Some Scientific and Regulatory Developments of National and International Significance in Food Microbiology*

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Many current and ongoing developments in the areas of food microbiology and hygiene have significant international impact. Some of these are programs of the FDA; others are programs of other countries and international organizations. Several of these activities may be of particular interest to food microbiologists and sanitarians working either in government or industrial organizations. Accordingly, I shall attempt to bring into focus the following topics of national and international interest: (a) Objective analytical criteria of quality and safety for foods; (b) Microbiological methods development and standardization; (c) Sampling and testing plans and acceptance criteria for salmonellae in foods; (d) Penicillin in milk supplies; and (e) Surveillance of import foods.

Objective Analytical Criteria of Quality and Safety for Foods

I am sure we all recognize the need for objective measures that will assist in making judgements of the wholesomeness and safety of foods. One of these measures utilizes microbial count limits as indicators of food manufacturing and handling practices; another utilizes limits for extraneous materials commonly called "filth".

Microbial Count Limits or Microbiological Criteria

Actually there are three such criteria in use today to a greater or lesser extent. These are illustrated as follows:

A microbiological specification is the maximum acceptable number of microorganisms or of specific types of microorganisms, as determined by prescribed methods, in a food being purchased by a firm or agency for its own use.

A recommended microbiological limit is the suggested maximum acceptable number of microorganisms or of specific types of microorganisms, as determined by prescribed methods, in a food.

A microbiological standard is that part of a law or administrative regulation designating the maximum acceptable number of microorganisms or of specific types of microorganisms, as determined by prescribed methods, in a food produced, packed, or stored, or imported into the area of jurisdiction of an enforcement agency.

To establish microbial limits or criteria for foods is not a simple undertaking. The facts of life in this regard become evident as soon as one recognizes the great diversity that exists in food products and in the processing operations by which they are prepared. This diversity is reflected in the differences in the microflora of various food products and thereby influences the extent of useful application of one or more of the criteria defined above. A great deal of knowledge about the microbiology of a product is needed in order to avoid undue consumer as well as producer risks. For example, without adequate knowledge, a standard might be established that would be so

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restrictive that a substantial percentage of producers could not meet it——in other words a standard that technologically is not feasible. The opposite also could occur——a standard having associated with it little or no significance relative to consumer benefit, in other words, an unrealistically high consumer risk.

Microbial counts standing by themselves are only one of several indicative criteria by which judgements can be made of the conditions under which a product was produced and handled. Furthermore, the degree to which a microbial count reflects the conditions of production and handling depends largely upon the product involved. For example, a severe microbial destructive treatment of the product near the final stage of its processing could render almost meaningless any relationship between the bacterial count of the finished product and the sanitary conditions that may have prevailed during its manufacture.

Time does not permit further expansion of the pros and cons of applying various microbiological and other criteria to foods. The point I wish to emphasize is that efforts toward developing and applying such criteria are rapidly increasing. The exciting challenge we face nationally and internationally is the development and application of meaningful criteria in the interest of consumer protection. Let me indicate first our approach to this problem and then what is happening elsewhere.

As we visualize the role of the Food and Drug Administration, we either have sole or a shared responsibility for 1) establishing a rationale for the setting of microbiological quality standards, 2) developing or identifying a data base for use in establishing a standard, 3) creating, publishing, and revising a proposed standard, 4) enforcing the standard and, finally, 5) updating the standard as appropriate to reflect current technology.

Rationale for microbiological quality standards. A variety of approaches has been used to identify microbiological limits of one type or another in foods. Several local or state governments have either set such things as maximum permissible total aerobic plate, coliform, *E. coli*, and *S. aureus* counts for selected foods or are considering such limits. Generally, the limits selected are based upon the experience the governmental unit has had with the product and a product that exceeds these limits is in violation of the law.

The Food and Drug Administration has had a series of microbiological guidelines available to our field force for many years. These guidelines consisted essentially of a series of microbiological limits based upon what was attainable with the product in question when good manufacturing practices were being used. These guidelines were used in conjunction with inspectional findings to support observations of insanitary practices.

These approaches, although useful, did present problems. We have had difficulties in bringing enforcement action on products having high bacterial counts if the bacteria present could not be demonstrated to include pathogens. The fact that the magnitude of the counts were indicative of conditions that were likely to result in contamination with pathogens or that could permit their growth was insufficient. Additional evidence showing unsanitary conditions directly related to the high counts was necessary. Also, the FDA guidelines were not available to the press or to the public. As a result, neither affected party had an opportunity to participate in their creation nor were the limits available for use by the processor and the public to measure product quality. The guidelines also were generally limited in their application in that they were useful in evaluating microbiological quality during production and not necessarily at the grocery store or retail level. Because to this, abuses of the product during distribution and display at the grocery store were not a consideration in the application of the guidelines. Since the consumer eats food that is most nearly of the quality found in grocery stores, and since abuses during distribution and display do occur, the agency came to the position that standards applicable at the grocery store would offer the consumer the greatest protection from food that had been produced under insanitary conditions and/or abused during distribution and display.

Microorganisms are in the environment in which foods are grown, harvested, stored, processed, and distributed. As a result, except

for foods that are processed, to make them sterile, finished foods may normally contain many bacteria. The total bacterial load of a finished food is generally a reflection of many things, including the degree of contamination of the raw products used in the food, the contamination of the food contact surfaces experienced by the food as it is prepared, and the control of bacterial growth during the harvesting, storage, processing, and distribution of the food.

Obviously there are exceptions to this general rule and these exceptions include fermented foods as well as foods that undergo a processing step that is lethal to bacteria. In the latter case the bacterial burden of the finished product would reflect the survivors of the process and those bacteria added by recontamination of the product after the process, as well as those that resulted from bacterial multiplication in the product after processing. Since the bacterial burden of most foods is controllable through good manufacturing practices, we believe that microbial content in most processed foods is a criterion of quality. Accordingly, FDA has proposed to incorporate reasonable microbiological limits in U. S. Standards of Quality. This is provided for in Section 401 of the Federal Food, Drug, and Cosmetic Act. A food would be considered misbranded under Section 403 of the Act if the number of the microorganisms exceeded the number specified in the Standard of Quality for that food.

The concept of selected microbiological characteristics as an indicator of an attribute of quality has been applied for years by industry. For example, such things as total plate, *E. coli*, coliform, and *S. aureus* counts as indicators of microbiological quality. These kinds of tests have been done for years by virtually all reputable food processors as an integral part of their quality control programs.

Some confusion has existed as to why our proposed microbiological quality standards do not contain criteria for pathogens. The Federal Food, Drug, and Cosmetic Act states that the presence of a harmful or deleterious substance in foods is prohibited. Thus, if follows that pathogenic microorganisms or their toxins are prohibited. Foods that contain pathogenic microorganisms or toxins are illegal and the people responsible are subject to criminal prosecution. Accordingly, pathogenic organisms or toxins in foods are factors of safety and for our purposes factors of safety are not included in a microbiological quality standard.

Development of a data base. To ascertain what are reasonable standards, we have conducted and are conducting microbiological surveys at the national level. Our published microbiological quality standards for frozen cream pies and gelatin are based upon data obtained from samples of all manufacturers known to be shipping their product in interstate commerce at the time of the survey. Our current surveys are done by a statistically based sampling plan. Generally, this calls for sample gathering from five grocery stores in each of 64 standard metropolitan statistical areas. Five samples of the food are taken from each store to give a total of 1,600 units of product.

Generally, our analytical program includes total aerobic plate, coliform, *E. coli* and *S. aureus* counts. For total aerobic plate count, we have been using two incubation temperatures for some products, namely 35 and 30°C. We believe that these data will be helpful in establishing the usefulness of 30°C as an incubation temperature for aerobic plate count of some foods, as well as in enabling us to compare our data with proposed microbiological standards that are being developed internationally.

To date we have completed surveys of 19 foods and 34 are currently in progress (Table 1).

Development and publication of a standard. Once the data base for a microbiological standard is completed, these data are analyzed and reviewed, and a tentative standard is identified through an iterative process. Operational characteristic curves are constructed, and consumer and producer risks are identified. For example, for frozen pies and gelatin, an operational characteristic curve was computed for aerobic plate count and another curve was computed for coliforms. Since each lot must pass both an aerobic plate count and a coliform standard, the overall probability of lot acceptance is the probability that the sample

Table 1. Microbiological Surveys Completed or in Progress

Frozen products completed
- Breaded scallops
- Fish sticks
- Fish cakes
- Crab cakes
- Breaded clams
- Breaded shrimp
- Breaded haddock
- Dungeness crab
- Fish-and-chip dinners
- Cream-type pies——
  - banana, coconut, lemon, chocolate—frozen

Other products completed
- Gelatin
- Cocoa
- Cream or custard-filled cakes
- Refrigerated biscuit dough
- Infant dry formulae
- Fresh oysters

In progress
- Macaroni with and without egg
- Frozen uncooked and cooked shrimp
- Frozen lobster tail
- Black pepper
- Oregano
- Rosemary
- Celery seed
- Thyme
- Potatoes——
  - frozen, raw french fried or shoestring
  - frozen, pre-cooked hash browns
  - dried mashed potato or potato buds
  - dried hash browns
- Onion rings
- Spices——
  - cinnamon
  - nutmeg
  - paprika
  - ginger
  - cloves
- Shrimp——frozen, in shell
- Shellfish——soft shell clams, hard shell clams
- Bluecrab meat, fresh
- Potato Salad——refrigerated
- Tuna pot pies——frozen
- Frozen green peas
- Frozen corn
- Frozen cauliflower
- Meat extender——unseasoned, seasoned
- Frozen dessert topping
- Coffee creamer
- Instant chocolate drink

geometric mean of 10 analytical units does not exceed the aerobic plate count standard times the probability that the geometric mean coliform count does not exceed that standard. Probabilities of lot acceptance related to true lot quality for frozen cream pies and gelatin are shown in Tables 2 and 3. As a further

Table 2. Cream-type Pies——Probability of Lot Acceptance[a]

APC/g[b]	Coliforms/g[b]		
	10	30	60
10,000	>0.9999	0.9736	0.2653
30,000	0.9991	0.9727	0.2651
60,000	0.1322	0.1287	0.0351

[a] Based upon a standard of APC≤50,000/g and coliforms ≤50/g.
[b] All values are the geometric mean of 10 analytical units.

Table 3. Gelatin——Probability of Lot Acceptance[a]

APC/g[b]	Coliforms/g[b]		
	4	8	12
1000	>0.9999	0.8337	0.2142
2000	0.9981	0.8321	0.2138
4000	0.0202	0.0168	0.0043

[a] Based upon a standard of APC≤3000/g and coliform ≤10/g.
[b] Geometric mean of 10 analytical units.

example, if the true mean of the coliform count is 30/g for frozen cream pies and the true aerobic plate count mean is 60,000/g, then the overall probability of lot acceptance is about 0.13.

Once a proposed standard is developed, it is published in the Federal Register as a proposal with provision for a comment period. The comments received are reviewed and each is responded to through subsequent publication in the Federal Register. Changes are made in the proposed standards as appropriate. Microbiological quality standards have been published for frozen banana, coconut, chocolate, and lemon cream type pies, and for gelatin and bottled water. The microbiological quality standards for these products are shown in

Table 4. Frozen Cream-Type Pies and Gelatin Microbiological Quality Standards

	Frozen[a,b] Cream Pies	Gelatin[b]
Aerobic plate count	≤50,000/g	≤3000/g
Coliforms	≤50/g	≤10/g

[a] Applies only to banana, coconut, lemon, and chocolate cream-type pies.

[b] Geometric mean of 10 analytical units.

Table 5. Bottled Water Microbiological Quality Standards

Multiple tube method
a) 9 of 10 analytical units <2.2 coliforms/100 ml; and
b) No analytical unit >9.2 coliforms/100 ml
Membrane filter method
a) 9 of 10 analytical units <4.0 coliforms/100 ml; and
b) Arithmetic mean of all analytical units shall not exceed 1 per 100 ml

Tables 4 and 5. As new standards are developed, they will be published as proposals in the Federal Register.

Enforcement. A food that contains numbers of microorganisms in excess of the microbiological quality standard requires substandard quality labeling. The general statement used for foods of substandard quality provided for in the regulations ("Below Standard in Quality——Good Food Not High Grade") is not appropriate for microbiological quality since the consumer usually cannot see this attribute of quality. Therefore, to promote honesty and fair dealing in the interest of the consumer a more specific statement is needed and is required. This is "Below Standard in Quality——Contains Excessive Bacteria."

The production of food within the microbiological limits identified by the standard does not substitute for other requirements of the Federal Food, Drug and Cosmetic Act. As an example, a Section of the Act requires that food not be prepared, packed, or held under insanitary conditions. Similarly, the law requires that food manufacturers must observe current good manufacturing practices. Violation of these requirements makes the food adulterated and therefore the introduction of the food into interstate commerce is a prohibited act even though the food may meet the microbiological quality standard.

Updating microbiological quality standards. If microbiological quality standards are to remain meaningful, provision must be made for review and updating of these standards. We plan to do this primarily through two mechanisms: 1) solicitation of data from the food industry, from state and local governments, and from other organizations concerned with the microbiology of foods, and 2) from statistically designed surveys.

Present status of FDA proposals. Our proposed microbiological quality standards have been challenged in the sense that requests for a hearing have been received from several affected organizations. In other words, through legal procedure we have been requested to justify the legal basis for establishment of microbiological quality standards. Until this matter is resolved FDA will not propose additional standards and any enforcement action relative to the previously proposed standards for cream type pies and gelatin will be held in abeyance.

Introduction of Microbiological Specifications into the Codex Alimentarius Standards Program

At the UN Conference on the Human Environment held in Stockholm in 1972, one of the resolutions passed recommended increased support to Codex Alimentarius in developing international standards for pollutants in food*[1]. This recommendation and other considerations led to the convening of a Joint FAO/WHO Expert Consultation on Microbiological Specifications for Foods. Its purpose was to assess the work already done by various bodies in the field of food microbiology and to make recommendations to the Codex Alimentarius Commission as to international microbiological

*[1] Resolution 82 of the UN Conference on the Human Environment "It is recommended that increased support be given to the Codex Alimentarius Commission to develop international standards for pollutants in food and a Code of Ethics for international food trade, and that the capabilities of the Food and Agriculture Organization of the United Nations and the World Health Organization in the field of food control be increased."

specifications for various foods. The Consultation would deal primarily with foods in international trade which represent microbiological hazards, related microorganisms, methods of sampling and examination, and microbiological end-product specifications.

So far, in the field of food hygiene, the Codex Alimentarius Commission has confined itself largely to the elaboration of codes of hygienic practice for various groups of foods. This work is done by its subsidiary bodies, mainly by the Codex Committee on Food Hygiene. This Committee has now commenced work on microbiological specifications for individual foods. A number of Commodity Committees have also begun to consider microbiological aspects of their work which will be reconsidered at a later stage by the Food Hygiene Committee. However, the controversial nature of microbiological sampling plans, methods of analysis and limits calls for recommendations being obtained at an independent expert level and followed by consideration of such recommendations by the Food Hygiene Committee and other concerned subsidiary bodies of the Codex Alimentarius Commission. The convening of regular meetings of experts concerning the microbiological aspects of food should enable the best scientific opinion to be made available directly to governments, at the same time providing a sound basis for intergovernmental consideration within the Joint FAO/WHO Food Standards Program, with a view to reaching agreement on microbiological specifications for foods.

The first Joint FAO/WHO Expert Consultation on Microbiological Specifications for Foods met in Geneva, Switzerland, April 7-11, 1975*[2].

The Consultation considered that the following foods deserved particular attention with respect to the formulation of microbiological specifications:

(1) *Vegetable products*

(a) *Cereal grains and flours* under inadequate dry storage support mold growth and hence the possibility of mycotoxin formation.

(b) *Cocoa products and chocolate* have occasionally been found to be contaminated with salmonellae.

(2) *Animal products*

(a) *Meats including poultry* are frequently vehicles for the dissemination of salmonellae. Although international trade in frozen meat is greatly impeded by the presence of agents of epizootic disease, the presence and significance of zoonotic agents such as salmonellae should not be overlooked.

(b) *Dairy products.* Dried milk products, although prepared from pasteurized milk, have not uncommonly contained *Staphylococcus aureus* or salmonellae. Cheese may on occasion contain toxigenic or pathogenic bacteria, and staphylococcal enterotoxin and enteropathogenic *Escherichia coli* have been of particular concern.

(c) *Egg products*, both dried and frozen, even when pasteurized, are frequently contaminated with salmonellae.

(d) *Pre-cooked, frozen seafoods*, particularly crustaceans, are of considerable microbiological concern. Contamination with salmonellae, staphylococci and *Vibrio* parahaemolyticus is common in some areas of the world.

(3) *Mixed products*

(a) *Infant foods* and foods for special dietary uses require very high levels of microbiological purity. Depending upon the nature of the food, microbiological specifications might be required for a number of pathogenic microorganisms.

The Codex Commission has not yet decided which cereals or cereal products need to be standardized. Cocoa products and chocolate are minor commodities compared with most others listed here. The range of cheese types makes standardization difficult at this time. Infant foods are already the subject of draft microbiological limits under process within the Codex Alimentarius.

The Consultation decided that a high priority be given to foods in the remaining categories, *viz.*, egg products, non-fat dried milk, precooked frozen seafoods and frozen meat, and considered that initially discussion should be confined to the first three, *i.e.*, the processed foods.

*[2] Report of the Joint FAO/WHO Expert Consultation on Microbiological Specifications for Foods, April 7-11, 1975. Geneva, Switzerland.

A series of general and specific recommendations were made by the Consultation. Particularly germane to this discussion was the recommendation of microbiological specifications for dried and frozen eggs for submission to the Codex Food Hygiene Committee. These specifications included sampling plans, methods and microbiological limits for the aerobic plate count, coliform count and the presence of salmonellae. Also included was a specification for salmonellae for other egg products. The proposed sampling plans and microbiological limits are as follows:

Dried and Frozen Whole Eggs

Salmonellae: *Salmonella* organisms should not be recovered from any of ten sample units examined when the test is carried out according to the method described. (n=10, c=0, m=0). In products intended for special dietary purposes, *Salmonella* organisms should not be recovered from any of thirty sample units examined (n=30, c=0, m=0).

Mesophilic aerobic bacteria: Mesophilic aerobic bacteria should not be recovered from any of five sample units examined when the test is carried out according to the method described in a number exceeding one million per gram, nor in a number exceeding 50,000 per gram from three or more of the five sample units examined. (n=5, c=2, m=$\times 10^4$, M=10^6).

Coliform bacteria: Coliform bacteria should not be recovered from any of five sample units examined, when the test is carried out according to the method described, in a number exceeding 1,000 per gram, nor in a number exceeding ten per gram from three or more of the five sample units examined. (n=5, c=2, m=10, M=10^3).

Other Egg Products

Salmonellae: *Salmonella* organisms should not be recovered from any of ten sample units examined when the test is carried out according to the method described. (n=10, c=0, m=0).

In products intended for special dietary purposes, *Salmonella* organisms should not be recovered from any of thirty sample units examined (n=30, c=0, m=0).

It is noteworthy that these are the first microbiological specifications that have been proposed by the Codex Food Hygiene Committee for inclusion in a Code of Hygienic Practice for a food or food commodity group. In all probability others will follow.

Extraneous Materials in Foods

In March 1972 the Food and Drug Administration published a document entitled "Current Levels for Natural or Unavoidable Defects in Foods for Human Use that Present No Health Hazard". In essence this document lists tolerances for various kinds of physical, biological and miscellaneous extraneous materials in foods. These tolerances are commonly referred to as Defect Action Levels (DAL). These extraneous materials consist of any foreign matter in a food that is associated with objectionable conditions or practices in production, storage, or distribution; included are decomposed material (decayed tissues due to microbial, parasitic or non-parasitic causes), miscellaneous matter such as sand, soil, glass, rust particles, etc., and filth*3. Amounts present in a food in excess of the DAL's listed constitutes the basis for legal action, *i.e.*, seizure of the product, an injunction or criminal prosecution.

Administrative guidelines for extraneous materials had been available to the field districts of the Food and Drug Administration for many years. These guidelines consisted of analytical criteria or limits based upon what was attainable when good manufacturing practices were observed. These limits were supported by one or more data bases consisting of studies correlating laboratory findings with factory conditions, extensive laboratory data on market samples, or years of regulatory experience associating laboratory findings with inspectional evidence of routes of contamination with filth or decomposition. The DAL listing was a blanket transposition of all objective analytical limits in the administrative guidelines. The listing includes a total of 77 individual products and commodity groups and

*3 Filth is the term having specific legal implication and consists of objectionable matter contributed by animal contamination of product such as rodent, insect parasite or bird matter (feces, hair, insect parts, etc.); or any other objectionable matter attributable to insanitary conditions.

identifies 199 specific defect levels for these products.

Five revisions of the DAL's have issued since the initial publication of March 30, 1972. The 5 revisions have contained changes in 25 specific defect levels involving 20 products. Twenty of the changes announced a lowering of maximum permissible levels requiring stricter controls for certain defects.

The DAL listing has been and is under continuous review on a product priority basis since its original publication. Data are being continually generated through surveys of the various products. Some of these surveys are being done by contract. Products that are under survey are listed in Table 6.

Table 6. Products Under Survey for Defect Action Levels

Wheat	Pineapple juice
Macaroni products	Cranberry sauce
Chocolate	Canned mushrooms
Cocoa	Canned peaches
Fig paste	Unpopped popcorn
Walnuts	Canned pineapple
Pecans	Tomato sauce
Brazil nuts	Tomato soup
Figs	Tomato juice, paste and puree
Products planned for in near future:	
Canned crabmeat	Spices
Canned tuna	Pickles and pickle relish
Canned sardines	

Methods Development and Standardization

For almost a century——beginning shortly after the work in Koch's laboratories in 1881-82 that laid the foundation for quantitative microbiology——work has steadily progressed toward developing and standardizing methods for the microbiological analysis of foods. As a consequence, we have methods that are reasonably accurate, sensitive, and capable of being used by well-trained analysts in properly equipped laboratories. However, there is need for additional or improved methods, especially for selective quantitative enumeration of certain groups and species of bacteria. Also, there is equal, if not greater, need for standardization of methods. One of the most important single factors contributing to minimum intra- and inter-laboratory variation in analytical results is use of standardized methods; in other words, methods that are adequate and which are universally used for the purpose that the method was designed to serve. If such methods are available and used in this manner variations in results between analysts and between laboratories are minimized. Minimum intra- and inter-laboratory variation is necessary because vital decisions based on analytical results are continually being made; for example: (a) judgements as to regulatory compliance of the output of a single food processor or manufacturer may be determined by analytical results from several laboratories located within any one country or in several countries; and (b) false or misleading results can cause destruction of a wholesome product (an injustice), or the movement of contaminated product to the consumer (a serious danger to health). Currently, much effort is being expended in the United States and internationally in developing and standardizing microbiological methods for the examination of foods.

International interest in methods development and standardization has been greatly stimulated by the Codex Food Hygiene Committee which, as previously discussed, has begun to include end product microbiological specifications in Draft Codes of Hygienic Practice for various commodities. Utilization of these specifications will require international agreement and uniform application of appropriate methods for the microbial groups and species included in the specifications.

Several international organizations have become deeply involved in methodology. The Association of Official Analytical Chemists (AOAC) was the first of these. It was organized in 1884. Fundamental in the AOAC program is the system of method validation through interlaboratory collaborative testing to determine the precision with which analysts can perform a method. Numerous microbiological methods applicable to foods have received official sanction through the AOAC and subsequent publication in the Official Methods of Analysis of the AOAC. The first

methods published were for examination of eggs. These appeared in 1939; the most recent is the method for viruses in foods which appeared in 1975. AOAC methods are utilized by those U.S. government agencies which must prove their allegations to a court of law when called upon to do so.

The International Dairy Federation (IDF) has published several methods as standards which are applicable to various dairy products. Although their interests are quite specialized, the pioneering work of IDF at the international level has been significant since their effort in methods development and application was a forerunner to similar efforts undertaken subsequently by other organizations.

The International Commission on Microbiological Specifications for Foods (ICMSF), a standing Commission of the International Association of Microbiological Societies, has been addressing itself to the problem of uniform methodology in microbiology from various viewpoints. ICMSF was formed in 1962 in response to the need for internationally acceptable and authoritative decisions on microbiological limits for foods commensurate with public health safety and quality, and particularly for foods in international commerce. Prerequisite to fulfilling this need are internationally acceptable methods for enumeration of the significant microbial species and groups. Accordingly, ICMSF initiated the preparation of a book which would contain a compendium of microbiological methods for the enumeration of the more important organisms of foodborne illness and certain of the microbial indicator species and groups, and which would contain discussions of the significance of these organisms in foods. The book was published in 1968*[4] and the second edition currently in preparation is scheduled for publication in 1976.

A second book was published by ICMSF in 1974.*[5] The subject matter is divided into two sections. Part I defines statistical concepts and terms, describes sampling plans available, outlines procedures for selecting the best plan for the purpose intended, and explains the principles of randomization. Part II describes the practical applications of these principles and includes recommended microbiological limits for various foods.

Additionally, ICMSF has sponsored and financially supported a series of comparative and collaborative studies of methods as bases for selection of the most appropriate method to recommend for use and to include in their publications. In this work close liaison with AOAC has been maintained.

More recently the International Standards Organization (ISO), in which 70 countries have official member status, initiated a program for selection of methods for microbiological examination of foods. The work of ISO is accomplished through a series of technical committees and subcommittees. In 1974 Subcommittee 9 (microbiology) of Technical Committee 34 (Agriculture and Food Products) was established to deal specifically with microbiological methods for foods. This work has been in the province of commodity subcommittees (*e.g.*, SC6—Meat and Meat Products). However, most microbiological methods are applicable to various foods. With few exceptions, they differ only in the manner of sample preparation prior to analysis. While not yet clearly delineated, the work of Subcommittee 9 will likely be similar to that done previously by the Commodity Subcommittees and should avoid duplication of effort. Any needs unique to a particular commodity undoubtedly would be referred to an appropriate commodity subcommittee. In any event, close coordination between Subcommittee 9 and concerned commodity subcommittees is necessary.

The role of ISO in microbiology methodology is viewed by many as critical. For example, the Codex Food Hygiene Committee and other concerned Codex subsidiary bodies undoubtedly will look to ISO for appropriate methods to include in various end-product microbiological specifications. ISO depends upon "consensus" as the basis for reaching decisions on methods to be accepted as standards. In contrast, AOAC and, to an increasing extent, ICMSF require

*[4] Microorganisms in Foods: Their significance and methods of enumeration. F.S. Thatcher and D.S. Clark, *Eds.*, University of Toronto Press, Toronto, Canada.

*[5] Microorganisms in Foods II. Sampling for microbiological analysis: Principles and specific applications. ICMSF., University of Toronto Press, Toronto, Canada.

comparative and collaborative studies to validate methods before their consideration of them for adoption. Thus, it is most important for ISO to have input from these two organizations relative to methods under consideration. Effective coordination between these organizations is essential.

Much already has been accomplished along these lines. For example, ISO has accepted liaison representatives from AOAC and ICMSF in "Observer" status at subcommittee meetings. Furthermore, individuals who are members of ICMSF and AOAC also actively participate in related work of ISO subcommittees. In the United States we have coordinated the methods requirements of government agencies in relation to the international organizations. This is being done through a Committee representing the four agencies concerned with microbiological methods for foods. Thus we are now able to speak with a single voice on such matters as review and comment on proposed methods. Also, IDF has taken an important step. On the suggestion of the General Secretariat, the IDF General Assembly in New Delhi in 1974 adopted a resolution urging IDF "to take the initiative in promoting closer cooperation between international organizations engaged in the development of standards for the chemical/bacteriological analysis of dairy products." In response to this resolution a Joint IDF/ISO/AOAC "Bacteriological Week" will be held April 5-8, 1976 in Brussels. This meeting will bring together all of the existing Joint IDF/ISO/AOAC Expert Groups. These groups have been quite successful in the coordination of efforts in the area of chemical methodology but less so in microbiology. Thus the purposes of the meeting include (a) review of the program of IDF in the development of bacteriological methods and to assess or reassess priorities in this field; and (b) assessement of bacteriological methods for dairy products in the broader context of food microbiology. It is expected that results of this meeting will serve materially in closing the "coordination gap" in the activities of the principal international organizations in the field of development and standardization of microbiological metods for foods.

Sampling and Testing Plans and Acceptance Criteria for Salmonellae in Foods

Salmonellosis remains as one of the most important communicable disease problems in many parts of the world. The FDA considers food containing salmonellae as adulterated. Accordingly, the sale of foods containing salmonellae cannot be condoned. However, it must be recognized that various foods present different degrees of hazard. Furthermore, salmonellae can be found in many foods if a sufficient number of tests are made. Surveillance programs that involve testing "lots" of food require that a decision be made as to when a food "lot" shall be rejected or accepted. When should the testing of a lot stop and a decision be rendered? To avoid confusion and uncertainty in this regard FDA has specified sampling and testing plans for the detection of salmonellae which serve as a basis for rejection or acceptance of food "lots". Recommendations of two committees have served as the basis for these sampling and testing plans.[4),5)]

Briefly, three product characteristics are used to judge the degree of potential health hazard that a food presents: (a) the product contains an ingredient that has been identified as a significant potential factor in salmonellosis, in other words, a sensitive ingredient; (b) the manufacturing process does not include a controlled step that would destroy salmonellae; and (c) there is substantial likelihood of microbiological growth if the product is mishandled or abused in distribution or consumer usage.

These three hazard characteristics can be combined into eight different configurations. This is illustrated in Table 7. Categories are defined as follows:

Category I——foods that are intended for infants, aged and the infirm, and that contain a sensitive ingredient; thus, not all foods consumed by these groups are included, but rather those with a significant *Salmonella* hazard.

Category II——foods with all three hazard characteristics (sensitive ingredient, no destructive treatment, abuse potential).

Category III——foods with two hazard

Table 7. Categories of Food Products Based on Product Hazard Characteristics

Type of food	Hazard characteristic[a]			Category
	A	B	C	
Intended for infants, aged, and infirm	+	+ or 0	+ or 0	I
Intended for general use	+	+	+	II
	+	+	0	III
	+	0	+	III
	0	+	+	III
	+	0	0	IV
	0	+	0	IV
	0	0	+	IV
	0	0	0	V

[a] See text for explanation of hazard characteristics A, B & C.
+=hazard present; 0=hazard not present

Table 8. Number of Analytical Units or Composite Units Tested & Criteria of Acceptance for Each Product Category

Product category	Anal. units	Total wt. tested	Composite units[a]	No. +[b]	Concn. of Salmonellae
I	60	1500 g	4	0	≥1/500 g
II	30	750 g	2	0	≥1/250 g
III-V	15	375 g	1	0	>1/125 g

[a] 375 g each. [b] criteria for FDA acceptance.

characteristics.

Category IV——foods with one hazard characteristic.

Category V——foods with none of these hazard characteristics.

Table 8 illustrates the testing and acceptance criteria we have adopted. Note the three different testing levels——one for each product category. The objective here was to have a sampling and testing plan that would provide adequate assurance that the number of salmonellae present, if any, is below a statistically defined limit that offers minimal hazard to the consumer. This limit is as given in the last column of Table 8, *i.e.*, if the test results provide 95% confidence that the salmonellae contamination level, if any, is no more than 1 in 500 g for Category I; no more than 1 in 250 g for Category II; and no more than 1 in the 125 g for Categories III, IV and V.

The appropriate sampling and testing plan is to be used in arriving at a final decision whether to accept or reject a particular lot in question.

Table 9. Definitions

Sample:	A series of sample units
Sample unit:	100 g minimum
Analytical unit:	25 g from sample unit
Composite unit:	375 g (15 Analytical units)

Table 9 gives the definition of several pertinent terms.

A sampled lot is acceptable only if analyses of all analytical units or composite units are negative for salmonellae. Analytical units may be composited. The maximum size of a composite unit is 375 g. The composite unit must consist of a series of 25 g analytical units, the maximum size of the composite being 375 g or fifteen 25 g analytical units. The advantage of compositing analytical units is obvious. To do so, however, requires that no significant decrease in sensitivity of the test for salmonellae would result. We are satisfied that this is the case. There are several published reports that support this position.

The acceptance criterion indicated above is applicable to any lot of product tested in con-

nection with any of our surveillance or compliance programs.

Penicillin in Milk Supplies

Milk and milk products containing penicillin may present a significant hazard to the health of individuals who are hypersensitive to penicillin. The domestic milk supply of many countries, is supplemented significantly by imports, usually nonfat dry milk. Milk from both sources may be contaminated with penicillin. Several facets of this problem will be discussed briefly including, (a) human hypersensitivity to penicillin, (b) the mechanism by which milk becomes contaminated with penicillin, (c) methods of analysis and acceptance criteria, (d) disposal or use of contaminated products, and (e) assurance that imports do not add to the problem.

Human Hypersensitivity to Penicillin

Among the more common hypersensitivity drug reactions (allergic) in man are those caused by penicillin. Approximately 10% of all individuals who receive repeated doses of the antibiotic can become so highly sensitized that a single and very small amount may elicit hypersensitive reactions manifested by several physical signs and symptoms. The empirical clinical use of penicillin during the past 30 years has resulted in a sensitized population of unknown proportions. Stewart and McGovern[10] reviewed the pertinent literature in 1965 and again in 1970 and concluded that it is not possible to determine a true figure of incidence but that it lies between 1 and 10%. In 1973 Stewart[9] further stated that nothing had been found in the intervening period to contradict that estimate. In 1959 Welch[12] had estimated the hypersensitive population in the U.S. to be 17 to 20 million individuals (based on a population of over 175 million at that time).

Penicillin hypersensitivity can be induced in two ways: (a) the immediate type involving reactions of humoral tissue with a specific antigen such as the penicilloyl-protein conjugates formed in tissues following intramuscular injection and the oral administration of the antibiotic, and possibly the ingestion of certain foods such as milk and milk products, and (b) the delayed type which is a form of immunologic response that is mediated by sensitized lymphoid cells rather than by humoral tissue. This type of sensitization can be the consequence of long exposure to and contact with penicillin not therapeutically administered,[8] *e.g.*, penicillin production plant workers, nurses and pharmacists. Similarly, the dermatophyte *Trichophyton*, an etiologic agent of cutaneous mycosis, and other ubiquitous fungi produce penicillin-like molecules which may also sensitize an individual who never received penicillin therapeutically.[8] It is conceivable that long term ingestion of milk containing low levels of penicillin could also sensitize in this way. However, regardless of the kind of exposure, the reactions are varied: mild skin rashes, often urticarial, to severe generalized urticaria, edema, anaphylactic reactions and sudden death. The most severe and critical reactions are caused by the parenteral application of this antibiotic in therapeutic use.

Anaphylactic reactions as a result of oral administration were at one time considered to be less severe and never fatal. Recently, however, it has been observed that serious reactions following the ingestion of penicillin can lead to serum sickness, the Arthus syndrome (humoral antigen-antibody complexes resulting in vascular injury) and death. In 1971 Spark[8] reported four fatal anaphylactic reactions following the therapeutic administration of oral penicillin. Three of the individuals had received prior penicillin therapy; similar information was not available for the fourth victim. The interval between ingestion and death in each case was 30 min to within 1.5 hr. All fatalities were the consequence of a single oral dose of between 400,000 and 1 million units.

Literature is sparse regarding reactions attributed to milk and milk products and those reported have been of the urticarial type. Generally these reactions occur in individuals who have been sensitized by therapeutic applications. Vickers, *et al.*,[11] reported one such case from England involving a woman who drank a liter of milk each day. Since the milk produced at her farm contained 4 units per *ml*, she may have ingested up to 4000 units

of penicillin daily; she was sensitive to 4 units of penicillin G intramuscularly. Therefore, the injection of 4 units would yield about 0.000013 unit per *ml* of blood. Some hypersensitized individuals have been reported to react severely to an intradermal injection of 0.000003 unit of the drug.[2] Similarly, Zimmerman[13] reported on 4 cases of chronic urticaria associated with the ingestion of dairy products. In each case the reactions cleared rapidly after the intramuscular injection of 800,000 units of neutrapen (penicillinase) and the subjects remained urticaria-free when dairy products were eliminated from the diet. However, the patients could include these products in their diets without subsequent allergic reactions when neutrapen was administered prophylactically prior to ingestion.

Later Rosanove,[7] with reference to Zimmerman's study, reported his observations of a patient at the Mayo Clinic who had a persistent oral and cutaneous blister-like eruption that clinically and histopathologically resembled pemphigus vulgaris (lesions of the mucocutaneous surfaces). This condition disappeared without specific treatment when milk and other dairy products were eliminated from the diet.

While it is clear that consumption of milk containing penicillin will elicit allergic reactions in the hypersensitive individual, there is no documented evidence that the consumption of milk or milk products containing penicillin can alone induce the hypersensitive state.

The outstanding clinical feature of penicillin hypersensitivity is its unpredictability. Paradoxically, it is not dose related: large doses may be well tolerated while small skin test doses may cause severe local or generalized reactions. No less paradoxical is the fact that one of the least toxic of all antibiotics has proven to be the most highly allergenic. It is suspected that individuals become hypersensitive to penicillenic acid derived from the 6-aminopenicillenic acid nucleus. Though not yet proved, this could well indicate that sensitization by one penicillin can and does result in hypersensitivity to other penicillins derived either fermentatively or bio-synthetically.

Intramammary Infusion of Penicillin

The presence of penicillin in milk and milk products occursalmost exclusively through the therapeutic treatment of mastitis in lactating or dry cows with penicillin. The principal method of administering the drug is by intramammary infusion.

In the United States there are numerous intramammary infusion products containing certifiable antibiotics intended for use in treating mastitis in milk producing animals. With few exceptions, penicillin is one of the antibiotic components of such preparations. These products are available as single entity antibiotics or antibiotics in combination with other drugs. Furthermore, some are over-the-counter and others are prescription-type drugs. In any event, for each there is specified the appropriate dosage, condition of use (*i.e.*, for lactating or dry cow therapy or both) and the withdrawal time (*i.e.*, the period of time after last administration of the drug that must elapse before either the milk or the meat may be used). As a practical matter, relative to minimizing the presence of penicillin in milk, it is most important to strictly adhere to the withdrawal time indicated for each drug. For lactating animals this ranges from 36 to 96 hr depending on the drug used. To date, these withdrawal periods have been based on residue studies that indicate the period necessary to assure the absence of detectable residues in the milk. It is conceivable that with the development of penicillin detection methods of greater sensitivity, withdrawal times currently designated may be modified. This matter is only one of several presently receiving attention by the FDA in the interest of more effective regulation of drugs intended for use in treating mastitis. In the final analysis, in the case of lactating animals, it is the person who performs the milking who determines, in fact, whether or not the appropriate withdrawal time is observed. Accordingly, the mechanism of enforcing adherence to a specified withdrawal time resolves itself largely to a combination of (a) "friendly persuasion" of the person who controls the milking operation, and (b) the use of an analytical method for assaying the presence of the drug in milk offered for sale.

FDA has established a tolerance of zero for penicillin in milk. In enforcing the regulation,

Table 10. Acceptance Criteria for Penicillin Residues in Dairy Products

Product	Sample dil.	Dil. factor	Method	Accept. criteria[a] (Units/ml or G of product)
Raw milk (ind. producer)	None	1	DA[b]	<0.05
Raw milk (comingled) Past. milk Buttermilk	None	1	CC[c]	<0.01
Cond. conc. evap.	1+1	2	CC	<0.02
Cheese, butter, ice cream	1+1	5	CC	<0.05
Dried milks	1+3	4	CC	<0.04

[a] See text. [b] "Disc assay". [c] "Cylinder cup"

we have taken the position that through use of a specified method of analysis, the finding of no detectable amount of penicillin in milk and milk products and in any milk containing processed food will be considered as in compliance with the specified zero tolerance for the respective product. Two methods of analysis have been approved for use; however, specific conditions governing the use of each method have been outlined.

Methods of Analysis and Criteria of Acceptance Based on Analytical Findings

The first of these is the Disk Assay Method-A as described in "Standard Methods,"[1] second, the *Sarcina lutea* cylinder cup method.[6] Table 10 lists several dairy products with indicated detection levels likely to be acceptable as based upon the sensitivity of the analytical method specified.

Studies have shown that the sensitivity in terms of units of penicillin per milliliter of suspension used in testing is approximately 0.01 unit for the "cylinder cup" method and 0.05 unit for the "disc assay" method. The sensitivities will vary slightly under normal usage of the methods. Accordingly when analyses are made to establish or support a legal action, the sensitivity of the method is established in each instance of use of the test method. Thus, for example, the acceptance criterion for a particular lot of dried milk could conceivably be less than a value somewhat below or above 0.04 units per gram of the product.

Disposal of Milk and Milk Products Containing Penicillin

FDA has taken the position that any manufactured product, if found to conform to the acceptance criterion as specified in Table 1, would be considered to be non-actionable, regardless of whether or not the raw material from which it was manufactured contained penicillin. We expect this policy to apply to all readily forseeable situations except in the case of blending if utilized to achieve non-detectable levels of penicillin. Any product of such blending would be considered adulterated. For example, milk containing penicillin could be separated and the cream churned. If the butter, when tested, was below the detectable level, *i.e.*, <0.05 units per g, the butter would be considered non-actionable. Also, dry milk containing penicillin may be used as an ingredient in medicated animal feeds containing penicillin provided the quantity of dry milk used in the feed does not result in raising the level of penicillin in the feed above the level approved for the feed. In general, any product resulting from further processing of milk or milk product containing penicillin and found to be below the detectable level of penicillin upon testing would be considered non-actionable; provided, that the further processing would in fact serve to remove or destroy the penicillin originally present (in contrast to merely diluting it) to the extent that the processed product when tested would be below the detectable level.

Dried Milk Products Offered for Import

FDA is charged with the responsibility of examining dried milk products offered for import into the United States. In the interest of fulfilling this responsibility with minimum

expense, we have undertaken the development of dried milk certification agreements with several exporting countries. Currently, agreements have been made with Belgium, Denmark, France, and The Netherlands. These agreements specify processing and quality control procedures including sampling plans and testing methods. Since the initiation of this program in December 1974, we have yet to find the first dried milk product entering with a detectable level of penicillin.

In conclusion, there is no question that penicillin in milk supplies is a serious problem for the dairy industry. It is a problem that originates at the dairy farm. Control measures must begin at that point. Furthermore, any country that imports dried milks might well consider negotiating certification agreements with appropriate dry milk exporting countries.

Surveillance of Import Foods

The FDA must assure the American public that imported foods in interstate commerce are safe, sanitary and properly labeled. Criteria used for these purposes are the same as those used to judge domestic foods with the exception that FDA does not inspect foreign plants. Rather, we depend largely upon inspection and analysis of foods offered at import on a case by case basis. For several reasons we consider this procedure inefficient and lacking considerably in effectiveness. For example, every "lot" of product presented for import cannot be examined simply because of lack of sufficient manpower and facilities to do so. Also, our knowledge of defects to which a particular food product may be susceptible often is lacking. This limits the likelihood of applying the most suitable methods to the testing of the product. Furthermore, in most instances, foreign producers or shippers have not designed their production processes and quality control systems to assure that their products meet our criteria of safety and quality.

To improve our surveillance over imported foods FDA has taken two approaches: first, we have provided technical assistance to foreign countries to improve their capability to provide satisfactory products to the U.S.; and second, we have negotiated agreements, certain of them formalized through "Memoranda of Understanding," with several countries for source certification of certain foods.

The first of these approaches may be illustrated by our work with the governments of Morocco, Turkey, Egypt and Indonesia in improving the sanitary quality of spices and other food products these countries export to the United State.

Our cooperative efforts with exporting countries have taken the form of visits to these countries by our technical people. These visits involve direct exchange of information between our scientists and personnel from industry and government of the foreign country; for example: explanation of the legal requirements imposed by the federal Food, Drug, and Cosmetic Act; discussions generated from joint plant inspections and direct observations relative to the adequacy of sampling and laboratory control procedures to assure compliance with the law; and indigenous sanitary aspects of production, processing, storage and distribution of a food product that may bear upon its safety, quality and integrity from the standpoint of regulatory compliance. Further, practices in the United States which have been successful in protecting food against contamination at the various stages of handling are explained and the application of such protective measures to local conditions are explored. Additional technical assistance has taken the form of short-term training of laboratory personnel within the country as well as more extensive training by arranging for foreign scientists to work at the Food and Drug Administration laboratories. Examples of such training are our efforts covering unique problems and specialized methods relative to mycotoxins and filth and decomposition in foods.

The second approach to improve our surveillance over imported foods is through negotiation of a Memorandum of Understanding (MOU) with an exporting country. A MOU is an agreement between FDA and its foreign counterpart agency upon the acceptable characteristics of a product, the kinds of controls the foreign agency must exercise to assure itself that the product meets the characteristics agreed upon, and the monitoring procedures FDA will use to assure itself that the terms

of the agreement are being met. Each product covered by a MOU will be certified by the foreign agency that the product meets the terms of the agreement or MOU. We speak of this as "source certification" of import foods. The advantages of participating in source certification agreements are largely twofold, namely, reduced time for processing the entry of products into the U.S. and reduced costs for the shipper as well as the U.S. These advantages accrue for several reasons. For example, entries are sampled on a predictable basis since the monitoring procedures are standardized, *i.e.*, based on known sampling and testing plans; fewer entries are detained or refused entry since the likelihood of defective entries is much reduced due principally to the surveillance by the exporting country; and transportation or freight costs are reduced due to fewer instances of products having to be returned or shipped elsewhere.

We have source certification agreements with Belgium, Netherlands and France for non-fat dry milk and with India for frog legs. Also, on October 24, 1962, an agreement was signed between the United States Public Health Service and the Japanese Ministry of Health and Welfare concerning fresh or frozen oysters, clams and mussels intended for shipment from Japan to the United States. Basically, this agreement stated that the Manual of Recommended Practice for the Sanitary Control of the Shellfish Industry approved by the United States Public Health Service and the Canadian Department of National Health and Welfare would henceforth apply to the sanitary regulation of shellfish production and certification of shellfish exporters in Japan, and that the sanitary quality of the shellfish certified for export to the United States would be equivalent to the sanitary quality of shellfish produced in the United States. Therefore, any shipper certified by the Japanese Government would be included in the Interstate Shellfish Shippers List of Certified Shellfish Shippers. The agreement further stated that whenever observations of production areas or processing plants are desired by either government the other government will facilitate such observations.

Currently, we are negotiating source certification agreements with several other countries including New Zealand and Australia (drymilk products); Norway (low acid canned fish and fish products); Brazil (chocolate liquor); Spain Morocco, Bulgaria and Hungary (paprika).

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