Development and use of microbiological criteria for foods

Guidance for those involved in using and interpreting microbiological criteria for foods

Preface

With the increased use of Hazard Analysis and Risk Assessment in the management of food safety and, to some extent, quality, it is reasonable to ask whether microbiological testing and criteria are still necessary, other than when required by legislation. Certainly, there is considerable debate on whether criteria should be published at all. In fact, information on the numbers of particular microbial populations and their safe limits is still needed in many instances.

Microbiological criteria are necessary to assist in setting critical limits in HACCP systems, verification of HACCP plans, and in shelf life studies where storage trials and challenge tests are needed. In microbiological risk assessments, knowledge of microbial population distribution and numbers in food forms an essential part of the information required, in conjunction with population exposure, infective dose and pathogenicity of organisms. When mathematical models are used to predict microbial growth, survival or decline, it is also necessary to appreciate the numbers that are inevitable and those that cause concern or signal the end of shelf life.

For larger companies, such information is generated in-house and is readily available on their databases, but this information is likely to remain confidential and will not be published for reasons of competitive advantage. Small-to-medium sized companies in supply, manufacture, catering and retailing have little or no such information readily available to them. The information, where it exists, can only be found in a wide diversity of literature, including ICMSF publications, industry codes of practice, legislation and peer-reviewed technical publications. Much of this information is now elderly and therefore incomplete with respect to current microbiological issues, because recently emerged pathogens and new products and processes are inevitably excluded.

In order to help redress the balance, this discussion of the development and use of criteria is offered for guidance. It includes a list of product-group related microbiological limits, compiled from the experience, knowledge and publications of many experts in food microbiology. It acknowledges that all tests included in each group are not always needed and that extra tests are sometimes applicable, but within that framework it gives an extensive array of information on what can be achieved by GMP and what represents the limit of shelf-life and safety.

This paper is intended for practical use. Debate and comment are encouraged and welcomed. The second edition will be published soon as an IFST Monograph. **If you wish your comments to be considered, please write to Dr C Bell, c/o IFST, 5 Cambridge Court, 210 Shepherd's Bush Road, London W6 7NJ by the end of November 1997.**

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1. Introduction

1.1 Aims and scope

The safety of food must be assured by a preventative approach based on the application of a Hazard Analysis Critical Control Point (HACCP) at all stages in the food supply system. The authors fully support this fundamental requirement.

It is recognised that microbiological criteria are widely used in the food industry but they have rarely been published. Whilst not advocating the introduction of further legal standards or the indiscriminate use of criteria, food industry experience of practical microbiological criteria needs to be shared.

This document discusses the role of microbiological testing and criteria in support of a safe food supply. It aims to provide guidance to all those involved in producing, using and interpreting microbiological criteria in the food and catering industries. It has been written by professional food microbiologists with experience in the food manufacturing, retailing, public health, testing and consultancy sectors in consultation with colleagues also expert in these fields.

Microbiological criteria are inevitably controversial and the figures given in the tables are derived

from the practical experience of the authors as well as existing guidelines and standards. Such a document can never be exhaustive and the food categories used encompass a wide range of products. Where microbiological testing and criteria are considered useful by a food business, the information and figures given in this document may be used as a basis for determining relevant criteria for individual products.

It is hoped that this document will stimulate a productive discussion on this important subject.

1.2 Background

Foodborne disease and microbial spoilage of food result from the failure or inability to control microorganisms at one or more stages of the food chain, from raw material production to consumption of the final product. The implications of situations that result in food poisoning outbreaks or food spoilage can be severe for food producers, retailers, consumers and regulatory authorities.

Traditionally, control of microorganisms in a food has been demonstrated by microbiological testing of samples at various stages of production and of the final product. Criteria have been developed to give some degree of assurance that food is safe and of suitable quality, and that it will remain so to the end of its shelf life provided it is handled appropriately. Conformance to microbiological criteria is determined by testing. It is recognised that microbiological testing can never give an absolute assurance of product safety or quality, particularly because of the problems involved in product sampling and the distribution of microorganisms in food. Used judiciously however, microbiological testing is a valuable tool in the food industry.

In the food industry today, microbiological testing can be effective when used alongside physical and chemical process control systems as part of hazard analysis approaches such as HACCP (ICMSF, 1988) and other HACCP-based systems. The HACCP system is a structured approach to identifying hazards and defining and implementing systems of control. Microbiological examination can be used to help verify that these critical control points remain under control.

1.3 Definition and practical application of microbiological criteria

Microbiological criteria are essentially of three types:

- Standard
- Guideline
- Specification

These terms, as defined by the Codex Alimentarius Commission (Codex, 1981), were principally for use in Codex standards and codes of practice. Microbiological standards, guidelines and specifications have been redefined by Codex (Codex, 1993), the International Commission on Microbiological Specifications for Foods (ICMSF, 1986) and the United States Subcommittee on Microbiological Criteria (NRC, 1985) and for simplicity the following working definitions are generally recognised:

Standard - This is a microbiological criterion contained in a law or a regulation where compliance is mandatory. These are introduced by governments or regulatory authorities. Typical examples include most criteria in European Community (EC)/European Union (EU) Directives and Statutory Instruments of England and Wales. The food industry must ensure full compliance with these standards which are monitored by enforcement agencies. As well as being an offence, products not complying with standards will be rejected as unfit for the intended use. Results not conforming to standards may be used by enforcement agencies as the basis for prosecution or for refusing importation of foods. Current EU microbiological standards are summarised in Section 7.

Guideline - A microbiological guideline is a criterion applied at any stage of food processing and retailing which indicates the microbiological condition of the sample and aids in identifying situations requiring attention for food safety or quality reasons. Results obtained from testing against a microbiological guideline also assist in trend analysis. Results that deviate significantly from the trend may indicate a tendency towards a situation which is out of control and highlight the need for attention before control is lost. Guidelines are usually self imposed by the food industry but may occasionally be included in legislation. Products not complying with guidelines should result in investigative action to identify and rectify the cause. Guidelines on the levels and types of microorganism relevant in specified foods produced under good manufacturing practice may also be provided by industrial associations for their members. In some EU Directives there are guidelines for indicator organisms e.g. Directive 92/46/EEC on milk and milk products. In the UK, the Public Health Laboratory Service has published microbiological guidelines (PHLS, 1996) to assist food examiners and enforcement officers in the determination of the microbiological quality of foods and to indicate levels of bacterial contamination considered to be a potential health risk in ready-to-eat foods at point of sale.

Specification - This is a microbiological criterion applied to raw materials, ingredients or the end product which is used in a purchase agreement. Criteria may include pathogens, toxins, indicator microorganisms or spoilage microorganisms where non-compliance may affect product safety and/or quality during shelf life. End product specifications

are usually more stringent than microbiological standards in order to provide a margin of safety.

Products not complying with specifications should be investigated to determine the cause. Rejection of products may occur even if they are not hazardous or unwholesome at the time of testing.

It is important to ensure that the components of microbiological specifications are relevant and realistic and fully understood by both parties in the agreement.

1.4 Components of microbiological criteria

A microbiological criterion consists of a statement of *at least* the following:

- the microorganism or microbial toxin of concern
- the food concerned and sample type
- the sampling plan (see 3.3.1)
- the microbiological limit(s)

Conformance to microbiological criteria should be monitored using specified, relevant methods which have been validated for the microorganism or toxin of concern in the food being examined (see also 4.9 and 6.1).

Microorganism or microbial toxin of concern

The contaminants detailed in the criterion may be foodborne pathogens, food spoilage organisms, indicator organisms or microbial toxins (ICMSF, 1978). Specified microorganisms must be relevant to the food and/or food process (see Appendix: Specific organisms and the principles of test methods). Pathogen testing is applied for reasons of public health where evidence exists of a potential hazard to health from a specific food/organism. Testing for indicator and spoilage microorganisms is used for the following reasons:

Cost effectiveness: More analyses can be conducted for microbial indicators of unhygienic practice and spoilage microorganisms than for specific pathogens because testing for pathogens is usually more expensive.

Simplicity: Test for indicator microorganisms are frequently simpler to conduct than tests for specific pathogens.

Rapidity: Tests for indicator organisms usually provide results more rapidly than those for pathogens, thus allowing for faster remedial action.

Trend analysis: Indicator and spoilage microorganisms are usually present in higher numbers than pathogens and indicate unsatisfactory conditions when levels increase significantly. Since levels can be monitored, trends can be established so that trend analysis can identify situations before they become out of control. Tests for pathogens or toxins usually result in numerous negative results; any positive results indicate situations which often require immediate remedial action.

Food concerned and sample type

Microbiological criteria must indicate the food and stage of processing to which they relate. Ideally, they should give a description of the food, indicating key processing features and conditions under which the material should be stored and used. Such factors significantly influence the content and validity of a microbiological criterion. For example, a specification for frozen food may allow higher aerobic plate counts at the point of production compared with the same chilled food since bacteria will not grow under frozen conditions but may grow during chilled storage. However, bacterial growth during or after defrosting should be taken into account for frozen products.

Also, the microbial levels in a perishable sample tested at the beginning of its shelf life are likely to differ from those at the end of shelf life. Such differences must be taken into account when specifying a microbiological criterion and it should be made clear at what point in the product shelf life the relevant criterion is to be applied. It is also important to indicate the nature of the sample if this is likely to influence the end result and its subsequent interpretation. Microbiologically sensitive components of multi-component foods e.g. sandwiches may need to be tested separately. In the food industry such details are usually contained in product specifications.

Microbiological limit

The microbiological limit defined in a criterion represents the level above which action is required. Levels must be realistic and should be determined from a knowledge of the microbiology of raw materials and the effects of processing, product handling, storage and end use on the microbiology of the final product. Limits may be derived by determining microbial levels in products where conditions of processing, storage and product characteristics are known. They should take into account the levels that indicate inadequate control and may represent a hazard to health.

It is not possible to prove the *absence* of an organism in a product and negative results from detection (presence/absence) tests should be declared as 'not detected' (ND) e.g. not detected in 25g or not detected in 1ml.

For quantitative tests in which no growth occurs, it is normal to record the result as less than the limit of detection of the test e.g. <10 cfu/g, which is equivalent to ND in 0.1g.

1.5 Limitations of microbiological criteria

The tests used to assess whether a food complies with a microbiological criterion are subject to a

number of factors which should always be clearly understood when using them:

(a) Methods

No microbiological method is capable of detecting all representatives of the target microorganism being sought; even the best are probably capable of recovering no more than 90% of representatives. The presence of sub-lethally injured cells or cells that may be viable but non-culturable, contaminants present in the food but at a level below the sensitivity of the method and variants not capable of growth under the conditions of the test will all result in less than total recovery of the target organism. Confidence limits for results from enumerative techniques are also relatively wide (BS 5763: Part 0: 1996).

(b) Sampling

Microorganisms are rarely distributed homogeneously in a sample or a product batch and pathogens, if present, are usually at low levels. Any single test for detection or enumeration only reflects the situation in that particular sub-sample of the batch. The effectiveness of the test is also dependent on sample size and type and sampling techniques; aseptic sampling procedures are of particular importance for preventing extraneous contamination.

(c) Laboratory competence

Laboratory staff competence and the operation of quality systems in laboratories are very important for achieving valid microbiological results. Independent auditing, use of external quality assurance schemes and accreditation of laboratories help to provide assurance of laboratory competence.

(d) Cost effectiveness

The only way microbiological testing and associated criteria can be totally reliable is by adoption of 100% inspection with methods of 100% reliability and accuracy. Since current methods are effectively destructive, 100% testing would leave no food to consume and whilst this would undoubtedly reduce foodborne illness it is not a feasible option!

In the absence of 100% inspection but by the application of statistical techniques to sampling, the degree of assurance derived from a result will be dependent on the number and size of samples taken. Detection of foodborne pathogens, present at low levels and distributed heterogeneously in a food, requires an exceptionally high number of samples to be analysed to achieve a reasonable degree of assurance (ICMSF, 1986).

Despite the limitations described (which are well understood by food microbiologists), use of microbiological testing may play an important part in the hazard analysis process. When used regularly, non-statistical sampling and testing schemes are cost effective in that they are a simple form of monitoring (trend analysis) that can allow trends to be identified and add to the assurance that the process is under control. This constructive use of microbiology alongside other process control systems helps to ensure that the food supply is safe and of good quality.

2. Factors affecting the microbiology of foods

2.1 General

The food industry uses a wide variety of technologies to produce an enormous range of foodstuffs, from chilled foods with a short shelf life to shelf stable intermediate moisture foods and long life packaged foods. Each technology involves a complex series of interlinked processes that combine to produce safe and wholesome products. It is essential to understand the interrelationship of these processes so that relevant criteria can be developed and applied. Monitoring procedures should be derived from HACCP-based assessments of all the processes involved.

Factory structure, manufacturing equipment, raw materials, process flows, temperature/time controls and hygiene systems (personnel and environment) all affect the microbiological nature of the product and each requires careful evaluation.

2.2 Factory structure, environment and personnel

In establishing criteria, consideration must be given to the potential for contamination of food from the environment. For example, a cooked, sliced meat will be more susceptible to environmental contamination than the same type of meat cooked in its final container; this should be reflected in the associated criteria. The IFST booklet *Food and Drink - Good Manufacturing Practice: A Guide to its Responsible Management* (1991) contains useful advice concerning premises, equipment and approaches to good manufacturing practice (GMP).

2.3 Raw materials

It is essential to understand the nature of potential hazards that may be presented by raw materials. Specifications for raw materials should contain relevant microbiological, toxicological and physicochemical parameters. Microbiological criteria for finished product should take account of the level and types of microorganism present in a raw material and the processing conditions which will affect the levels of organisms throughout the process. Even where a manufacturing process may be expected to eliminate a particular potential microbial hazard from the raw material, the possible production by

the microorganism of heat-stable toxins which may cause illness or enzymes (proteases and lipases) which may cause spoilage must be taken into account in microbiological criteria.

2.4 Process technology

2.4.1 General

A clear understanding of the effect of food process technologies on microorganisms and their survival and growth characteristics in foods is necessary before relevant criteria can be assigned (ICMSF, 1996).

2.4.2 Temperature

Foods may be chilled, frozen, mildly heat treated (thermised, pasteurised, sous vide) or exposed to high heat treatments (Ultra High Temperature (UHT) or those used in some drying or canning processes).

Freezing to temperatures below approximately minus 10°C will stop microbiological growth but enzyme activity can still occur and may cause organoleptic changes. The microbial population at the point of freezing generally remains stable throughout the frozen shelf life although some organisms are more susceptible to freezing temperatures and may die.

Chill temperatures prevent the growth of many microorganisms, but some pathogens and a wide variety of food spoilage organisms may grow, albeit relatively slowly. Criteria applied at the point of manufacture must take account of the potential for subsequent growth of psychrotrophic microorganisms during shelf life.

Mild heat treatments such as pasteurisation reduce the levels of vegetative microorganisms in foods but thermotolerant organisms and spores may survive. Criteria must take account of these organisms together with post-process contaminants.

High temperature processes such as UHT and some canning processes are used to produce shelf stable products. Vegetative microorganisms should be completely destroyed and only a few types of spore forming bacteria are able to survive such processes although pathogenic strains are killed.

2.4.3 Water activity

Water activity (a_w) is a measure of the water available for microbial growth; reducing the a_w in a food can restrict the growth of many microorganisms. Water activity may be reduced from the maximum (1.0 for pure water) to low levels (<0.75) by direct removal of water e.g. drying, or by the addition of solutes such as salt or sugar. The microorganisms capable of growth or survival at the a_w of a particular food should be considered when setting criteria. For example, most bacteria cannot grow below a_w 0.9 but *Staphylococcus aureus (S. aureus)* can grow down to approximately a_w 0.85 and may be important in criteria for some dried products whilst some yeasts and moulds can grow at levels as low as a_w 0.6.

2.4.4 pH and acidity

The pH/acidity of a product affects the range of organisms that can survive and grow. Many products have a low pH i.e. <pH 4.5 resulting from natural acidity, fermentation or pickling processes. Spoilage organisms included in criteria for such products are likely to be yeasts, moulds and acid tolerant bacteria. Pathogenic bacteria may survive, but few are likely to grow. At a product pH greater than 4.5, *Clostridium botulinum* may grow and produce toxin. It may therefore be necessary to apply a high heat process (F_0>3.0 see section 5.5) or acidify the product to ensure its safety. The possibility of outgrowth of organisms that may survive any heat treatment and raise the product pH to a level at which pathogens may grow should also be taken into account.

2.4.5 Atmosphere

The atmosphere within product packaging is often altered to help improve product keeping quality and increase shelf life. Air may be removed altogether to create a vacuum as with some meat joints, or air may be replaced by other gases e.g. nitrogen and/or carbon dioxide in various proportions as with many sliced cooked meats, fresh salad mixes and fish products. Most of these latter types are termed modified atmosphere packs (MAP) and the atmosphere within these packs changes over time because of the dynamic processes which occur between the food and the atmosphere.

Strictly controlled atmosphere packs (CAP) are those in which these dynamic processes are balanced throughout the storage life of the product, the atmosphere therefore remaining constant. Some novel packaging systems incorporate a chemical scavenging system to keep oxygen levels low. In each particular case, the packaging material is carefully selected to ensure its permeability characteristics are appropriate for the required atmospheric conditions to be maintained. Such packaging systems are often used for chilled foods and although the changed atmospheric conditions contribute to the inhibition of some microbiological growth, lactic acid bacteria and yeasts in particular may cause spoilage. Consideration must be given to the potential for some modified atmospheres to provide conditions which select for the growth of clostridia including *C. botulinum* (ACMSF, 1992).

2.4.6 Combination of factors

The combined effect of non-optimal conditions of temperature, a_w, pH and packaging atmosphere on the growth and survival of microorganisms usually

has a greater effect than an individual factor used at the same level (ICMSF, 1996). Preservatives can also be used in combination with these factors to enhance microbial suppression. The ICMSF book *Factors Affecting Life and Death of Microorganisms* (1980a) gives extensive information on each of the above factors in addition to listing some 'limits for growth' of some microorganisms for a_w and pH values.

2.5 Shelf life

The potential for growth and/or toxin production of residual microbial populations in finished products depends on the types of organisms present and their ability to grow to a level of concern under the storage conditions applied during the product shelf life.

Microbiological criteria should take account of any organisms likely to be present. The levels of tolerance applied at the point of manufacture should be such that allowing for predictable growth of these organisms, the product will remain safe and wholesome to the end of shelf life provided it is stored under the correct conditions. It is industry practice to build in a safety margin for chilled products which allows for mild temperature abuse of product during storage.

2.6 Application of microbiology

Microbiological testing, even using rapid methods, takes time (sometimes many days) and costs money. There is a tendency to include too many organisms and to apply over-stringent limits in microbiological criteria in a misguided attempt to achieve product safety. Inappropriate criteria are costly in time and resources (human and financial) which could be put to better use in the practical application of HACCP and GMP systems. It is therefore important to ensure that any such testing is both relevant and meaningful within the context of the food production operation. Answering the following questions will assist in obtaining the most sensible, practical and cost effective use of microbiology:

- what information is needed?
- why is this information necessary?
- what is the best means of obtaining the information?
- if a microbiological test is to be used, where is it best applied?
- what criteria are appropriate and practicable?
- what response is appropriate to 'out of limit' results?

For example, tens of thousands of finished product examinations for *S. aureus* are carried out each year in industry on extensively handled foods and the organism is rarely detected. In order to minimise the level of *S. aureus* in the finished product, it may be more effective to apply a structured personnel hand-swabbing and training programme for those involved in direct handling of the product and/or its components. This application of the test could yield more positive benefits in heightened personnel awareness and improved personal hygiene practices and handling procedures than would be accrued from so many tests on products.

Product safety and due diligence obligations will be supported more cost effectively by the application of directly relevant microbiological testing regimes and criteria based on sound product and process knowledge in association with HACCP-based systems and the operation of GMP.

3. Sampling for microbiological testing

3.1 Introduction

No amount of testing will ever make a food safe. The sheer volume and variety of food produced, the distribution and state of microorganisms in the food, and industry resource limitations make it essential to ensure that food production systems are inherently safe. Microbiological testing should be regarded as a helpful tool in support of these systems, not as a means whereby a 'satisfactory' or 'unsatisfactory' result from an end product is used to demonstrate the safety of the food.

The reliability of a result from the analysis of a food is dependent upon the accuracy and precision of the method, the quality of the aseptic sampling procedures used and the extent of sampling.

Inappropriate sampling can lead to invalid conclusions being reached. The purpose and limitations of sampling and the relevance of statistical techniques to sampling need to be understood if the results from microbiological testing are to be correctly interpreted.

3.2 Terms

The term 'sample' refers to the material removed for examination from an identified batch or operation. This sample may consist of one or more sample units. The individual sample unit may be split into one or more test portions, or sample units may be combined for testing. The test portion will usually be only part of the sample unit and it is essential to understand that the microorganisms in a sample unit are unlikely to be distributed evenly. For the following discussion, unless otherwise indicated, no distinction is made between sample, sample unit or test portion.

Statistical significance is used to describe the confidence in any decision made concerning the safety or quality of the food. A high level of confidence (significance) is assumed to be greater than 95% certainty of being right. Most microbiological sampling plans in common use work with less

confidence than this. This does not make these plans invalid, but the user should be aware of a plan's performance when using it. A full discussion of statistical sampling and methods is given in the ICMSF book *Sampling for Microbiological Analysis: Principles and Specific Applications* (1986).

The criteria given by the ICMSF are generally recommended for port-of-entry sampling and examination of foods, circumstances in which very little may be known concerning the food production processes (see 2.1). Food manufacturers however, should have full knowledge of raw materials, processing technology and other factors contributing to the product's microbiological characteristics. With this knowledge, product sampling and microbiological testing in food businesses can be applied in a more practical way (see 1.2).

The size, number, composition and method of taking samples will affect the outcome of a test and should be specified. Attention to consistency with respect to these points will maximise repeatability, but uneven microbial distribution may still cause results to vary significantly between tests.

Sampling should also take account of the nature of the hazard and the likelihood of it occurring given the raw materials, the process and process controls. Hazard categories and associated sampling plans have been detailed (ICMSF, 1986 and 1994). Historical data demonstrating the absence of a hazard are often used in determining a sampling plan, but in the absence of a hazard analysis approach assumptions based on such data are unsound.

3.3 Selection of sampling plan

3.3.1 Sampling plan structure

The sampling plan should describe the sampling strategy used in assessing the microbial target and indicate the action to be taken in response to test results. The principles of 2-class and 3-class attribute plans are described by ICMSF (1986) and these are commonly applied in industry.

Sampling plan definitions may be summarised as follows:

Two-class sampling plan

n = number of samples analysed
m = maximum acceptable level of target organism(s) or toxin(s)
c = the number of samples allowed to exceed m

In industry, 2-class sampling plans are applied mainly when testing for specific pathogens using detection (presence/absence) tests and c=0.

Example: In a two-class sampling plan for *Salmonella* in a ready meal, where 5 samples each of 25g are taken and tested and the presence of any positives is unacceptable, the following applies:

n=5, c=0, m=not detected in 25g

This is a simple test protocol. A specified sample size and number are examined. If a 'not detected' result in these samples is achieved, the test requirements are satisfied. At a given level of contamination for organisms distributed homogeneously, the larger the sample examined e.g. 100g, the greater the chance of detecting the target organism. If organisms are unevenly distributed, a greater confidence is achieved by specifying larger numbers of samples for examination. Table 1 illustrates this approach.

Table 1: Probability of accepting a batch containing specific percentages of defective product

Number of samples examined	% Probability of acceptance			
	Actual % of defective samples			
	10%	20%	30%	40%
3	73	51	34	22
5	59	33	17	8
10	35	11	3	<0.5
20	12	1	<0.5	<0.5

(Modified from ICMSF, 1986)

The figure given is the percentage probability of obtaining negative results and accepting a batch containing 10-40% actual defectives (positives). For example, in a two-class plan where c=0, if five samples are tested and 30% of all samples are actually defective, then the chance of obtaining negative results for all five samples is 17%. If 10 samples are tested then, for the same level of defective samples, the chance of getting all 10 samples negative is only 3% i.e. the larger the number of samples examined, the smaller the probability (chance) of accepting the batch.

Three-class sampling plan

n = number of samples analysed
m = maximum level of target organism(s) or toxins acceptable under conditions of good manufacturing practice
M = level of target organism(s) or toxin(s) which, if exceeded, is considered unacceptable i.e. defective
c = the number of samples that can fall between m and M without the batch being considered unacceptable

In industry, 3-class sampling plans are applied when using enumeration tests.

3.3.2 Detection (presence/absence) tests

These tests are used to detect a particular organism in a defined quantity of sample. Typically, these are used for detecting pathogens in foods. The size and

number of samples are the two main considerations when determining the probability of detecting a target organism with a given frequency of contamination. This type of test is not used to quantify bacteria in a food. If a high degree of confidence is required to detect very low numbers of positives (defects) in a batch, many samples must be taken (ICMSF, 1986). For practical and economic reasons, it is common practice to combine a number of samples to reduce the number of tests required. Continuous sampling devices are useful for removing large numbers of small samples, particularly for powders and liquids, which are then mixed thoroughly before removal of test portions for examination.

3.3.3 Enumeration (counts) of microorganisms

Enumeration procedures are normally carried out using a minimum test portion of 10g. A homogenate and further dilutions are prepared from the test portion and used to enumerate the level of organisms in the portion; counts are typically expressed in results as colony forming units per gram (cfu/g). Estimates based on counts/g assume that perfect mixing has occurred at all stages of sampling before any aliquot is removed for examination.

ICMSF three-class sampling plans are often used to describe specifications for microbiological counts. For example, where five samples of a ready meal are taken and it is acceptable for the aerobic plate count to exceed 10^3 cfu/g on 2 occasions but not to exceed 10^4 cfu/g at all, the following applies:

$n = 5$, $m = 10^3$ cfu/g, $M = 10^4$ cfu/g, $c = 2$.

In many manufacturing situations the sample numbers do not readily lend themselves to sample attributes plans; this may occur for instance, when a microbiological specification relates to one sample only ($n = 1$). In such cases, practical strategies are sometimes employed in industry in which samples from different batches are examined and results used for trend analysis.

3.3.4 Censored values

A censored value is one that is outside a measurable range. These are common in food microbiology. The commonest examples are 'less than' estimates e.g.<100/g, although 'greater than' examples (>10^3/g) do occur.

For statistical analyses of results, it is important to avoid censored values; this can be done by extending the dilution ranges examined in 'count' tests.

4. Considerations in choosing microbiological methods

4.1 General

Factors to be considered when choosing a microbiological method include:

1) Proven capability of recovering the target organism(s) from the product being examined (validation).
2) How soon the result is required.
3) Ability to achieve the sensitivity and specificity required.
4) Cost.

The microorganisms being detected or enumerated are effectively defined by the method used. For example, an aerobic plate count (APC; also referred to as the standard plate count or total viable count) will only determine the number of organisms capable of forming visible colonies after a specified time on the growth medium used and under the specific temperature and atmosphere used for incubation. Thus an APC at 30°C for 48 hours may give different information from an APC at 22°C or 37°C for 48 hours.

4.2 Choice of test

Information about relevant target organisms and the basis for their use in microbiological specifications can be found in the Appendix: Specific organisms and the principles of test methods.

(a) Indicator and spoilage organisms

Examination of a product for indicator or spoilage organisms can provide simple, reliable and rapid information about processing failure, post-processing contamination, contamination from the environment and the general level of hygiene. Methods normally involve estimation of numbers of organisms in the food. These methods cannot replace examination for specific pathogens where suitable methods exist and where such testing is appropriate, but usually provide information in a shorter time than that required for isolation and identification of specific organisms or pathogens.

(b) Pathogens

Most methods of examination for pathogens aim to detect very low numbers e.g. a single viable cell of the target organism in a defined amount of sample. Although detection tests are also referred to as presence/absence tests, absence of a pathogen from a food cannot be proven; microorganisms can only be demonstrated to be 'not detected' by the test method used. Classical microbiological methods are often slow and labour intensive requiring resuscitation of sub-lethally injured organisms, selective enrichment and isolation of the target organism using media which encourage the growth of the target organism whilst suppressing growth of the background flora. This is then followed by confirmatory tests on suspect isolates. Because detection tests generally incorporate a resuscitation stage they allow better recovery of stressed organisms than most direct plating methods.

4.3 Accuracy of the result

Accuracy depends on the methods used, the technical competence of the laboratory staff, and the number, physiological state and distribution of the target organisms present. ISO 7218, *Microbiology - General guidance for microbiological examinations* (1985, revised 1994), tabulates the statistical variability of counts arising from plating methods and most probable number (MPN) procedures. An understanding of the capability of the method and the error margins of the results is essential for the sensible application of microbiological criteria and interpretation of results obtained.

A count of 10,000 cfu/g appears very exact but could have been calculated from only ten colonies on a medium inoculated with 1ml from a 1 in 1000 dilution of the test portion. There are clearly defined rules relating to the number of colonies acceptable for counting under such circumstances, but these cannot always be followed. The lower accuracy of estimates made outside this acceptable range, e.g. low numbers of colonies (typically lower than 20) needs to be recognised. The confidence limits of 'counts' ranging from 3 – 320 are illustrated in Table 2.

Most probable number estimates are often used by food microbiologists but are not precise estimates of numbers of organisms. Nevertheless, MPN techniques are widely used (Mossel et al, 1995) for samples in which levels of target organisms are expected to be low i.e. <10 per gram.

4.4 Limit of detection of the method

The lower limit of detection of a test method is often referred to as its sensitivity. Plate counts are generally used for counts of more than 10 cfu/g but are more accurate when levels exceed 100 cfu/g. Most probable number determinations are suitable for low counts e.g. <100 per gram, and are widely used for estimations of levels below 10 per gram. Detection (presence/absence) tests are used if information concerning the presence of an organism in a specified quantity of food such as 1g or 25g is required. The sensitivity of these tests is then defined by the quantity of food examined.

4.5 Recovery of target organisms

Microorganisms may be damaged by a variety of factors, including heating, freezing, drying, exposure to high osmotic pressure, low pH, disinfectants and other chemical and physical antimicrobial factors. Injured cells recover poorly if exposed directly to selective media or elevated temperatures without first being allowed to undergo cell repair using resuscitation techniques. The degree of resuscitation appropriate will depend on the target organism and the product. Surface methods of inoculation may be preferable to pour plate methods because of the possible injurious effect of the temperature of the molten agar used for pouring plates. In addition, some food-associated organisms are obligately aerobic e.g. pseudomonads, and their growth may be impaired by the relatively anaerobic conditions obtained in pour plates. These factors may result in a reduced count in comparison with surface methods of enumeration. The use of a liquid medium will normally provide better conditions for recovery than a similar solid medium.

There is evidence that some bacteria can become 'viable but non-culturable', that is, they cannot be grown *in vitro* but are able to grow *in vivo*. *Campylobacter* and *Vibrio* are examples of genera containing species for which this has been demonstrated. Currently there are no routine laboratory methods for detection of organisms in this state.

Table 2: Confidence limits associated with numbers of colonies on plates

Colony count	95% confidence intervals for the count	
	Lower	Upper
3	<1	9
5	2	12
10	5	18
12	6	21
15	8	25
30	19	41
50	36	64
100	80	120
200	172	228
320	285	355

(Modified from BS 5763: Part 0: 1996 and Cowell & Morisetti, 1969

4.6 Comparability of results between different methods

A wide variety of methods are available for the detection of individual microorganisms. It is important to recognise that results obtained from the use of different methods for a particular organism may not be comparable. This may not matter for the purposes of in-house trend analyses, but it is essential if the results are to be measured against, for example, industry criteria. To this end some uniformity of methods and sampling procedures is desirable, but this should not prevent development of new methods.

Results obtained by different methods should not be assumed to be equivalent without evidence e.g. by performing the different methods in parallel on the same samples.

4.7 Inhibition of growth of microorganisms by the constituents of the food tested

Growth of microorganisms is often affected by the chemical composition of the food; if insufficiently diluted or neutralised, these factors may prevent growth of the target microorganisms during the test procedure. Salt or sugar content, concentrations of chemical preservatives and the acidity of the product are examples of factors that may influence the test results. Chocolate, many herbs and spices, garlic and onion contain inhibitory compounds and many flavourings may contain high levels of salt and acids. These inhibitory compounds may affect the results of tests. Examination of samples inoculated with known levels of representative strains of the target organisms can help to verify both media and method efficacy (Mossel *et al*, 1995).

4.8 Competitive growth

The microflora of foods can vary widely, for example between the fresh product and the same product at the end of shelf life. Growth of the target organism may be suppressed or the organisms may be outgrown by background flora. Validation studies may be useful to demonstrate that the method used detects the target organism in the presence of the competitive flora in the products to be examined.

4.9 Test methods

It is important to ensure that the method used detects the organism of concern in the food being examined. It may be necessary to initially validate the method, and in routine use, quality assurance systems must be employed to verify test efficacy.

Reference and industry test methods are available from several sources e.g. British Standards Institution, International Standards Organisation, International Dairy Federation, International Commission on Microbiological Specifications for Foods, Public Health Laboratory Service (UK), Association of Official Analytical Chemists (USA), Campden and Chorleywood Food Research Association (UK). Many microbiological standards in legislation also prescribe methods. The appendix describes the principles of classical test methods and discusses the relevance and limitations for some of the more commonly specified target organisms. New rapid test methods are becoming available for many bacteria and their toxins, and their performance is generally measured by comparison with results obtained by classical methods. For some microorganisms e.g. viruses and protozoa, there are currently no methods appropriate for routine application to foods.

5. Food categories

5.1 Introduction

In establishing microbiological criteria it is important to consider the technologies applied in the preparation of the food. The following sections discuss some aspects of the technologies and their effect on microorganisms.

5.2 Frozen foods

Frozen foods are microbiologically stable i.e. there is no growth of microorganisms providing the temperature is below about minus 10°C. Most bacteria do not grow below 0°C, but some psychrotrophic organisms, particularly some fungi, will grow slowly at a few degrees below freezing point. The shelf life of frozen foods is determined by physical and chemical/enzymic changes e.g. dehydration and oxidation, and not by microbiological growth. Some bacteria and fungi die in a frozen food and others become sublethally damaged. However, most microorganisms are preserved by freezing; *Salmonella* for example has been recovered from frozen foods after years of storage. Therefore, it must not be assumed that freezing can improve the microbiological safety or quality of a food. Freezing does not inactivate the toxins of microorganisms but can reduce the infectivity of some pathogenic protozoa and nematodes e.g. in raw meat and fish (ICMSF, 1980a).

The application of microbiological criteria to frozen foods is dependent on the intended use of the food e.g. stringent criteria may be applied to frozen foods which are ready-to-eat after being thawed.

Food should be handled and stored hygienically before freezing so that its quality does not deteriorate. Microbiological testing is occasionally used to monitor the efficacy of such handling processes. The rate of freezing and thawing can significantly affect the microbiology of a food and factors affecting the rate of freezing such as pack size and temperature gradients must be considered.

Frozen foods are often examined after controlled defrosting of the sample and microbiological tests used should incorporate a resuscitation procedure to maximise the recovery of any damaged cells.

5.3 Dried foods, low water activity foods and other ambient stable foods

Dried foods with an a_w of <0.6 are microbiologically stable. The shelf life of these foods is not limited by microbial spoilage provided they remain dry. A wide range of foods often termed 'intermediate moisture foods' or 'semi-moist' foods (jams, dried and fermented sausages, dried fruits) have a higher a_w. These foods are stored at ambient temperature and may be preserved by a combination of factors such as a_w, pH and preservatives; their shelf life may be limited by microbiological, chemical and/or physical spoilage.

Drying without heat preserves many bacteria so the process cannot be relied upon to improve microbiological quality; in fact the net effect can be to increase the concentration of microorganisms. Numbers of organisms generally decline slowly in dried foods (over a period of months or years) although *Salmonella* has been shown to survive for years in chocolate (ICMSF, 1980b). However some foodborne pathogens, notably *Campylobacter*, do not survive well in a dry environment.

Microbiological criteria for dried foods are varied and take account of the intended use of the food (ready-to-eat or not), the intended consumer (baby food or not) and the type of food (heat processed, fermented, dried).

The inhibitory factors in many products (see Section 4.7) must be diluted out or neutralised prior to microbiological examination. Pathogens present in dried foods may be stressed and a resuscitation stage must be incorporated into detection procedures.

5.4 Fresh & chilled foods

Most fresh foods are not microbiologically stable and some commodities are highly perishable. The associated microflora and consequent shelf life will be affected by the quality and hygienic conditions of husbandry, slaughtering, harvesting, storage and processing. The application of rigorous hygiene measures at all stages will help to prevent product contamination and achieve a realistic shelf life. Extension of shelf life of many fresh foods can be achieved by chilling, and the type of packaging used may also have an effect on shelf life. During chilled storage the microbial profile will change from predominantly mesophilic to psychrotrophic, and spoilage will be due to the nature and activity of these psychrotrophic microorganisms. In contrast, the microbiological quality of foods such as nuts and citrus fruits that have a natural protective outer casing depends on maintaining the integrity of that outer barrier and the control of moisture during storage. The contents are comparatively microbiologically stable unless penetration of the outer casing occurs.

The occasional presence of pathogens can be expected in most raw foods. When selecting relevant microbiological criteria, the type of food, the required shelf life, storage conditions, intended use and destination of the commodities must all be taken into account. An aerobic plate count may be used to indicate the quality and the probable shelf life of many raw fresh foods; whilst normally this is performed at 30°C to measure the mesophilic population, a temperature of 20-25°C may be preferable for chilled foods in order to estimate the levels of psychrotrophic organisms present and hence the potential spoilage organisms. Yeast or mould counts are more relevant indicators of shelf life than bacteria for commodities with a low pH such as fruits and fruit juices. Indicator organisms may be used to monitor the hygiene of processing, and may also give information about the temperature conditions of storage. In addition, *E. coli* levels may be used to assess the quality of irrigation water used for fruit and vegetables, to monitor slaughterhouse hygiene and the conditions for production and marketing of live bivalve molluscs (Directive 91/492/EEC).

5.5 Heat-treated foods

Heat-treated food may be ready to eat either hot or cold, may require reheating or further cooking before consumption, or may undergo further cooking as an ingredient in a composite food product. The degree of heat treatment it has received will affect its microbiological profile and stability. Some forms of heat treatment such as blanching or scalding are not designed to cook the food, but to inactivate enzymes or assist in skin or shell removal. This type of mild heat treatment and others such as pasteurisation and sous-vide processes will kill most vegetative bacteria, leaving only heat resistant strains and spores. Pasteurisation or an equivalent process also kills the majority of common foodborne yeasts and moulds but does not destroy mycotoxins.

Growth of any surviving organisms in many heat processed foods is minimised by chilled storage, acidity, chemical preservation, or some other controlling factor.

A more severe heat treatment such as the Ultra High Temperature (UHT) process used for certain liquids results in a shelf stable product at room temperature provided that aseptic filling occurs after heat treatment or that the liquid is heated in the final packaging. Oven baking of biscuits and certain cakes may also result in stable products at ambient temperature as long as contact with moisture and surface mould contamination can be prevented. Cooking of meats and meat products may allow the survival of bacterial spores, and so chilled storage will be necessary after cooking.

Long-term microbiological stability without the use of refrigeration for non-dried foods is obtained by prolonged heating in hermetically sealed containers such as cans; the heat process will need to be more severe for low acid foods (Hersom & Hulland, 1980). Low acid foods (pH greater than 4.5) must receive a minimal thermal process of F_0 3 (121.1°C for 3 minutes) known as a 'botulinum cook' or an equivalent heat process. To minimise spoilage due to thermophilic spore forming bacteria in canned foods destined for or manufactured in countries with hot climates, it may be necessary to apply a very much higher thermal process e.g. $F_0>10$.

The bactericidal effect of heat will depend on the nature of the food, for example the fat or sugar content, moisture content or acidity, as well as the temperature and exposure time employed. The parameters of the heat treatment must take account of the characteristics of the food. Measuring the temperature and duration of heat treatment in all

these processes is of prime importance; the integrity of the containers is also essential to prevent ingress of contamination after heat treatment. The main purpose for microbiological testing of heat processed foods is to assess post processing contamination. Ingredients added after heat processing may affect the microbiological status of the product and hence the criteria that are applicable.

The nature and extent of contamination introduced by personnel and equipment after heat treatment will affect the resulting shelf life and safety of the food. These criteria should reflect the intended use of the commodity; for example the specification for a cooked ingredient of a composite product such as pizza which receives a further cooking process might be less rigorous than if no further heat process is to be applied. The type of packaging and expected shelf life should also be taken into consideration, for example *Listeria monocytogenes* should be absent from products cooked in hermetically sealed containers and/or products which allow its growth and have long refrigerated shelf lives. However, a low level of *Listeria* might be tolerated in a chilled food likely to be eaten within a few days.

5.6 Fermented foods

The term fermentation describes the process of intended chemical change in foods catalysed by microbial enzymes. Fermented foods may be produced with the use of starter microorganisms or by the action of natural fermentative microorganisms present in the raw materials or production environment. Many microorganisms are used in fermentation processes but those most widely used in the food industry include lactic acid bacteria and yeasts (*Saccharomyces* spp.) (see Appendix: Specific organisms and the principles of test methods).

The metabolic products of fermentation processes often extend the shelf life and enhance the safety of food products because of their inhibitory effect on pathogenic and spoilage microorganisms. Lactic acid bacteria used for yogurt production produce organic acids which decrease the pH and act to restrict the growth of many natural spoilage organisms such as *Pseudomonas* spp. Some starter culture organisms also produce bacteriocins which have an inhibitory effect on other bacteria. Yeasts used for wine and beer production produce organic acids and ethanol, which create an extremely harsh environment where few microorganisms can grow.

The raw materials used as substrates for fermentation reactions e.g. milk, wort and meat are equally capable of supporting the growth of many pathogenic and spoilage microorganisms if present. The safety and stability of the fermented product is therefore dependent on both the growth of the fermentative microorganism to produce inhibitory compounds and the rate at which these compounds are produced. Strict process control is essential to ensure that the desired fermentation profiles are achieved. Slow growth or no growth of the fermentative microorganisms may allow pathogenic or spoilage microorganisms to predominate resulting in unsafe products or ones that do not meet the required organoleptic qualities.

Fermentations are often monitored using simple indicators of microbial growth such as decreases in pH or sugar concentrations or increases in cell numbers (turbidity, direct microscopic examination), acidity or alcohol concentrations. Fermentation alone can lead to inhibition of microbial growth and, in some cases, to microbial death although this will be dependent on both the type and concentration of metabolic products produced by the fermenting organisms.

The application of microbiological criteria to fermented foods is dictated by the nature of the product and its intended use. Criteria are frequently applied to a product at stages during the process e.g. *S. aureus* in the production of salami, and to the final product. Levels of significance of specific organism groups in/on a particular food must be understood e.g. psychrotrophic moulds such as some *Penicillium* spp. can be commercially significant for block matured, vacuum packed cheese if bags are punctured and the organism grows to spoil the product. In this circumstance, a significant contamination rate could be as low as one spore on the entire surface of a block of the cheese and a specification with criteria for moulds of <10 per gram would be meaningless. Control requires ensuring the vacuum remains intact and the storage temperature is maintained.

In some cases the acidity or alcohol content in fermented foods may need to be neutralised or diluted out prior to enumeration or detection of microorganisms as they may interfere with the indicator dyes used in some agar test methods e.g. Violet Red Bile Glucose Agar used for the enumeration of Enterobacteriaceae. In addition, test procedures for the detection of pathogens should incorporate a resuscitation stage as the effect of pH, acidity or alcohol content in the food may stress the organisms.

6. Microbiological limits

6.1 Introduction

Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. If, after due practical consideration of the issues raised and discussed in previous chapters it is considered useful to use microbiological tests and criteria for clearly specified purposes then the following tables of microbiological information may help as a further guide to the use of relevant criteria.

The figures given in the following tables do not represent microbiological criteria in themselves but should be used as the basis for the development of

criteria. Use of internationally recognised or equivalent validated industry methods is assumed.

For easy reference, products are listed in the tables by type and technology, and then broadly split into groups based on their use e.g. to be cooked, ready-to-eat. A comprehensive list of foods with associated combinations of technology, packaging and use is not the aim of this document.

Guidance on limits and some key points concerning pathogens and indicator organisms relevant to each food grouping is given. Important toxins are also noted for some groups.

Some of the levels given in the Tables are based on existing published criteria (see Chapter 7 and PHLS, 1996). Others are derived from industry specifications, practice and experience. The limits given in these tables are considered by the authors to be practical, realistic and relevant to the use of the food and take account of the potential hazards associated with the food and risk to the consumer.

It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins.

Although it may be desirable to minimise the level of specific microorganisms, criteria for organisms in foods that require cooking may be less stringent than those for ready-to-eat foods. In particular, criteria for *Escherichia coli* (not verocytotoxigenic *E. coli* - VTEC) and *S. aureus* are often over-specified and seldom justified in terms of food safety or food spoilage. High levels ($>10^4$ cfu/g - PHLS, 1996) of *S. aureus* may be relevant due to their ability to produce heat resistant enterotoxins although low levels of the organism are usually of little significance in foods prior to cooking.

Any raw food may be contaminated by pathogens such as *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and VTEC. Criteria requiring the absence of pathogens in raw foods are generally impractical, particularly in raw meats. In addition, food poisoning is regularly associated with drinking raw milk; therefore, the consumption of raw meat or raw milk is not advocated. The application of HACCP-based systems and GMP to minimise the presence of such organisms coupled with clear advice to consumers about how to handle such food will help minimise the risk of foodborne illness. Absence of pathogens in products which are eaten raw is desirable but cannot be guaranteed.

6.2 Use of tables

For practical reasons these tables represent broad product categories and inevitably some product groups may not conform to the limits specified for indicator microorganisms. Also, for some multi-component products e.g. sandwiches containing cooked meat and salad ingredients, the information in more than one table will need to be considered.

The figures given in the tables are under two headings: *GMP* (good manufacturing practice) and *Maximum*.

GMP values are those expected immediately following production of the food under good manufacturing conditions. Occasional samples may exceed these limits and such results should not be used as rejection criteria without consideration of all the factors important in an entire sampling plan (Chapters 1 and 3).

Maximum values are those regarded as the maximum acceptable at any point in the shelf life of the product. Higher levels of pathogens indicate a possible health risk whilst higher levels of indicators signify failure of the process or hygiene procedures.

In many products, organisms may continue to grow throughout shelf life. If 'Maximum' levels are approached early in the intended life of the product, it is likely to become unacceptable i.e. hazardous or spoiled within its shelf life. This must be taken into account when using these tables.

The levels of any specific indicator organism may vary according to food material and processing conditions. Maximum levels given in the tables indicating process failure may therefore differ to reflect these variations. For example, products which have been processed to achieve a 'botulinum cook' are expected to remain safe throughout their shelf life; low 'Maximum' criteria are therefore usually applicable to these products.

The values are intended to be applied to a representative sample of the food, which in most cases would be not less than 10g (25g for detection (presence/absence) tests for pathogens) and would include surface material, core material and a selection of the components in the case of a multi-component product. Ingredients added after the processing stage indicated in the tables are not considered and must be taken account of separately. Sampling plans and methods are not indicated in these tables and should be developed and used in line with the considerations discussed in Chapters 3 and 4.

The European Union and some individual nations currently have legislation containing microbiological criteria applicable to some foodstuffs at defined points in the production process e.g. for milk, dairy products, shellfish, egg products, mineral waters, minced meat and meat products (Chapter 7). It is essential for food businesses to ensure that products conform to relevant legislation.

6.3 Index for foods

FOOD	TABLE
Milk shake	H
Muesli	L
Mussels	C & J
Noodles	D & K
Nut oil	R
Nuts	L
Offal	B
Oils	R
Oyster	C
Part-baked products	D
Part-cooked foods	I
Pasta	D & K
- fresh filled	I
Pasties	J
Pastry	D
Pickles	Q
Pies - (meat, fish and/or vegetable)	I & J
Pizzas	I & J
Pork	B & J
'Pot' snacks	N
Pouched foods	P
Poultry - raw (whole, portions, minced)	A
- cooked	J
- canned	P
- marinaded	A
- smoked	J
Powdered desserts	N
Prawns - raw	C
- cooked	J
Preserved foods	Q
Processed cheese	G
Pulses	K
- cooked	J
Quiches	J
Recipe dishes - raw	I
(ready meals) - part cooked	I
- heat processed	J & P
Relishes	Q
Rice	K
Rice products	J
Roll mop herrings	C
Salad vegetables - raw and prepared	F & I
Salami	J & Q
Sandwiches & sandwich fillings	F & J
Sauces	J & Q
Sauce mixes	N
Sausages	B
Scombroid fish	C & J
Shellfish	C & J
Smoked salmon	C
Snacks	M & N
Soft drinks	S
Soup	
- mixes	N
- canned	P

FOOD	TABLE
Sous vide	J
Spaghetti	D & K
Spices	K, L & M
Sugar	M
Tagliatelli	D & K
UHT dairy products	H
Vegetables - heat treated	J
- raw and prepared	F
- dried	K
- preserved	Q
- frozen	F
- stir-fry	F
- canned	P
Virgin olive oil	R
Wine	S
Yoghurt - live	G
- pasteurised	G

6.4 Abbreviations

APC: Aerobic Plate Count

a_w: Water activity

B. cereus: *Bacillus cereus*

cfu: Colony Forming Units

C. perfringens: Clostridium perfringens

DSP: Diarrhetic Shellfish Poisoning

E. coli: Escherichia coli

F_03: see Glossary of Terms

GMP: Good Manufacturing Practice

HACCP: Hazard Analysis Critical Control Point

IMF: Intermediate Moisture Foods

L. monocytogenes: Listeria monocytogenes

ND: Not Detected

PSP: Paralytic Shellfish Poisoning

S. aureus: Staphylococcus aureus

UHT: Ultra Heat Treatment

V. parahaemolyticus: Vibrio parahaemolyticus

VTEC: Verocytotoxigenic *E. coli*

Y. enterocolitica: Yersinia enterocolitica

10^n: 1 x 10^n where n = 2, 3, 4, 5, 6, 7, 8 etc.

6.5 Tables

All counts/levels in the following tables represent colony forming units per gram or per ml unless otherwise specified.

A: RAW POULTRY

Product examples: Whole birds, portions, minced/reformed poultry meat, marinaded products

Storage: Frozen or chilled

Use: To be cooked

Pathogens
Salmonella, Campylobacter and other pathogens may be present; monitoring the incidence may be useful for trend analysis.

Organism	GMP	Maximum
Bacterial pathogens	Criteria for absence not generally applicable.	

Indicators & spoilage organisms
APC can be used to indicate quality. Generally, higher counts are found in minced/reformed poultry meats than on whole birds or portions. Pseudomonads are also frequently used to indicate quality.

Organism	GMP	Maximum
APC	$<10^5$	10^7
Pseudomonas spp.	$<10^5$	10^7
Yeasts (marinaded products)	$<10^4$	10^6

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

B: RAW MEAT

Product examples: Joints, mince, diced meats, offal, burgers, sausages, bacon, marinaded products, cured products

Storage: Frozen or chilled

Use: To be cooked

Pathogens
Salmonella, Campylobacter, VTEC and parasites may be present; *Y. enterocolitica* may also be important in pork and *S. aureus* in bacons. Monitoring the incidence of bacterial pathogens may be useful for trend analysis.

Organism	GMP	Maximum
Bacterial pathogens	Criteria for absence not generally applicable.	

Indicators & spoilage organisms
APC can be used to indicate quality. Counts are generally higher in minced meats than on whole cuts. *E. coli* may be useful as an indicator of hygienic slaughterhouse practices. Coliforms/Enterobacteriaceae may be useful for trend analysis.

Organism	GMP	Maximum
APC	$<10^5$	10^7
E. coli	$<10^2$	10^4
Yeasts (sausages & marinaded products)	$<10^4$	10^6

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

C: RAW FISH & SHELLFISH

Product examples: Whole, fillets, smoked salmon, mussels, oysters, prawns, gravadlax, roll mop herrings, cold smoked, marinaded or pickled (acidified) products

Storage: Frozen or chilled

Use: To be cooked or ready-to-eat

Pathogens & toxins

Pathogens may be present; in particular, *Vibrio* may occur in warm water fish/shellfish. Monitoring the incidence of pathogenic bacteria may be useful for trend analysis. As filter feeders, bivalve molluscs concentrate contaminants from the water in which they grow, including bacterial pathogens, viruses and algal toxins. Monitoring the incidence of bacterial pathogens and algal toxins may be necessary. Presence of histamine (scombrotoxin) in scombroid fish should also be considered.

Use	Organism/Toxin	GMP	Maximum
To be cooked	Bacterial pathogens	Criteria for absence not generally applicable.	
	Histamine (scombroid fish)	<50ppm (<5mg/100g)	50ppm (5mg/100g)
	PSP (bivalve mollusc flesh)	ND/100g	<80µg/100g
	DSP (bivalve mollusc flesh)	ND in bioassay	ND in bioassay
Ready-to-eat	*Salmonella* spp.	ND in 25g	ND in 25g
	V. parahaemolyticus (warm water fish)	ND in 25g	10^2
	L. monocytogenes	ND in 25g	10^3
	S. aureus (cold smoked fish)	$<10^2$	10^3
	Histamine (scombroid fish)	<50ppm (<5mg/100g)	50ppm (5mg/100g)
	PSP (bivalve mollusc flesh)	ND/100g	<80µg/100g
	DSP (bivalve mollusc flesh)	ND in bioassay	ND in bioassay

Indicators & spoilage organisms

APC is often high; monitoring may be useful for trend analysis. *E. coli* is commonly used as an indicator of faecal contamination in shellfish.

Organism	GMP	Maximum
APC	$<10^6$	10^7
E. coli	<10	10^3
Yeasts (pickled/marinaded)	$<10^4$	10^6

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

D: DOUGHS, PASTA & BATTERS

Product examples: Bread dough, pastry, part-baked products, spaghetti, tagliatelli, noodles

Storage: Frozen or chilled

Use: To be cooked

Pathogens

Salmonella and Listeria may be present occasionally in raw products but usually only at low levels and should be killed by cooking. *B. cereus* spores and/or toxin and *S. aureus* toxin could survive cooking.

Organism	GMP	Maximum
B. cereus	$<10^2$	10^4
S. aureus (pasta and batters)	$<10^2$	10^4

Indicators & spoilage organisms

APC and Enterobacteriaceae levels may be very high and variable. *E. coli* may be used to indicate hygiene of production. Lactic acid bacteria may be used to indicate spoilage (except for sour doughs).

Organism	GMP	Maximum
E. coli	$<10^2$	10^4
Lactic acid bacteria (doughs/pastry)	$<10^5$	10^7

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

E: FRUIT AND FRUIT JUICES

Product examples: Whole, sliced or chopped fruits, freshly squeezed juices, pasteurised juices, fruit cocktail

Storage: Frozen, chilled or ambient

Use: To be cooked or ready-to-eat

Pathogens & toxins

Pathogens may be present but are not common. Growth is unlikely in an acidic environment. Monitoring for mycotoxins may be appropriate e.g. patulin in apple juice.

Use	Organism	GMP	Maximum
Any	Bacterial pathogens	Criteria for absence not generally applicable.	
In chilled desserts (non heat processed fruit)	*Salmonella* spp.	ND in 25g	ND in 25g
	L. monocytogenes	ND in 25g	10^3

Indicators & spoilage organisms

TVC ~~APC~~ could be high in raw products. Yeast & mould levels are important for spoilage and visual monitoring is particularly relevant. If fruit is to be used for juices the level of yeasts should be low to obtain a realistic shelf life. *E. coli* may be useful as an indicator of poor agricultural hygiene in harvesting and processing.

Use	Organism	GMP	Maximum
In chilled desserts (non heat processed fruit)	Yeasts	$<10^3$	10^6
Fruit juice (Unpasteurised)	Yeasts	$<10^3$	10^5
Fruit juice (pasteurised)	Yeasts	<10	10^6

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

F: RAW AND PREPARED VEGETABLES (INCLUDING SALAD VEGETABLES)

Product examples: Crudites, prepared salads, stir-fry vegetables, blanched vegetables, fresh herbs, bean sprouts, sandwich fillings

Storage: Frozen, chilled or ambient

Use: To be cooked or ready-to-eat

Pathogens

Pathogens may be present on raw vegetables and washing procedures are unlikely to eliminate them. However, only low incidence and levels may be expected and criteria for specific pathogens are commonly applied.

Use	Organism	GMP	Maximum
To be washed or cooked	Bacterial pathogens	Criteria for absence not generally applicable.	
Prepared (ready-to-eat)	*Salmonella* spp.	ND in 25g	ND in 25g
	L. monocytogenes	ND in 25g	10^3

Indicators & spoilage organisms

High levels of APC and Enterobacteriaceae/coliforms are probable and could be derived from the soil or poor handling. *E. coli* levels may be used to monitor the quality of irrigation water and hygiene of handling. If vegetables are to be used in further processing e.g canning, monitoring for bacterial spores may be useful. Visual inspection is particularly important for bacterial rots and mould contamination.

Use	Organism	GMP	Maximum
To be washed or cooked	APC, coliforms/ Enterobacteriaceae	Criteria for absence not generally applicable	
Prepared (ready-to-eat)	*E. coli*	$<10^2$	10^3

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

G: MILK, CREAM & DAIRY PRODUCTS

Product examples: Liquid milk, cream, cheese, ice cream, butter, dairy desserts, yoghurts and other fermented products

Storage: Frozen or chilled

Use: Ready-to-eat

Pathogens

Salmonella, Campylobacter, VTEC, *Listeria* and *S. aureus* will occasionally be present in raw milk. Other pathogens and parasites may also occur. Vegetative pathogens will be absent in properly pasteurised milk and cream; testing for *Salmonella* and *Listeria* is not conducted routinely in these products. Low levels of spores and other thermoduric organisms may occasionally be present.

Organism	GMP	Maximum
Salmonella spp.	ND in 25ml/g	ND in 25ml/g
L. monocytogenes	ND in 25ml/g	10^3
S. aureus	<20	10^3
VTEC (raw milk based products)	ND in 25ml/g	ND in 25ml/g

Indicators & spoilage organisms

Criteria applicable depend on the level of heat treatment applied. Coliforms/Enterobacteriaceae and *E. coli* are most commonly used as indicators of the quality and hygienic processing of dairy products. APC may be useful for trend analysis but is not applicable to live fermented products.

Products	Organism	GMP	Maximum
Soft cheese (raw milk)	*E. coli*	$<10^2$	10^4
Processed cheese	Aerobic plate count	$<10^2$	10^5
	Anaerobic plate count	<10	10^5
Other cheeses	Coliforms/ Enterobacteriaceae	$<10^2$	10^4
	E. coli	<10	10^3
Pasteurised milk and cream	Coliforms/ Enterobacteriaceae	<1	10^2
Other pasteurised milk products	Coliforms/ Enterobacteriaceae	<10	10^4
	E. coli	<10	10^3
	Yeasts (yoghurt)	<10	10^6

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

H: UHT MILK, CREAM & DAIRY PRODUCTS

Product examples: Long life milks including flavoured products, milk shakes, sterilised milk

Storage: Ambient

Use: Ready-to-eat

Pathogens
Pathogens should be absent.

Organism	GMP	Maximum
Bacterial pathogens	Criteria for absence not generally applicable	

Indicators & spoilage organisms
UHT products should be microbiologically stable. Control of the process is essential. Microbiological testing of the finished product is not considered appropriate. Incubation of finished packs followed by examination for signs of microbial growth (pH change, organoleptic defects, blown packs) is most commonly used in industry. Direct inoculation of agar with incubated product or alternative methods such as impedance/ conductance and ATP-bioluminescence are also used to detect growth.

Organism	GMP	Maximum
All (after incubation of pack)	No evidence of growth	No evidence of growth

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

I: PART COOKED FOODS

Product examples: Prepared meals, pizzas, coated fish and meat products, fresh filled pastas

Storage: Frozen or chilled

Use: To be cooked

Pathogens
Salmonella, Campylobacter and *Listeria* could be present (particularly from raw meats, poultry and fish). Spores of *C. perfringens* and *B. cereus* may also be present and monitoring for trend analysis may be useful. Monitoring for *S. aureus* should be considered for products receiving significant handling during processing e.g. pizzas and pastas.

Organism	GMP	Maximum
Bacterial pathogens	Criteria for absence not generally applicable	

Indicators & spoilage organisms
Meals of different components, some perhaps partly cooked, cannot easily be regarded as a single sample likely to show a consistent microbial flora; APC and Enterobacteriaceae limits are not generally applicable. Individual components of meals may merit separate consideration. *E. coli* may be used to indicate hygiene of production.

Organism	GMP	Maximum
E. coli	$<10^2$	10^4

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

J: PROCESSED FOODS

Product examples: Ready meals, cooked meats and fish products, pies, pasties, quiches, flans, sandwiches, sous vide products, fermented meats, cured meats, desserts, moist bakery products (muffins, crumpets)

Storage: Frozen or chilled

Use: Ready-to-eat or to be re-heated

Pathogens & toxins

Pathogens may be present in some raw fermented meat products. Spores of psychrotrophic strains of *Clostridium botulinum* may present a hazard in products given an extended shelf life at refrigeration temperatures. Monitoring the incidence of algal toxins may be necessary in molluscs and the presence of histamine (scombrotoxin) in scombroid fish should also be considered.

Organism	GMP	Maximum
Salmonella spp.	ND in 25g	ND in 25g
L. monocytogenes	ND in 25g	10^3
C. perfringens (cooked meat, vegetables and pulses)	$<10^2$	10^3
B. cereus (rice products, highly spiced products and bakery products)	$<10^2$	10^4
S. aureus	<20	10^3
V. parahaemolyticus (warm water fish)	ND in 25g	10^2
VTEC (raw fermented meats)	ND in 25g	ND in 25g
Histamine (scombroid fish)	<50ppm (<5mg/100g)	50ppm (5mg/100g)
PSP (bivalve mollusc flesh)	ND/100g	<80µg/100g
DSP (bivalve mollusc flesh)	ND in bioassay	ND in bioassay

Indicators & spoilage organisms

APC varies greatly between products depending on processing and stage of shelf life. Most non-fermented products will meet a limit of 10^5 at point of production if hygienically prepared; many will easily meet 10^4. Monitoring of APC may be useful for trend analysis but is not appropriate for live fermented products. Enterobacteriaceae are a useful monitor of hygiene practice. For some cooked sliced meats the APC can reach 10^8 during shelf life due to the presence and growth of lactic acid bacteria without detrimental effect to the product. Presence of Gram negative bacilli in high numbers ($>10^7$) may cause spoilage. Care is therefore required in the use and interpretation of APC levels.

Organism	GMP	Maximum
APC (heat treated)	$<10^4$	variable depending on product
Enterobacteriaceae	$<10^2$	10^4
E. coli	<10	10^3

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

K: DRIED FOODS, TO BE COOKED

Product examples: Rice, pulses, cereals, grains, flour, vegetables, fruit, pasta, coconut, dry mixes, meats, herbs, spices

Storage: Ambient

Use: To be cooked

Pathogens & toxins
Pathogens may be present; monitoring may be useful for trend analysis. Spores may survive cooking. Heat stable toxins of *S. aureus* and *Bacillus* spp. may not be inactivated by cooking. The presence of mycotoxins should be considered.

Organism	GMP	Maximum
S. aureus (pasta)	$<10^2$	10^4
B. cereus	$<10^2$	10^4
C. perfringens	$<10^2$	10^3

Indicators & spoilage organisms
APC and levels of Enterobacteriaceae and spore forming bacteria will vary according to commodity; monitoring may be of value for trend analysis. Bacterial spore levels should be low for some uses e.g. canning.

Organism	GMP	Maximum
E. coli	$<10^2$	10^3
Yeasts (fruits)	$<10^3$	10^6
Moulds	$<10^3$	10^5

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

L: DRIED RAW FOODS, READY-TO-EAT

Product examples: Nuts, fruits, spices, herbs, muesli, coconut

Storage: Ambient

Use: Ready-to-eat

Pathogens & toxins
Pathogens may be present; bacterial sporeformers are common in herbs and spices. Aflatoxin testing may be relevant for nut products.

Organism	GMP	Maximum
Salmonella spp.	ND in 25g	ND in 25g
B. cereus (herbs and spices)	$<10^2$	10^4
C. perfringens (herbs and spices)	$<10^2$	10^3
Aflatoxin (nuts)	<4ppb	<4ppb

Indicators & spoilage organisms
APC and levels of Enterobacteriaceae may be as high as 10^7 in herbs and spices. Monitoring may be useful for trend analysis of individual products.

Organism	GMP	Maximum
E. coli	<10	10^3
Yeasts (fruits)	$<10^3$	10^6
Moulds	$<10^3$	10^4

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

M: DRIED HEAT PROCESSED FOODS

Product examples: Breakfast cereals, crisps, snacks, confectionery, filled or topped biscuits and cakes, herbs, spices

Storage: Ambient

Use: Ready-to-eat

Pathogens

Bacterial sporeformers are common in herbs and spices. Spores may survive heat processes.

Organism	GMP	Maximum
Salmonella spp.	ND in 25g	ND in 25g

Indicators & spoilage organisms

APC is normally low ($<10^3$). Monitoring may be useful for trend analysis.

Organism	GMP	Maximum
APC	$<10^3$	variable depending on product
Enterobacteriaceae	<10	10^3
Yeast (in fondants)	$<10^2$	10^5
Moulds	$<10^2$	10^4

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

N: DRIED HEAT PROCESSED FOODS, READY-TO-EAT AFTER REHYDRATION

Product examples: Soup mixes, dessert mixes, milk powder, pot snacks

Storage: Ambient

Use: Ready-to-eat after rehydration

Pathogens

These products may contain bacterial sporeformers. If reconstituted product is held at warm temperatures (30-50°C) then pathogens may grow to levels causing illness. This must be considered when determining appropriate criteria as more stringent limits than those indicated below may be applicable.

Organism	GMP	Maximum
Salmonella spp.	ND in 25g	ND in 25g
S. aureus	<20	10^3
B. cereus	$<10^2$	10^4
C. perfringens	$<10^2$	10^3

Indicators & spoilage organisms

For some foods, monitoring the APC may be useful for trend analysis but it may be high in products containing herbs and spices.

Organism	GMP	Maximum
APC	$<10^3$	variable depending on product
Enterobacteriaceae	$<10^2$	10^4
E. coli	<10	10^3

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

O: DRIED BABY FOODS

Product examples: Dry mixes, milk powder

Storage: Ambient

Use: Ready-to-eat after rehydration

Pathogens
Because babies are more susceptible to food poisoning than adults, these products are normally made to very stringent criteria. Low microbial limits are applied together with an increased level of sampling. If reconstituted product is held at warm temperatures (30-50°C) then pathogens may grow to levels causing illness. This must be considered when determining appropriate criteria as even more stringent limits than those indicated below may be applicable.

Organism	GMP	Maximum
Salmonella spp.	ND in 250g*	ND in 250g*
VTEC (products containing beef)	ND in 250g*	ND in 250g*
C. perfringens	<10	10^2
B. cereus	<10	10^2
S. aureus	ND in 1g	10^2

*usually 10 x 25g samples examined

Indicators & spoilage organisms
APC and Enterobacteriaceae may be useful indicators of process hygiene.

Organism	GMP	Maximum
APC	$<10^3$	10^4
Enterobacteriaceae	<10	10^2
E. coli	ND in 1g	10

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

P: CANNED, POUCHED OR BOTTLED FOODS (>$F_0$3 process)

Product examples: Canned meats, fish, vegetables, soups, ready meals in pouches, baby foods in jars

Storage: Ambient

Use: Any

Pathogens & toxins
Bacterial spores may survive the heat process. Control of processing is paramount including post-heat treatment cooling and container handling hygiene. Microbiological monitoring of processed product is not recommended. Histamine (scombrotoxin) testing may be relevant for scombroid fish products.

Organism	GMP	Maximum
Bacterial pathogens	Criteria for absence not generally applicable	
Histamine (scombroid fish)	<50ppm (<5mg/100g)	50ppm (5mg/100g)

Indicators & spoilage organisms
Bacterial spores may survive processing. Control of the process is essential. Microbiological testing of the finished product is not considered appropriate. Incubation of finished packs followed by examination for signs of microbial growth or spoilage (pH change, organoleptic defects, blown packs) is most commonly used in industry.

Organism	GMP	Maximum
All (after incubation of pack)	No evidence of growth	No evidence of growth

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

Q: PRESERVED FOODS - HEAT TREATED (<$F_0$3 process), INTERMEDIATE MOISTURE (IMF) OR LOW pH

Product examples: Salami, dry cured hams, bresaola, pastrami, vegetables and herbs in oil, canned or bottled fruits, jams, relishes, pickles, sauces.

Storage: Ambient

Use: Ready-to-eat

Pathogens & toxins

Bacterial spores may be present from raw materials e.g. vegetables and herbs. Control of product physico-chemical characteristics e.g. pH, water activity during the process and maintenance of these conditions throughout product shelf life are paramount for ensuring safety. Histamine (scombrotoxin) testing may be relevant for scombroid fish products.

Organism	GMP	Maximum
Salmonella spp. (not heat treated e.g. herbs in oil)	ND in 25g	ND in 25g
L. monocytogenes	ND in 25g	10^3
C. perfringens	$<10^2$	10^3
S. aureus (meat products)	<20	10^3
VTEC (fermented and dried meats)	ND in 25g	ND in 25g
Histamine (scombroid fish)	<50ppm (<5mg/100g)	50ppm (<5mg/100g)

Indicators & spoilage organisms

APC and Enterobacteriaceae may be useful for monitoring process hygiene and spoilage potential. APC is not applicable to live fermented products. Yeasts and moulds (see Appendix - 'Yeasts and Moulds') may also be significant in the spoilage of some products.

Organism	GMP	Maximum
Enterobacteriaceae (not heat processed)	<10	10^4
Enterobacteriaceae (heat processed)	<10	10^2
E. coli (not heat processed)	<10	10^3
Yeasts	$<10^3$	10^5
Moulds (not mould ripened)	$<10^2$	10^4

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

R: NON-DAIRY FATS AND OILS

Product examples: Cooking oils, fat spreads, lard, virgin olive oil, nut oils, nut butters, cocoa butter

Storage: Ambient or chilled

Use: Any

Pathogens & toxins

Pathogens should be absent in hot refined oils and 100% fat products packed under good hygienic conditions and monitoring is not generally required. Control of product physico-chemical properties (emulsion characteristics, salt, pH) and hygienic processing conditions is important for ensuring the safety of fat spreads and margarines. Monitoring may be required for some cold pressed products. Mould contaminating nuts may produce mycotoxins which can carry through to the finished product. Raw material nuts should be routinely screened for mycotoxin contamination.

Organism	GMP	Maximum
Bacterial pathogens (heat treated)	Criteria for absence not generally applicable	
Salmonella spp. (not heat treated)	ND in 25g	ND in 25g
L. monocytogenes	ND in 25g	10^3

Indicators & spoilage organisms

APC and *Enterobacteriaceae* are normally low and may be useful for trend analysis for monitoring process hygiene. Lipolytic organisms may cause rancidity in fat spreads and testing for these organisms may be appropriate. Control of moisture during processing, packing and shelf life is important for solid fat products as the presence of moisture can allow spoilage by surface mould growth.

Organism	GMP	Maximum
Enterobacteriaceae (fat spreads)	<10	10^3

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

S: SOFT DRINKS AND ALCOHOLIC BEVERAGES

Product examples: Fruit squashes, fruit drinks, beers, wines, colas, lemonade

Storage: Ambient

Use: Ready to drink

Pathogens

Pathogens are unlikely to be present but could survive in low or no alcohol products.

Organism	GMP	Maximum
Bacterial pathogens	Criteria for absence not generally applicable	

Indicators & spoilage organisms

Raw material quality, processing hygiene and control of the product formulation are important in spoilage prevention for these products. Yeasts, moulds and lactic acid bacteria are the main causes of spoilage. Incubation of finished packs followed by visual examination for signs of microbial spoilage e.g. haze, sediment or gas is usually sufficient.

Organism	GMP	Maximum
Yeasts (filtered or heat processed)	<1/100ml	Evidence of growth
Lactic acid bacteria (filtered or heat processed)	<1/100ml	Evidence of growth
Spoilage yeasts (not filtered or heat processed)	<10	Evidence of growth
Lactic acid bacteria (not filtered or heat processed)	<10	Evidence of growth
E. coli (low alcohol beers)	<1/100ml	10/100ml

REMINDER: Indiscriminate application of microbiological testing and criteria is not advocated and should be avoided. It may not be necessary to apply all the tests listed for a product category and on some occasions it may be relevant to test for additional microorganisms and/or toxins. **GMP** values are those expected immediately following production of the food under good manufacturing conditions. **Maximum** values are those regarded as the maximum acceptable at any point in the shelf life of the product (see 6.1 and 6.2). When applying microbiological tests and criteria, all elements of the microbiological criterion including the sampling plan must be clearly specified (see chapters 1 & 3).

7. European Union directives

A number of European directives containing microbiological criteria have already been implemented for a variety of foodstuffs. The following tables (p167-172) are intended to provide an overview of information on microbiological criteria in the existing Directives. ***To ensure the correct context for application, the criteria should be read in conjunction with the appropriate legislation.***

EU Council Directives consulted
(* Principal UK Statutory Instrument implementing the Directive)

Council Directive 89/437/EEC on hygiene and health problems affecting the production and the placing on the market of **egg products.** (Official Journal of the European Communities 22.7.89 No. L212/87) amended by Council Directives 89/662/EEC (OJ No. L395, 30.12.89, p13) and 91/684/EEC (OJ No. L376, 31.12.91, p. 38)
* The Egg Products Regulations, 1993, S. I. No. 1520.

Council Directive 91/492/EEC laying down the health conditions for the production and placing on the market of **live bivalve molluscs**. (Official Journal of the European Communities 24.9.91 No. L268/1).
* The Food Safety (Live Bivalve Molluscs and other Shellfish) Regulations, 1992, S. I. No. 3164 and The Food Safety (Live Bivalve Molluscs and other Shellfish) (Import Conditions and Miscellaneous Amendments) Regulations, 1994, S. I. No. 2782.

Council Directive 91/493/EEC laying down the health conditions for the production and the placing on the market of **fishery products**. (Official Journal of the European Communities 24.9.91 No. L268/15).
* The Food Safety (Fishery Products) Regulations, 1992, S. I. No. 3163 and The Food Safety (Fishery Products) (Import Conditions and Miscellaneous Amendments) Regulations, 1994, S. I. No. 2783.

Commission Decision 93/51/EEC on the microbiological criteria applicable to the production of **cooked crustaceans and molluscan shellfish**. (Official Journal of the European Communities 21.1.93 No. L13/11).
* The Food Safety (Fishery Products) (Import Conditions and Miscellaneous Amendments) Regulations, 1994, S. I. No. 2783.

Council Directive 92/46/EEC laying down the health rules for the production and placing on the market of **raw milk, heat-treated milk and milk-based products.** (Official Journal of the European Communities 14.9.92 No. L268/1).
* The Dairy Products (Hygiene) Regulations, 1995, S. I. No. 1086.

Council Directive 80/777/EEC on the approximation of the laws of the member states relating to the exploitation and marketing of **natural mineral waters.** (Official Journal of the European Communities 30.8.80 No. L229/1).
* The Natural Mineral Waters Regulations, 1985, S. I. No. 71.

Council Directive 80/778/EEC relating to the quality of water intended for human consumption, in relation to **drinking water which is bottled or sold in a bottle.** (Official Journal of the European Communities 30.8.80 No. L229/11).
* The Drinking Water in Containers Regulations, 1994, S. I. No. 743.

Council Directive 94/65/EC laying down the requirements for the production and placing on the market of **minced meat and meat preparations**. (Official Journal of the European Communities 31.12.94 No. L368/10).
* The Minced Meat and Meat Preparations (Hygiene) Regulations, 1995, S. I. No. 3205.

Definitions:

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

n = number of units making up the sample

c = number of units in the sample giving values between m and M; the sample being considered acceptable if the values of the other sample units are = or < m.

S = the microbic limit at which a product must be considered toxic or tainted

8. Developing technologies

A variety of technologies such as irradiation, high pressure and ultra filtration are being investigated for application in specific food market sectors. All such technologies aim to reduce microbial loads. If required, microbiological criteria in relation to such foods can be developed from a consideration of all the factors discussed in this guideline.

The effect of the technologies used and subsequent product handling including storage, distribution and consumer handling, all contribute to the safety and wholesomeness of the food. Account must be taken of all these stages for the determination of relevant microbiological criteria.

DIRECTIVE	PRODUCTS	MICROORGANISM	LEVEL (per g or ml unless otherwise specified)	STATUS/ACTION
Egg products 89/437/EEC sampled in treatment establishments	All egg products after treatment	*Salmonella* spp.	absent in 25g or ml	Only products meeting the requirements may be used as foodstuffs
"	"	*Staphylococcus aureus*	absent in 1g	"
"	"	Mesophilic aerobic bacteria	M=100 000	"
"	"	*Enterobacteriaceae*	M=100	"
Live Bivalve Molluscs 91/492/EEC intended for immediate human consumption	Live bivalve molluscs	*Salmonella* spp.	absent in 25g	Withhold from market
"	"	*Escherichia coli*	<230 /100g	"
"	"	Faecal coliforms	<300 /100g	"
"	"	Paralytic shellfish poison (PSP)	< or = 80µg /100g	"
"	"	Diarrhetic shellfish poison (DSP)	Negative in bioassay	"
Fish products 91/493/EEC and Commission Decision 93/51/EEC	Cooked crustaceans and molluscan shellfish	*Salmonella* spp.	absent in 25g, n=5, c=0	Compulsory criteria; withhold from the market
"	"	Other pathogens and toxins thereof	Not present in quantities such as to affect health	Compulsory criteria
"	Whole products	Mesophilic aerobic bacteria (30°C)	m=10 000, M=100 000, n=5, c=2	Indicator organisms: to help manufacturers decide whether plants are operating satisfactorily (Guidelines)
"	Shelled or shucked products	*Staphylococcus aureus*	m=100, M=1000, n=5, c=2	Analytical criteria: organisms indicating poor hygiene
"	Shelled or shucked products	*Escherichia coli* (on solid medium)	m=10, M=100, n=5, c=1	Analytical criteria
"	Shelled or shucked products	Thermotolerant coliforms (44°C on solid medium)	m=10, M=100, n=5, c=2	"
"	Shelled or shucked products except crabmeat	Mesophilic aerobic bacteria (30°C)	m=50 000, M=500 000, n=5, c=2	Indicator organism (Guidelines)
"	Crabmeat	Mesophilic aerobic bacteria (30°C)	m=100 000, M=1 000 000, n=5, c=2	"

DIRECTIVE	PRODUCTS	MICROORGANISM	LEVEL (per g or ml unless otherwise specified)	STATUS/ACTION
Milk products 92/46/EEC: criteria apply on removal from the processing establishment	All milk products	Pathogenic microorganisms and their toxins	Must not be present in quantities such as to affect health	If standards exceeded, foodstuff to be excluded from human consumption and withdrawn from market
"	All milk products except milk powder	*Salmonella spp.*	absent in 25g, n=5, c=0	"
"	Cheese made from raw/thermised milk	*Staphylococcus aureus*	m=1000, M=10 000, n=5, c=2	Analytical criteria. If standards are exceeded there must be a review of monitoring methods. If strains of enterotoxigenic *Staphylococcus aureus* or *Escherichia coli* that may be pathogenic are identified all batches involved should be withdrawn. Where M is exceeded, there must be testing for presence of toxins.
"	Cheese made from raw/thermised milk	*Escherichia coli*	m=10 000, M=100 000, n=5, c=2	"
"	Fresh cheese, powdered milk, frozen milk-based products (including ice-cream)	*Staphylococcus aureus*	m=10, M=100, n=5, c=2	Analytical criteria. Review HACCP - if pathogenic *E.coli*/ toxigenic *S.aureus*, then withdraw from market
"	Cheese other than hard cheese	*Listeria monocytogenes* *	absent in 25g, n=5, c=0	If standards exceeded, foodstuff to be excluded from human consumption and withdrawn from market
"	Other milk products	*Listeria monocytogenes**	absent in 1g	"
"	Soft cheese (from heat treated milk)	*Staphylococcus aureus*	m=100, M=1000, n=5,c=2	Analytical criteria. Review HACCP - if pathogenic *E.coli*/ toxigenic *S.aureus*, then withdraw from market
"	"	*Escherichia coli*	m=100, M=1000, n=5, c=2	"
"	"	Coliforms 30°C	m=10 000, M=100 000, n=5, c=2	Indicator organisms (Guidelines)
"	Milk powder	*Salmonella* spp.*	absent in 25g, n=10, c=0	If standards exceeded, foodstuff to be excluded from human consumption and withdrawn from market
"	All except milk powder	*Salmonella* spp.*	absent in 25g, n=5, c=0	"

* In addition, pathogenic microorganisms and their toxins must not be present in quantities such as to affect the health of consumers

DIRECTIVE	PRODUCTS	MICROORGANISM	LEVEL (per g or ml unless otherwise specified)	STATUS/ACTION
Milk products 92/46/EEC: criteria apply on removal from the processing establishment (continued)	Powdered milk-based products	Coliforms 30°C	m=0, M=10, n=5, c=2	Indicator organisms (Guidelines)
"	Liquid milk-based products	Coliforms 30°C	m=0, M=5, n=5, c=2	Indicator organisms (Guidelines)
"	Liquid heat-treated unfermented milk-based products	Plate count (21°C) after product incubation at 6°C / 5days	m=50 000, M=100 000, n=5, c=2	Indicator organisms (Guidelines)
"	Frozen milk-based products (including ice-cream)	Coliforms 30°C	m=10, M=100, n=5, c=2	Indicator organisms (Guidelines)
"	"	Plate count (30°C)	m=100 000, M=500 000, n=5, c=2	"
"	Pasteurised milk	Pathogenic microorganisms	Absent in 25g m=0, M=0, n=5, c=0	Where standards exceeded investigate and take appropriate action
"	"	Coliforms	m=0, M=5, n=5, c=1	"
"	"	Plate count (21°C) after product incubation at 6°C / 5days	m=50 000, M=500 000, n=5, c=1	"
"	Butter from pasteurised milk or cream	Coliforms 30°C	m=0, M=10, n=5, c=2	Indicator organisms (Guidelines)
"	Sterilised and UHT milk	Plate count (30°C) after product incubation at 30°C for 15 days or 55°C for 7 days	<or=10 per 0.1ml	Where standards exceeded investigate and take appropriate action
"	Raw cows' milk for drinking in that state	*Salmonella* spp.	absent in 25ml, n=5, c=0	"
"	"	*Staphylococcus aureus*	m=100, M=500, n=5, c=2	"
"	"	Plate count (30°C)	<or=50 000	"
Milk products 92/46/EEC: requirements for the manufacture of heat-treated milk and milk-based products	Raw cows' milk for production of heat-treated milk, if not treated within 36 hours of acceptance at the treatment plant	Plate count (30°C) (in the raw milk)	<or=300 000	Must meet this standard otherwise must not be used for production of heat treated drinking milk

DIRECTIVE	PRODUCTS	MICROORGANISM	LEVEL (per g or ml unless otherwise specified)	STATUS/ACTION
Milk products 92/46/EEC: requirements for the manufacture of heat-treated milk and milk-based products (continued)	Pasteurised milk for production of heat-treated drinking milk.	Plate count (30°C) (in the pasteurised milk)	<or=100 000	Must meet this standard otherwise must not be used for production of heat treated drinking milk
"	Thermised milk for production of pasteurised, UHT or sterilised milk	Plate count (30°C) (in the thermised milk)	<or=100 000	"
Milk products 92/46/EEC: standards to be met for collection of raw milk from the production holding or for acceptance at the treatment or processing establishment	Raw cows' milk intended for direct human consumption or for the production of heat-treated drinking milk or products "made with raw milk"	Plate count (30°C)	<or=100 000	Where standards exceeded investigate and take appropriate action
"	Raw cows' milk intended for direct human consumption or for the manufacture of products "made with raw milk"	*Staphylococcus aureus*	m=500, M=2000, n=5, c=2	"
"	Raw cows' milk for the manufacture of other milk-based products	Plate count (30°C)	<or= 400 000 (to be reduced to <or= 100 000 from 1.1.98)	Where standards exceeded investigate and take appropriate action
"	Raw buffaloes' milk for the manufacture of milk-based products	Plate count (30°C)	<or= 1 000 000	"
"	Raw buffaloes' milk intended for the manufacture of products "made with raw milk"	Plate count (30°C)	<or= 500 000	"
"	"	*Staphylococcus aureus*	m=500, M=2000, n=5, c=2	"
"	Raw goats' milk or sheep's milk intended for the production of heat-treated drinking milk or the manufacture of heat-treated milk-based products.	Plate count (30°C)	<or= 1 000 000	"
"	Raw goats' milk or sheep's milk intended for the manufacture of products "made with raw milk".	Plate count (30°C)	<or= 500 000	"
"	"	*Staphylococcus aureus*	m=500, M=2000, n=5, c=2	"

DIRECTIVE	PRODUCTS	MICROORGANISM	LEVEL (per g or ml unless otherwise specified)	STATUS/ACTION
Natural mineral waters 80/777/EEC	Mineral water at source and thereafter, up to and including the point of sale	Coliforms	Absent in 250ml	Must meet this standard
"	"	*Escherichia coli*	Absent in 250ml	Must meet this standard
"	"	Faecal streptococci	Absent in 250ml	"
"	"	*Pseudomonas aeruginosa*	Absent in 250ml	"
"	"	Sporulated sulphite reducing anaerobes	Absent in 50ml	"
"	Mineral water at source	Aerobic plate count (37°C, 24h; 20-22°C,72h)	Normal for the source	"
"	Mineral water at source (within 12 hours after bottling; water maintained at 4°C ± 1°C)	Aerobic plate count (37°C, 24 hour)	<or=20	"
"	"	Aerobic plate count (20-22°C, 72 hour)	<or=100	"
"	Mineral water	Pathogenic microorganisms	Absent	"
"	"	Parasites	Absent	"
Quality of water intended for human consumption 80/778/EEC	Drinking water in containers (not mineral water)	Total coliforms	Absent in 100ml	Must meet this standard
"	"	Faecal coliforms	Absent in 100ml	"
"	"	Faecal streptococci	Absent in 100ml	"
"	"	Sulphite reducing clostridia	Absent in 20ml	"
"	"	Colony count (37°C) Colony count (22°C) (measure within 12 hours of bottling; sample water to be kept at a constant temperature)	<or=20 <or=100	"
"	"	Colony count (sampled between 12 hours after bottling and point of sale)	Any increase shall not be greater than expected	"

DIRECTIVE	PRODUCTS	MICROORGANISM	LEVEL (per g or ml unless otherwise specified)	STATUS/ACTION
Minced meat and meat preparations 94/65/EC	Minced meat	Aerobic mesophilic bacteria	m=500 000, M=5 000 000, S = 500 000 000, n=5, c=2	Compulsory criteria; values >M are unsatisfactory (M = acceptability threshold above which results are no longer considered satisfactory where M = 10m (solid medium) or 30m (liquid medium). S = the microbic limit value at which product must be considered toxic or tainted.
"	"	*Escherichia coli*	m=50, M=500, n=5, c=2, S=50 000	"
"	"	*Staphylococcus aureus*	m=100, M=5 000, n=5, c=2, S=50 000	"
"	"	*Salmonella*	Absent in 10g, n=5, c=0	Unacceptable if any sample shows presence in 10g
"	Meat preparations	*Escherichia coli* (solid media) *Escherichia coli* (liquid media)	m=500, M=5 000, n=5, c=2 m=500, M=15 000, n=5, c=2	Compulsory criteria; values >M are unsatisfactory
"	"	*Staphylococcus aureus* (solid medium) *Staphylococcus aureus* (liquid medium)	m=500, M=5 000, n=5, c=1 m=500, M=15 000, n=5, c=1	"
"	"	*Salmonella*	absent in 1g, n=5, c=0	Unacceptable if any sample shows presence in 1g

APPENDIX
Specific organisms and the principles of test methods

Aerobic plate count

Enumeration of microorganisms by the aerobic plate count is one of the most common methods used to indicate the microbiological quality of a food. It can indicate whether a product has been contaminated, temperature abused or spoilt. The temperature and length of incubation should be defined. The most common incubation regime is 30°C for 48 or 72 hours for mesophilic organisms, but other temperatures and times may be more appropriate e.g. 55°C for 48-72 hours for thermophilic organisms or 20-25°C for up to 5 days for psychrotrophic organisms. Different temperatures of incubation will lead to isolation of different flora; many organisms that develop during chilled storage for example will not grow well above 30°C, and so a lower temperature of incubation is preferable i.e. 20-25°C.

Consideration should be given to the method used for performing an aerobic plate count. Pour plate methods are frequently specified, but microorganisms commonly found in foods may be injured by the temperature of the molten agar used especially if they are already stressed. In these circumstances, surface inoculation methods may be preferable.

Anaerobic plate counts can be used for foods that provide anaerobic conditions for microbial growth e.g. processed cheese and vacuum packed products.

Aeromonas hydrophila and related species

The role of *Aeromonas* in causing food poisoning has not been clearly established, but some strains of certain species are thought to be capable of causing diarrhoea. They are aquatic organisms but have been isolated from many foods. *Aeromonas* spp. grow at refrigeration temperatures. Enumeration techniques on solid media and enrichment methods to establish presence or absence are both appropriate. This group of organisms is not normally included in microbiological specifications.

Bacillus cereus and other *Bacillus* species

The members of the Bacillaceae produce spores as well as vegetative cells. The spores can survive adverse conditions such as drying and pasteurisation. These organisms are common in the environment and in many foods. Some strains produce enterotoxins if allowed to grow, for example *Bacillus cereus* in rice dishes and high moisture flour products and members of the *B. subtilis - licheniformis* group in meat and pastry products and meat or seafood rice dishes (Kramer & Gilbert, 1989). The emetic toxin of *B. cereus* is particularly heat resistant (126°C for 90 minutes) whereas the diarrhoeagenic toxin is inactivated by exposure to 56°C for 30 minutes. High levels (>10^6 per gram) are necessary to produce enough toxin to cause illness. *Bacillus* species can also cause spoilage.

Recovery and enumeration of *Bacillus* species can be accomplished on many media, but media designed specifically for presumptive enumeration of *B. cereus* are usually used. These media may allow resuscitation, growth from spores and presumptive identification of *B. cereus* if present. Atypical strains of *B. cereus* can occur and could lead to a false negative result. In some circumstances mesophilic or thermophilic spore counts may be required; methods incorporate heat treatment of the sample prior to spore enumeration at appropriate temperatures. Biochemical tests (including ammonium salt sugars (glucose, arabinose, mannitol and xylose) and a nitrate reduction test) can be used to distinguish the most common food poisoning *Bacillus* species (Roberts *et al.* 1995).

Campylobacter species

Campylobacter jejuni and other thermotolerant campylobacters are some of the most common causes of diarrhoea but the routes of transmission are still unclear. Campylobacters are found in the intestinal tracts of many animals, especially poultry and birds, and can therefore contaminate natural water and raw milk as well as meat and poultry. These organisms have fastidious temperature and atmospheric requirements for growth. They do not grow in foods under normal storage conditions. As the infectious dose is low, the presence of low numbers in ready-to-eat foods is significant. Where detection is required, enrichment methods and selective agars are used for their isolation.

Clostridium perfringens and sulphite reducing clostridia

Clostridium perfringens is a spore-forming anaerobe commonly found in mammalian faeces and soil. The spores persist in the environment and often contaminate raw food materials. Spores may survive cooking and rapid growth may occur if the food is not chilled promptly. If large numbers of the organism are eaten in a food, they can sporulate in the gut and produce an enterotoxin which causes diarrhoea.

Isolation of *C. perfringens* on solid media requires anaerobic conditions of incubation, and usually the use of selective agents, followed by confirmation of suspect colonies. Vegetative cells of *C. perfringens* do not survive well in foods at low temperatures, and spore counts may be more appropriate on such foods.

The sulphite reducing clostridia (SRCs) include the pathogens *C. perfringens* and *C. botulinum* (the causative organism of botulism). The SRCs may be used as an indicator of plant hygiene and for the presence of other clostridia that may cause food poisoning or food spoilage. There are no routine laboratory tests for confirming the presence of *C. botulinum*.

Coliforms, faecal coliforms and *Escherichia coli*

Coliforms were one of the first groups of bacterial indicators used in the water and dairy industries.

They are those members of the Enterobacteriaceae that ferment lactose, and include *Enterobacter,* most *Escherichia, Klebsiella* and *Citrobacter.* These organisms are not exclusively of faecal origin. Thermotolerant coliforms are those coliforms that can multiply at 44°C. This group includes *E. coli* types I and II and occasional strains of *Klebsiella* and *Enterobacter.* Most faecal coliforms are also able to multiply at 44°C and in practice the terms 'faecal' and 'thermotolerant' are used synonymously.

Absence of *E. coli* is normally required in processed foods, and so a detection method or MPN method is required to detect low levels. Direct enumeration methods for coliforms and *E. coli* may be more suitable when contamination might be expected e.g. raw meats. Caution should be used in comparing results obtained by different methods and it is important to clearly define the target organism(s) and method used.

Whilst the presence of *E. coli* in food is generally undesirable because it indicates poor hygienic conditions, certain serotypes are pathogenic and may cause gastroenteritis. Serotyping of strains normally requires specialist facilities.

Verocytotoxin-producing strains of *E. coli* (VTEC) e.g. *E. coli* 0157 may be particularly virulent and can cause symptoms ranging from mild diarrhoea to severe bloody diarrhoea (haemorrhagic colitis), sometimes progressing to haemolytic uraemic syndrome (HUS) and kidney failure. Because of the increasing significance of VTEC strains in foodborne gastroenteritis, specific methods are being developed for their detection. Selective agar media currently used for the isolation of *E. coli* 0157 are based on this strain's inability to ferment sorbitol (most other members of the *E. coli* group are able to ferment sorbitol). Conventional methods used for *E. coli* isolation are usually unsuitable for this serotype because of its poor growth at 44°C and absence of glucuronidase activity.

Enterobacteriaceae

The family Enterobacteriaceae includes bacteria that naturally inhabit the mammalian gut but can also occur and multiply in other environments e.g. species of *Escherichia, Citrobacter, Enterobacter, Proteus,* and also some of the most important enteric pathogens such as *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica,* and pathogenic *E. coli.* Enterobacteriaceae are useful indicators of hygiene and of post-processing contamination of heat processed foods. Detection methods are usually based on direct enumeration using media containing bile salts and glucose.

***Enterococcus* species / faecal streptococci**

The enterococci, which include faecal streptococci, survive and grow well in factory environments. Because they are more resistant to heat and adverse conditions than the Enterobacteriaceae, they are used as hygiene indicators, particularly in dried products and frozen foods. Methods for the detection of enterococci usually involve isolation on selective agar.

Lactic acid bacteria

This group includes bacteria capable of producing lactic acid from fermentable substrates. Some strains grow poorly in aerobic conditions. Genera commonly found in/on food include *Lactobacillus, Pediococcus, Leuconostoc, Lactococcus* and *Streptococcus.* They are ubiquitous in the environment and are notable for their ability to grow at low pH levels. Starter cultures for fermented foods such as cheese and salami commonly include this group of organisms, and so their presence in high numbers should be expected. However, in some foods they may cause spoilage. Direct enumeration methods on solid media are usually used to detect these organisms.

Listeria monocytogenes

Listeria monocytogenes is a foodborne pathogen causing the disease listeriosis. The organism is ubiquitous in the environment, and scrupulous hygiene is required to minimise its presence in food production premises. It is not yet clear how many organisms are required to cause illness, but as the organism is able to grow at refrigeration temperatures, its absence or very low levels in ready to eat foods is desirable. Although the other members of the *Listeria* group rarely cause illness, they are often used as an indicator for the presence of *L. monocytogenes.*

Detection (presence/absence) tests for *Listeria* species involve selective enrichment followed by subculture to selective agar. Enumeration of *Listeria* is usually performed on solid selective media.

Protozoan parasites

The most important waterborne or foodborne protozoan parasites which infect humans are *Cryptosporidium parvum, Giardia intestinalis (lamblia), Sarcocystis* spp. and *Toxoplasma gondii.* Transmission of *Giardia* and *Cryptosporidium* is mainly by ingestion of contaminated water, but contamination of food and person-to-person transmission can also occur. For the detection of these parasites in water, methods involving concentration by filtration of large volumes (>50 litres) of water are required; methods for detection in food are poorly developed.

Transmission of *Sarcocystis* results from ingestion of raw or undercooked meat containing mature cysts; these may be detected by the naked eye or microscopic examination of histological sections. The main host of *Toxoplasma gondii* is the cat which excretes oocysts into the environment. These can lead to infection in food animals. Ingestion of raw or undercooked meat may cause infection as may direct contact with infected cats and consumption of contaminated water. These parasites are rarely sought in food and water unless they are implicated in illness.

International specifications for bottled waters may require absence of parasites, but examination of potable water used in the food industry for the presence of parasites is carried out infrequently.

More recently, a large outbreak of diarrhoeal illness in the USA was diagnosed as infection with *Cyclospora cayetanensis;* a coccidian parasite believed to have been associated with consumption of fresh fruit.

Human infection by parasites carried on foods or in water is of growing concern and because infection may possibly be caused by ingestion of very few infective units, strict codes of hygiene control in growing and harvesting areas are most important.

Pseudomonas species

Pseudomonas species are ubiquitous in the environment and water, and some species are psychrotrophic. *Pseudomonas aeruginosa* is of clinical significance as an opportunistic pathogen but has rarely been implicated in gastroenteric infection. Other pseudomonads are significant in food spoilage, particularly in chilled food. Levels higher than 10^7 cfu/g or ml of food may result in off flavours, off odours and visual defects. Direct enumeration methods on selective solid media are used.

Salmonella species

Salmonella spp. are organisms of faecal origin, and the majority of species are regarded as potentially pathogenic to humans. These organisms survive and may grow well in factory environments. *Salmonella* spp. cause significant numbers of food poisoning cases each year. Classical methods for detection of *Salmonella* spp. involve testing a defined amount of food, usually 25g. This quantity should be increased for food products for which increased test sensitivity is required e.g. some dried foods, or if the food is destined for a susceptible population e.g. baby foods.

Methods must be capable of detecting low levels of *Salmonella* spp. in the presence of high levels of background flora. Traditional procedures used to achieve this are lengthy, and typically involve a pre-enrichment resuscitation stage in a non-selective medium, enrichment in selective media, and subculture to solid selective media. Atypical (and rare) lactose-fermenting *Salmonella* spp. may not be detected by many common test methods. No single medium or method is optimal for growth of all serotypes of *Salmonella*; two formulations of enrichment and solid selective media are often used in industry. Biochemical and serological confirmation of suspect colonies are required.

More rapid methods have been developed for screening out negative samples and automating the procedures, which should be assessed by each laboratory for the types of foods that they are required to examine.

Shigella species

Shigella species are members of the Enterobacteriaceae. They are highly infectious enteric pathogens, easily spread by the faecal-oral route. The infective dose is known to be very low so enrichment methods of detection are used for isolation. Food may be contaminated by food-handling personnel or by contaminated irrigation water. Foodborne illness due to *Shigella* is relatively uncommon in the UK and *Shigella* species are rarely included in microbiological criteria.

Staphylococcus aureus

Staphylococcus aureus (S. aureus) may be associated with the skin, nose and throat of healthy individuals as well as being the cause of boils and many skin and wound infections. Given suitable conditions it is capable of growth in foods, and some strains may produce a heat-stable enterotoxin. It is used as an indicator of general hygiene and adherence to good food handling procedures. Its presence, particularly in high numbers ($>10^4$ per gram), indicates the potential presence of enterotoxin and a consequent risk of food poisoning. If poor temperature control allowing growth has occurred prior to heat treatment, then the enterotoxin may remain despite the absence of viable *S. aureus* cells. *S. aureus* is normally sought by direct selective enumeration. Methods are also available for the detection of staphylococcal enterotoxin.

Vibrio parahaemolyticus and other *Vibrio* species

Vibrio parahaemolyticus is a marine organism capable of causing profuse diarrhoea and is most often associated with seafood and shellfish from warm waters. The infective dose is not known; high numbers e.g. $>10^2$ per gram are regarded as unacceptable. Enrichment methods are normally used for their isolation although enumeration can be useful. *V. cholerae* is the causative organism of cholera and is usually transmitted via contaminated water and the faecal-oral route. The organism is not usually sought except in response to specific public health concerns. A number of other species of *Vibrio* are of pathogenic significance e.g. *V. vulnificus,* and also appear to be associated with consumption or handling of seafood. The presence of vibrios in cooked seafood indicates either inadequate heat treatment or post-processing contamination.

Viruses

Foodborne viral illness is caused mainly by small round structured viruses (SRSVs also known as Norwalk-like viruses) which cause gastroenteritis; and hepatitis A, which causes infectious hepatitis. Viruses cannot grow in foods, but the infectious dose of these viruses is very low. Primary contamination of the food at source is caused mainly by polluted water. Consumption of molluscan shellfish harvested from sewage polluted waters has been implicated in many outbreaks of gastroenteritis; contaminated soft fruits, vegetables and salad items which do not receive a heat process may also cause illness. However, most viral foodborne illness results from secondary contamination of food by infected food

handlers during preparation of items which do not receive further heat treatment. Detection of viruses in food may be achieved using complex extraction methods and cell culture or the polymerase chain reaction (PCR), but these techniques are not suitable for routine screening and microbiological specifications for foods rarely include viruses.

Yeasts and moulds

The yeasts are a common cause of food spoilage, particularly of acid foods such as fruit and fruit juices, and foods of reduced water activity (a_w) such as confectionery. They have not been implicated in food poisoning. Moulds are also a significant cause of food spoilage, and some strains are able to grow at very low a_w values e.g. 0.6-0.7, causing spoilage of otherwise microbiologically stable commodities such as bakery goods. Although moulds do not cause food poisoning, some strains are able to produce mycotoxins which can cause serious chronic illness if consumed.

Moulds can rarely be successfully enumerated when growing in foods. A focus of mould growth may consist either of hyphae or of hyphae producing spores. 'Counts' obtained from hyphae will vary depending on the degree of fragmentation caused by macerating the sample and can range from tens to many hundreds per gram. If spores are present, the 'count' may rise to millions per gram. In these circumstances, 'counts' are usually meaningless.

Selective agars are commonly used for the detection of both yeasts and moulds. The brewing industry use specific agars for detecting 'spoilage yeasts' . Surface inoculation methods of enumeration are preferable to pour plate methods because of the need to maximise aerobic conditions for mould growth and to enhance colony morphology for identification purposes. Incubation is normally lengthy and can take 5 days (or considerably longer for xerophiles) to obtain a result.

Standard enumeration tests are inappropriate when the significant organism is present as a very low proportion of the contaminant flora e.g. heat resistant moulds on fruit; or when significant contamination is below the detection limit of the method e.g. a few yeasts in a 200 litre drum of fruit juice concentrate. In such circumstances and if deemed necessary, enrichment or concentration procedures may be employed for detecting relevant organisms.

***Yersinia enterocolitica* and related species**

Yersinia species are also members of the Enterobacteriaceae, and are capable of psychrotrophic growth. *Yersinia enterocolitica* and related organisms are widely distributed in animals of all types and are found in many environments. The recognised pathogenic serotypes belong to *Y. enterocolitica* and are mainly associated with pigs. These strains can cause gastroenteritis and many other symptoms. The infectious dose is not known; however presence of pathogenic serotypes should be regarded as potentially hazardous. Other serotypes and species have been found in a wide variety of foods, and their presence in heat processed foods indicates post-processing contamination. Isolation is usually accomplished using enrichment methods. This organism is not normally included in microbiological criteria.

Glossary of terms

Commercial sterility - A stable product condition in which no microbial growth occurs under ambient storage conditions.

$F_0$3 - A heat process of 121.1°C for 3mins or an equivalent heat process (designed to achieve a 12 log cycle reduction in *C. botulinum.)*

Pasteurisation - A form of heat treatment that kills vegetative pathogens and spoilage microorganisms in milk and other foods e.g. for milk a common pasteurisation process is 71.7°C for 15 secs.

Psychrotrophic - Organisms that can grow at temperatures as low as minus 5°C but which have an optimum growth temperature in the mesophilic range (20-30°C).

Thermisation - A mild heat treatment (57-68°C for 15 sec) applied to raw milk to extend its storage life prior to use in further processing - usually into cheese.

Sous vide - Usually composite foods pasteurised in a vacuum pack intended for catering outlets. Such products are often given an extended shelf life at refrigeration (< or = 2°C) temperatures.

Verocytoxigenic - Organisms which produce a toxin capable of killing vero cells which is an established cell line derived from African Green Monkey kidney.

Ultra Heat Treatment - A high temperature heat treatment (138-142°C for 2-5 secs) applied to liquid foods usually followed by aseptic packaging for the production of long life ambient stable products.

Water activity (a_w) - A measure of the availability of water for the growth and metabolism of microorganisms. It is expressed as the ratio of the water vapour pressure of a food or solution to that of pure water at the same temperature.