instruments for sampling of cereals and other commodities covered in this international standard.

Table C1. Instruments for different types of product and storage states.

Storage State	Cereal grain and pulses.	Flour and other milled products.
Static bulks in silos, bins and ware houses.	1. Concentric hand spears: Open handler, single aperture, open handle multi aperture, open handle, sequentially-opening slots, multi aperture, 2. Gravity spear with extension rods and T-Handle: Concentric type , Cup type 3. Mechanical Samplers: Gravity sampler, suction (or Vacuum) sampler, air-assisted sampler.	Mechanical samplers: electric screw auger, gravity sampler.
Rail wagons, barges and bulk freight containers	1. Concentric hand spears: Open handler, single aperture, open handle multi aperture, open handle, sequentially-opening slots, multi aperture, 2. Gravity spear with extension rods and T-Handle: Concentric type , Cup type 3. Mechanical Samplers: Gravity sampler, suction (or Vacuum) sampler, air-assisted sampler.	Mechanical samplers: electric screw auger, gravity sampler.
Tote bins and rigid containers	1. Concentric hand spears: Open handler, single aperture, open handle multi aperture, open handle, sequentially-opening slots, multi aperture, 2. Gravity spear with extension rods and T-Handle: Concentric type , Cup type 3. Mechanical Samplers: Gravity sampler, suction (or Vacuum) sampler, air-assisted sampler.	Mechanical samplers: electric screw auger, gravity sampler.
Bags and sacks (woven fibre, paper and plastic)	Gravity spear with extension rods and T-Handle: Concentric type; Cup type. Sack Samplers: Dynamic sack spear; Walking stick type; open handle, single a multi aperture; with compartments, single and multi aperture; conical sampler; portable screw auger.	Walking stick type: With compartments, single and multi aperture; conical sampler; portable screw auger. Mechanical samplers: electric screw auger, gravity sampler.

Flour and Flour-Milling

A flour spatula or slick, boot trier and sieves with deep-bottom pans will be needed as equipment for flourmill inspection.

Raw materials:

At the start of the sampling, prior to checking any of the equipment, the FSO should examine uncleaned wheat-cleaning equipment for rodent excreta; mouldy, treated or weevil-cut kernels; dirt; filth; excreta etc. He should next examine coarse separates for rodent excreta and fine screenings for insects, especially weevils. The examination of wheat for ergot-infested kernels (black, discoloured kernels) should be done by the FSO with confirmation in the laboratory by someone trained in this particular examination.

Sampling:

Unless otherwise directed, samples of bulk flour submitted to the laboratory for examination for rodent filth, insect filth, fungus and pesticides should consist of 10 separate portions of about 500g each.

Noodles, Spaghetti, Pasta

Usually, noodles contain eggs, while spaghetti or macaroni do not. Practices vary from country to country. The major problems with noodles are contamination by insect infestation and bacteria, especially *Salmonella* and *Staphylococcus*. All such products can become insect-infested if production equipment is not kept clean. *Salmonella*-contaminated raw materials, particularly eggs, will result in *Salmonella* contamination of the finished product. The FSO should check the use of insecticide and rodenticide chemicals for possible contamination of raw materials and finished products.

When examining for filth, for retail packages of 500g or more the FSO should, unless otherwise directed, sample the square root of the number of packages in the lot, with a minimum of three and a maximum of six packages. Enough smaller packages should be collected for each subsample to provide 500g for bacteriological analysis, the FSO should collect a minimum of 10 packages of 100g each. If bulk noodles are collected, aseptic technique should be used.

Nuts and Nut Products

Nuts in the shell:

The major problems with nuts in the shell are insect infestation and mould growth due to excess moisture. The FSO should check storage conditions for evidence of insects, and crack open shell nuts for evidence of mould and shriveled nuts.

Shelled nuts:

The major problems with shelled nuts are bacteriological contamination and insect infestation. These are normally caused by unsatisfactory processing after cracking.

Groundnuts:

The FSO should observe the steps taken to prevent contamination by aflatoxin and the tests made to determine whether the nuts are aflatoxin-free. He should also observe whether any preservatives or antioxidants are added, either directly or with the roasting oil.

Sampling:

Samples of shelled nuts, groundnuts, groundnut products (including peanut butter) and in-shell pistachio nuts, to be analysed for aflatoxin, may consist of 2-3 kg collected at random to represent the condition of the lot, unless otherwise directed.

Sampling of Alcoholic Drinks

(As per IS:3735-1984)(Reaffirmed-2000)

ITEMS: Beer, Wine, Whisky, Rum, Gin, Brandy.

EQUIPMENTS: The following types of sampling instruments may be used:

- a) Sampling can or weighted sampling can for taking samples from various depths of large tanks.
- b) Sampling tube.

PROCEDURE:

- a) **Sampling can:** it consists of a metal container of suitable capacity (about 1 litre) attached to a suitable lead. For taking a sample, the chain is lowered into the required depth and the stopper is removed by means of chain for filling the container.
- b) **Weighted sampling can:** it can be made of a suitable metal with a suitable capacity for example, 1 litre, and of such dimensions that it will pass freely through the tank dip-hatch. The can should be of such a mass as to sink rapidly in the material to be sampled.
- c) **Sampling tube:** it is made of metal or thick glass and is 20-40mm in diameter and 0.5-1m in length. For taking a sample, the tube is first closed at the top with a thumb or a stopper and lowered until the desired depth is reached. It is then opened for a short time to admit the material and finally closed and withdrawn.

NO. OF SAMPLES/ AMOUNT TO BE TAKEN:

- 1) Fermented alcoholic beverages (Beer, Wines) - The entire quantity of beer manufactured and stored at a time in each bottling vat just prior to bottling shall constitute a batch.
- 2) Distilled Alcoholic Beverages (Whisky, rum, gin and randy) - The quantity of material manufactured at a time in the compounding/ dilution/ distillation tank shall constitute a batch.

LOT SAMPLING:

LOT INSPECTION: If the manufacturer has maintained an adequate and satisfactory system of quality control in the manufacture of alcoholic drinks, the resulting data and information

may be made available to the purchaser alongwith the material supplied to enable him to judge the acceptability of the consignment. When it is not possible to provide information or if the purchaser so desires, the procedure laid down in the following clauses shall be followed for determining the conformity of the material to the requirements of the specification.

Scale of sampling:

- a) Lot- The quantity of packed alcoholic drink of the same type, belonging to the same batch of manufacture and packed in a day, and packed in a day, shall constitute a lot.
- b) For ascertaining the conformity of the material to the requirements of the relevant specification, samples shall be tested from each lot separately.
- c) The number of bottles to be selected from a lot shall depend on the size of the lot and shall be according to Table 1. The bottles selected for net volume according to column 2 of Table1.

TABLE 1 SCALE OF SAMPLING

NO. OF BOTTLES IN THE LOT	SAMPLE SIZE	
	Requirements Other Then Net Volume	Net Volume
(1)	(2)	(3)
Up to 5000	9	36
5001to 10 000	12	36
10 001 to 15 000	15	72
15 001 and above	21	108

- d) These bottles shall be chosen at random from the lot. In order to ensure the randomness of selection, procedures given in IS: 4905- 1968 may be followed.
- e) Initially the number of cartons equal to the number of bottles to be taken from the lot in one set, shall be opened and the bottles in these cartons examined visually for the condition of packing, the external appearance and the fill. The lot shall be considered satisfactory for inspection of other characteristics given in the specification, if all the bottles in the cartons opened are found meeting the requirements for these characteristics.
- f) In case any defective bottle is found according to (e), twice the number of cartons shall be opened and the bottles examined for similar characteristics. If no defective bottle is found, the lot shall be considered satisfactory for inspection of other characteristics given in the specification.

Sampling of Fish and Fishery Products

(As per IS:11427:2001)

ITEMS: Canned Fish, Fresh Fish, Frozen Fish, Dried and Dry- Salted Fish, Fish Oils.

Sampling of Canned Fish:

- a) Lot- Cans of the same type of pack containing material of the same variety, same grade and packed at the same place on the same day shall constitute a lot.
- b) Scale of Sampling:-
 - For ascertaining the conformity of the material to the requirements of the relevant standard, samples shall be tested from each lot separately.
 - The number of cartons/cans to be selected from a lot shall depend on the size of the lot and shall be according to Table 1.

TABLE 1 Scale of Sampling

Number of Cartons in the lot (1)	Sample Size (2)
Up to 24	5
25 to 50	8
51 to 100	10
101 to 250	12
251 to 500	14
501 to 1000	18
1001 to above	24

- If sampling is done before packing the number of cartons shall be computed as 24 cans to a carton up to 150 g pack and 12 cans to a pack if the type of pack is more than 150 g and sampling scale of Table 1 shall be applied. In order to ensure the randomness of selections, procedure given in IS 4905 shall be followed.

Sampling of Fresh Fish:

Scale of Sampling:

- (a) Lot:- All the containers of the material of the same variety, same size grade and packed on the same day and at the same unit shall constitute a lot.
- (b) For ascertaining the conformity of the material to the requirements of the relevant specification, samples shall be according to Table 2.

TABLE 2 Selection of Containers for Fresh Fish

Number of Containers in the Lot (1)	Sample Size (2)
Up to 8	2
9 to 25	3
26 to 50	5
51 to 100	7
101 to 150	8
151 to 300	9
301 and above	10

- (c) These containers shall be selected at random from the lot. In order to ensure the randomness of selection, procedures given in IS 4905 may be followed.
- (d) In order to select at random the required number of fishes, from each of the containers, Table 2 may be applied. In this case, column 1 may be taken to represent the number of cartons, and column 2 the number of fishes to be selected from each carton. The selection of fishes shall be done at random and in order to ensure the randomness of selection, procedures given in IS 4905 may be followed.
- (e) In addition to the fishes selected from each selected carton (see (c)), one fish shall be selected at random from the carton for testing microbiological requirements. In order to ensure the randomness of selection, procedure given in IS 4905 may be followed.
- (f) Representative portions from the tail, middle and upper middle portion of the fishes so selected at (e) shall be aseptically cut and transferred into sterile containers for testing for microbiological parameters. Five such composite sample shall be made from each lot.

Sampling of Frozen Fish:

Scale of Sampling:

- (a) Lot:-
All the blocked packages cartons of the same type of pack containing the material of the same variety (species) and packaged on the same day and at the same unit shall constitute a lot.
- (b) For ascertaining the conformity of the material to the requirements of the relevant specification, samples shall be tested from each lot separately.
- (c) The number of blocks/ packages/ cartons to be selected from a lot shall depend on the size of the lot and shall be according to column 1 and 2 of Table 3.

Table 3 Selection of Blocks/ Packages/ Cartons and Permissible Number of Defectives

Number of Cartons in the lot (1)	Sample Size (2)
Up to 20	2
21 to 50	3
51 to 100	5
101 to 350	8
301 to 500	13
501 and above	16

- (d) In case, more than 10 blocks/ packages are packed in cartons, the sampling scale at Table3 shall be applied to select the number of blocks/ packages.
- (e) The cartons and blocks/ packages from the cartons shall be selected at random. In order to ensure the randomness of selection, procedures given in IS 4905 may be followed.
- (f) From the blocks/ packages selected, 5 composite samples shall be drawn aseptically in sterile containers from each lot for testing for microbiological requirement.

Special Conditions required:

Samples in the container shall be stored and transported in such a manner that there is no deterioration of the material. For frozen fish, temperature of storage transportation shall be -20° C. If samples are not immediately analysed, these shall be stored ad transported without any direct contact with ice. In the case of canned produce the sample cans shall be

stored and transported to the laboratory without any appreciable difference in the temperature.

Sampling of Dried and Dry-Salted Fish

Scale of Sampling

- (a) Lot:-
All the bundles/ bags/ packages in a single consignment, of the same size, grade and material of same variety and packed on the same day, in a single unit shall constitute a lot.
- (b) For ascertaining the conformity of the material to the requirements of the relevant specification, samples shall be tested from each lot separately.
- (c) The number of bundles/ bags/ packages to be selected from a lot shall depend on the size of the lot and shall be according to Table 4.

Table 4 Number of Bundles/ Packages to be Selected for Dried and Dry- Salted Fish

Number of Bundles/ Bags/ Packages in the Lot	Sample Size
(1)	(2)
Up to 8	2
9 to 25	3
26 to 50	4
51 to 100	5
101 to 150	6
151 and above	7

- (d) These bundles/ bags/ packages shall be selected at random from the lot. In order to ensure the randomness of selection, procedures given in IS 4905 may be followed.

Sampling of Fish Oils

Scale of Sampling

- (a) Lot:-
All the containers, in a single consignment, of the same size, containing the material of same type and belonging to the same batch of manufacture shall constitute a lot. If a consignment is declared to consist of different batches of manufacture. The batches shall be marked separately and the groups of containers in each batch shall constitute separate lots.
- (b) For ascertaining the conformity of the material to the requirements of the relevant material specification, samples shall be tested from each lot separately.
- (c) The number of containers to be selected from the lot shall depend on the size of the lot and shall be according to Table 5.

Table 5 Scale of Sampling for Fish Oils

Number of Containers In the Lot	Sample Size
(1)	(2)
Up to 25	5
26 to 50	8
51 to 100	13
101 and above	20

- (d) These containers shall be selected at random. In order to ensure the randomness of selection, procedures given in IS 4905 may be followed.

Sampling of Bakery Products

(as per IS 12471:1989)

1. Items:

- Biscuits
- Wafers
- Bread Rusks
- Ice Cream Cones
- Breads & Buns
- Cakes

2. Sampling Procedure:

3.1 Group I : Biscuits, Wafers, Bread Rusks & Ice Cream Cones	3.2 Group II : Breads & Buns	3.3 Group III: Cakes
<p>Test Samples & Referee Samples</p> <p>3.1.1 From each lot, draw the number of containers of biscuits as given col. 2 of Table 1 in Lot sampling (6.1.3). These containers shall be opened & mixed. From each selected container, about 600 g of biscuits shall be taken from different packets/ portions. This quantity of 600 g shall be, after proper mixing, divided into two equal parts of 300 g biscuits each. The first part of 300 g shall be divided into three equal parts of 100 g each. One of them shall be for the purchaser, another for the vendor & the third for the referee. These biscuits shall be packed in air tight dry containers & labelled with the particulars. Each of these containers of 100 g shall constitute individual test sample. These individual test samples shall be separated into three identical sets of test samples in such a way that each set has a sample representing each selected container.</p> <p>3.1.2 The second part of 300 g shall be suitably powdered & divided into two equal parts of 150 g each for analysis (for detail, refer to IS 12741:1989)</p>	<p>The number of loaves/ buns given under col .2 & 3 of Table 2 in Lot Sampling (6.2.3) combined together (that is $n_1 + n_2$) shall be selected at random from a lot & then randomly divided into two groups so that the number of loaves/ buns given in col. 2 & 3 of Table 2 are obtained separately.</p> <p>Test Samples & Referee Samples</p> <p>3.2.1 Loaves buns selected according to col. 2 of Table 2 in Lot Sampling (6.2.3) shall constitute test samples for visual examination, volume/ mass ratio & mass.</p> <p>3.2.2 For each of the loaves/ buns selected from the lot according to col. 3 of Table 2 in Lot Sampling (6.2.3) , the individual sample for testing total solid content & crude fibre (for detail, refer to IS 12741:1989)</p>	<p>The number of cakes given under col 2 & 3 of Table 3 in Lot Sampling (6.3.3) combined together (that is $n_1 + n_2$) shall be selected at random from a lot & then randomly divided into two groups so that the number of cakes given in col. 2 & 3 of Table 3 are obtained separately.</p> <p>Test Samples & Referee Samples</p> <p>3.3.1 The cakes selected from the lot according to col .2 of Table 3 in Lot Sampling (6.3.3) shall constitute test samples for visual requirements & mass.</p> <p>3.3.2 For each of the cakes selected from the lot according to col. 3 of Table 3 in Lot Sampling (6.3.3) , the individual sample for testing moisture content shall be prepared according to the procedure given in IS 12741:1989</p>

3. Amount of sample to be taken

- Biscuits : 600 g

4. Special Conditions

- Sample shall be taken in a protected place which is free of odour & not exposed to damp, air, dust or soot.
- The samples shall be placed in air tight, clean & dry glass, metallic containers suitably lacquered or lined & stored in such a manner that the material is not unduly affected.
- The samples shall be stored at room temperature.
- While powdering the biscuits , the following precautions shall be observed:
 - (i) A sample of plain biscuits shall be ground as quickly as possible.
 - (ii) The cream, chocolate, jam, jelly or any other filling between biscuits should be removed by gently scraping before powdering the sample.
 - (iii) As far as possible, the coatings & fillings should be removed before powdering the biscuits.
 - (iv) As the biscuits are highly hygroscopic, the preparation of the sample should be done very quickly, preferably in a closed & dry place.

5. Lot Sampling

6.1 Group 1 Biscuits, Wafers, Bread Rusks & Ice Cream Cones

- 5.1.1 All the containers in a single consignment belonging to the same batch of manufacture shall constitute a lot.
- 6.1.2 For ascertaining the conformity of the material to the requirements of the specification, sample shall be tested from each lot separately.
- 6.1.3 The number of containers to be sampled from a lot shall depend upon the size of the lot & shall be in accordance with Table 1

Table 1 Number of Containers to be Selected

Lot Size	Sample Size
Up to 50	3
51 to 150	4
151 to 300	5
301 to 500	6
501 to 1000	8
1001 & above	10

- 6.1.4 The containers shall be selected from the lot at random & to ensure the randomness of selection, procedures given in IS 4905:1968 may be followed.

6.2 Group II Breads & Buns

- 6.2.1 All the loaves of breads/ buns, in a single consignment, of the same type & belonging to the same batch of manufacture, not exceeding 10000 loaves shall constitute a lot.
- 6.2.2 For ascertaining conformity of the material to the requirements of the relevant material specification, samples shall be tested from each lot separately.
- 6.2.3 The number of loaves of breads/ buns to be tested from a lot shall depend on the size of the lot as given in col.1 shall be in accordance with col.2 & 3 of Table 2

Table 2 Number of Loaves of Breads/ Buns to be selected

Number of Loaves in the Lot	Sample Size	
	For Visual ,Volume/ Mass Ratio & Mass n ₁	Chemical Analysis n ₂
Up to 1000	5	3
1001 to 3000	8	3
3001 to 5000	13	6
5001 to 10000	20	9

6.2.4 The loaves/ buns shall be selected from the lot at random. In order to ensure the randomness of selection, procedures of simple random sampling or systematic sampling as given in IS 4905: 1968 may be followed.

6.3 Group III Cakes

6.3.1 All the cakes, in a single consignment, of the same type & belonging to the same batch of manufacture shall constitute a lot.

6.3.2 For ascertaining conformity of the material to the requirements of the relevant material specification, samples shall be tested from each lot separately.

6.3.3 The number of cakes to be tested from a lot shall depend on the size of the lot as given in col.1 & shall be in accordance with col.2 & 3 of Table 3

Table 3 Number of Cakes to be selected

Number of Cakes in the Lot	Sample Size	
	For Visual & Mass n ₁	Chemical Analysis n ₂
Up to 100	3	3
101 to 300	5	3
301 to 500	8	6
501 & above	13	9

6.3.4 The cakes shall be selected from the lot at random. In order to ensure the randomness of selection, procedures of simple random sampling or systematic sampling as given in IS 4905: 1968 may be followed.

Sampling of Spices & Condiments

(as per IS 13145:1993 reaffirmed 1998)

1. Items:

- Black pepper
- Chillies
- Cardamom (small & large)
- Ginger & turmeric
- Powders (including curry powder)
- Saffron

- Vanilla
- Others (including spices)

2. Equipments

3.1 A suitable sampling instrument, depending on the nature of the material shall be used. However, the following sampling instruments are recommended.

i. Sampling from Bags

Sack-type spears or triers.

ii. Mixing & Dividing

Shovels & dividing apparatus

3. Sampling Procedure:

- From each of the containers selected from the lot according to 7.3, equal number of increments shall be taken & thoroughly mixed so as to form the gross sample. The increments, from each of the containers sampled from the lot, shall be taken from different sides & depths (top, middle & bottom) so as to obtain representative quantity of material in the gross sample. One sample shall be for the buyer, another for the supplier, third for the referee & fourth sample will be sent to laboratory as laboratory sample. The referee sample bearing the seals of the buyer & seller (or their representatives) shall be kept at a place acceptable to both the parties so as to be used in case of dispute.
- If required, the containers selected from the lot shall be completely emptied & content mixed before taking increments.
- If the containers are packed in case, 5% of the cases subject to minimum of 2 shall be selected & approximately equal number of containers selected from each so as to constitute the requisite sample size.
- The quantity of material obtained in the gross sample shall be suitably reduced by the procedure of coning & quartering so as to obtain the laboratory sample.
 - For coning & quartering, the material shall be heaped into the shape of a cone by pouring scoopful of the material one after another at the apex of the cone till entire gross sample has been coned. The material shall be allowed to slide down the slides of the cone only under the influence of gravity. The cone shall be evenly flattened so that it forms a low circular pile. The pile shall be cut into four quarters along two diameters which intersect at right angles, one pair of opposite quarters shall be retained & the other one rejected. The procedure shall be repeated till the laboratory sample of requisite size is obtained.
- The quantity of material in the gross sample & laboratory sample shall be in accordance with 4.1. However, for some spices & condiments, the recommended quantities are given in 5
- In the case of cardamom for estimation of litre mass, a sample of 200 g shall be taken & the result shall be multiplied by 5 to get the required mass per litre.
- To estimate light berries in black pepper by flotation method, a sample of 100 g shall be taken & multiplied by 10 to get the required mass per litre.

h. Bulk Sampling

Whenever the material is supplied in bulk, the material of the same variety, same year of production & same grade, offered for inspection at a time shall constitute a lot. Equal number of increments shall be taken from different sides & depths of

each heap in the lot. The increments from different heaps shall be thoroughly mixed so as to constitute the gross sample from the lot. The gross sample shall be reduced to laboratory sample by the procedure of coning & quartering.

4. Amount of sample to be taken

Recommended quantities in Gross & Laboratory Sample for various Spices & Condiments

S.No.	Name of Spices & Condiments	Gross Sample	Laboratory Sample	
			Whole	Ground
1	Black Pepper	1 kg	1 kg	250 g
2	Chillies	1 kg	1 kg	250 g
3	Cardamom (small & large)	1 kg	500 g	250 g
4	Ginger & turmeric	5 kg	2 kg	250 g
5	Powders (including curry powder)	800 g	-	200 g
6	Saffron	100 g	-	15 g
7	Vanilla	100 g	-	25 g
8	Others (including seed spices)	1 kg	-	250 g

5. Special Conditions

- The laboratory sample shall be stored in such a manner that the temperature of the product does not vary unduly from the normal atmospheric temperature. Samples which are required to be kept for a long time shall be stored in a cool & dark place.
- Laboratory samples on which the analysis is to be carried out shall be despatched to the testing laboratory as soon as possible.

6. Lot Sampling

- All the containers in a single consignment of spice or condiment pertaining to the same variety year of production & grade & not exceeding 1000 containers shall constitute a lot. If a consignment is declared or is known to include different varieties, different classes or different years of production, or if it appears that the lot is heterogeneous, the containers holding products of similar characteristics shall be grouped together, & each group obtained shall constitute a separate lot.
- For ascertaining the conformity of material in a lot to the requirements given in the individual material specification, samples shall be tested from each lot separately.
- The number of containers to be taken from a lot shall depend on the size of the lot & shall be in accordance with Table as shown below:

Number of Containers to be Selected

Lot Size	Sample Size
Up to 50	5
51 to 100	8
101 to 300	13
301 to 500	20

- i. These containers shall be selected at random from the lot. In order to ensure the randomness of selection, procedures of simple random sampling or systematic sampling as given in IS 4905:1968 may be followed.
- ii. When the product is in movement, samples may be taken at the time of loading or unloading of the containers.
- iii. Increments shall be taken by means of an appropriate sampling instrument from different parts of each container selected.

Sampling of Processed fruits and vegetables.

(As per IS:2860-1964)(Reaffirmed 2001, 2005)

2. Items: Processed fruits and vegetables.

3. Sampling

3.1 General requirements of sampling

3.1.1 Sampling shall be done by a person agreed to between the purchaser and the vendor and if desired by any of them, in the presence of the purchaser (or his representative) and the vendor (or his representative).

3.1.2 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature.

3.2 Scale of sampling.

3.2.1 Lot- In any consignment, all cans of the same size containing material of the same type, style and grade shall constitute a lot.

3.2.2 Selection of sample- The number of packing cases to be selected from a lot for drawing the sample cans shall depend on the size of the lot and shall be in accordance with col 1 and 2 of table 1.

Table 1 Scale of Sampling		
No of cans in the lot (1)	No. Of packing cases to be opened (2)	No. Of cans to be Selected (3).
Up to 200	3	6
201 to 300	4	8
301 to 500	5	10
501 o 800	6	12
801 to 1300	7	14
1301 to 3200	8	16
3201 and above	10	20

3.2.3 The packing cases shall be chosen at random from the lot and for this purpose some random number table as agreed to between the purchaser and the vendor shall be used. In case a table is not available, the following procedure shall be adopted.

3.2.3.1 Arrange all the packing cases in a systematic manner and count them as 1, 2, 3..... Etc, up to r and so on. Every r^{th} case so counted shall be withdrawn,

R being the integral part of N/n

Where

N = total number of cases in the lot, and

n = number of cases to be chosen.

If r turns to be a fractional number, its value should be taken as equal to its integral part.

3.2.4 From each of the packing cases selected (3.2.3.1), to cans shall be drawn at random so as to get the total number of can from the lot as shown in col 3 of table 1.

3.2.5 In addition to the cans selected as in 3.2.4 select 8 can at random as far as possible from all the cases selected (3.2.3) for testing the microbiological requirements.

Bottled Water

The water being bottled should be from an approved source and regularly tested chemically and microbiologically for conformity to prescribed standards.

The FSO should check any treatment of the water by distillation, ion exchange, filtration, UV treatment, reverse osmosis, carbonation, mineral addition, or other treatment or purification steps. Samples for laboratory analysis should consist of at least 18 retail units with the same code number. This should be sufficient for both chemical and bacteriological assay.

Food-Canning Plant

Canned food products should be stored in such a manner that they do not become frozen or exposed to high humidity or excessive heat. These conditions can all cause stress on the container closure system.

Sampling. For canned foods the following schedule may be used. Can sizes have been standardized in most countries, and are referred to by number (3, 10, etc.). A No. 3 can has diameter of 108 mm and its height is 123.8 mm.

For products that are likely to be non-uniform throughout the lot, because of variations from standards of quality, identity, or fill-of-container, samples for analysis should be collected as follows:

Can size	Minimum number of subsamples
108-mm diameter x 123.8mm height and Smaller:	
Small lots Up to 1000 cases 1001 to 3600 cases Larger cans: Up to 600 cases 601 to 900 cases 90 to 1300 cases	24 cans 48 cans, each can from a different case 72 cans, each can from a different case 24 cans, each can from a different case 30 cans, each can from a different case 36 cans, each can from a different case

When inspecting abnormal cans, the FSO should examine at least the square root of the number of cases, or all if there are 10 or less. He should report the number of cases examined; the number of cans in each, the number of swells, springers and flippers, and the number of normal cans, as well as other pertinent facts, such as age of shipment.

No field examination should involve more than 1152 containers. However, a minimum of 288 containers should be examined if five or more abnormal containers are found.

The FSO should adhere to the following schedule for the field examination:

No. of containers examined	No. of abnormal containers requiring collection of samples	No. of abnormal containers requiring examination to be continued
288 cases (12 of 24 cans each)	5 or more	4 or less
576 cases (24 of 24 cans each)	7 or more	6 or less
864 cases (36 of 24 cans each)	9 or more	8 or less
1152 cases (48 of 24 cans each)	11 or more	

1. Sampling of Meat & Meat Products (as per IS/ISO 3100-1:1991)

2. Items:

Meat & meat products prepared or packed as individual units of any size & meat in pieces not exceeding 2 kg in mass

- Fresh Meat
- Frozen Meat
- Semi-preserved products
- Shelf-stable packaged or non-packaged products

Carcasses, cuts of carcass or meat in pieces exceeding 2 kg in mass & mechanically separated meat or dried meat

- Fresh meat
- Frozen Meat
- Dried meats
- Drip

3. Equipments

3.1 Waterproof, greaseproof, insoluble & non-absorbent container of a capacity & shape suited to the size of the sampling units

3.2 Stoppers for bottles- either suitable rubber or plastic stopper or a new cork stopper or by a screw cap made of metal or plastic. Stoppers shall be covered with a foil made of inert material before being pressed into the sample container. Screw caps have a liquid –tight liner made of inert material.

3.3 Oven for dry sterilization or Autoclave for wet sterilization

- Dry sterilization at not less than 170 °C for not less than 1 h, using an oven with efficient air circulation to ensure that the stated temperature is maintained in all parts of the oven.
- Wet sterilization at not less than 121°C for not less than 20 min.

3.4 Refrigerator, capable of being maintained at 2 °C & a **freezer** capable of being maintained at or below -24°C , for the storage of samples

3.5 Instruments (sterilizable), for opening packages of meat & cutting up samples, for example can-openers, scissors, knives & forceps.

3.6 Swabs, made of cotton or alginate

3.7 Tubes or flasks , with glass beads, in which swabs can be shaken

3.8 Flasks, for drips from samples.

3.9 Pipettes or syringes, for removing drips from the thawed or packaged meat samples.

Materials & equipments shall not influence the results of the examination to be carried out. It may be necessary to minimize the effect(s) of light &/or oxygen.

4. Sampling Procedure:

4.1 Meat or meat products prepared or packed as individual units of any size, or meat in pieces not exceeding 2 kg in mass

Take units or whole pieces as primary sampling units. Take the required number of primary sampling units from each lot according to the sampling plan mentioned in Number of sampling units to be taken

4.2 Carcasses, cuts of carcasses or cured meat in pieces exceeding 2 kg in mass , & mechanically separated meat or dried meat

Take the required number of primary sampling units from each lot according to the sampling plan mentioned in Number of sampling units to be taken & isolate them either for the removal of secondary sampling units for destructive examination in the laboratory (for example chemical or microbiological examination) or for non-destructive examination (for example visual inspection, sensory assessment, microbiological tests using the swab technique).

No single sample taken from a carcass or other large piece of meat can be truly representative of the whole but, equally, it is impracticable to analyse the entire meat unit. Therefore, in taking primary or secondary samples, the purpose for which they are being taken will determine the procedure to be followed.

Thus, in general, samples shall be taken as follows:

- (a) Surface sampling units (for example for the detection of coliforms or Salmonellae) shall be taken by wiping over the entire meat unit (or selected areas) with large moist swabs or (for making microbiological counts) by defining areas using a template & exercising or, in the case of frozen meat, scraping those areas;
- (b) Excised sampling units having a mass between 500 g & 1 kg for chemical or microbiological examination in the laboratory shall be taken , wherever possible, from an existing cut surface & in such a way as to cause minimum damage;
- (c) Deep muscle sampling units for microbiological examination [for example for the determination of the causes of deep putrefaction at bone level (“ bone taint”)] shall be taken from the affected part of the carcass with a sterile stainless steel myectome or, in the case of frozen meat, with a brace & bit;
- (d) Fat sampling units (for example for the estimation of fat-soluble compounds such as certain pesticides) shall, wherever possible , be taken from the kidney fat;
- (e) Drip sampling units , for example from vacuum –packed refrigerated meat, shall be taken aseptically through the foil or after opening of the package , using sterile syringes & /or flasks or bottles . If the meat is returned to the lot, this shall be done after repacking under vacuum.

5. Number of sampling units to be taken

The number of sampling units to be taken in order to obtain a primary sample which is as representative as possible of the consignment or lot(s) shall be in accordance with the sampling plan specified in the contract or otherwise agreed between the parties concerned.

If different types of test (e.g. chemical, microbiological, physical & sensory) are to be carried out, separate sampling units shall be taken for each type of test.

- consignments or lots of meat & meat products prepared or packed as individual units of any size (for example sausages, vacuum –packed minced meat, sliced sausages, canned cooked ham) ,or meat in pieces not exceeding 2 kg in mass;
- carcasses, cuts of carcass or cured meat in pieces exceeding 2 kg in mass(for example bacon joints, sides of bacon, fresh or frozen boneless meat, beef sides or quarters, pork sides lamb carcasses, venison), & mechanically separated meat or dried meat

6. Special conditions required

Storage & receipt

6.1 General

Samples shall be stored at the prescribed temperature, protected from direct sunlight or other sources of heat.

Contamination shall be prevented

Start the examination as soon as possible after receipt of the samples, & in any case, within the limits given in 6.2 & 6.3

6.2 Meat & meat products prepared or packed as individual units of any size & meat in pieces not exceeding 2 kg in mass

6.2.1 Fresh meat

Store the samples in refrigerator (3.4) on receipt & examine them within 24 h.

If a longer storage period is absolutely unavoidable, freeze them in the freezer (3.4) as soon as possible.

If a sample has been frozen, indicate this in the test report & state the temperature & duration of frozen storage.

6.2.2 Frozen meat

The sample shall reach the laboratory in a frozen condition & the temperature laid down by any legislation in force or, in any case, at a temperature of -24 °C or lower. Store the samples in the freezer (3.4)

6.2.3 Semi- preserved products

The sample shall be stored in the refrigerator (3.4).

Defective samples shall be placed in sealed containers (for example plastic bags), so as to avoid environmental contamination.

6.2.4 Shelf-stable packaged or non-packaged products

Apparently normal samples shall be stored, protected from direct sunlight or other sources of heat, at a temperature not exceeding 25 °C. Visibly defective samples shall be placed in sealed containers (for example plastic bags), so as to avoid environmental contamination, & shall be stored in the refrigerator (3.4).

Dried meat shall be stored in an airtight container.

Examination shall take place within 3 days.

In case of doubt, treat as in 6.2.1

6.3 Carcasses, cuts of carcass or meat in pieces exceeding 2 kg in mass & mechanically separated meat or dried meat

6.3.1 Fresh meat

See 6.2.1

6.3.2 Frozen meat

See 6.2.2

6.3.3 Dried meat

See 6.3.4

6.3.4 Drips

Store the sample in the refrigerator (3.4)

The sample shall be examined as soon as possible, but in any case on the day of receipt.

6.3.4 Swabs

Store the cotton or alginate swabs in the refrigerator (3.4) on receipt.

The samples shall be examined as soon as possible, but in any case on the day of receipt.

Sampling of Food Colours

(As per IS 1699:1995, Reaffirmed 2005)

2. Items: Food colours

3. Equipments: Air tight containers of inert material, spatula, scoop.

4. Procedure

- a) To draw a representative sample, the contents of each container selected for sampling shall be mixed as thoroughly as possible by suitable means. The sample shall be placed in clean, dry, air-tight glass containers or other suitable containers on which the material has no action.
- b) Draw with an appropriate sampling instrument, small quantities of the material from different parts of each container selected according to col 2 of table 1. Mix all the portions so drawn, thoroughly, to form a composite sample weighing not less than 30 g. Divide the composite sample into three equal parts to form test samples. Each part thus obtained shall constitute the test sample weighing not less than 10 g and shall be sufficient to conduct all the tests. The test samples shall be transferred immediately to thoroughly clean and dry containers which shall be sealed air-tight.
- c) Each sample container shall be sealed air-tight with a stopper after filling, and marked with full details of sampling, date of sampling, batch and code number.

5. Size of test sample: The test sample should be not less than 10 g.

6. Special conditions:

- a) The samples shall be taken in a protected place not exposed to damp air, dust or soot.
- b) The sampling instrument shall be clean and dry.
- c) The sample, the material being sampled, the sampling instrument and the containers for samples should be protected from adventitious contamination.
- d) The samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal atmospheric temperature.

7. Lot sampling

- a) Lot – all the containers of the same material produced under the same conditions of manufacture shall be grouped together to constitute a lot.
 - i. The sample shall be tested from each lot for ascertaining the conformity of the material to the requirements of the.
 - ii. The number of containers to be selected from the lot shall depend on the size of the lot and shall be in accordance with col 1 and 2 of Table 1.

Table 1 Number of containers to be selected for sampling

Lot Size	Number of containers to be selected
2 to 15	2
16 to 50	3
51 to 150	5
151 and above	8

- iii. These containers shall be selected at random from the lot. For this purpose, reference may be made to IS 4905 : 1968.

1. Sampling of Sugar Confectionery

(As per IS: 6287:1985, Reaffirmed 2005)

2. Items: Sugar confectionery

3. Equipments: Air tight glass containers.

4. Procedure

- a) From each of the selected containers, with the help of suitable sampling instrument, approximately equal quantity of material shall be taken out so as to make a composite sample of about 1 kg. This sample shall be thoroughly mixed and divided into three equal parts and transferred to clean and dry glass containers, sealed air-tight and labelled with particulars. One of these composite samples shall be for the purchaser and another for the vendor and the third for the referee.
- b) In case the materials of various types are packed in the same container, the material of each type shall be separated. The sample shall be prepared as mentioned above in 4 (a).
- c) Each sample shall be placed in clean and dry glass containers. The sample containers shall be of such size that they are almost completely filled by the sample. The samples shall be filled loose and not pressed in the container.
- d) Each sample container shall be sealed air-tight with a stopper after filling, and marked with full details of sampling, date of sampling, batch and code number, name of the manufacturer and other important particulars of the consignment.

5. Size of test sample: The test sample should be not less than 1/3 kg.

6. Special conditions:

- a) The samples shall be taken in a protected place not exposed to damp air, dust or soot.
- b) The sampling instruments shall be clean and dry when used.
- c) The sample, the material being sampled, the sampling instrument and the containers for samples should be protected from adventitious contamination.
- d) The samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal atmospheric temperature.

7. Lot sampling

- a) Lot – all the containers in a single consignment of the material drawn from a single batch of manufacture shall constitute a lot. If the consignment is declared to consist of different batches of manufacture. The batches shall be marked separately and the groups of containers in each shall constitute separate lots
 - i. The sample shall be tested from each lot for ascertaining the conformity of the material to the requirements of the corresponding specification.
 - ii. The number of containers to be sampled from each lot shall depend on the size of the lot and shall be done according to Table 1.
 - iii. These containers shall be selected at random from the lot and for this purpose a random number table (see IS 4905-1968 Methods of random sampling) as agreed between the purchaser and the supplier shall be used. If such table is not available, the following procedure shall be adopted:

Starting from any container in the lot count them as 1, 2, 3,.. up to r in a systematic manner, where r is the integral part of N/n , N being the total number of containers in the lot, n the number of containers to be selected (see Table 1). Every r th container thus counted shall be separated until the requisite number n container is obtained from the lot to give the sample for the test.

Table 1 Number of containers to be selected for sampling

Lot Size N (1)	Number of containers to be selected (n) for size of the containers	
	500 g and Above (2)	Less than 500 g (3)
Up to 25	3	6
26 to 100	4	6
101 to 300	5	9
301 to 500	7	12
501 and above	9	15

1. Sampling of Cocoa Beans

(As per IS 9314:2003/ ISO 2292:1973)

2. Items: Cocoa Beans

3. Equipments

- a) Sampling spear (open trier) – for sampling from bags.
- b) Hand scoops, triers etc – for drawing small samples periodically from a flow of cocoa beans.
- c) Conical divider, quartering irons – for mixing and dividing of samples.
- d) Containers and closures – they should be perfectly clean and dry. They should be of a material not likely to affect the odour, taste or composition of the product; for e.g.: fabric with a close weave, strong paper, board, metal, suitable plastics material, glass.
- e) Hermetically sealable and water-tight containers – for samples for moisture determination.

4. Procedure

- a) Primary samples – According to circumstances, primary samples should be taken from bulk products or from bags as indicated in 4 (a) i and 4 (a) ii. A minimum of 300 beans should be taken per tonne or fraction of tonne.

- i. Bags – Primary samples should be taken from at least one-third of the bags in each lot, the bags being drawn at random throughout the lot. They should be taken by means of an open trier, at random, from the top part, the centre and the bottom part of bags in good condition.
- ii. Bulk – at least five primary samples should be taken per tonne or fraction of a tonne.

When sampling is carried out while the product is in motion, the primary samples should be taken through the whole section of the flow of beans, at intervals determined by the rate of flow.

When the sampling of beans in bulk takes place when the product is on a clean surface, the primary samples should be drawn from the upper part; the middle and the lower part of the heap after the beans in the lot have been carefully mixed.

- b) Bulk sample – to obtain the bulk sample, combine the primary samples and mix them carefully.
- c) Reduced sample (Final lot sample) – Reduce the bulk sample by division, using the mixing and dividing apparatus, until a reduced sample is obtained of a size depending on the number of final lot samples necessary and on their mass. The number of final lot samples to be made up for examination and arbitration should be specified in the contract or should otherwise be the subject of an agreement between buyer and seller; it may also be specified by an official agency concerned with checking. A mass of 2 kg per final lot sample is usually sufficient. A larger or

smaller sample may be requested in certain cases depending on the tests which have to be carried out.

- d) The samples shall be representative of lots and since the composition of the lots is usually to some extent heterogeneous, a bulk sample shall be taken from each lot by drawing a certain number of primary samples and carefully mixing them. The sample for laboratory examination shall be obtained by successive reductions of this bulk sample.
- e) The sampling of beans which are sea-damaged or otherwise damaged in transit, or in poor condition, as well as loose collected (leaked from its original container but not unduly contaminated) or rejects, shall be carried out separately from the sampling of sound beans. These products shall not be mixed with sound material, but shall be assessed separately.
- f) The following minimum information should be given on the labels, legibly and indelibly:
 - i. Ship, vehicle or warehouse
 - ii. From (in case of transport by ship or vehicle)
 - iii. To g
 - iv. Arrived g
 - v. Quantity
 - vi. Bulk/bags (number)
 - vii. Goods
 - viii. Identification mark or lot no.
 - ix. No. And date of the bill of lading or of the contract
 - x. Date of sampling
 - xi. Place and point of sampling (in the case of products in motion in particular, indicate whether sampling took place on entry to, or on leaving, the transit system)
 - xii. Sampled by g

5. Size of test sample: 2 kg per final lot sample.

6. Special conditions:

- a) The complete consignment shall be examined in lots of not more than 25 tonnes on despatch and not more than 200 tonnes on arrival
- b) All sampling apparatus shall be clean, dry and free from foreign odours.
- c) Sampling shall be carried out in such a way as to protect the samples of cocoa beans, the sampling instruments and the containers in which the samples are placed from adventitious contamination such as rain, dust etc.
- d) Matter adhering to the outside of the sampling instrument shall be removed before the instrument is emptied of its contents.

7. Lot sampling

- a) Lot – A quantity of merchandise assumed to be of uniform characteristics, taken from the consignment and permitting the quality of the merchandise to be

assessed. Lot should not exceed the sizes (25 tonnes on despatch and 200 tonnes on arrival) and each final sample should represent only one lot.

1. Sampling of Tea (as per IS 3611:2000)

2. Items:

- Containers containing more than 20 kg of loose tea
- Containers containing not more than 1 kg of loose tea
- Containers containing 1 to 20 kg of loose tea

3. Equipments

3.1 Spoons, scoops, bores or other instruments, suitable for taking samples from the interior of containers.

3.2 Dividing apparatus, suitable for the purpose of reducing the bulk sample to obtain the laboratory samples.

4. Procedure for Random Sampling

The containers to be sampled shall be taken at random, and, for this purpose, use should be made of random number tables. If such tables are not available, the following procedure may be used:

Let N be the number of containers in the lot & n the number of containers to be taken. Starting from any container, count the containers in orders as 1, 2, ... etc. up to r , where $r = N/n$. (If N/n is not a whole number, take r as the integral part of it). Select the r^{th} container as a sample. Continue counting & selecting every r^{th} container, until the required number of containers has been taken.

In the case of containers containing less than 1 kg of loose tea, if the containers are packed in outer cases, carton or crates containing a convenient number of units, approximately 20% (but, not fewer than two) of the outer packages shall be taken at random. From these, small containers shall be taken in equal numbers, at random, so as to make up the required number of containers to be sampled as specified in 7.2

5. Amount of sample to be taken

Primary Samples

Quantity of loose tea in a container	Amount of primary sample to be taken
More than 20 kg	50 g
Not more than 1 kg	(i) If the amount of tea in each container taken from the lot as described in 4 (Procedure of Random Sampling) does not exceed 50 g, each of containers shall constitute a primary sample. (ii) If the amount of tea in each container exceed 50 g, it shall be taken carefully mixed & a primary sample 50g shall then be taken by means of apparatus mentioned in 3.1 (iii) If the amount of tea in each container is less than 100 g, select a sufficient number of containers to obtain the minimum mass of each laboratory sample.
1 – 20 kg	If appropriate i.e. particularly in the case of small containers within the range 1 to 20 kg, the contents of

the container shall be well mixed. Then, by means of the apparatus mentioned in 3.1, a primary sample of 50 g , representative of the contents, shall be taken from each container selected as described in not more than 1 kg (ii). Otherwise proceed as described in (iii)

The size of each laboratory sample shall be not less than 100 g for the purposes of chemical analysis & not less than 50 g for sensory tests, unless otherwise agreed.

6. Special Conditions

- Sampling shall be carried out in a covered place, in such a manner that the

Number of containers in lot	Number of containers to be sampled
2 to 10	2
11 to 25	3
26 to 100	5
101 & over	7

samples of tea, the sampling instruments & the sample containers are protected from adventitious contamination & other factors likely to affect the samples, for example moisture, dust, radiation etc.

- Handling of the samples (for example combining of primary samples into the bulk sample, packaging of the sample) shall be carried out with care in order to avoid changing the original characteristics of the tea.
- Samples shall be packed in clean, dry, odour – free aluminium or tin – plate containers with close- fitting lids, of such a size that they are almost completely filled by the sample. It is imperative that containers for samples for sensory tests are seasoned to avoid taint. Seasoning of sample containers involves exposure of the inside to the atmosphere (“airing off”) or storage with tea of the same type as the sample before use to eliminate taint whether from the container itself or from tea previously contained in it.
- The samples shall be protected from light during storage.

7. Lot Sampling

Number of containers to be sampled

7.1 Containers containing more than 20 kg of loose tea ¹ (for example, tea chests)

In the case of containers containing not more than 20 kg of loose tea, the minimum number of containers to be sampled from a lot shall be as shown in table 1.

Table 1

Number of containers in lot	Number of containers to be sampled
2 to 10	2
11 to 25	3
26 to 100	5
101 & over	7

7.2 Containers containing not more than 1 kg of loose tea

In the case of containers containing not more than 1 kg of loose tea, the minimum number (see 5 -Amount of sample to be taken) of containers to be sampled from a lot shall be as shown in table 2 , provided that the mass specified for each laboratory sample is obtained.

Table 2

Number of containers in lot	Number of containers to be sampled
Upto 25	3
26 to 100	5
101 to 300	7
301 to 500	10
501 to 1000	15
1001 to 3000	20
3001 & over	25

7.3 Containers containing 1 to 20 kg of loose tea

The minimum number of containers to be sampled from a lot shall be that shown in table 1 or table 2 according to agreement between the interested parties.

- 1) "Loose tea" means tea in containers not otherwise packeted.

1. Sampling of instant tea in solid form

(As per IS: 13861:1993)

2. Items: Instant tea in solid form

3. Equipments

- a) Powder scoop – For method A and B.
- b) A suitable trier – For method C.
- c) Polyethylene bag (large enough to hold all primary samples) – For method A, B, C and D.
- d) Polyethylene bag (at least equal in volume to those in which the instant tea is received from the manufacturer) – For method B.
- e) Polyethylene heat sealer (optional).

4. Procedure

Sampling from immediate containers:

- a) Number of immediate containers to be sampled – unless otherwise specified or in a contract, the number of immediate containers to be sampled shall be given as follows:

1. Immediate containers containing more than 20 kg of loose instant tea: In this case, the no. of containers to be sampled from a lot shall be as shown in table 1.

Table 1

Number of immediate containers in lot	Number of immediate container to be sampled
2 to 10	2
11 to 25	3
25 to 100	5
101 and over	7

2. Immediate containers containing not more than 1 kg of loose instant tea: in this case the minimum no. of containers to be sampled from a lot shall be as shown in table 2, provided that the mass specified for each laboratory sample is obtained.

Table 2

Number of immediate containers in lot	Number of immediate container to be sampled
Up to 25	3
26 to 100	5
101 to 300	7
301 to 500	10
501 to 1000	15
1001 to 3000	20
3001 and over	25

3. Immediate containers containing 1 to 20 kg of instant tea: the minimum no. of immediate containers to be sampled from a lot shall be that shown in table 1 or table 2, according to agreement between the interested parties.
- b) Procedure for random sampling: The containers shall be selected at random from the lot and for this purpose a random number table (see IS 4905-1968 Methods of random sampling). If such table is not available, the following procedure may be used: Starting from any container in the lot count them as 1, 2, 3,.. up to r in a systematic manner, where r is the integral part of N/n , N being the total number of containers in the lot, n the number of containers to be selected. Select the r th container, until the required no. of immediate containers has been taken.

In the case of immediate containers containing not more than 1 kg of instant tea, if the containers are packed in outer cases, cartons or crates containing a convenient no. of units, approx. 20% (but not fewer than two) of these outer packages shall be taken at random. From these, immediate containers shall be

taken in equal numbers, at random, so as to make up the required no. of immediate containers to be sampled as specified in table 2.

- c) Primary samples – the method of taking primary samples depends on the point in the manufacturing and distribution chain at which sampling is to be undertaken and can depend on the methods of analysis that will be made on the samples.

When sampling at point of manufacture, method A shall be used.

When sampling at any point after the point of manufacture, provided that the instant tea is not packed in retail packages.

- Method B shall be used when the samples are required for determination of bulk density, flowability and particle size. The samples shall not be used for the determination of moisture content but may be used for any other determinations.
- Method C shall be used when the samples are required for the determination of moisture content. The samples shall not be used for determination of bulk density, flowability and particle size but may be used for any other determinations.
- When sampling retail packs, method D shall be used. The samples so obtained may be used for any determination.

1. Method A

Using scoop, take primary sample from each immediate container in the lot as it is being filled or when filled but before it is sealed. Place the primary samples in the polythene bag. Minimize the risk of take up or loss of moisture by keeping the polyethylene bag holding the primary samples closed except when inserting further samples, and with as little air inside as possible. Use the primary samples to obtain a bulk sample.

2. Method B

The number of immediate containers to be sampled from a given lot or consignment shall either be subject to prior agreement between the interested parties, or, in the absence of such agreement.

The required number of immediate containers shall be withdrawn from the lot or consignment according to a system of random numbers.

Operating preferably in an air-conditioned room, open completely one of the outer containers & the immediate containers inside it. Gently pour at full flow the entire contents of the immediate container into a polyethylene bag so that the contents are mixed & any layers destroyed.

Using the scoop, take a primary sample from the top surface of this bag & place it in another polyethylene bag (i.e. the bag which will contain the bulk sample). Place the filled bag in its outer container & seal it using the heat sealer or other effective method of sealing.

Repeat the procedure on all the other immediate containers to be sampled.

Use the primary samples to obtain a bulk sample.

Iii Method C

The number of immediate containers to be sampled from a given lot or consignment shall either be subject to prior agreement between the interested parties, or, in the absence of such agreement.

The required number of immediate containers shall be withdrawn from the lot or consignment according to a system of random numbers.

Open each outer container & its immediate container, causing as little damage as possible. Using the trier, take a primary sample from the immediate container & place it in the polyethylene bag. Reseal the immediate container using the heat sealer or other effective method of sealing & reseal the outer container.

Minimize the risk of take up or loss of moisture by keeping the polyethylene bag holding the primary samples closed except when inserting further samples, & with as little air inside as possible.

Repeat the procedure on all the other immediate containers to be sampled.

Use the primary samples to obtain a bulk sample.

Method D

The number of immediate containers to be sampled from a given lot or consignment shall either be subject to prior agreement between the interested parties, or, in the absence of such agreement.

The required number of immediate containers shall be withdrawn from the lot or consignment according to a system of random numbers.

If the amount of instant tea in each immediate container does not exceed 50 g, each container shall constitute a primary sample. (The containers shall be opened & their contents collected together & treated as the bulk sample).

If the amount of instant tea in each immediate container exceed 50 g, invert the container several times to mix the contents. Open the container & pour out about 50 g of instant tea into the polyethylene bag. Minimize the risk of take up or loss of moisture by keeping the polyethylene bag holding the primary samples closed except when inserting further samples, & with as little air inside as possible.

Repeat the procedure on all the other immediate containers to be sampled.

Use the primary samples to obtain a bulk sample.

5. Special instructions:

- a) Sampling should be carried out in a covered place, in such a manner that the samples of instant tea, the sampling apparatus and the sample containers are protected from adventitious contamination and other factors likely to affect the samples like dust, moisture etc.
- b) The sampling instrument shall be clean, dry and do not impart any taint or contamination to the sample.
- c) Handling of the sample (e.g. combining of primary samples into the bulk sample, packaging of the sample) shall be carried out with care in order to avoid changing the original characteristics of the instant tea.

As per IS: 4905-1968, methods of random sampling are as follows:

3. RANDOM SAMPLING METHODS

3.1 Simple Random Sampling

3.1.0 In case the lot consists of a number of items such that each item is easily identifiable and, apart from the lot size, no other information about the composition of the lot is available, the method of simple random sampling may be followed for selecting the items for the sample.

3.1.1 According to this method, the sample of the requisite size n is drawn from a lot of size N in such a manner that, while selecting an item, the chance for any item of the lot being included in the sample is the same. An item once drawn is not placed back in the lot.

NOTE- In case the item drawn is put back in the lot before the next item is selected, thereby allowing for the possibility of the same item being chosen more than once for inclusion in the sample, the method is usually referred to as simple random sampling with replacement. However, this method of sampling is not commonly used in industrial practice and hence it has been left out from further consideration in this standard.

3.1.2 For the selection of a simple random sample of n items from a lot of N . The first requisite is to obtain n random numbers (see 4) which lie in the range 1 to N . For this purpose, starting from any number of the random number table (see Appendix B) and continuing on with the numerals in any direction, right or left, up or down, the succeeding numerals are copied out one-by-one till n different numerals are obtained. The numerals zero or those which are greater than N or which have already occurred shall be omitted. The numerals noted down in this manner shall then be arranged in ascending order of magnitude. Starting from any item in the lot and counting them in one order, the items corresponding to the numerals already noted down shall be withdrawn to constitute the required sample of size n .

3.2 Stratified Sampling

3.2.0 When a lot consists of items which can be divided into a certain number of more homogenous groups or strata, the method of stratified random sampling may be followed according to which each group of stratum is sampled *separately* so as to obtain a sample representative of the entire lot. In such cases, this method of sampling may be generally more efficient than the random sampling as the latter may not always result in the selection of the items from each stratum of the lot, thereby affecting the representativeness of the sample drawn.

3.2.1 The application of the stratified sampling method would require the division of a lot into a suitable, number of strata and then the selection of a simple random sample from each of the stratum to make up the desired sample size. For this purpose, the division of a lot into the strata may be undertaken on the basis of the homogeneity of the items within a lot, convenience of sampling or such other considerations which would make the items within each stratum as much alike as possible whereas those between the strata may also be as much different as possible. The allocation of the number of items to be selected from each stratum is sometimes done on the basis of the variability of the items within a stratum. But in most of the industrial applications such a knowledge is hardly available in advance and hence the number of items to be selected from the stratum is generally taken to be proportionate to the stratum size, that is, the number of items in the stratum. This procedure known as proportional allocation would have the added advantage of

considerably simplifying the estimation of the lot mean proportion of defectives. It would also be advisable to ensure that a minimum of two items are selected from each stratum.

3.2.2 The selection of the sample items from each of the stratum shall be done on the same lines as given in 3.1.2.

3.3 Systematic Sampling

3.3.0 When the items in a lot are presented in an orderly manner (such as piles of asbestos sheets or stacks of cement bags) it is possible to considerably simplify the selection of a random sample of the required size. Instead of choosing the desired number of random numerals and then drawing the items corresponding to these numerals as illustrated in simple random sampling (see 3.1.2), one item is chosen at random from the lot and thereafter the items are selected regularly at predetermined intervals. It has been established that this method of systematic sampling is quite a good approximation to the simple random sampling method described earlier, provided there is no deliberate attempt to manipulate the sequence of the items in the lot in any desired manner while the lot is presented for inspection. Because of its simplicity of operation and the appealing nature of the 'spread' of the sample items all through the lot, the systematic sampling has found a very wide application in industry as well as in other fields like agricultural and socio-economic surveys.

3.3.1 The method consists of first selecting a single sample item from the population of N items and thereafter selecting items at regular predetermined intervals to make up the desired sample of size n . For this purpose, the integral part of N/n (say r) is taken as the interval and then the items are counted in one order and every r th item thus counted is withdrawn until the sample of required size is obtained.

3.4 Cluster Sampling

3.4.0 When the lot submitted for inspection consists of certain groups of clusters of items, it is sometimes advantageous and economical to select a few clusters and then examine all the items in the selected clusters. This would be the case, for example, when the lot consists of items packed in cartons and it is either impracticable or costly to repack the cartons opened for selecting sample items. The method has also extensive applications in agriculture and socio-economic surveys. In the former case, for example, if the problem is to estimate the area under a certain crop in a district, it may be simpler and more economical to ascertain the area under crop in all fields of a selected village (cluster) rather than a few fields in each of the villages of the district. It may however be mentioned here that unlike the stratified sampling, satisfactory results for cluster sampling would be obtained when the items within a cluster are quite heterogeneous.

3.4.1 The method consists of selecting a few of the clusters at random without replacement in the first instance. Thereafter, all the items in each of the selected clusters are pooled to obtain the required sample from the lot.

3.4.2 The selection of the sample clusters from the lot shall be done on the same lines as given in 3.1.2.

3.5 Two-Stage Sampling

3.5.0 When a lot submitted for inspection consists of a large number of packages each consisting of a number of items, it may not be quite economical and feasible to open each of the packages for drawing sample items (as in the case of stratified sampling described in 3.2), or to open only a few packages and inspect all the items in these packages (as in case of cluster sampling described in 3.4). In such cases, it may be desirable to first select an adequate number of packages and then to choose the necessary number of items from each

of these selected packages. Because of the two stages involved in this method of sampling it is referred to as two-stage sampling. The first and second stage units are also sometimes called as 'primary' and 'ultimate' units.

3.5.1 The method consists in selecting the items for the sample in two stages: in the first stage a desired number of primary units is selected at random and in second stage, the required number of items are chosen at random from the selected units.

3.5.2 The selection of the primary units in the first stage as also the selection of items from the chosen primary units is done on the same lines as described in **3.1.2**.

Need for Food Analysis

Food analysis is the discipline dealing with the development, application & study of analytical procedures for characterizing the properties of foods & their constituents. All food products whether raw or processed are analyzed to provide information about a wide variety of different characteristics, including their composition, structure, physicochemical properties & sensory attributes. The food is analyzed for several reasons, e.g. compliance with legal & labeling requirements, assessment of product quality, determination of nutritive value, detection of adulteration, research & development.

Food safety is an issue of prime importance. With the growing concerns about the food & health safety, the food regulatory authorities in different countries have imposed stringent mandatory norms for the presence of various toxicants, which if present beyond a prescribed residual level might prove hazardous to human health. Moreover, with the implementation of WTO & globalization, it has become important that all food products for export out of the country should meet the regulatory norms of the prescribed limits of different toxicants in various food products. As, government bodies regulate the permitted levels of contaminant compounds; much of this advancement has been driven by increased sensitivity & specificity of determination technique e.g. using analytical instruments.

Food Analysis serves as a unique & invaluable tool for all food scientists, technologists & regulatory authorities for quality assurance & control of food products, to study the different aspects of food products.

Food is a complex matrix consisting of different components. These components can be categorized into different categories which are listed as given below:

- 1) **Nutrients:** e.g. Proteins, Amino acids, Total cholesterol, Trans fats & Lipid profile, Carbohydrates. Sugars, Dietary fiber, Vitamins, Minerals etc. Depending upon the food product some of them may be present at high concentration levels while others may be present at low concentration levels of parts per million.
- 2) **Additives:** e.g. Colors, Dyes, Stabilizers, Antioxidants, Flavors & Fragrance, Preservatives, etc.

The additives are added to the food products for the purpose of giving the food products desired appearance, texture, flavor & extending the shelf life. The additives are usually present at very low concentration levels.

- 3) **Adulterants:** They are added intentionally to the food products mostly for the purpose of cost benefits & they may be present at higher as well as lower amounts. They may be safe or sometimes highly toxic, such as, argemone in mustard oil, sudan red in chilies, animal cholesterol in ghee, low cost vegetable oil in high cost vegetable oil etc.
- 4) **Contaminants & Toxicants:** Toxicants can be classified into:
 - a) Physical toxicants- e.g. glass, wood, metal, insect matter etc.
 - b) Biological toxicants- e.g. microbes & pathogens
 - c) Chemical toxicants- e.g. residual pesticides, residual antibiotics, mycotoxins & environmental pollutants like PAH (polycyclic aromatic hydrocarbons), PCB (polychlorinated biphenyls), Dioxins, toxic metals etc.

Most of the times these contaminants are not added intentionally but find their way into the food products from environmental pollution or if proper practices are not being followed during agriculture, animal breeding, storage or processing. The various toxicants are present at low levels of concentration & if present beyond a certain prescribed level of concentration in food products may prove to be highly toxic or carcinogenic to humans.

Accreditation of Food Laboratory

Accreditation is defined as a procedure by which the accrediting body gives formal recognition that a laboratory or an organization is competent to carry out specific tasks.

Food lab accreditation certifies that a lab is using the best test tools and methods for the need and performing those tests correctly under ideal conditions. Laboratory Accreditation provides formal recognition of competent laboratories, thus providing a ready means for customers to find reliable testing and calibration services in order to meet their demands.

Laboratory Accreditation enhances customer confidence in accepting testing / calibration reports issued by accredited laboratories.

In India, accreditation of laboratory is done by National Accreditation Board for Testing and Calibration Laboratories (NABL). NABL is an autonomous body under the aegis of Department of Science & Technology, Government of India. NABL provides laboratories accreditation services to laboratories that are performing tests / calibrations in accordance with ISO/IEC 17025. Hence the laboratories will be required to establish and implement full systems as per the requirements of ISO 17025 and the specific criteria as per NABL 102 (Microbiology) and NABL 103 (Chemical) within a period of one year.

Recognition and accreditation of laboratories, research institutions and referral food laboratories (Section 43)

Authority may notify and recognise food laboratories and research institutions accredited by NABL for purposes of carrying out analysis of samples by Food Analysts, Recognition of referral food laboratories and Recognition of agencies for food safety audit and checking compliances under Food Safety Management Systems.

Process of Accreditation

Stage I

- Prepare your laboratory's application for NABL accreditation, giving all desired information and enlisting the test(s) / calibration(s) along with range and measurement uncertainty for which the laboratory has the competence to perform. Laboratory can apply either for all or part of their testing / calibration facilities. Formats NABL 151, NABL 152 & NABL 153 are to be used by Testing, Calibration & Medical Laboratories respectively for applying to NABL for accreditation.
- Laboratory has to take special care in filling the scope of accreditation for which the laboratory wishes to apply. In case, the laboratory finds any clause (in part or full) not applicable to the laboratory, it shall furnish the reasons.
- Laboratories are required to submit three sets of duly filled in application forms for each field of testing / calibration along with two sets of Quality Manual and Application Fees.
- NABL Secretariat on receipt of application will issue acknowledgement to the laboratory. After scrutiny of application for it being complete in all respects, a unique Customer Registration Number will be allocated to laboratory for further processing of application.
- NABL Secretariat shall then nominate a Lead Assessor for giving Adequacy Report on the Quality Manual / Application submitted by the laboratory. A copy of Adequacy

Report by Lead Assessor will be provided to Laboratory for taking necessary corrective action, if any. The laboratory shall submit Corrective Action Report.

After satisfactory corrective action by the laboratory, a Pre-Assessment audit of the laboratory will be organised by NABL. Laboratories must ensure their preparedness by carrying out its internal audit before Pre-Assessment.

Stage II

- NABL Secretariat shall organise the Pre-Assessment audit, which shall normally be carried by Lead Assessor at the laboratory sites.
- The pre-assessment helps the laboratory to be better prepared for the Final Assessment. It also helps the Lead Assessor to assess the preparedness of the laboratory to undergo Final Assessment apart from Technical Assessor(s) and Total Assessment Man-days required vis-à-vis the scope of accreditation as per application submitted by the laboratory.
- A copy of Pre-Assessment Report will be provided to Laboratory for taking necessary corrective action on the concerns raised during audit, if any.
- The laboratory shall submit Corrective Action Report to NABL Secretariat.
- After laboratory confirms the completion of corrective actions, Final Assessment of the laboratory shall be organised by NABL.

Stage III

- NABL Secretariat shall organise the Final Assessment at the laboratory site(s) for its compliance to NABL Criteria and for that purpose appoint an assessment team.
- The Assessment Team shall comprise of a Lead Assessor and other Technical Assessor(s) in the relevant fields depending upon the scope to be assessed.
- Assessors shall raise the Non-Conformance(s), if any, and provide it to the laboratory in prescribed format so that it gets the opportunity to close as many Non-Conformance(s) as they can before closing meeting of the Assessment.
- The Lead Assessor will provide a copy of consolidated report of the assessment to the laboratory and send the original copy to NABL Secretariat.
- Laboratory shall take necessary corrective action on the remaining Non-Conformance(s) / other concerns and shall submit a report to NABL within a maximum period of 2 months.

Stage IV

- After satisfactory corrective action by the laboratory, the Accreditation Committee examines the findings of the Assessment Team and recommends additional corrective action, if any, by the laboratory.
- Accreditation Committee determines whether the recommendations in the assessment report is consistent with NABL requirements as well as commensurate with the claims made by the laboratory in its application.
- Laboratory shall have to take corrective action on any concerns raised by the Accreditation Committee.
- Accreditation Committee shall make the appropriate recommendations regarding accreditation of a laboratory to NABL Secretariat.
- Laboratories are free to appeal against the findings of assessment or decision on accreditation by writing to the Director, NABL.
- Whenever possible NABL will depute its own technical personnel to be present at the time of assessment as Coordinator and NABL Observer. Sometimes, NABL may at its own cost depute a newly trained Technical Assessor as "Observer" subject to convenience of the laboratory to be accessed.

Stage V

- Accreditation to a laboratory shall be valid for a period of 2 years and NABL shall conduct periodical Surveillance of the laboratory at intervals of one year.
- Laboratory shall apply for Renewal of accreditation to it at least 6 months before the expiry of the validity of accreditation.

Criteria's for the Accreditation of food laboratories

The technical criteria common to testing of both chemical and microbiological parameters have been given under the heading "General" and criteria specific to chemical or microbiological testing have been separately given under the heading "Chemical" or "Microbiological" against each of the technical requirements.

Note 1: The term "shall" used throughout this document indicates those provisions which are mandatory in nature. The term "should" is used to indicate guidance which, although not mandatory, is provided as a means of meeting the requirements of the approval criteria. In case the laboratories use an alternative means of meeting the requirements, they would have to provide suitable and adequate justification.

Note 2: The laboratory shall be required to establish documents in the form of manual, procedures, work instructions etc wherever the criteria has referred to a documented procedure or system. In all other cases the laboratory shall be required to demonstrate compliance to the requirements of the criteria. However, even in these cases the laboratories are encouraged to document their system.

1 Administrative Requirement

1 Organization

1.1.1 Organization Structure The organization structure of the laboratory shall be defined and documented. It shall include the reporting and supervisory structure. The size, structure and composition of the laboratory, taken together should be suitable for the competent performance of the technical and administrative functions of the laboratory. The responsibilities and authorities of key personnel of the lab shall be documented along with job descriptions for all personnel. Deputies shall be appointed for key managerial personnel. The laboratory should appoint a member of staff with defined responsibility and authority for ensuring that the system related to quality as described vide this criteria is established, implemented and followed at all times.

1.1.2 Supervision - The laboratory shall have a documented system for providing effective supervision of the testing activities carried out by individual analysts in different testing sections like chemical, microbiology, residue, etc, as applicable. The supervision should be provided by persons familiar with the test methods and analysis work in the particular testing section. Bigger laboratories, that is, laboratories having more than six analyst, should provide for a three level supervisory structure.

1.1.2 Integrity, Impartiality, etc – The laboratory shall have policies and procedures in place to avoid involvement in any activities that would diminish confidence in its competence, impartiality, judgment or operational integrity.

1.2 Document Control - The laboratory shall establish system for approval and issue of all the internal procedures, SOPs, work instructions, formats, etc, established in line with the requirements of this criteria. A system shall also be established for controlling distribution of external origin documents like standard test methods, etc. The laboratory shall also ensure that authorised editions of appropriate documents are available at all locations where operations essential to the effective functioning of the laboratory are performed and invalid or obsolete documents are promptly removed from all points of issue or use, or otherwise assured against unintended use.

1.3 Review of Requests for tests

1.3.1 - Based on the facilities available, the laboratory shall maintain detailed data on the types of tests that are required to be carried out for various food products as per regulatory requirements. While preparing these lists, consideration shall be given to various circulars and orders received from the FSSAI, Central Food Laboratories, etc. Further, based on the facilities available in house, the lab should also list out the parameters it is capable of testing for various food commodities as per the regulatory requirements. There shall be a system of sharing this information with other sister laboratories.

1.3.2 - The laboratory shall establish a system for review of request letters accompanying the samples received for testing. Based on the review and the capability as determined above, it shall decide on the tests that would be carried out in house and inform the testing section of the same. In case of any temporary incapacity due to equipment breakdown or temporary non availability of testing staff, the same may be taken in to consideration for deciding about subcontracting of the tests.

1.4 Subcontracting – When a laboratory is required to subcontract testing work, because it does not have testing facilities for testing of some of the parameters for certain food products as per the regulatory requirements, then the lab shall ensure that the testing work is subcontracted to a laboratory which is similarly approved or accredited against ISO 17025 for those tests.

2. Technical Requirements

2.1 Personnel

2.1.1 General

2.1.1.1 The laboratory management shall ensure the competence of all those who operate specific equipment, perform tests and/or calibrations, evaluate results, and sign test reports and calibration certificates. The requirements with respect to educational qualification, training, experience and/or demonstrated skills shall be as per individual testing section requirements as stated in subsequent clauses.

2.1.1.2 The laboratory shall have a system for authorising testing personnel for specific tests based on in house, on the job training or external training and subsequent evaluation. Further the laboratory shall have a system for authorising specific personnel to perform certain specific tasks like issue of test reports, to give opinions and interpretations, to handle sample receipt and coding, to operate particular types of equipment, etc. The laboratory management shall ensure that all personnel have received adequate training for the competent performance of the assigned tasks. Personnel may only perform tests on samples if they are either recognized as competent to do so, or working under adequate supervision.

2.1.1.3 Authorised signatory should fulfil either of the following requirements listed below:

- a) Five years experience in similar area out of which at least two years experience should be at supervisory level.
- b) Postgraduate/higher degree in the relevant field or equivalent with a minimum of two years experience in the relevant scope of testing.

The competence of authorised signatory will be assessed during the assessment before being approved by FSSAI. Relaxation in minimum qualification and/or experience requirements for authorised signatory can be considered by FSSAI on specific recommendation by the assessment team on competence with objective evidences for proven competency. Signatories must be able to oversee the operations and cope with any problems that may arise in their work or that of their colleagues or subordinates. All tests must be carried out under the supervision of concerned signatory. After hours, weekend,

statutory holidays and during short absences a signatory needs to be available on-call (able to be contacted at all times and able to arrive back at the laboratory within the same day).

2.1.1.4 The laboratory shall establish a system for initial and ongoing training of the individuals working in the laboratory whose work has influence on quality of testing. Internal training alone is not considered adequate to make the staff knowledgeable on the latest status of science and technology and while introducing specialized testing like residue testing, pathogen testing, etc. The laboratory shall document a procedure for identifying training needs and providing training to its personnel. The training programme shall be relevant to the present and anticipated tasks of the laboratory. It should include specialised training in different fields of food testing like general chemical, residue and microbiological testing as relevant to the laboratory. The effectiveness of the training actions taken shall be evaluated. Evidence of effective training in specific field should be available in terms of performance in quality checks.

2.1.1.5 On-going competence should be monitored objectively with provision for retraining where necessary. Where a method or technique is not in regular use, verification of personnel performance is necessary before the testing is undertaken. The critical interval between performances of non-routine tests should be established and documented by the laboratory.

2.1.1.6 If the laboratory has a system for employing personnel on contract, then they shall be appointed on long-term contract basis. The laboratory shall also ensure that such personnel are supervised and competent and that they work in accordance with the laboratory's established systems. For the purpose of criteria, minimum period of contract shall be 3 years.

2.1.1.7 The laboratory shall maintain current job descriptions for managerial, technical and key support personnel involved in tests and should also include contracted personnel.

2.1.2 Chemical

2.1.2.1 The chemical testing section of the laboratory shall be headed by a person preferably having a post graduate degree in Chemistry or equivalent or Bachelor's degree in chemistry, food technology or equivalent with adequate experience (at least 5 years) in the relevant area especially in the analysis and testing of relevant products. The requirements for authorized signatory in chemical testing section should be similar to that specified above. The minimum qualification for the testing staff in a chemical testing laboratory shall be Graduate in Science with chemistry as one of the subjects or equivalent. The staff shall have sufficient training and exposure in analytical chemistry and in analysis and testing of appropriate products.

2.1.2.2 Chemical testing laboratory involved in testing variety of general chemical testing parameters and residue parameters should have a group in-charge for both the testing areas. The group in-charge should have adequate relevant experience in addition to the minimum qualification.

2.1.3 Microbiological

2.1.3.1 Microbiological testing of food samples involves pathogen testing and as such shall be headed by a qualified microbiologist (graduate/post graduate in Microbiology) with 2-3 years of experience in pathogen testing in case of person with post graduate degree and 5 years in case of person with graduate degree. The requirements for authorized signatory in microbiological testing section should be similar to that specified above.

2.1.3.2 The minimum qualification for the testing staff in a biological testing laboratory shall be graduate or postgraduate in microbiology, food science or equivalent with at least 1 year working experience in similar area as covered by the scope of testing as proven by demonstrated competence on records. Fresher's can be put under training with adequate supervision. In addition to test methods, in some cases, it may be more appropriate to relate competence to a particular technique or instrument, for example use of approved biochemical, serological kits or microbial identification kits.

2.1.3.3 The interpretation of test results for identification and verification of microorganisms is strongly connected to the experience of the performing analyst and should be monitored for each analyst on a regular basis.

2.2 Accommodation and Environmental Conditions

2.2.1 General

2.2.1.1 Laboratory facilities for testing shall be such as to facilitate correct performance of the tests as per the scope of the laboratory. These facilities shall include accommodation, space, layout, environmental conditions, energy and water sources, biological sterility, dust, humidity, electrical supply, temperature, etc. Adequate and proper lighting should be available. Bench and floor surfaces should be appropriate for the work being performed.

2.2.1.2 The laboratory shall document, monitor, control and record environmental conditions as required by the relevant specifications, methods and procedures or where they influence the quality of the results. Tests and calibrations shall be stopped when the environmental conditions jeopardise the results of the tests.

2.2.1.3 There shall be effective separation between neighbouring areas in which there are incompatible activities. Measures shall be taken to prevent cross-contamination. Storage facilities of the laboratories should be sufficient enough to allow the sample retention and if required segregation of samples for designated periods and provide conditions that maintain sample integrity.

2.2.1.4 Access to and use of areas affecting the security of the sample and quality of the tests and/or calibrations shall be controlled. Access should be controlled by but is not limited to: issuance of key cards for entrance/ issuance of identification badges/ escorting visitors/ use of security guards. The laboratory shall determine the extent of control based on its particular circumstances.

2.2.1.5 Measures shall be taken to ensure good housekeeping in the laboratory.

2.2.1.6 When a change of premises occurs the laboratory shall notify FSSAI. In such cases, revalidation might be necessary in every aspect of environmental and analytical issues.

2.2.2 Chemical

2.2.2.1 Effective separation shall be ensured between residue testing sections, especially the areas used for sample extraction, Certified Reference Material (CRM) dilution and similar activities and the rest of the general chemical testing sections.

2.2.2.2 The chemical testing sections where the heat producing equipments are located should be separated from rest of the testing areas. Proper ventilation should be ensured through the use of exhaust fans and adequate number of fume chambers should be available for carrying out activities, generating excessive fumes.

2.2.2.3 Proper equipment and material like fire extinguishers of suitable type, sand buckets, etc should be available, located at appropriate locations, for handling chemical spillages, fires, etc. Laboratory personnel should be trained in fire fighting. The laboratory should have, as appropriate, safety showers and eye wash equipment, located at suitable locations and in good working condition. Sufficient exhaust hoods and fume cabinets should be available to maintain a safe work environment. Sufficient first aid kits should be available and strategically located. Personal protective equipments like laboratory coats/gowns, gloves, protective eye wear/masks should be made available as relevant. Appropriate storage must be provided for volatile, flammable, explosive and other hazardous materials. General cleanliness and good housekeeping should be apparent. Gas cylinders should be secured and should be kept outside the testing area.

2.2.3 Microbiological

2.2.3.1 The laboratory should be arranged in such a way, that the risks of cross contamination can be reduced. This can be achieved by carrying out the test procedures in a sequential manner using appropriate precautions to ensure test and sample integrity and by segregating the activities by time or space. It is generally considered as a good practice to have separate areas for Sample receipt and sample storage, Sample preparation, Handling and storage of reference cultures/Reference materials, Media preparation and Sterilization, Inoculation and plating, Incubation, Decontamination and any other Incompatible activities.

2.2.3.2 For laboratories engaged in specialized testing like GMO Testing, additional requirements relevant to the activities involved shall apply.

2.2.3.3 For procedures that involve the handling of pathogens and reference stock cultures, they should generally be operated within a safety cabinet of a class commensurate with the risk level of the microorganism handled. Most of the microbes encountered in a non-clinical testing laboratory belong to Risk Group 2 microorganisms e.g. *Salmonellae*, *Staphylococcus aureus*.

2.2.3.4 Laboratories should devise appropriate environmental monitoring program with respect to the type of tests being carried out and records shall be maintained for the same. The laboratory environment, where relevant, shall be microbiologically monitored through exposure plate method for air borne contamination and surface swabbing of sampling and testing benches, utensils, balances stomachers, etc. Based on the results of such monitoring, where relevant, environmental contamination should be controlled by appropriate air-filters and air-exchange systems. Where air conditioners are used filters should be appropriate, inspected maintained and replaced according to the type of work being carried out. The laboratory shall have pest control program, as appropriate.

2.3 Test methods

2.3.1 General

2.3.1.1 The laboratory shall use appropriate methods and procedures for all tests within its scope. The laboratory shall have instructions on the use and operation of all relevant equipment, and on the handling and preparation of samples for testing, where the absence of such instructions could jeopardise the results of tests and/or calibrations. If the standard test methods used contain sufficient and concise information on how to perform the test, then they may not be rewritten as internal procedures. It may however be necessary to provide additional documentation for optional steps or additional details.

2.3.1.2 All instructions, standards, manuals and reference data relevant to the work of the laboratory shall be kept up to date and shall be made readily available to personnel.

2.3.1.3 Normally the standard methods shall not be deviated unless the deviations have been technically justified, validated, documented and authorised. All modified, lab developed or non standard test methods used by the laboratory shall be validated before use. Following information shall be provided for the accreditation of an in-house test method:

- A copy of the fully documented test method
- Details of the origin of the in-house test method
- Details of the reason for its development and application e.g. the specification against which the product/products is being tested
- The results of comparative tests with standard method (if possible) and /or with other laboratories.

2.3.2 Chemical

2.3.2.1 For testing of general chemical parameters, additives, etc, only standard methods as specified in DGHS Manual or standard methods as prescribed in relevant BIS specifications and Association of Official Analytical Chemists (AOAC) standard methods manual, or similar international publications like FDA Macro analytical Procedures Manual (MPM), the Pesticide Analytical Manual (PAM), the Food Additives Analytical Manual, and the Food Chemicals Codex shall be used.

2.3.2.2 For specialized tests like residue testing the laboratory would generally require to use standard methods, modified to suit the sample matrices of food products to be tested for specific residues and laboratory specific aspects like type of equipment used and their sensitivities, extraction technique used and competence and skill development of the testing personnel, in order to achieve the desired detection levels matching the MRL's. Consequently the lab would require to validate the modified test methods. The validation of chemical (specifically residue) test methods should be carried out as per the guidance provide in NABL 103. Some of the relevant aspects to be complied with are given below.

2.3.2.3 The laboratory should develop a detailed procedure for method validation and lab performance validation, covering the Procedure for design of experiment to cover all required method validation parameters, Procedure for carrying out actual validation experiments the number of replicates, number of data points for different types of method performance parameters like recovery, linearity, etc. The laboratory should also establish acceptance criteria. Based on the validation results the lab should document SOP for regular analysis, which should include aspects like system for repeating calibration curves, recovery data, etc.

2.3.2.4 Based on the above the laboratory should establish the laboratory performance characteristics like Selectivity & specificity, Range, Linearity, Sensitivity, LOD, LOQ, Accuracy/recovery, repeatability, etc, for analysis of different residue parameters in different food matrices.

2.3.3 Microbiological

2.3.3.1 The laboratory shall normally use only standard methods as prescribed in DGHS Manual, BIS specifications, AOAC test method manual or any other international publications like USFDA BAM, American Public Health Association (APHA) Compendium of Methods for the Microbiological Examination of Foods.

2.3.3.2 Where standard methods are prescribed and followed, the laboratory is expected to maintain current versions of the standard methods (reference texts) and up-date laboratory bench methods in accordance with these. Although full validation is not required, a laboratory must verify that it can properly operate the method, and can demonstrate (where specified) the limits of detection, selectivity, repeatability and reproducibility. Laboratories shall pay attention to the limitations, concentrations range and sample matrix specified in the test standards.

2.3.3.3 The use of commercial test systems (kits) shall normally be avoided unless absolutely necessary. In case these are used, they shall require further validation if the laboratory is unable to source the validation data. When the manufacturer of the test kits supplies validation data, the laboratory will only perform secondary validation (verification). Laboratories should retain validation data on commercial test systems (kits) used in the laboratory. These validation data may be obtained through collaborative testing, from the manufacturers and subjected to third party evaluation (e.g. AOAC. Refer www.aoac.org for information on methods validation). If the validation data is not available or not applicable, the laboratory should be responsible for completing the primary validation of the method. It has been found in some cases (e.g. veterinary microbiological testing) that a specific test kit performs differently under local environmental conditions, to that of the original environmental conditions it was subjected to primary validation. In such cases the laboratory should conduct the validation to prove that the kit performs under local environmental conditions.

2.3.3.4 The validation of microbiological tests methods should be carried out as per the guidance provide in NABL 102

Uncertainty of Measurement and Limits of Detection

Laboratories need to make a formal estimate of measurement uncertainty for all tests in the scope of accreditation that provide numerical results. Where results of tests are not numerical or are not based on numerical data e.g. detected/not detected, pass/fail, positive/negative, or based on visual, tactile or other qualitative examinations, estimates of uncertainty are not required.

Where an estimate of measurement uncertainty is required, laboratories must document their procedures and processes on how this is to be done. There are various published approaches to the estimation of uncertainty in testing (Eurachem Guide, APLAC, CITMAC, Nordic Committee on Food Analysis (NMKL)). All approaches which give a reasonable estimate and are considered valid within the chemical and biological testing communities are equally acceptable and no one approach is favoured over others.

Once a documented procedure is established, the laboratory should implement a programme for applying this procedure to all relevant tests within the scope of accreditation.

Laboratories that are performing analyses at low analyte levels such as residue testing and low level micronutrient testing, method detection limits need to be determined. Laboratories could follow the methodologies published by CODEX, AOAC, etc. or the specific industry sector it is operating within. However, laboratories should document procedure on how they determine their detection limits. The procedure should be consistent with the source of methodologies normally used (e.g. if American Public Health Association, APHA details detection limit determination methodology, then the laboratory should adopt these recommended practices for method detection limit determination for methods sourced from APHA), or the conventions used within the industry sector.

In the absence of industry or test methodology conventions, laboratories will determine method detection limits from a series of independent analyses of the analyte concerned in the matrix of interest at a level near the expected limit of detection. The method detection limit is calculated from the variation of results at this level, and is not to be confused with a so-called instrument level of detection obtained from readings of a series of blanks. In some cases, the sensitivity of the method is such that the lowest calibration standard is more than two orders of magnitude below the regulatory limit. In this situation, laboratories may sometimes set the limit of detection at the level of the lowest calibration standard, but this approach does require that the laboratory consider the manner in which they report low positive results below the artificially high limit of detection, to ensure that reports are not misleading to users.

2.4 Equipment management, Calibration and Traceability

2.4.1 General

2.4.1.1 The laboratory shall be furnished with all items of test equipment required for the correct performance of the tests contained in the laboratories scope of testing, which may cover General Chemical testing, residue testing and microbiology testing or a combination thereof for a variety of food products as per the Food regulation. Equipment used for testing shall be capable of achieving the accuracy required and shall comply with specifications relevant to the tests as given in the test methods used.

2.4.1.2 All equipments that have direct or indirect effect on the results of analysis shall be calibrated or checked before being placed into service, establish that they meet the laboratory's specification requirements and comply with the relevant standard specifications. There after they shall be calibrated at regular intervals as per laboratories established calibration program. The calibration results shall be verified against the equipment specification/acceptance criteria to confirm continued suitability.

2.4.1.3 Equipment shall be operated by authorised personnel. Up-to-date instructions on the use and maintenance of equipment (including any relevant manuals provided by the

manufacturer of the equipment) shall be readily available for use by the appropriate laboratory personnel.

2.4.1.4 Each item of equipment and its software used for testing and calibration and significant to the result shall, when practicable, be uniquely identified and appropriate records shall be maintained, which should include as relevant the equipment details and specification; the current location, where appropriate; the manufacturer's instructions, manuals, etc; dates, results and copies of reports and certificates of all calibrations, adjustments, acceptance criteria, and the due date of next calibration; the maintenance plan, where appropriate, and maintenance carried out to date; any damage, malfunction, modification or repair to the equipment. If practicable the status of calibration, including the date when last calibrated and the date or expiration criteria when recalibration is due should be indicated on the equipment.

2.4.1.5 Maintenance of essential equipments used in the laboratory shall be carried out at specified intervals as determined by factors such as the frequency of use, ruggedness, etc. All sophisticated equipments available in the laboratory like Atomic absorption spectrophotometers, Gas chromatographs, HPLCs, etc should be serviced and maintained through Annual Maintenance Contracts with the supplier of the equipment/authorised service agents.

2.4.1.6 Equipment that has been subjected to overloading or mishandling, gives suspect results, or has been shown to be defective or outside specified limits, shall be taken out of service. It shall be isolated to prevent its use or clearly labelled or marked as being out of service until it has been repaired and shown by calibration or test to perform correctly.

2.4.2 Chemical

2.4.2.1 General Service Equipment - General service equipment like hotplates, stirrers, non volumetric glass wares including measuring equipments, etc, which do not have direct or indirect effect on analysis results, do not require calibration and should be maintained by appropriate cleaning and checks for safety as necessary. Other equipments like Ovens, furnaces, where the setting can significantly affect the test or analysis result, shall be calibrated using an NABL accredited external labs.

2.4.2.2 Volumetric equipment - The correct use of volumetric equipment is critical to analytical measurements and shall be suitably maintained and calibrated. The volumetric equipment like burette, pipette shall generally be calibrated/verified in house using calibrated analytical balance and thermometers, by density method.

2.4.2.3 Measuring instruments/equipment - Equipment like analytical balances, spectrophotometers, pH meters, Chromatographs, etc, shall be subjected to periodic servicing and calibration. The calibration of these equipment may involve periodic performance checks (to check the response, stability and linearity of sources, sensors and detectors, the separating efficiency of chromatographic systems, the resolution, alignment and wavelength accuracy of spectrometers etc) and calibration (Operational checking) using CRM's (e.g. based on the levels of expected detector or sensor response to calibrants, the resolution of calibrants in separating systems, the spectral characteristics of calibrants etc). The laboratory should establish frequency of such checks/calibrations based on the ruggedness of the equipment, frequency of usage, test method requirements, etc. Equipment like thermometers, etc, should also be covered under the calibration program established by the laboratory.

2.4.3 Microbiological

2.4.3.1 The equipment which do not have direct or indirect bearing on microbiology test results shall be maintained by cleaning and servicing, inspecting for damage, general verification and, where relevant, sterilizing. These are General service equipment like water baths, incubators, microbiological cabinets, autoclaves, homogenizers, fridges, freezers and Accessories like filtration apparatus, glass or plastic containers (bottles, test tubes), glass or plastic Petri-dishes, sampling instruments, wires or loops of platinum, nickel/chromium or disposable plastic.

2.4.3.2 Other commonly used equipments in microbiology labs, which are likely to have direct/indirect effect on test results are balances, thermometers, pH meter, timer, ovens, incubators, autoclaves, water bath, Laminar Flow chamber, Bio safety cabinets and volumetric glassware and these shall be subjected to periodic calibration and/or performance verification.

2.5 Reference materials, Chemicals, Media and Cultures

A RM is a material sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

CRMs are defined as a reference material that is characterised by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value(s) of the specified property (ies), its (their) uncertainty (ies) and a statement of metrological traceability.

2.5.1 Chemical

2.5.1.1 Certified Reference Materials – All the Certified Reference Materials (CRMs) required to cover the scope of testing of the laboratory should be procured from traceable (National Accreditation Board For Testing & Calibration Laboratories, NABL in India, National Institute of Standards and Technology, NIST in USA, Bureau Communautaire de Référence, BCR in Europe, National Measurement Institute Australia etc) sources. In case traceable CRMs are not available, like that for pesticides, etc, then they should be procured from reputed, reliable and industry accepted sources. The laboratory should establish and implement system for handling, storage and maintenance of CRMs, their dilution and preparation of secondary CRM's their maintenance and retention and maintain appropriate records.

2.5.1.2 Reagents and chemicals - The laboratory shall purchase reagents, chemicals, and other consumables only from reliable and reputed manufacturers with proven track record. The laboratory shall also ensure that the quality (grade, etc) of the reagents/chemicals/media used is appropriate for the tests concerned. The solutions made out of chemicals shall be made using standard procedures and when being stored for long term use should be appropriately labelled to identify the substance, strength/concentration, solvent (where not water), any special precautions or restrictions of use, and date of preparation and/or expiry. The person responsible for the preparation of the reagent shall be identifiable either from the label or from records. Acids, alkalies and other solutions prepared for volumetric analysis should be periodically (as per defined system) checked for their strength and documented properly. Reagents used as primary standards for volumetric and gravimetric methods should have traceability to National and International standards.

2.5.2 Microbiological

2.5.2.1 Reference Cultures – These shall be procured for all the microbiological parameters tested by the lab and shall be procured from with traceable and authorized sources (like Microbial Type Cell Culture, MTCC in India, ATCC in USA, NCTC in UK, NZRCC in NZ). The laboratory should establish and document system for handling and effective maintenance of cultures and their appropriate usage.

2.5.2.2 Media, reagents and solutions for microbiology – All the media, reagents and chemicals required for complete range of microbiological tests (including biochemical and serological tests) as per laboratory's scope of testing as prescribed in respective standard test method shall be available. The laboratory should establish and implement a system (or quality control programme) for suitability and efficacy checks (on all media whether it be in-house prepared from basic ingredients, or purchased pre-pared media) on the received media.

2.6 Sample Handling

2.6.1 General

2.6.1.1 On receipt of the test sample, any visible abnormalities, leakages, or departures from normal or specified conditions, as described in the test request as well as test method, shall be recorded. When there is doubt as to the suitability of the sample received, or when a sample does not conform to the description provided, or the test required is not specified in sufficient detail, or the details on the sample and the accompanying letter do not match, the laboratory shall consult the source from where the sample was received for further instructions before proceeding and shall record the discussion. In case the sample is observed to be unfit for testing and it is apparent that the testing would not serve any useful purpose, the lab should decide not to undertake testing and suitably inform the source from where the sample is received.

2.6.1.2 On receipt, a sample shall be registered into laboratory records. The system for registration and the type of records used may vary from lab to lab, however use of a sample register is the most prevalent method for registration purposes. The laboratory shall follow all the defined (as per regulatory norms) system for sample coding, in order to ensure that the source of sample and other details are not be revealed to the testing personnel and others in the laboratory. All staff concerned with administration of the sample handling system should be appropriately trained.

2.6.1.3 The laboratory shall have a system for unique and clear identification of test samples. The identification shall be retained throughout the life of the item in the laboratory. The system shall be designed and operated so as to ensure that sample cannot be confused physically or when referred to in records or other documents. The sample identification number shall be fixed in such a fashion that it does not get obliterated during its journey within the lab. The system identification system shall be extended in case, if appropriate, accommodate a sub-division of the sample and the transfer of sample within different laboratory sections.

2.6.1.4 Frequently, it is necessary to split or transfer samples for testing of different properties. Sub-sampling by the laboratory immediately prior to testing is considered as part of the test method. It should be performed as per national/international standards, where they exist, or by validated in-house methods. It is essential that procedures are available for preventing spread of contamination, delivery of samples including special transportation such as refrigeration and exclusion of light, disposal and decontamination processes and unbroken chain of identification of the sub-samples/samples shall be provided.

2.6.1.5 The laboratory shall have procedures and appropriate facilities for avoiding deterioration, loss or damage to the test samples during storage, handling and preparation. When samples are required to be stored or conditioned under specified environmental conditions, these conditions shall be maintained, monitored and recorded. After completion of testing of the sample the laboratory may require to retain the sample. The laboratory should establish system for retention based on stability and storage considerations of different materials received for testing.

2.6.2 Chemical

2.6.2.1 Sample containers should be leak-proof and impervious to possible contamination during transport. Where specified, samples should be maintained within set temperature or other environmental tolerances during transfer to the laboratory and prior to testing. In some cases, it may be necessary for sample containers to be pretested prior to use to ensure freedom from contamination, especially samples to be tested for residue parameters

2.6.3 Microbiological

2.6.3.1 Laboratory shall examine and record the conditions and appearance of samples upon receipt. Where appropriate time of receipt of the sample, storage temperature of sample on receipt, conditions of sample container, etc should be recorded. If there is insufficient sample or the sample is in poor condition due to physical deterioration,

incorrect temperature, torn packaging or deficient labelling, laboratory should either refuse the sample or carry out the tests as instructed by the sender of the sample and shall indicate the conditions on test reports.

2.6.3.2 Samples awaiting test shall be stored under suitable conditions as relevant for microbiology testing for avoiding modifications to any microbial population present. Storage conditions and maximum holding times for different samples shall be documented and shall fulfil the requirements of test standards. Where a sample has to be held secure, the laboratory must have arrangements for storage and security that protect the condition and integrity of the secured samples concerned.

2.7 Quality Assurance Measures & Proficiency Testing

2.7.1 General

2.7.1.1 The laboratory shall have quality control procedures for monitoring the validity of tests and calibrations undertaken. These should include Internal Quality Control measures like use of Certified Reference Samples/materials, replicate tests or calibrations using the same or different methods, retesting or recalibration of retained items, correlation of results for different characteristics of an item. This monitoring should be carried out as per predetermined plan and should cover all the tests under the laboratories scope of testing, in a defined time period.

2.7.1.2 As external quality assurance measure, the laboratory shall periodically participate in self initiated Inter-laboratory comparison programmes or programmes initiated by other laboratories like delete CFL.

2.7.1.3 In addition to the above, the laboratory should also participate, in independently and professionally organized national/international Proficiency Testing Programmes (such as run by NABL, Asia Pacific Laboratory Accreditation Co-operation (APLAC), as per plan to cover all the tests under the laboratory's scope of testing in a defined period, of say 3 years.

2.7.1.4 Quality control data obtained from Internal Quality Control measures and the External Quality Assurance measures shall be analysed and, where they are found to be outside pre-defined criteria (acceptance criteria), planned action shall be taken to correct the problem and to prevent incorrect results from being reported. If unsatisfactory results are obtained, laboratories shall be able to show that the problems are promptly investigated and rectified, and satisfactory performance for the test/method in question can be achieved afterward. All findings in connection with unsatisfactory performance shall be recorded.

2.7.2 Chemical

2.7.2.1 For Internal Quality Control the level adopted should be demonstrably sufficient to ensure the validity of the results. As a guide, for routine analysis the level of internal QC typically should be not less than 5% of the sample throughout, i.e. 1 in every 20 samples analyzed should be a QC sample. For more complex procedures, 20% is not unusual and on occasions even 50% may be required. For analyses performed infrequently, a full system validation should be performed on each occasion. This may typically involve the use of a reference material containing a certified or known concentration of analyte, followed by replicate analyses of the sample and spiked sample (a sample to which a known amount of the analyte has been deliberately added). Those analyses undertaken more frequently should be subject to systematic QC procedures incorporating the use of control charts and check samples.

2.7.3 Microbiological - The Internal Quality Control measures specific to microbiology testing are given below. The interval between these checks will be influenced by the construction of the program and by the number of actual tests. It is recommended that, where possible, tests should incorporate controls to monitor performance.

2.7.3.1 Sterility controls – Un-inoculated samples are to be run at a minimum of once for every test run. Sterility controls are used to detect the presence or absence of possible laboratory contamination.

2.7.3.2 Split samples (Duplicates) for quantitative tests - Split samples comprise a sample divided into 2 sub-samples. Analyses of split samples are normally expected to be conducted at a frequency of once per test run.

2.7.3.3 Confirmation/verification of presumptive positive samples - Positive and negative characteristic strains, if applicable, shall be tested concurrently with any biochemical, serological and morphological tests for confirmation of presumptive microorganisms. The number or percentage of colonies that stipulated in test standard required for confirmation process shall be followed. Laboratories can also define the minimum number of colonies for confirmation if such requirements are not specified.

2.7.3.4 Verification of continuing competence - Laboratories shall establish schedules, in compliance with the verification frequency stipulated in test standards, for checking the continuing competence to perform positive tests for each test method if no positive samples are encountered. Reference stocks should be maintained for all tests conducted, and suitable suspensions of fresh subcultures should be spiked into appropriate matrix and run through each entire test procedure. The analyst is required to make parallel analyses with another analyst. Criteria shall be set for maximum allowance difference between the counts based on precision of test methods.

2.8 Laboratory Data and Record Management

2.8.1 General

2.8.1.1 The laboratory shall establish a system for identification, collection, indexing, access, filing, storage, maintenance and disposal of technical records. It shall also ensure that all records are legible and stored in a suitable environment to prevent damage or deterioration and to prevent loss. Retention times of records shall be established as per regulatory requirements.

2.8.1.2 The technical records shall cover all original observations, derived data and sufficient information to establish traceability and reconstruction of results; calibration certificates and other instrument related records; relevant records pertaining to CRMs, medias, reference cultures, chemicals, glass wares and other consumables; staff related records; Quality assurance related records; test reports issued and related records; sample receipt, entry and coding related records; various types of monitoring records; etc. The records shall include the identity of personnel responsible for the performance of each test and checking of results.

2.8.1.3 Observations, data and calculations shall be recorded at the time they are made and shall be identifiable to the specific task. When mistakes occur in records, each mistake shall be crossed out, not erased, made illegible or deleted and the correct value entered alongside. All such alterations to records shall be signed or initialed by the person making the correction. In the case of records stored electronically, equivalent measures shall be taken to avoid loss or change of original data. Electronic records and data files are backed up on a regular basis to safeguard against the loss of information due to equipment malfunctions or human error

2.8.1.4 Calculations and data transfers shall be subject to appropriate checks in a systematic manner

2.8.1.5 Access to all records must be restricted to prevent unauthorized use and amending of information.

2.8.2 Chemical

2.8.2.1 Computer controlled automated systems, generally used with sophisticated equipments like AAS, GC, etc, should normally be validated by checking for satisfactory operation and establishing the reliability of the system before it is allowed to run unattended. Where possible the controlling software should be tailored to identify and highlight any such malfunctions and tag associated data. The use of quality control

samples and standards run at intervals in the sample batches should then be sufficient to monitor correct performance on a day-to-day basis. Calculation routines can be checked by testing with known parameter values. Electronic transfer of data should be checked to ensure that no corruption has occurred during transmission. This can be achieved on the computer by the use of verification files' but wherever practical, the transmission should be backed up by a hard copy of the data.

2.8.3 Microbiological

2.8.3.1 An adequate test record system, which provides evidence of all the activities performed during the entire duration of the microbiological tests, like daily observation recording system shall be established and implemented. The laboratory can develop appropriate formats for recording all the observations relevant to the different microbiological tests performed in the laboratory. System for maintaining all other records with respect to media and reagent preparing, culture handling and maintenance, etc shall be established and implemented.

2.9 Reporting

4.9.1 General

2.9.1.1 The results of each test, or series of tests carried out by the laboratory shall be reported accurately, clearly, unambiguously and objectively, and in accordance with any specific instructions in the test methods.

2.9.1.2 The results shall be reported, usually in a test report, and shall include all the information intended by the requirements of the regulatory testing and necessary for the interpretation of the test or calibration results and all information required by the method used. The food testing laboratories working under regulatory regime are required to report as per the requirements of the regulation, which specifies the information required to be contained in the test report and covers all essential information like Unique identification number, Description of the sample, Physical appearance, Label, Quality Characteristics, Name of method of test used, Result, Prescribed standard, Signature and date. Other essential information like dates of start of test and completion of test, deviations from, additions to, or exclusions from the test method, and information on specific test conditions, such as environmental conditions, shall be available in other records like observation work book, etc. For ease and consistency in reporting the labs should establish product wise report format in line with the regulatory requirements.

2.9.1.3 Opinions and interpretations - When opinions and interpretations are included, the laboratory shall document the basis upon which the opinions and interpretations have been made. Opinions and interpretations shall be clearly marked as such in a test report.

2.9.1.4 When the test report contains results of tests performed by subcontractors, these results shall be clearly identified. The subcontractor shall report the results in writing or electronically.

2.9.1.5 Electronic Reporting: In the case of transmission of test results by telephone, telex, facsimile or other electronic or electromagnetic means, (*delete- the requirements of this criteria shall be met.*) issuing laboratory must ensure what is transmitted electronically is what is received by the customer.

While sending reports as email attachment laboratories need to consider whether customers will have the appropriate software and version to open attachments without corruption.

Transmission: Laboratories should verify (at least initially, and periodically thereafter is recommended) the integrity of the electronic link e.g. by asking the customer to supply a copy of what was received and comparing it with what was transmitted. It is also important that the laboratory and its customer agree as to which part of the electronic transfer system they are responsible for and the laboratory must be able to demonstrate data integrity at the point the data comes under the control of the customer. The laboratory should document what this check involves and record when it has been carried out.

Security: Laboratories should avoid sending test reports in an electronic format that can be readily amended by the recipient. Examples would be in word processing or spreadsheet software. Where possible, reports should be in an image format e.g. the image format option for pdf files. Where this is not possible e.g. the customer may wish to transfer the reported results file into a larger database, then laboratories are recommended to indicate these electronic reports have an interim status and are followed-up by a hard copy (or more secure) final report. Laboratories must retain an exact copy of what was sent. This may be a hard copy (recommended) or non-editable electronic copy. These copies must be retained securely and be readily available for the time specified in the laboratory's documented policies.

Signatures: All reports (whether hard copy or electronic) must not be released to the customer until authorised by individuals with the authority to do so. For electronic reports there must be a clear audit trail with a positive authorisation record to demonstrate this is the case. Where this is managed through password access levels in the laboratory's electronic system, appropriate procedures should be in place to prevent abuse of password access.

2.9.1.6 Amendments to test reports and calibration certificates - After a test report is already released by the laboratory and an amendment is required to be issued then it shall be made only in the form of a further document, or data transfer, which shall clearly state that the amendment is a Supplement to the previously issued test report and shall also include the test report identification number. In case the amendment to the report is substantial and relates to test results reported, then it may be desirable to issue a fresh test report. The fresh test report may have new identity created by prefixing the old report number with a letter "R". It shall also contain reference to the old number stating that it replaced the old test report.

2.9.2 Chemical

2.9.2.1 For residue parameter, if the result of analysis indicates that the analyte tested is not present, then the result shall be reported as "Not detected" or "less than detection limit" and at the same time the laboratory's "Method Detection Limit" as estimated through method validation experiments shall be reported along side.

2.9.3 Microbiological

2.9.3.1 The reporting of test results for the microbiological parameters shall be strictly as per the reporting requirements specified in the relevant test method. Further the results shall be reported in the same units as the specified requirements for the food product/matrix tested.

2.9.3.2 In microbiological testing if the result of the enumeration is negative, it should be reported as "not detected for a defined unit" or less than the detection limit for a defined unit". Qualitative test results should be reported as "detected/not detected in a defined quantity or volume"

Referral Labs

Referral Laboratory (as per Food Safety & Standards Rule,2009; Part 3.5)

Rule 3.5.1 Functions – In addition to the functions entrusted to it under the Act, the Referral Laboratory shall carry out the following functions, namely:

Article

1. analysis of samples of food sent by any officer or authority authorized by the Food Authority for the purpose and submission of the certificate of analysis to the authorities concerned;
2. investigation for the purpose of fixation of standard of any article of food;

3. investigation in collaboration with the laboratories of Public Analysts in the various States and such other laboratories and institutions which the Food Authority may approve in this behalf, for the purpose of standardizing methods of analysis.

4. ensuring that the laboratory follows the scientific protocols laid down for handling/testing the articles of food.

5. maintaining high standards of accuracy, reliability and credibility in the operation of the laboratory and achieving and maintaining the required levels of accreditation and reliability.

6. laying down mechanism for ensuring that personnel of the laboratory adhere to high professional standards and discipline.

7. Such other conditions, as the Authority may lay down for Referral Laboratories.

Rule 3.5.2: Local area of Referral Laboratory

Article

1. The laboratory specified in Col.(1) of Table I below, shall carry out the functions entrusted to it by the Act or these rules in respect of the local areas specified in the corresponding entry in Col.(2) thereof.

Table-I

S.NO.	Name of Referral Laboratory (initially known as Central Food Laboratory as per PFA)	Local Areas
1.	Referral Food Laboratory, Kolkata	Haryana, Himachal Pradesh, Punjab, Jammu & Kashmir, Goa, Union Territory of Chandigarh, Union Territories of Dadra & Nagar Haveli, Daman & Diu
2.	Referral Food Laboratory, Mysore	Delhi, Bihar, Jharkhand, Chhattisgarh, Rajasthan, Uttar Pradesh, Uttarakhand, Arunachal Pradesh
3.	Referral Food Laboratory, Pune	Madhya Pradesh, West Bengal, Orissa, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Tripura and Union Territories of Andaman & Nicobar Islands & Lakshadweep
4.	Referral Food Laboratory, Ghaziabad	Andhra Pradesh, Assam, Kerala, Gujarat, Karnataka, Maharashtra, Tamil Nadu, Union Territory of Puduchery

2. The certificate of analysis to be provided by the central food laboratory shall be as per part B of **Form VII**.

Rule 3.5.3: Notified Laboratories

Article

1. In case the authorized officer takes a sample of any imported article of food for analysis, he shall send the sample to such of Food Analyst of any of the following notified laboratories having jurisdiction over the area in which the sample was taken.

Name of Central Food Lab	Local Areas
Central Food Laboratory, Kolkata	<ol style="list-style-type: none"> 1. All seaports/airports/ container depots in the Union Territories/States of (i) the Andaman & Nicobar Islands,(ii) Andhra Pradesh,(iii) Arunachal Pradesh,(iv) Assam,(v) Bihar,(vi) Manipur,(vii) Meghalaya,(viii) Mizoram,(ix) Nagaland,(x) Orissa,(xi) Sikkim,(xii) Tripura, & (xiii) West Bengal & (xiv) Jharkhand 2. International borders in the States of (i) Arunachal Pradesh, (ii) Assam,(iii) Bihar,(iv) Manipur,(v) Meghalaya,(vi) Mizoram, (vii) Nagaland,(viii) Sikkim,(ix) Tripura & (x) West Bengal
Central Food Laboratory, Ghaziabad	<ol style="list-style-type: none"> 1. All airports/inland container depots in the Union Territories /States of –(i) Chandigarh,(ii) Delhi,(iii) Haryana, (iv) Himachal Pradesh,(v) Jammu & Kashmir,(vi) Madhya Pradesh,(vii) Punjab,(viii) Rajasthan & (ix) Uttar Pradesh,(x) Chhattisgarh,(xi) Uttaranchal 2. All International borders in the States of (i) Himachal Pradesh,(ii) Rajasthan, (iii) Jammu & Kashmir,(iv) Punjab,(v) Uttar Pradesh& Uttaranchal
Central Food Laboratory, Mysore	All airports/inland container depots in the Union Territories /States of –(i) Karnataka, (ii) Kerala, (iii) Lakshadweep,(iv) Pondicherry, &(v) Tamil Nadu
Central Food Laboratory, Pune	<ol style="list-style-type: none"> 1. All airports/inland container depots in the Union Territories /States of –(i) Dadra & Nagar Haveli,(ii) Daman & Diu ,(iii) Goa, (iv) Gujarat, & (v) Maharashtra 2. All International borders in the State of Gujarat.

Recognition & accreditation of laboratories, research institutions & referral food laboratory. (as per section 43 of FSS Act,2006)

(1) The Food Authority may notify food laboratories & research institutions accredited by National Accreditation Board for Testing & Calibration Laboratories or any other Accreditation agency for the purposes of carrying out analysis of samples by the Food Analysts under this Act.

(2)The Food Authority shall, establish or recognize by notification, one or more referral food laboratory or laboratories to carry out the functions entrusted to the referral food laboratory by this Act or any rules & regulations made thereunder.

(3)The Food Authority may frame regulations specifying –

- (a) The functions of food laboratory & referral food laboratory & the local area or areas within which such functions may be carried out;
- (b) The procedure for submission to the said laboratory of samples of articles of food for analysis or tests , the forms of the laboratory’s reports thereon & the fees payable in respect of such reports ; &
- (c) Such other matters as may be necessary or expedient to enable the said laboratory to carry out its functions effectively.

Functions of Food Analyst, their coordination

Food Analysts (as per section 45 of FSS Act, 2006)

The Commissioner of Food Safety may, by notification, appoint such persons as he thinks fit, having the qualifications prescribed by the Central Government, to be Food Analysts for such local areas as may be assigned to them by the Commissioner of Food Safety:

Provided that no person, who has any financial interest in the manufacture or sale of any article of food shall be appointed to be a Food Analyst under this section:

Provided further that different Food Analysts may be appointed for different articles of food.

Functions of Food Analyst. (as per section 45 of FSS Act,2006)

(1) On receipt of a package containing a sample for analysis from a Food Safety Officer or any other person, the Food Analyst shall compare the seal on the container and the outer cover with specimen impression received separately and shall note the conditions of the seal thereon:

Provided that in case a sample container received by the Food Analyst is found to be in broken condition or unfit for analysis, he shall within a period of seven days from the date of receipt of such sample inform the Designated Officer about the same and send requisition to him for sending second part of the sample.

(2) The Food Analyst shall cause to be analysed such samples of article of food as may be sent to him by Food Safety Officer or by any other person authorised under this Act.

(3) The Food Analyst shall, within a period of fourteen days from the date of receipt of any sample for analysis, send—

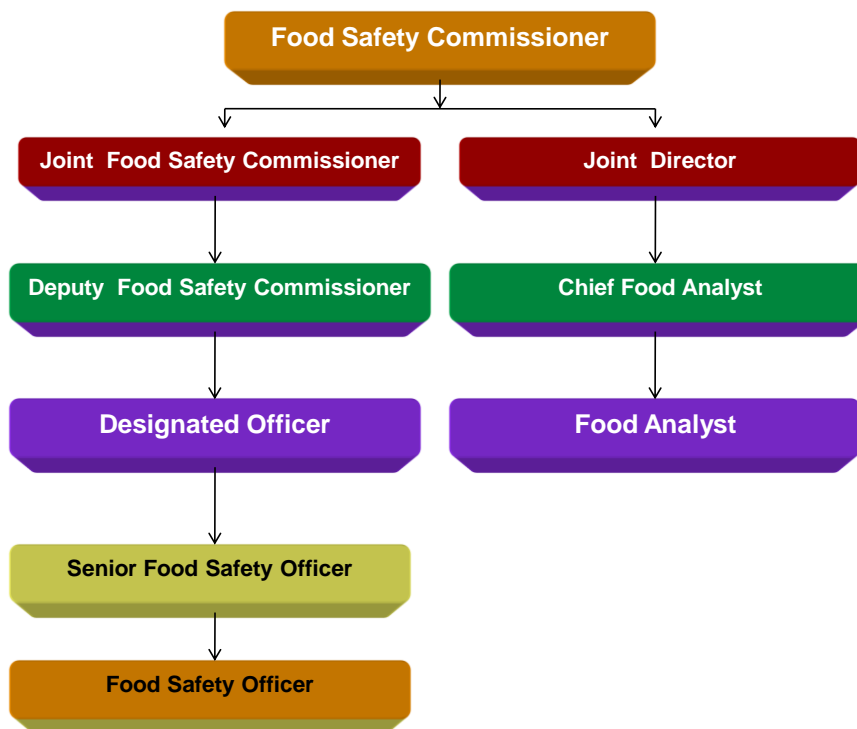
(i) where such sample is received under section 38 or section 47, to the Designated Officer, four copies of the report indicating the method of sampling and analysis; and

(ii) where such sample is received under section 40, a copy of the report indicating the method of sampling and analysis to the person who had purchased such article of food with a copy to the Designated Officer:

Provided that in case the sample cannot be analysed within fourteen days of its receipt, the Food Analyst shall inform the Designated Officer and the Commissioner of Food Safety giving reasons and specifying the time to be taken for analysis.

(4) An appeal against the report of Food Analyst shall lie before the Designated Officer who shall, if he so decides, refer the matter to the referral food laboratory as notified by the Food Authority for opinion.

7.4 Hierarchy with the Food Safety Authorities



Analysis of food samples and Reports

Analysis of food samples by food analyst (as per Rule 3.4.2 of Food Safety & Standards Rule,2009)

Article

1. On receipt of the package containing a sample of food for analysis, the Food Analyst or an officer authorized by him shall compare the seals on the container and the outer cover with specimen impression received separately and shall note the condition of the seal thereon.
2. If the sample container received by the Food Analyst is found to be in broken condition or unfit for analysis, he shall, within a period of seven days from the date of receipt of such sample inform the Designated Officer about the same and request him to send the second part of the sample for analysis.
3. On receipt of requisition from the Food Analyst pursuant to rule 3.4.2.2 the Designated Officer, shall by the immediate succeeding working day dispatch to the Food Analyst for analysis one part of the samples sent to him by the Food Safety Officer.
4. On receipt of the sample, the Food Analyst shall analyse or cause to be analysed the sample and send the analysis report mentioning the method of sampling and analysis. The analysis report shall be in as per Part A **Form VII** and four copies of the same shall be sent to the Designated Officer under whose jurisdiction the Food Safety Officer functions. The analysis report shall be signed by the Food Analyst and such report shall be sent within fourteen days of the receipt of the sample by the Food Analyst.

FORM VII
Part A
[Refer rule 3.4.4(2)]

Report of the Food Analyst

Report No. _____.

Certified that I _____ (name of the Food Analyst) duly appointed under the provisions of Food Safety and Standards Act, 2006 (34 of 2006), for _____ (name of the local area) received from _____* a sample of _____, bearing Code number and Serial Number _____ of Designated Officer of _____ area* on _____ (date of receipt of sample) for analysis.

The condition of seals on the container and the outer covering on receipt was as follows:

_____ I found the sample to be (category of the sample) falling under item No. _____ of Appendix B of Food Safety and Standards Rules, 2009/ proprietary food**. The sample **was in a condition fit for analysis and has been analysed on _____ (give date of starting and completion of analysis) and the result of its analysis is given below/** was not in a condition fit for analysis for the reason given below:

Reasons:

.....

Analysis Report
Refer rule 3.4.2 (4)

(i) Sample Description

.....

(ii) Physical Appearance

.....

(iii) Label

.....

Sl No	Quality characteristics	Nature of Method of test used	Result	Prescribed standards as per (a) Item A of Appendix B (b) As per label declaration for proprietary food (c) As per provisions of the Act , rules and regulations for both the above.
1				
2				
3				
4				
5				
6				

Opinion***

Signed this _____ day of _____ 20

(Sd/-)
Food Analyst.
Address:

*Give the details of the senders

** Strike out whichever is not applicable

*** When opinion and interpretation are included, document the basis upon which the opinions/interpretations have been made.

FORM VII
Part B
(Refer rule 3.5.2. (2))

CERTIFICATE OF ANALYSIS BY THE Referral FOOD LABORATORY

Certificate No.

Certified that the sample , bearing numberpurporting to be a sample/of was received on with Memorandum No..... Dated From [Name of the Court] for analysis. The condition of seals on the container and the outer covering on the receipt was as follows:

..... I (name of the Director)..... found the sample to be..... (Category of food sample) falling under item No..... of *Appendix B of Food Safety and Standards Regulations/*proprietary food. The sample was in a condition fit for analysis and has been analyzed on (Give date of starting and completion of analysis)..... and the result of its analysis is given below / *was not in a condition fir for analysis for the reason given below:-

Reasons:-

.....
.....

Analysis Report:-

(i) Sample Description:-

.....
.....

(ii) Physical Appearance:-

.....
.....

(iii) Label:-

.....
.....

Sl No	Quality characteristics	Nature of Method of test used	Result	Prescribed standards as per (a) Item A of Appendix B (b) As per label declaration for proprietary food (c) As per provisions of the Act , rules and regulations for both the above.
1				
2				
3				
4				
5				
6				

Options **

Place:

(Signature)

Date:

Director
Referral Food Laboratory
(Seal)

PROVIDED that in case the sample cannot be analysed within fourteen days of its receipt, the Food Analyst shall inform the Designated Officer and the Commissioner of Food Safety giving the reasons and specifying the time to be taken for analysis.

After test or analysis, the certificate thereof shall be supplied forthwith to the sender in Form VII Part(B)

The fees payable in respect of such a certificate shall be Rs. 1000/= per sample of food analysed.

Certificates issued under these rules by the laboratory shall be signed by the Director.

The manuals of the method of analysis brought out by the ministry of Health and family welfare shall be adopted for analyzing the samples of food articles. However, in case the method for analyzing any parameter is not available in these manuals, the method of analysis prescribed in the AOAC/ISO/pearson's/JACOB/IUPAC/Food chemicals codex/BIS/Woodmen/Winton-Winton/Joslyn shall be adopted. Further, in case of non-availability of method of analysis in these manuals, the method prescribed in other standard published literature or publication shall be adopted.

Rule 3.4.3: Purchasers may have the food analysed

Article

1. A Purchaser of food article may, if he so desires, have the article analysed by the Food Analyst.
2. If the Purchaser desires to have the food article purchased by him to be analysed by the Food Analyst, he shall give a notice in writing, then and there, in Form VII of his intention to have it so analysed to the person from whom he has purchased the food article.
3. The purchaser shall follow the same procedure prescribed in **rule 3.4.1** in so far they are applicable for taking sample by the Food Safety Officer or the authorized officer.
4. The provisions **of 3.4.1** shall mutatis mutandis apply in respect of samples taken for analysis by the Purchaser.
5. The Purchaser shall pay the prescribed fee to the Food Analyst for carrying out the analysis.
6. The Food Analyst shall send to the Purchaser his report on analysis of the article of food and if the findings of the report is to the effect that the article of food is adulterated/misbranded/contaminated or does not conform to the standards prescribed under the Act or the Regulations, the Food Analyst shall also send a copy of his report to the Designated Officer of the area in which the article of food was purchased, in addition to sending a copy of the Report to the Purchaser.
7. The report of the Food Analyst shall be sent within 14 days of the receipt of the article of food for analysis and such report shall be in Part A of **Form No. VII**.
8. If the report of the Food Analyst shows that the article of food is not in compliance with the provision of the Act or the rules or the regulations made there under, the Purchaser shall be entitled to get refund from the Designated Officer, the amount of fees paid by him to the Food Analyst.

Provided that the purchaser may request the Designated officer with justification why sampling is required to take appropriate samples for testing. Designated officer shall consider the application on merit and take appropriate Action

Rule 3.4.4: Food business operator's right to have the food Analysed

Article

1. In case the Food business operator from whom the sample has been taken or the person whose name and address and other particulars have been disclosed under **Rule 3.6** of these rules, desires to have the fourth part of the sample analysed, he shall request the Food Safety Officer to send the sample to any accredited laboratory for analysis under intimation to the Designated officer.

2. The Food Safety Officer shall send the sample to an accredited laboratory, under intimation to the Designated officer immediately, but not later than next succeeding day.

3. The Food analyst of the accredited laboratory shall analyse the sample within fourteen days from the date of the receipt of the sample

Provided that in case the sample can not be analysed within fourteen days from the date of its receipt, the Food analyst of the accredited laboratory, shall inform the Designated officer and the Commissioner of Food Safety giving reasons and specify the time to be taken for analysis.

4. The Food Analyst shall send four copies to the Designated officer, in the proforma given in Part A of **Form VII**, indicating the method of analysis

Rule 3.4.5: Appeal to the Designated Officer

Article

1. On receipt of analysis report from the Food Analyst, to the effect that the sample of food sent for analysis is adulterated/misbranded/contaminated/does not conform to standards prescribed under the **Regulations**, the Food business operator or the person whose name and address and other particulars have been disclosed under **Rule 3.6** of these rules, may prefer an appeal before the Designated Officer against the findings of the Food Analyst that the sample of food sent for analysis is adulterated/misbranded/does not conform to standards prescribed under the **Regulations**. Such appeal shall be in **Form VI** and the same shall be filed within 30 days from the date of the receipt of the copy of the analysis report from the Designated Officer. The appellants in the appeal, may, require the Designated Officer to send to the referral food laboratory one part of the sample which is with him for analysis and the report of the referral laboratory shall be final and binding on the Appellants.

2. The Designated Officer shall fix a date of hearing of the appeal after giving notice of such hearing to the Appellants.

3. If on a consideration of materials placed before him, the Designated Officer is of the opinion that the matter be referred to the referral laboratory for opinion, he shall forward one part of the sample with him to the referral laboratory and the report of the referral laboratory shall be final and binding.

Visits to Food Lab

Analytical Methods

Analytical Methods covers fundamental and specific aspects of the development, optimization, and practical implementation in routine laboratories, and validation of food analytical methods for the monitoring of food safety and quality

The laboratory shall normally use only standard methods as prescribed in DGHS Manual, BIS specifications, AOAC test method manual or any other international publications like USFDA BAM, American Public Health Association (APHA) Compendium of Methods for the Microbiological Examination of Foods. etc. as per the product & laboratory requirement.

The laboratory should develop a detailed procedure for method validation and lab performance validation, covering the Procedure for design of experiment to cover all required method validation parameters, Procedure for carrying out actual validation experiments the number of replicates, number of data points for different types of method performance parameters like recovery, linearity, etc. The laboratory should also establish acceptance criteria. Based on the validation results the lab should document SOP for regular analysis, which should include aspects like system for repeating calibration curves, recovery data, etc. The validation of microbiological tests methods should be carried out as per the guidance provide in NABL 102 & the validation of chemical tests methods should be carried out as per the guidance provide in NABL 103.

Validation

Laboratory, whenever using non-standard methods or a standard method beyond the stated limits of operation is required to validate such test methods. The guidance document on Validation of Test Methods, NABL 212 may be referred. Validation of a method establishes, by systematic laboratory studies, that the performance characteristics of the method meet the specifications related to the intended use of the analytical results. The performance characteristics determined include:

- Selectivity & specificity
- Range
- Linearity
- Sensitivity
- Limit of Detection
- Limit of Quantitation
- Ruggedness
- Accuracy
- Precision

These parameters should be clearly stated in the documented method so that the user can assess the suitability of the method for their particular needs.

Good Lab Practices

Good laboratory practice or **GLP** generally refers to a system of management controls for laboratories and research organizations to ensure the consistency and reliability of results - as outlined in the Organisation for Economic Co-operation and Development (OECD) Principles of GLP and national regulations.

Good Laboratory Practice (GLP) embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported and archived. These studies are undertaken to generate data by which the hazards and risks to users, consumers and third parties, including the environment, can be assessed for pharmaceuticals (only preclinical studies), agrochemicals, cosmetics, food additives, feed additives and contaminants, novel foods, biocides, detergents etc. GLP helps assure regulatory authorities that the data submitted are a true reflection of the results obtained during the study and can therefore be relied upon when making risk/safety assessments.

GLP is a quality system concerned with the organisational processing process and conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.

GLP ensures the quality, integrity, and reliability of safety data.

GLP principles include

1. Organization and Personnel
 - Management-Responsibilities
 - Sponsor-Responsibilities
 - Study Director-Responsibilities
 - Principle Investigator-Responsibilities
 - Study Personnel-Responsibilities
2. Quality Assurance Program
 - Quality Assurance Personnel
3. Facilities
 - Test System Facilities
 - Facilities for Test and Reference Items
4. Equipments, reagents and Materials
5. Test systems
 - Physical/Chemical
 - Biological
6. Test & Reference items
7. Standard operating procedures
8. Performance of Study
 - Study Plan
 - Conduct of Study
9. Reporting of results
10. Storage of Records and Reports

The laboratories will be required to establish and implement full systems as per the requirements of ISO 17025 and the specific criteria as per NABL 102 (Microbiology) and NABL 103 (Chemical) within a period of one year, unless decided otherwise by FSSAI.

1 Organization

1.1.1 Organization Structure The organization structure of the laboratory shall be defined and documented. It shall include the reporting and supervisory structure. The size, structure and composition of the laboratory, taken together should be suitable for the competent performance of the technical and administrative functions of the laboratory. The responsibilities and authorities of key personnel of the lab shall be documented along with job descriptions for all personnel. Deputies shall be appointed for key managerial personnel. The laboratory should appoint a member of staff with defined responsibility and authority for ensuring that the system related to quality as described vide this criteria is established, implemented and followed at all times.

1.1.2 Supervision - The laboratory shall have a documented system for providing effective supervision of the testing activities carried out by individual analysts in different testing sections like chemical, microbiology, residue, etc, as applicable. The supervision should be provided by persons familiar with the test methods and analysis work in the particular testing section. Bigger laboratories, that is, laboratories having more than six analyst, should provide for a three level supervisory structure.

1.1.2 Integrity, Impartiality, etc – The laboratory shall have policies and procedures in place to avoid involvement in any activities that would diminish confidence in its competence, impartiality, judgment or operational integrity.

1.2 Document Control - The laboratory shall establish system for approval and issue of all the internal procedures, SOPs, work instructions, formats, etc, established in line with the requirements of this criteria. A system shall also be established for controlling distribution of external origin documents like standard test methods, etc. The laboratory shall also ensure that authorised editions of appropriate documents are available at all locations where operations essential to the effective functioning of the laboratory are performed and invalid or obsolete documents are promptly removed from all points of issue or use, or otherwise assured against unintended use.

1.3 Review of Requests for tests

1.3.1 - Based on the facilities available, the laboratory shall maintain detailed data on the types of tests that are required to be carried out for various food products as per regulatory requirements. While preparing these lists, consideration shall be given to various circulars and orders received from the FSSAI, Central Food Laboratories, etc. Further, based on the facilities available in house, the lab should also list out the parameters it is capable of testing for various food commodities as per the regulatory requirements. There shall be a system of sharing this information with other sister laboratories.

1.3.2 - The laboratory shall establish a system for review of request letters accompanying the samples received for testing. Based on the review and the capability as determined above, it shall decide on the tests that would be carried out in house and inform the testing section of the same. In case of any temporary incapacity due to equipment breakdown or temporary non availability of testing staff, the same may be taken in to consideration for deciding about subcontracting of the tests.

1.4 Subcontracting – When a laboratory is required to subcontract testing work, because it does not have testing facilities for testing of some of the parameters for certain food products as per the regulatory requirements, then the lab shall ensure that the testing work is subcontracted to a laboratory which is similarly approved or accredited against ISO 17025 for those tests.

2. Technical Requirements

2.1 Personnel

2.1.1 General

2.1.1.1 The laboratory management shall ensure the competence of all those who operate specific equipment, perform tests and/or calibrations, evaluate results, and sign test reports and calibration certificates. The requirements with respect to educational qualification, training, experience and/or demonstrated skills shall be as per individual testing section requirements as stated in subsequent clauses.

2.1.1.2 The laboratory shall have a system for authorising testing personnel for specific tests based on in house, on the job training or external training and subsequent evaluation. Further the laboratory shall have a system for authorising specific personnel to perform certain specific tasks like issue of test reports, to give opinions and interpretations, to handle sample receipt and coding, to operate particular types of equipment, etc. The laboratory management shall ensure that all personnel have received adequate training for the competent performance of the assigned tasks.

Personnel may only perform tests on samples if they are either recognized as competent to do so, or working under adequate supervision.

2.1.1.3 Authorised signatory should fulfil either of the following requirements listed below:

- c) Five years experience in similar area out of which at least two years experience should be at supervisory level.

- d) Postgraduate/higher degree in the relevant field or equivalent with a minimum of two years experience in the relevant scope of testing.

The competence of authorised signatory will be assessed during the assessment before being approved by FSSAI. Relaxation in minimum qualification and/or experience requirements for authorised signatory can be considered by FSSAI on specific recommendation by the assessment team on competence with objective evidences for proven competency. Signatories must be able to oversee the operations and cope with any problems that may arise in their work or that of their colleagues or subordinates. All tests must be carried out under the supervision of concerned signatory. After hours, weekend, statutory holidays and during short absences a signatory needs to be available on-call (able to be contacted at all times and able to arrive back at the laboratory within the same day).

2.1.1.4 The laboratory shall establish a system for initial and ongoing training of the individuals working in the laboratory whose work has influence on quality of testing. Internal training alone is not considered adequate to make the staff knowledgeable on the latest status of science and technology and while introducing specialized testing like residue testing, pathogen testing, etc. The laboratory shall document a procedure for identifying training needs and providing training to its personnel. The training programme shall be relevant to the present and anticipated tasks of the laboratory. It should include specialised training in different fields of food testing like general chemical, residue and microbiological testing as relevant to the laboratory. The effectiveness of the training actions taken shall be evaluated. Evidence of effective training in specific field should be available in terms of performance in quality checks.

2.1.1.5 On-going competence should be monitored objectively with provision for retraining where necessary. Where a method or technique is not in regular use, verification of personnel performance is necessary before the testing is undertaken. The critical interval between performances of non-routine tests should be established and documented by the laboratory.

2.1.1.6 If the laboratory has a system for employing personnel on contract, then they shall be appointed on long-term contract basis. The laboratory shall also ensure that such personnel are supervised and competent and that they work in accordance with the laboratory's established systems. For the purpose of criteria, minimum period of contract shall be 3 years.

2.1.1.7 The laboratory shall maintain current job descriptions for managerial, technical and key support personnel involved in tests and should also include contracted personnel.

2.1.2 Chemical

2.1.2.1 The chemical testing section of the laboratory shall be headed by a person preferably having a post graduate degree in Chemistry or equivalent or Bachelor's degree in chemistry, food technology or equivalent with adequate experience (at least 5 years) in the relevant area especially in the analysis and testing of relevant products. The requirements for authorized signatory in chemical testing section should be similar to that specified above. The minimum qualification for the testing staff in a chemical testing laboratory shall be Graduate in Science with chemistry as one of the subjects or equivalent. The staff shall have sufficient training and exposure in analytical chemistry and in analysis and testing of appropriate products.

2.1.2.2 Chemical testing laboratory involved in testing variety of general chemical testing parameters and residue parameters should have a group in-charge for both the testing areas. The group in-charge should have adequate relevant experience in addition to the minimum qualification.

2.1.3 Microbiological

2.1.3.1 Microbiological testing of food samples involves pathogen testing and as such shall be headed by a qualified microbiologist (graduate/post graduate in Microbiology) with 2-3 years of experience in pathogen testing in case of person with post graduate degree and 5

years in case of person with graduate degree. The requirements for authorized signatory in microbiological testing section should be similar to that specified above.

2.1.3.2 The minimum qualification for the testing staff in a biological testing laboratory shall be graduate or postgraduate in microbiology, food science or equivalent with at least 1 year working experience in similar area as covered by the scope of testing as proven by demonstrated competence on records. Fresher's can be put under training with adequate supervision. In addition to test methods, in some cases, it may be more appropriate to relate competence to a particular technique or instrument, for example use of approved biochemical, serological kits or microbial identification kits.

2.1.3.3 The interpretation of test results for identification and verification of microorganisms is strongly connected to the experience of the performing analyst and should be monitored for each analyst on a regular basis.

2.2 Accommodation and Environmental Conditions

2.2.1 General

2.2.1.1 Laboratory facilities for testing shall be such as to facilitate correct performance of the tests as per the scope of the laboratory. These facilities shall include accommodation, space, layout, environmental conditions, energy and water sources, biological sterility, dust, humidity, electrical supply, temperature, etc. Adequate and proper lighting should be available. Bench and floor surfaces should be appropriate for the work being performed.

2.2.1.2 The laboratory shall document, monitor, control and record environmental conditions as required by the relevant specifications, methods and procedures or where they influence the quality of the results. Tests and calibrations shall be stopped when the environmental conditions jeopardise the results of the tests.

2.2.1.3 There shall be effective separation between neighbouring areas in which there are incompatible activities. Measures shall be taken to prevent cross-contamination. Storage facilities of the laboratories should be sufficient enough to allow the sample retention and if required segregation of samples for designated periods and provide conditions that maintain sample integrity.

2.2.1.4 Access to and use of areas affecting the security of the sample and quality of the tests and/or calibrations shall be controlled. Access should be controlled by but is not limited to: issuance of key cards for entrance/ issuance of identification badges/ escorting visitors/ use of security guards. The laboratory shall determine the extent of control based on its particular circumstances.

2.2.1.5 Measures shall be taken to ensure good housekeeping in the laboratory.

2.2.1.6 When a change of premises occurs the laboratory shall notify FSSAI. In such cases, revalidation might be necessary in every aspect of environmental and analytical issues.

2.2.2 Chemical

2.2.2.1 Effective separation shall be ensured between residue testing sections, especially the areas used for sample extraction, Certified Reference Material (CRM) dilution and similar activities and the rest of the general chemical testing sections.

2.2.2.2 The chemical testing sections where the heat producing equipments are located should be separated from rest of the testing areas. Proper ventilation should be ensured through the use of exhaust fans and adequate number of fume chambers should be available for carrying out activities, generating excessive fumes.

2.2.2.3 Proper equipment and material like fire extinguishers of suitable type, sand buckets, etc should be available, located at appropriate locations, for handling chemical spillages, fires, etc. Laboratory personnel should be trained in fire fighting. The laboratory should have, as appropriate, safety showers and eye wash equipment, located at suitable

locations and in good working condition. Sufficient exhaust hoods and fume cabinets should be available to maintain a safe work environment. Sufficient first aid kits should be available and strategically located. Personal protective equipments like laboratory coats/gowns, gloves, protective eye wear/masks should be made available as relevant. Appropriate storage must be provided for volatile, flammable, explosive and other hazardous materials. General cleanliness and good housekeeping should be apparent. Gas cylinders should be secured and should be kept outside the testing area.

2.2.3 Microbiological

2.2.3.1 The laboratory should be arranged in such a way, that the risks of cross contamination can be reduced. This can be achieved by carrying out the test procedures in a sequential manner using appropriate precautions to ensure test and sample integrity and by segregating the activities by time or space. It is generally considered as a good practice to have separate areas for Sample receipt and sample storage, Sample preparation, Handling and storage of reference cultures/Reference materials, Media preparation and Sterilization, Inoculation and plating, Incubation, Decontamination and any other Incompatible activities.

2.2.3.2 For laboratories engaged in specialized testing like GMO Testing, additional requirements relevant to the activities involved shall apply.

2.2.3.3 For procedures that involve the handling of pathogens and reference stock cultures, they should generally be operated within a safety cabinet of a class commensurate with the risk level of the microorganism handled. Most of the microbes encountered in a non-clinical testing laboratory belong to Risk Group 2 microorganisms e.g. *Salmonellae*, *Staphylococcus aureus*.

2.2.3.4 Laboratories should devise appropriate environmental monitoring program with respect to the type of tests being carried out and records shall be maintained for the same. The laboratory environment, where relevant, shall be microbiologically monitored through exposure plate method for air borne contamination and surface swabbing of sampling and testing benches, utensils, balances stomachers, etc. Based on the results of such monitoring, where relevant, environmental contamination should be controlled by appropriate air-filters and air-exchange systems. Where air conditioners are used filters should be appropriate, inspected maintained and replaced according to the type of work being carried out. The laboratory shall have pest control program, as appropriate.

2.3 Test methods

2.3.1 General

2.3.1.1 The laboratory shall use appropriate methods and procedures for all tests within its scope. The laboratory shall have instructions on the use and operation of all relevant equipment, and on the handling and preparation of samples for testing, where the absence of such instructions could jeopardise the results of tests and/or calibrations. If the standard test methods used contain sufficient and concise information on how to perform the test, then they may not be rewritten as internal procedures. It may however be necessary to provide additional documentation for optional steps or additional details.

2.3.1.2 All instructions, standards, manuals and reference data relevant to the work of the laboratory shall be kept up to date and shall be made readily available to personnel.

2.3.1.3 Normally the standard methods shall not be deviated unless the deviations have been technically justified, validated, documented and authorised. All modified, lab developed or non standard test methods used by the laboratory shall be validated before use. Following information shall be provided for the accreditation of an in-house test method:

- A copy of the fully documented test method

- Details of the origin of the in-house test method
- Details of the reason for its development and application e.g. the specification against which the product/products is being tested
- The results of comparative tests with standard method (if possible) and /or with other laboratories.

2.3.2 Chemical

2.3.2.1 For testing of general chemical parameters, additives, etc, only standard methods as specified in DGHS Manual or standard methods as prescribed in relevant BIS specifications and Association of Official Analytical Chemists (AOAC) standard methods manual, or similar international publications like FDA Macro analytical Procedures Manual (MPM), the Pesticide Analytical Manual (PAM), the Food Additives Analytical Manual, and the Food Chemicals Codex shall be used.

2.3.2.2 For specialized tests like residue testing the laboratory would generally require to use standard methods, modified to suit the sample matrices of food products to be tested for specific residues and laboratory specific aspects like type of equipment used and their sensitivities, extraction technique used and competence and skill development of the testing personnel, in order to achieve the desired detection levels matching the MRL's. Consequently the lab would require to validate the modified test methods. The validation of chemical (specifically residue) test methods should be carried out as per the guidance provide in NABL 103. Some of the relevant aspects to be complied with are given below.

2.3.2.3 The laboratory should develop a detailed procedure for method validation and lab performance validation, covering the Procedure for design of experiment to cover all required method validation parameters, Procedure for carrying out actual validation experiments the number of replicates, number of data points for different types of method performance parameters like recovery, linearity, etc. The laboratory should also establish acceptance criteria. Based on the validation results the lab should document SOP for regular analysis, which should include aspects like system for repeating calibration curves, recovery data, etc.

2.3.2.4 Based on the above the laboratory should establish the laboratory performance characteristics like Selectivity & specificity, Range, Linearity, Sensitivity, LOD, LOQ, Accuracy/recovery, repeatability, etc, for analysis of different residue parameters in different food matrices.

2.3.3 Microbiological

2.3.3.1 The laboratory shall normally use only standard methods as prescribed in DGHS Manual, BIS specifications, AOAC test method manual or any other international publications like USFDA BAM, American Public Health Association (APHA) Compendium of Methods for the Microbiological Examination of Foods.

2.3.3.2 Where standard methods are prescribed and followed, the laboratory is expected to maintain current versions of the standard methods (reference texts) and up-date laboratory bench methods in accordance with these. Although full validation is not required, a laboratory must verify that it can properly operate the method, and can demonstrate (where specified) the limits of detection, selectivity, repeatability and reproducibility. Laboratories shall pay attention to the limitations, concentrations range and sample matrix specified in the test standards.

2.3.3.3 The use of commercial test systems (kits) shall normally be avoided unless absolutely necessary. In case these are used, they shall require further validation if the laboratory is unable to source the validation data. When the manufacturer of the test kits supplies validation data, the laboratory will only perform secondary validation (verification). Laboratories should retain validation data on commercial test systems (kits) used in the laboratory. These validation data may be obtained through collaborative testing, from the manufacturers and subjected to third party evaluation (e.g. AOAC. Refer www.aoac.org for information on methods validation). If the validation data is not available or not applicable, the laboratory should be responsible for completing the primary validation of the method. It has been found in some cases (e.g.

veterinary microbiological testing) that a specific test kit performs differently under local environmental conditions, to that of the original environmental conditions it was subjected to primary validation. In such cases the laboratory should conduct the validation to prove that the kit performs under local environmental conditions.

2.3.3.4 The validation of microbiological tests methods should be carried out as per the guidance provide in NABL 102

Uncertainty of Measurement and Limits of Detection

Laboratories need to make a formal estimate of measurement uncertainty for all tests in the scope of accreditation that provide numerical results. Where results of tests are not numerical or are not based on numerical data e.g. detected/not detected, pass/fail, positive/negative, or based on visual, tactile or other qualitative examinations, estimates of uncertainty are not required.

Where an estimate of measurement uncertainty is required, laboratories must document their procedures and processes on how this is to be done. There are various published approaches to the estimation of uncertainty in testing (Eurachem Guide, APLAC, CITMAC, Nordic Committee on Food Analysis (NMKL). All approaches which give a reasonable estimate and are considered valid within the chemical and biological testing communities are equally acceptable and no one approach is favoured over others.

Once a documented procedure is established, the laboratory should implement a programme for applying this procedure to all relevant tests within the scope of accreditation.

Laboratories that are performing analyses at low analyte levels such as residue testing and low level micronutrient testing, method detection limits need to be determined. Laboratories could follow the methodologies published by CODEX, AOAC, etc. or the specific industry sector it is operating within. However, laboratories should document procedure on how they determine their detection limits. The procedure should be consistent with the source of methodologies normally used (e.g. if American Public Health Association, APHA details detection limit determination methodology, then the laboratory should adopt these recommended practices for method detection limit determination for methods sourced from APHA), or the conventions used within the industry sector.

In the absence of industry or test methodology conventions, laboratories will determine method detection limits from a series of independent analyses of the analyte concerned in the matrix of interest at a level near the expected limit of detection. The method detection limit is calculated from the variation of results at this level, and is not to be confused with a so-called instrument level of detection obtained from readings of a series of blanks. In some cases, the sensitivity of the method is such that the lowest calibration standard is more than two orders of magnitude below the regulatory limit. In this situation, laboratories may sometimes set the limit of detection at the level of the lowest calibration standard, but this approach does require that the laboratory consider the manner in which they report low positive results below the artificially high limit of detection, to ensure that reports are not misleading to users.

2.4 Equipment management, Calibration and Traceability

2.4.1 General

2.4.1.1 The laboratory shall be furnished with all items of test equipment required for the correct performance of the tests contained in the laboratories scope of testing, which may cover General Chemical testing, residue testing and microbiology testing or a combination thereof for a variety of food products as per the Food regulation. Equipment used for testing shall be capable of achieving the accuracy required and shall comply with specifications relevant to the tests as given in the test methods used.

2.4.1.2 All equipments that have direct or indirect effect on the results of analysis shall be calibrated or checked before being placed into service, establish that they meet the laboratory's specification requirements and comply with the relevant standard

specifications. There after they shall be calibrated at regular intervals as per laboratories established calibration program. The calibration results shall be verified against the equipment specification/acceptance criteria to confirm continued suitability.

2.4.1.3 Equipment shall be operated by authorised personnel. Up-to-date instructions on the use and maintenance of equipment (including any relevant manuals provided by the manufacturer of the equipment) shall be readily available for use by the appropriate laboratory personnel.

2.4.1.4 Each item of equipment and its software used for testing and calibration and significant to the result shall, when practicable, be uniquely identified and appropriate records shall be maintained, which should include as relevant the equipment details and specification; the current location, where appropriate; the manufacturer's instructions, manuals, etc; dates, results and copies of reports and certificates of all calibrations, adjustments, acceptance criteria, and the due date of next calibration; the maintenance plan, where appropriate, and maintenance carried out to date; any damage, malfunction, modification or repair to the equipment. If practicable the status of calibration, including the date when last calibrated and the date or expiration criteria when recalibration is due should be indicated on the equipment.

2.4.1.5 Maintenance of essential equipments used in the laboratory shall be carried out at specified intervals as determined by factors such as the frequency of use, ruggedness, etc. All sophisticated equipments available in the laboratory like Atomic absorption spectrophotometers, Gas chromatographs, HPLCs, etc should be serviced and maintained through Annual Maintenance Contracts with the supplier of the equipment/authorised service agents.

2.4.1.6 Equipment that has been subjected to overloading or mishandling, gives suspect results, or has been shown to be defective or outside specified limits, shall be taken out of service. It shall be isolated to prevent its use or clearly labelled or marked as being out of service until it has been repaired and shown by calibration or test to perform correctly.

2.4.2 Chemical

2.4.2.1 General Service Equipment - General service equipment like hotplates, stirrers, non volumetric glass wares including measuring equipments, etc, which do not have direct or indirect effect on analysis results, do not require calibration and should be maintained by appropriate cleaning and checks for safety as necessary. Other equipments like Ovens, furnaces, where the setting can significantly affect the test or analysis result, shall be calibrated using an NABL accredited external labs.

2.4.2.2 Volumetric equipment - The correct use of volumetric equipment is critical to analytical measurements and shall be suitably maintained and calibrated. The volumetric equipment like burette, pipette shall generally be calibrated/verified in house using calibrated analytical balance and thermometers, by density method.

2.4.2.3 Measuring instruments/equipment - Equipment like analytical balances, spectrophotometers, pH meters, Chromatographs, etc, shall be subjected to periodic servicing and calibration. The calibration of these equipment may involve periodic performance checks (to check the response, stability and linearity of sources, sensors and detectors, the separating efficiency of chromatographic systems, the resolution, alignment and wavelength accuracy of spectrometers etc) and calibration (Operational checking) using CRM's (e.g. based on the levels of expected detector or sensor response to calibrants, the resolution of calibrants in separating systems, the spectral characteristics of calibrants etc). The laboratory should establish frequency of such checks/calibrations based on the ruggedness of the equipment, frequency of usage, test method requirements, etc. Equipment like thermometers, etc, should also be covered under the calibration program established by the laboratory.

2.4.3 Microbiological

2.4.3.1 The equipment which do not have direct or indirect bearing on microbiology test results shall be maintained by cleaning and servicing, inspecting for damage, general verification and, where relevant, sterilizing. These are General service equipment like water baths, incubators, microbiological cabinets, autoclaves, homogenizers, fridges, freezers and Accessories like filtration apparatus, glass or plastic containers (bottles, test tubes), glass or plastic Petri-dishes, sampling instruments, wires or loops of platinum, nickel/chromium or disposable plastic.

2.4.3.2 Other commonly used equipments in microbiology labs, which are likely to have direct/indirect effect on test results are balances, thermometers, pH meter, timer, ovens, incubators, autoclaves, water bath, Laminar Flow chamber, Bio safety cabinets and volumetric glassware and these shall be subjected to periodic calibration and/or performance verification.

2.5 Reference materials, Chemicals, Media and Cultures

A RM is a material sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

CRMs are defined as a reference material that is characterised by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value(s) of the specified property (ies), its (their) uncertainty (ies) and a statement of metrological traceability.

2.5.1 Chemical

2.5.1.1 Certified Reference Materials – All the Certified Reference Materials (CRMs) required to cover the scope of testing of the laboratory should be procured from traceable (National Accreditation Board For Testing & Calibration Laboratories, NABL in India, National Institute of Standards and Technology, NIST in USA, Bureau Communautaire de Référence, BCR in Europe, National Measurement Institute Australia etc) sources. In case traceable CRMs are not available, like that for pesticides, etc, then they should be procured from reputed, reliable and industry accepted sources. The laboratory should establish and implement system for handling, storage and maintenance of CRMs, their dilution and preparation of secondary CRM's their maintenance and retention and maintain appropriate records.

2.5.1.2 Reagents and chemicals - The laboratory shall purchase reagents, chemicals, and other consumables only from reliable and reputed manufacturers with proven track record. The laboratory shall also ensure that the quality (grade, etc) of the reagents/chemicals/media used is appropriate for the tests concerned. The solutions made out of chemicals shall be made using standard procedures and when being stored for long term use should be appropriately labelled to identify the substance, strength/concentration, solvent (where not water), any special precautions or restrictions of use, and date of preparation and/or expiry. The person responsible for the preparation of the reagent shall be identifiable either from the label or from records. Acids, alkalies and other solutions prepared for volumetric analysis should be periodically (as per defined system) checked for their strength and documented properly. Reagents used as primary standards for volumetric and gravimetric methods should have traceability to National and International standards.

2.5.2 Microbiological

2.5.2.1 Reference Cultures – These shall be procured for all the microbiological parameters tested by the lab and shall be procured from with traceable and authorized sources (like Microbial Type Cell Culture, MTCC in India, ATCC in USA, NCTC in UK, NZRCC in NZ). The laboratory should establish and document system for handling and effective maintenance of cultures and their appropriate usage.

2.5.2.2 Media, reagents and solutions for microbiology – All the media, reagents and chemicals required for complete range of microbiological tests (including biochemical and

serological tests) as per laboratory's scope of testing as prescribed in respective standard test method shall be available. The laboratory should establish and implement a system (or quality control programme) for suitability and efficacy checks (on all media whether it be in-house prepared from basic ingredients, or purchased pre-prepared media) on the received media.

2.6 Sample Handling

2.6.1 General

2.6.1.1 On receipt of the test sample, any visible abnormalities, leakages, or departures from normal or specified conditions, as described in the test request as well as test method, shall be recorded. When there is doubt as to the suitability of the sample received, or when a sample does not conform to the description provided, or the test required is not specified in sufficient detail, or the details on the sample and the accompanying letter do not match, the laboratory shall consult the source from where the sample was received for further instructions before proceeding and shall record the discussion. In case the sample is observed to be unfit for testing and it is apparent that the testing would not serve any useful purpose, the lab should decide not to undertake testing and suitably inform the source from where the sample is received.

2.6.1.2 On receipt, a sample shall be registered into laboratory records. The system for registration and the type of records used may vary from lab to lab, however use of a sample register is the most prevalent method for registration purposes. The laboratory shall follow all the defined (as per regulatory norms) system for sample coding, in order to ensure that the source of sample and other details are not be revealed to the testing personnel and others in the laboratory. All staff concerned with administration of the sample handling system should be appropriately trained.

2.6.1.3 The laboratory shall have a system for unique and clear identification of test samples. The identification shall be retained throughout the life of the item in the laboratory. The system shall be designed and operated so as to ensure that sample cannot be confused physically or when referred to in records or other documents. The sample identification number shall be fixed in such a fashion that it does not get obliterated during its journey within the lab. The system identification system shall be extended in case, if appropriate, accommodate a sub-division of the sample and the transfer of sample within different laboratory sections.

2.6.1.4 Frequently, it is necessary to split or transfer samples for testing of different properties. Sub-sampling by the laboratory immediately prior to testing is considered as part of the test method. It should be performed as per national/international standards, where they exist, or by validated in-house methods. It is essential that procedures are available for preventing spread of contamination, delivery of samples including special transportation such as refrigeration and exclusion of light, disposal and decontamination processes and unbroken chain of identification of the sub-samples/samples shall be provided.

2.6.1.5 The laboratory shall have procedures and appropriate facilities for avoiding deterioration, loss or damage to the test samples during storage, handling and preparation. When samples are required to be stored or conditioned under specified environmental conditions, these conditions shall be maintained, monitored and recorded. After completion of testing of the sample the laboratory may require to retain the sample. The laboratory should establish system for retention based on stability and storage considerations of different materials received for testing.

2.6.2 Chemical

2.6.2.1 Sample containers should be leak-proof and impervious to possible contamination during transport. Where specified, samples should be maintained within set temperature or other environmental tolerances during transfer to the laboratory and prior to testing. In some cases, it may be necessary for sample containers to be pretested prior to use to ensure freedom from contamination, especially samples to be tested for residue parameters

2.6.3 Microbiological

2.6.3.1 Laboratory shall examine and record the conditions and appearance of samples upon receipt. Where appropriate time of receipt of the sample, storage temperature of sample on receipt, conditions of sample container, etc should be recorded. If there is insufficient sample or the sample is in poor condition due to physical deterioration, incorrect temperature, torn packaging or deficient labelling, laboratory should either refuse the sample or carry out the tests as instructed by the sender of the sample and shall indicate the conditions on test reports.

2.6.3.2 Samples awaiting test shall be stored under suitable conditions as relevant for microbiology testing for avoiding modifications to any microbial population present. Storage conditions and maximum holding times for different samples shall be documented and shall fulfil the requirements of test standards. Where a sample has to be held secure, the laboratory must have arrangements for storage and security that protect the condition and integrity of the secured samples concerned.

2.7 Quality Assurance Measures & Proficiency Testing

2.7.1 General

2.7.1.1 The laboratory shall have quality control procedures for monitoring the validity of tests and calibrations undertaken. These should include Internal Quality Control measures like use of Certified Reference Samples/materials, replicate tests or calibrations using the same or different methods, retesting or recalibration of retained items, correlation of results for different characteristics of an item. This monitoring should be carried out as per predetermined plan and should cover all the tests under the laboratories scope of testing, in a defined time period.

2.7.1.2 As external quality assurance measure, the laboratory shall periodically participate in self initiated Inter-laboratory comparison programmes or programmes initiated by other laboratories like delete CFL.

2.7.1.3 In addition to the above, the laboratory should also participate, in independently and professionally organized national/international Proficiency Testing Programmes (such as run by NABL, Asia Pacific Laboratory Accreditation Co-operation (APLAC), as per plan to cover all the tests under the laboratory's scope of testing in a defined period, of say 3 years.

2.7.1.4 Quality control data obtained from Internal Quality Control measures and the External Quality Assurance measures shall be analysed and, where they are found to be outside pre-defined criteria (acceptance criteria), planned action shall be taken to correct the problem and to prevent incorrect results from being reported. If unsatisfactory results are obtained, laboratories shall be able to show that the problems are promptly investigated and rectified, and satisfactory performance for the test/method in question can be achieved afterward. All findings in connection with unsatisfactory performance shall be recorded.

2.7.2 Chemical

2.7.2.1 For Internal Quality Control the level adopted should be demonstrably sufficient to ensure the validity of the results. As a guide, for routine analysis the level of internal QC typically should be not less than 5% of the sample throughout, i.e. 1 in every 20 samples analyzed should be a QC sample. For more complex procedures, 20% is not unusual and on occasions even 50% may be required. For analyses performed infrequently, a full system validation should be performed on each occasion. This may typically involve the use of a reference material containing a certified or known concentration of analyte, followed by replicate analyses of the sample and spiked sample (a sample to which a known amount of the analyte has been deliberately added). Those analyses undertaken more frequently should be subject to systematic QC procedures incorporating the use of control charts and check samples.

2.7.3 Microbiological - The Internal Quality Control measures specific to microbiology testing are given below. The interval between these checks will be influenced by the construction of the program and by the number of actual tests. It is recommended that, where possible, tests should incorporate controls to monitor performance.

2.7.3.1 Sterility controls – Un-inoculated samples are to be run at a minimum of once for every test run. Sterility controls are used to detect the presence or absence of possible laboratory contamination.

2.7.3.2 Split samples (Duplicates) for quantitative tests - Split samples comprise a sample divided into 2 sub-samples. Analyses of split samples are normally expected to be conducted at a frequency of once per test run.

2.7.3.3 Confirmation/verification of presumptive positive samples - Positive and negative characteristic strains, if applicable, shall be tested concurrently with any biochemical, serological and morphological tests for confirmation of presumptive microorganisms. The number or percentage of colonies that stipulated in test standard required for confirmation process shall be followed. Laboratories can also define the minimum number of colonies for confirmation if such requirements are not specified.

2.7.3.4 Verification of continuing competence - Laboratories shall establish schedules, in compliance with the verification frequency stipulated in test standards, for checking the continuing competence to perform positive tests for each test method if no positive samples are encountered. Reference stocks should be maintained for all tests conducted, and suitable suspensions of fresh subcultures should be spiked into appropriate matrix and run through each entire test procedure. The analyst is required to make parallel analyses with another analyst. Criteria shall be set for maximum allowance difference between the counts based on precision of test methods.

2.8 Laboratory Data and Record Management

2.8.1 General

2.8.1.1 The laboratory shall establish a system for identification, collection, indexing, access, filing, storage, maintenance and disposal of technical records. It shall also ensure that all records are legible and stored in a suitable environment to prevent damage or deterioration and to prevent loss. Retention times of records shall be established as per regulatory requirements.

2.8.1.2 The technical records shall cover all original observations, derived data and sufficient information to establish traceability and reconstruction of results; calibration certificates and other instrument related records; relevant records pertaining to CRMs, medias, reference cultures, chemicals, glass wares and other consumables; staff related records; Quality assurance related records; test reports issued and related records; sample receipt, entry and coding related records; various types of monitoring records; etc. The records shall include the identity of personnel responsible for the performance of each test and checking of results.

2.8.1.3 Observations, data and calculations shall be recorded at the time they are made and shall be identifiable to the specific task. When mistakes occur in records, each mistake shall be crossed out, not erased, made illegible or deleted and the correct value entered alongside. All such alterations to records shall be signed or initialed by the person making the correction. In the case of records stored electronically, equivalent measures shall be taken to avoid loss or change of original data. Electronic records and data files are backed up on a regular basis to safeguard against the loss of information due to equipment malfunctions or human error

2.8.1.4 Calculations and data transfers shall be subject to appropriate checks in a systematic manner

2.8.1.5 Access to all records must be restricted to prevent unauthorized use and amending of information.

2.8.2 Chemical

2.8.2.1 Computer controlled automated systems, generally used with sophisticated equipments like AAS, GC, etc, should normally be validated by checking for satisfactory operation and establishing the reliability of the system before it is allowed to run unattended. Where possible the controlling software should be tailored to identify and highlight any such malfunctions and tag associated data. The use of quality control samples and standards run at intervals in the sample batches should then be sufficient to monitor correct performance on a day-to-day basis. Calculation routines can be checked by testing with known parameter values. Electronic transfer of data should be checked to ensure that no corruption has occurred during transmission. This can be achieved on the computer by the use of verification files' but wherever practical, the transmission should be backed up by a hard copy of the data.

2.8.3 Microbiological

2.8.3.1 An adequate test record system, which provides evidence of all the activities performed during the entire duration of the microbiological tests, like daily observation recording system shall be established and implemented. The laboratory can develop appropriate formats for recording all the observations relevant to the different microbiological tests performed in the laboratory. System for maintaining all other records with respect to media and reagent preparing, culture handling and maintenance, etc shall be established and implemented.

2.9 Reporting

4.9.1 General

2.9.1.1 The results of each test, or series of tests carried out by the laboratory shall be reported accurately, clearly, unambiguously and objectively, and in accordance with any specific instructions in the test methods.

2.9.1.2 The results shall be reported, usually in a test report, and shall include all the information intended by the requirements of the regulatory testing and necessary for the interpretation of the test or calibration results and all information required by the method used. The food testing laboratories working under regulatory regime are required to report as per the requirements of the regulation, which specifies the information required to be contained in the test report and covers all essential information like Unique identification number, Description of the sample, Physical appearance, Label, Quality Characteristics, Name of method of test used, Result, Prescribed standard, Signature and date. Other essential information like dates of start of test and completion of test, deviations from, additions to, or exclusions from the test method, and information on specific test conditions, such as environmental conditions, shall be available in other records like observation work book, etc. For ease and consistency in reporting the labs should establish product wise report format in line with the regulatory requirements.

2.9.1.3 Opinions and interpretations - When opinions and interpretations are included, the laboratory shall document the basis upon which the opinions and interpretations have been made. Opinions and interpretations shall be clearly marked as such in a test report.

2.9.1.4 When the test report contains results of tests performed by subcontractors, these results shall be clearly identified. The subcontractor shall report the results in writing or electronically.

2.9.1.5 Electronic Reporting: In the case of transmission of test results by telephone, telex, facsimile or other electronic or electromagnetic means, (*delete- the requirements of this criteria shall be met.*) issuing laboratory must ensure what is transmitted electronically is what is received by the customer.

While sending reports as email attachment laboratories need to consider whether customers will have the appropriate software and version to open attachments without corruption.

Transmission: Laboratories should verify (at least initially, and periodically thereafter is recommended) the integrity of the electronic link e.g. by asking the customer to supply a copy of what was received and comparing it with what was transmitted. It is also important that the laboratory and its customer agree as to which part of the electronic transfer system they are responsible for and the laboratory must be able to demonstrate data integrity at the point the data comes under the control of the customer. The laboratory should document what this check involves and record when it has been carried out.

Security: Laboratories should avoid sending test reports in an electronic format that can be readily amended by the recipient. Examples would be in word processing or spreadsheet software. Where possible, reports should be in an image format e.g. the image format option for pdf files. Where this is not possible e.g. the customer may wish to transfer the reported results file into a larger database, then laboratories are recommended to indicate these electronic reports have an interim status and are followed-up by a hard copy (or more secure) final report. Laboratories must retain an exact copy of what was sent. This may be a hard copy (recommended) or non-editable electronic copy. These copies must be retained securely and be readily available for the time specified in the laboratory's documented policies.

Signatures: All reports (whether hard copy or electronic) must not be released to the customer until authorised by individuals with the authority to do so. For electronic reports there must be a clear audit trail with a positive authorisation record to demonstrate this is the case. Where this is managed through password access levels in the laboratory's electronic system, appropriate procedures should be in place to prevent abuse of password access.

2.9.1.6 Amendments to test reports and calibration certificates - After a test report is already released by the laboratory and an amendment is required to be issued then it shall be made only in the form of a further document, or data transfer, which shall clearly state that the amendment is a Supplement to the previously issued test report and shall also include the test report identification number. In case the amendment to the report is substantial and relates to test results reported, then it may be desirable to issue a fresh test report. The fresh test report may have new identity created by prefixing the old report number with a letter "R". It shall also contain reference to the old number stating that it replaced the old test report.

2.9.2 Chemical

2.9.2.1 For residue parameter, if the result of analysis indicates that the analyte tested is not present, then the result shall be reported as "Not detected" or "less than detection limit" and at the same time the laboratory's "Method Detection Limit" as estimated through method validation experiments shall be reported along side.

2.9.3 Microbiological

2.9.3.1 The reporting of test results for the microbiological parameters shall be strictly as per the reporting requirements specified in the relevant test method. Further the results shall be reported in the same units as the specified requirements for the food product/matrix tested.

2.9.3.2 In microbiological testing if the result of the enumeration is negative, it should be reported as "not detected for a defined unit" or less than the detection limit for a defined unit". Qualitative test results should be reported as "detected/not detected in a defined quantity or volume"

Advance Lab Equipments for food analysis

- **Gas chromatography**

Gas chromatography (GC), is a common type of chromatography used in analytic chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture.

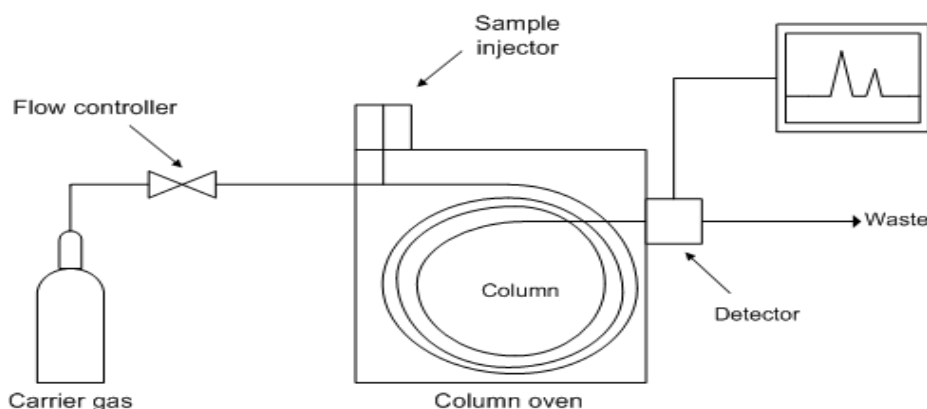


Principle of GC

Gas chromatography (GC) is based on a partition equilibrium of analyte between a solid stationary phase (often a liquid silicone-based material) and a mobile gas (most often Helium). The stationary phase is adhered to the inside of a small-diameter glass tube (a capillary column) or a solid matrix inside a larger metal tube (a packed column). It is widely used in analytical chemistry; though the high temperatures used in GC make it unsuitable for high molecular weight biopolymers or proteins (heat will denature them), frequently encountered in biochemistry, it is well suited for use in the petrochemical, environmental monitoring, and industrial chemical fields. It is also used extensively in chemistry research.

Procedure

In a GC analysis, a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a micro syringe (or, solid phase micro extraction fibers, or a gas source switching system). As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase materials. Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column.



Applications of Gas Chromatography

It is used for analysis & determination of different compounds in food products such as:

- Cholesterol , Fatty Acid profiling & Trans fat analysis
- Analysis of residual pesticides & environmental contaminants
- Antioxidants & Preservatives like TBHQ, Benzoic acid, Sorbic acid, Acetic acid, etc.
- Characterization of flavours & fragrances
- The GC profiling of the essential volatile oils gives a reasonable 'fingerprint' which can be used to characterize the identity of the particular oil.

High Performance Liquid Chromatography

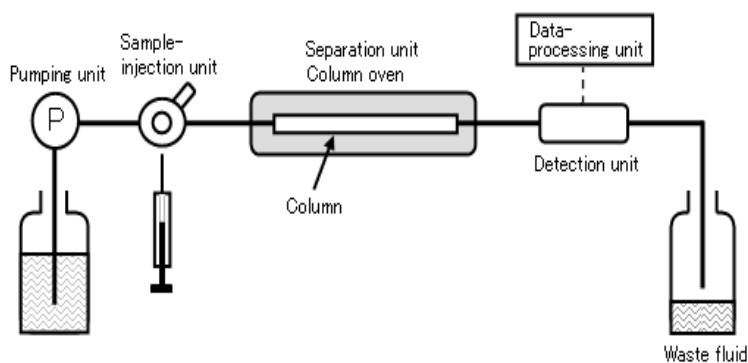
Principle

High performance liquid chromatography (or **high pressure liquid chromatography, HPLC**) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds based on their idiosyncratic polarities and interactions with the column's stationary phase. HPLC utilizes different types of stationary phase (typically, hydrophobic saturated carbon chains), a pump that moves the mobile phase(s) and analyte through the column, and a detector that provides a characteristic retention time for the analyte. The detector may also provide other characteristic information (i.e. UV/Vis spectroscopic data for analyte if so equipped). Analyte retention time varies depending on the strength of its interactions with the stationary phase, the ratio/composition of solvent(s) used, and the flow rate of the mobile phase.

With HPLC, a pump (rather than gravity) provides the higher pressure required to propel the mobile phase and analyte through the densely packed column. The increased density arises from smaller particle sizes. This allows for a better separation on columns of shorter length when compared to ordinary column chromatography.

Procedure

The sample to be analyzed is introduced in small volume to the stream of mobile phase. The analyte's motion through the column is slowed by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. How much the analyte is slowed depends on the nature of the analyte and on the compositions of the stationary and mobile phases. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time; the retention time under particular conditions is considered a reasonably unique identifying characteristic of a given analyte. The use of smaller particle size column packing (which creates higher backpressure) increases the linear velocity giving the components less time to diffuse within the column, leading to improved resolution in the resulting chromatogram. Common solvents used include any miscible combination of water or various organic liquids (the most common are methanol and acetonitrile). Water may contain buffers or salts to assist in the separation of the analyte components, or compounds such as trifluoroacetic acid which acts as an ion pairing agent.



A further refinement to HPLC has been to vary the mobile phase composition during the analysis; this is known as *gradient elution*. A normal gradient for reversed phase

chromatography might start at 5% methanol and progress linearly to 50% methanol over 25 minutes; the gradient chosen depends on how hydrophobic the analyte is. The gradient separates the analyte mixtures as a function of the affinity of the analyte for the current mobile phase composition relative to the stationary phase. This partitioning process is similar to that which occurs during a liquid-liquid extraction but is continuous, not step-wise. In this example, using a water/methanol gradient, the more hydrophobic components will elute (come off the column) when the mobile phase consists mostly of methanol (giving a relatively hydrophobic mobile phase). The more hydrophilic compounds will elute under conditions of relatively low methanol/high water.

The choice of solvents, additives and gradient depend on the nature of the stationary phase and the analyte. Often a series of tests are performed on the analyte and a number of trial runs may be processed in order to find the HPLC method which gives the best separation of peaks.

Applications of HPLC

It is used for profiling & analyzing of various components in food products such as:

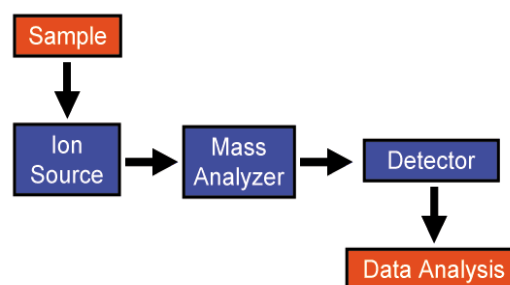
- Amino acids profiling, peptides & proteins
- Carbohydrates & carbohydrate profiling , sweeteners
- Lipids & alcohols
- Fat soluble & water soluble vitamins , carotenoids
- Organic acids & organic bases
- Residues of Mycotoxin, Antimicrobial & veterinary drugs, pesticides etc.
- Pigments, colorants & phenolic compounds
- Bittering substances
- Additives, preservatives, antioxidants & stabilizers in processed food products

Mass Spectrometry

Mass spectrometry (MS) is an analytical technique for the determination of the elemental composition of a sample or molecule. It is also used for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. The MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass-to-charge ratios.

Procedure:

1. a sample is loaded onto the MS instrument, and undergoes vaporization.
2. the components of the sample are ionized by one of a variety of methods (e.g., by impacting them with an electron beam), which results in the formation of positively charged particles (ions)
3. the positive ions are then accelerated by an electric field
4. computation of the mass-to-charge ratio (m/z) of the particles based on the details of motion of the ions as they transit through electromagnetic fields, and
5. detection of the ions, which in step 4 were sorted according to m/z .



MS instruments consist of three modules:

- an *ion source*, which can convert gas phase sample molecules into ions (or, in the case of electrospray ionization, move ions that exist in solution into the gas phase);

- a *mass analyzer*, which sorts the ions by their masses by applying electromagnetic fields; and
- a *detector*, which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present.

The technique has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds

Applications of Mass Spectrometry

- Identifying unknown compounds by the mass & mass fragmentation pattern
- Used for elucidating the chemical structures of molecules, such as peptides and other chemical compounds.
- Quantifying the amount of a compound

Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample.



Principle of GC-MS

The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A “library” of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector.

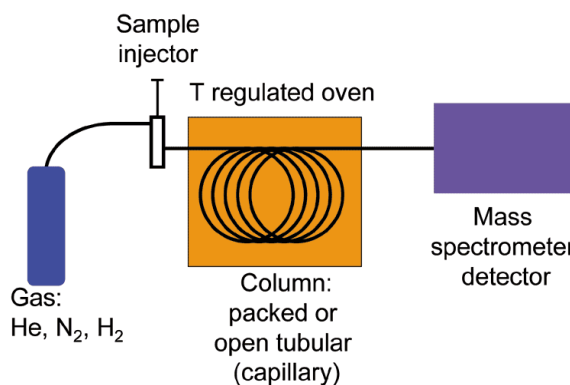
GC-MS Equipment

The GC-MS is composed of two major building blocks:

- ✚ the gas chromatograph and
- ✚ the mass spectrometer.

The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules take different amounts of time (called the retention time) to come out of (elute from) the gas chromatograph, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio.

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame Ionization Detector) detects multiple molecules that happen to take the same amount of time to travel through the column (*i.e.* have the same retention time) which results in two or more molecules to co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes makes it extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically lends to increased certainty that the analyte of interest is in the sample.



Split/Splitless GC-MS inlets

Samples are introduced to the column via an inlet. This inlet is typically injection through a septum. Once in the inlet, the heated chamber acts to volatilize the sample. In a split system, a constant flow of carrier gas moves through the inlet. A portion of the carrier gas flow acts to transport the sample into the column. Another portion of the carrier gas flow gets directed to purge the inlet of any sample following injection (septum purge). Yet another portion of the flow is directed through the split vent in a set ratio known as the split ratio. In a splitless system, the advantage is that a larger amount of sample is introduced to the column. However, a split system is preferred when the detector is sensitive to trace amounts of analyte and there is concern about overloading the column.

Purge and Trap GC-MS

For the analysis of volatile compounds a Purge and Trap (P&T) concentrator system may be used to introduce samples. The target analytes are extracted and mixed with water and introduced into an airtight chamber. An inert gas such as Nitrogen (N₂) is bubbled through the water; this is known as purging. The volatile compounds move into the headspace above the water and are drawn along a pressure gradient (caused by the introduction of the purge gas) out of the chamber. The volatile compounds are drawn along a heated line onto a 'trap'. The trap is a column of adsorbent material at ambient temperature that holds the compounds by returning them to the liquid phase. The trap is then heated and the sample compounds are introduced to the GC-MS column via a volatiles interface, which is a split inlet system. P&T GC-MS is particularly suited to volatile organic compounds (VOCs) and BTEX compounds (aromatic compounds associated with petroleum).

Types of Mass Spectrometer Detectors

The most common type of mass spectrometer (MS) associated with a gas chromatograph (GC) is the quadrupole mass spectrometer, sometimes referred to by the Hewlett-Packard (now Agilent) trade name "Mass Selective Detector" (MSD). Another relatively common detector is the ion trap mass spectrometer. Additionally one may find a magnetic sector mass spectrometer, however these particular instruments are expensive and bulky and not typically found in high-throughput service laboratories. Other detectors may be encountered such as time of flight (TOF), tandem quadrupoles (MS-MS) (see below), or in the case of an ion trap MSⁿ where n indicates the number mass spectrometry stages.

Analysis

A mass spectrometer is typically utilized in one of two ways:

- Full Scan
- Selective Ion Monitoring (SIM).

The typical GC/MS instrument is capable of performing both functions either individually or concomitantly, depending on the setup of the particular instrument.

- **Full scan MS**

When collecting data in the full scan mode, a target range of mass fragments is determined and put into the instrument's method. An example of a typical broad range of mass fragments to monitor would be m/z 50 to m/z 400. The determination of what range to use is largely dictated by what one anticipates being in the sample while being cognizant of the solvent and other possible interferences. A MS should not be set to look for mass fragments too low or else one may detect air (found as m/z 28 due to nitrogen), carbon dioxide (m/z 44) or other possible interferences. Additionally if one is to use a large scan range then sensitivity of the instrument is decreased due to performing fewer scans per second since each scan will have to detect a wide range of mass fragments.

Full scan is useful in determining unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method.

- **Selected ion monitoring**

In selected ion monitoring (SIM) certain ion fragments are entered into the instrument method and only those mass fragments are detected by the mass spectrometer. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan. More scans can take place each second. Since only a few mass fragments of interest are being monitored, matrix interferences are typically lower. To additionally confirm the likelihood of a potentially positive result, it is relatively important to be sure that the ion ratios of the various mass fragments are comparable to a known reference standard.

The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum. Two kinds of analysis are possible, comparative and original. Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. This is best performed by a computer because there are a myriad of visual distortions that can take place due to variations in scale. Computers can also simultaneously correlate more data (such as the retention times identified by GC), to more accurately relate certain data.

Another method of analysis measures the peaks in relation to one another. In this method, the tallest peak is assigned 100% of the value, and the other peaks being assigned proportionate values. All values above 3% are assigned. The total mass of the unknown compound is normally indicated by the parent peak. The value of this parent peak can be used to fit with a chemical formula containing the various elements which are believed to be in the compound. The isotope pattern in the spectrum, which is unique for elements that have many isotopes, can also be used to identify the various elements present. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be identified, and must be consistent with the characteristics recorded by GC/MS. Typically, this identification done automatically by programs which come with the instrument, given a list of the elements which could be present in the sample.

A "full spectrum" analysis considers all the "peaks" within a spectrum. Conversely, selective ion monitoring (SIM) only monitors selected peaks associated with a specific substance. This is done on the assumption that at a given retention time, a set of ions is characteristic of a certain compound. This is a fast and efficient analysis, especially if the analyst has

previous information about a sample or is only looking for a few specific substances. When the amount of information collected about the ions in a given gas chromatographic peak decreases, the sensitivity of the analysis increases. So, SIM analysis allows for a smaller quantity of a compound to be detected and measured, but the degree of certainty about the identity of that compound is reduced.

Applications of GC-MS

- Pesticide residue analysis in all raw & processed food products
- Analysis of environmental contaminants such as polychlorinated biphenyls, polyaromatic hydrocarbons, dioxins, etc. in food products
- Flavours & fragrance in food products
- Fatty acid profiling in oils & fats
- Volatiles & other residual solvents in food packaging material

GC-MS/MS

When a second phase of mass fragmentation is added, for example using a second quadrupole in a quadrupole instrument, it is called MS/MS or Tandem MS. Tandem mass spectrometry (MS/MS) is a more powerful technique to quantitate low levels of target compounds in the presence of a high sample matrix background.

The first quadrupole (Q1) is connected with a collision cell (q2) and another quadrupole (Q3). Both quadrupoles can be used in scanning or static mode, depending on the type of MS/MS analysis being performed. Types of analysis include product ion scan, precursor ion scan, Single Reaction Monitoring (SRM) and Multiple Reaction Monitoring (MRM) and Neutral Loss Scan. For example: When Q1 is in static mode (looking at one mass only as in SIM), and Q3 is in scanning mode, one obtains a so-called product ion spectrum (also called "daughter spectrum"). From this spectrum, one can select a prominent product ion which can be the product ion for the chosen precursor ion. The pair is called a "transition" and forms the basis for SRM (MRM is sometimes used as term). SRM is highly specific and virtually eliminates matrix background.

Applications

✚ Food, Beverage and Perfume Analysis

Foods and beverages contain numerous aromatic compounds, some naturally present in the raw materials and some forming during processing. GC-MS is extensively used for the analysis of these compounds which include esters, fatty acids, alcohols, aldehydes, terpenes etc. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful and which is often controlled by governmental agencies, for example pesticides

✚ Environmental Monitoring and Cleanup

✚ Criminal Forensics

✚ Law Enforcement

✚ Medicine

In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of ^{13}C as the labeling and the measurement of $^{13}\text{C}/^{12}\text{C}$ ratios with an isotope ratio mass spectrometer (IRMS); an MS with a detector designed to measure a few select ions and return values as ratios.

Liquid Chromatography/Mass Spectroscopy

Liquid chromatography / Mass Spectroscopy (LC / MS) is a technique which combines high performance liquid chromatography HPLC, a powerful analytical separation technique with mass spectroscopy, a powerful analysis & detection technique. LC-MS is a powerful technique used for many applications which has very high sensitivity and specificity.

Generally its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture).

Working of LCMS

The liquid chromatography scale used in LC-MS is usually much smaller i.e. internal diameter of the column and with respect to flow rate since it scales as the square of the diameter. For a long time, 1 mm columns were typical for LC-MS work (as opposed to 4.6 mm for HPLC). More recently 300 μm and even 75 μm capillary columns have become more prevalent. At the low end of these column diameters the flow rates approach 100 nL/min and are generally used with nanospray sources.



Flow splitting

When standard bore (4.6 mm) columns are used the flow is often split ~10:1. This can be beneficial by allowing the use of other techniques in tandem such as MS and UV. However splitting the flow to UV will decrease the sensitivity of spectrophotometric detectors. The mass spectrometry on the other hand will give improved sensitivity at flow rates of 200 $\mu\text{L}/\text{min}$ or less.

Mass spectrometry

Mass analyzer

There are a lot of mass analyzers that can be used in LC/MS. Single Quadrupole, Triple Quadrupole, Ion Trap, TOF (time of Flight) and Quadrupole-time of flight (Q-TOF).

Interface

The interface is most often an electrospray ion source or variant such as a nanospray source; however fast atom bombardment, thermospray and atmospheric pressure chemical ionization interfaces are also used. Various deposition and drying techniques have also been used such as using moving belts.

Applications of LC-MS

- Routinely Used for detection of Mycotoxins; toxins produced by different fungi, e.g. *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., etc. Some of the mycotoxin regularly analysed in food samples include Aflatoxin B1, B2, G1, G2, ochratoxin A, etc.
- Residual drugs & antibiotics in different food products
- Residual pesticides in raw & processed food products
- Banned dyes & colourants e.g. Sudan dyes in different food products.

Inductively coupled Plasma Mass Spectroscopy (ICP-MS)

Inductively coupled plasma mass spectroscopy (ICP-MS) is a technique which combines the easy sample introduction and quick analysis of ICP technology with the accurate and low

detection limits of a mass spectrometer. The resulting instrument is capable of trace multi element analysis, often at the part per trillion level. ICP-MS has been used widely in a number of different fields including drinking water, wastewater, natural water systems/hydrogeology, geology and soil science, mining/metallurgy, food sciences, and medicine.

Principle of ICP-MS

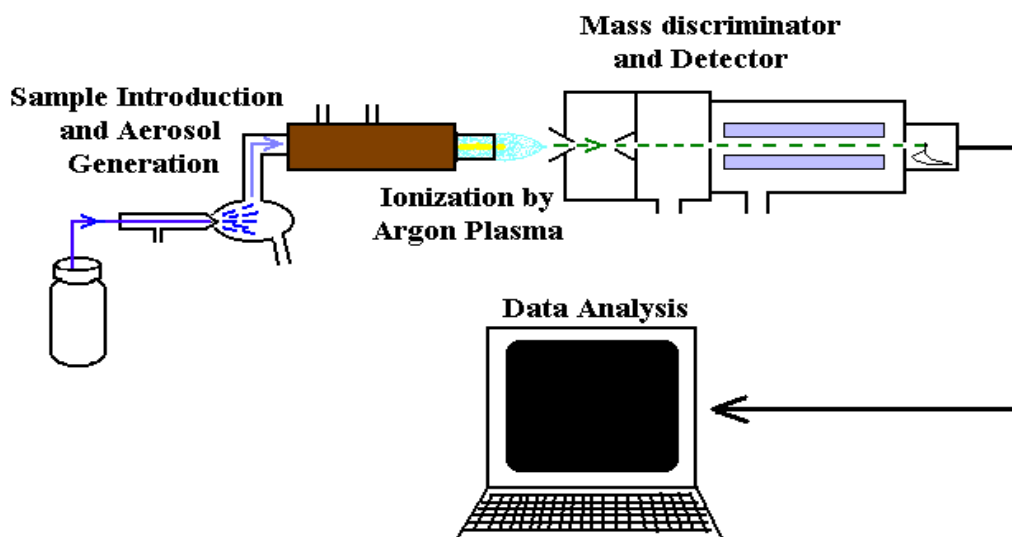
ICP –MS is based on the principles used in atomic emission spectrometry. Samples are decomposed to neutral elements in a high temperature argon plasma and analyzed based on their mass to charge ratios.

Process:

An ICP-MS consists of four main processes:

- sample introduction and aerosol generation,
- ionization by an argon plasma source,
- mass discrimination,
- detection system.

The schematic below illustrates this sequence of processes.



Applications of ICP-MS

- Used for the detection of trace metals such as Al, As, Sb , Cr, Mn, Ni, Pb Hg etc. in food products. ICP-MS has the capability to scan for all elements simultaneously
- Used for quantification of proteins & biomolecules

Atomic Absorption Spectroscopy

Atomic absorption spectroscopy is a technique for determining the concentration of a particular metal element in a sample. The technique can be used to analyze the concentration of over 70 different metals in a solution.

Principle:

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It relies therefore heavily on Beer-Lambert law.

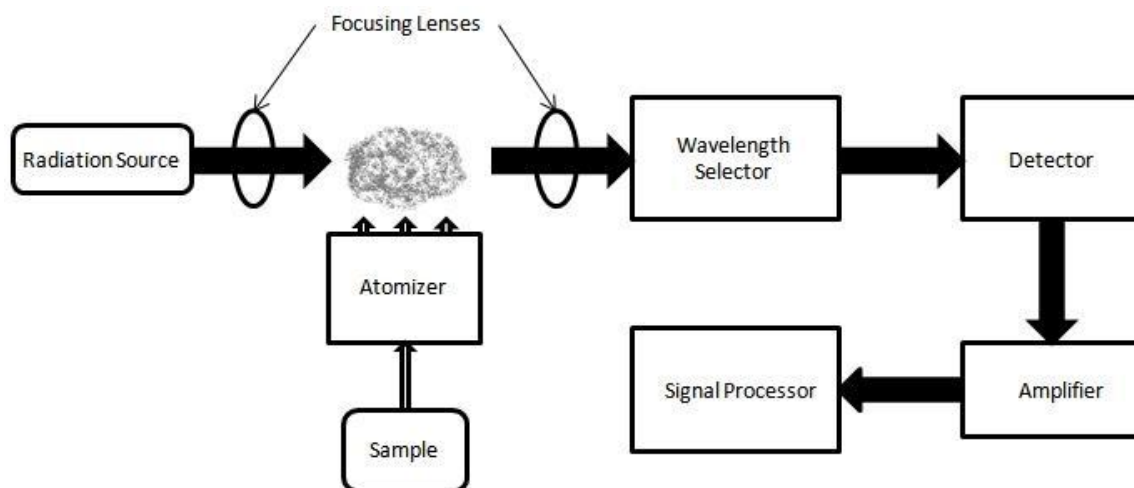
In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals for a short amount of time by absorbing a set quantity of energy (i.e. light of a given wavelength). This amount of energy (or wavelength) is specific to a particular electron transition in a particular element, and in general, each wavelength corresponds to only one element. This gives the technique its elemental selectivity.

As the quantity of energy (the power) put into the flame is known, and the quantity remaining at the other side (at the detector) can be measured, it is possible, from Beer-Lambert law, to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured.



Procedure

In order to analyze a sample for its atomic constituents, it has to be atomized. The sample should then be illuminated by light. The light transmitted is finally measured by a detector. In order to reduce the effect of emission from the atomizer (e.g. the black body radiation) or the environment, a spectrometer is normally used between the atomizer and the detector.



Application of AAS

- Used for assessing the concentration of metals & minerals that may be present in food products such as Fe, Pb, As, Cd ,Zn etc.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Principle:

Reverse transcription polymerase chain reaction (RT-PCR) is a variant of polymerase chain reaction (PCR), a laboratory technique commonly used in molecular biology to generate many copies of a DNA sequence, a process termed "amplification". In RT-PCR, however, an RNA strand is first reverse transcribed into its DNA complement (*complementary DNA*, or *cDNA*) using the enzyme reverse transcriptase, and the resulting cDNA is amplified using traditional or real-time PCR. Reverse transcription PCR is not to be confused with real-time polymerase chain reaction (Q-PCR/qRT-PCR), which is also sometimes (incorrectly) abbreviated as RT-PCR.

Procedure:

RT-PCR utilizes a pair of primers, which are complementary to a defined sequence on each of the two strands of the cDNA. These primers are then extended by a DNA polymerase and a copy of the strand is made after each cycle, leading to logarithmic amplification .

RT-PCR includes three major steps. The first step is reverse transcription (RT), in which RNA is reverse transcribed to cDNA using reverse transcriptase and primers. This step is very important in order to perform PCR since DNA polymerase can act only on DNA templates. The RT step can be performed either in the same tube with PCR (one-step PCR) or in a separate one (two-step PCR) using a temperature between 40°C and 50°C, depending on the properties of the reverse transcriptase used.

The next step involves the denaturation of the dsDNA at 95°C, so that the two strands separate and the primers can bind again at lower temperatures and begin a new chain reaction. Then, the temperature is decreased until it reaches the annealing temperature which can vary depending on the set of primers used, their concentration, the probe and its concentration (if used), and the cations concentration. The main consideration, of course, when choosing the optimal annealing temperature is the melting temperature (T_m) of the primers and probes (if used). The annealing temperature chosen for a PCR depends directly on length and composition of the primers. This is the result of the difference of hydrogen bonds between A-T (2 bonds) and G-C (3 bonds). An annealing temperature about 5 degrees below the lowest T_m of the pair of primers is usually used.



The final step of PCR amplification is DNA extension from the primers. This is done with thermostable Taq DNA polymerase, usually at 72°C, the temperature at which the enzyme works optimally. The length of the incubation at each temperature, the temperature alterations, and the number of cycles are controlled by a programmable thermal cycler. The analysis of the PCR products depends on the type of PCR applied. If a conventional PCR is used, the PCR product is detected using agarose gel electrophoresis and ethidium bromide (or other nucleic acid staining).

Conventional RT-PCR is a time-consuming technique with important limitations when compared to real-time PCR techniques. This, combined with the fact that ethidium bromide has low sensitivity, yields results that are not always reliable. Moreover, there is an increased cross-contamination risk of the samples since detection of the PCR product

requires the post-amplification processing of the samples. Furthermore, the specificity of the assay is mainly determined by the primers, which can give false-positive results. However, the most important issue concerning conventional RT-PCR is the fact that it is a semi- or even a low-quantitative technique, whereas the amplicon can be visualized only after the amplification ends.

Real-time RT-PCR provides a method in which the amplicons can be visualized as the amplification progresses using a fluorescent reporter molecule. There are three major kinds of fluorescent reporters used in real time RT-PCR, which are general non-specific DNA Binding Dyes such as SYBR Green I, TaqMan Probes and Molecular Beacons (including Scorpions).

The real-time PCR thermal cycler has a fluorescence detection threshold, below which it cannot discriminate the difference between an amplification generated signal and background noise. On the other hand, the fluorescence increases as the amplification progresses and the instrument performs data acquisition during the annealing step of each cycle. The number of amplicons will reach the detection baseline after a specific cycle, which depends on the initial concentration of the target DNA sequence. The cycle at which the instrument can discriminate the amplification generated fluorescence from the background noise is called the threshold cycle (Ct). The higher the initial DNA concentration, the lower its Ct will be.

Applications of RT-PCR

- Used for the detection and quantitation of VT 1 and VT 2 toxin genes in *E. coli* O157:H7
- Used in the GMO quantification