

**WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES**

No. 543

Food-Borne Disease : Methods of Sampling and Examination in Surveillance Programmes

Report of a WHO Study Group

This report contains the collective views of
an international group of experts and does not necessarily
represent the decisions or the stated policy of the
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ISBN 92 4 120543 1

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PRINTED IN SWITZERLAND

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**WHO STUDY GROUP ON FOOD-BORNE DISEASE : METHODS OF SAMPLING
AND EXAMINATION IN SURVEILLANCE PROGRAMMES**

Geneva, 17-23 July 1973

Members :

- Professor M. Ingram, Director, Meat Research Institute, Langford, Bristol, England
- Professor E. H. Kampelmacher, Head, Laboratory for Zoonoses and Food Microbiology, National Institute of Public Health, Bilthoven, Netherlands
- Dr J. Menšík, Head, Department of Infectious Diseases, Veterinary Research Institute, Brno, Czechoslovakia
- Dr S. N. Mitra, Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta, India
- Professor H. A. Neshat, Dean, Veterinary Faculty, Teheran University, Iran
- Professor G. Sakaguchi, Department of Veterinary Science, University of Osaka Prefecture, Osaka, Japan
- Professor M. A. Shiffman, School of Public Health, University of North Carolina at Chapel Hill, N.C., USA (*Chairman*)
- Professor N. Skovgaard, Institute of Hygiene and Microbiology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark (*Rapporteur*)
- Dr J. Takács, Head, Central Laboratory, and Director, Veterinary Meat Control Service, Budapest, Hungary (*Vice-Chairman*)
- Dr F. S. Thatcher, Chairman, International Commission on Microbiological Specifications for Foods, Merrickville, Ontario, Canada (*Vice-Chairman*)

Secretariat :

- Dr M. Abdussalam, Chief, Veterinary Public Health, WHO, Geneva, Switzerland
- Dr W. H. Barker, Chief, Enteric Diseases Section, Bacterial Diseases Branch, Epidemiology Program, Center for Disease Control, Atlanta, Ga., USA (*Temporary Adviser*)
- Professor Z. Matyáš, Department of Food Hygiene and Technology, University of Veterinary Medicine, Brno, Czechoslovakia (*Temporary Adviser*)
- Dr L. Reinius, Food Hygienist, Veterinary Public Health, WHO, Geneva, Switzerland (*Secretary*)

FOOD-BORNE DISEASE : METHODS FOR SAMPLING AND EXAMINATION IN SURVEILLANCE PROGRAMMES

Report of a WHO Study Group

A WHO Study Group on Food-Borne Disease met in Geneva from 17 to 23 July 1973 to discuss methods of sampling and examination in surveillance programmes. Dr M. Takabe, Director, Division of Communicable Diseases, opened the meeting and stressed the importance of food hygiene methodology in public health, food control, and food standardization.

INTRODUCTION

Food-borne health hazards have increased as a result of the centralization of food production, the increase in communal eating, and the expansion of international trade and tourism, and there is thus a great need for improved sampling procedures, laboratory methods for the examination of food and for trained personnel to carry out these tests.

Many outbreaks of food-borne disease are not reported and even in outbreaks that are reported the causative agent is often not discovered because of deficiencies in the surveillance system and the lack of suitable laboratory methods. Uniform laboratory methods are also indispensable for the development of microbiological standards for particular food products and in connexion with standards for other disease-producing agents of biological origin such as parasites.

1. MICROBIOLOGICAL FOOD STANDARDS

1.1 General

Since the safety and keeping qualities of foods are related to their microbial content, microbiological criteria¹ have been proposed for a variety of foods but only those for pasteurized milk have been widely adopted.

¹Defined on p. 9.

1.2 The appropriate use of microbiological standards¹

Attempts to establish microbiological food standards have given rise to some controversy. It is argued that numerical microbiological standards are frequently arbitrary and may not be related to the microbiological status of the food. However, experience in a number of countries has shown that the establishment of microbiological standards, even on an arbitrary basis, has led to an improvement in the microbiological status of the food concerned, since processors are stimulated to make improvements in plant sanitation and quality control. Standards can be used to supplement programmes of plant inspection in the development of a comprehensive sanitation programme.

Although counts of "indicator organisms" (see page 18) or total microbial counts in certain foods may not be related to hazards to the consumer or to keeping quality, a satisfactory standard may be established if the specific types of organism that can spoil the particular food, and the prevailing conditions of storage are taken into account.

Although low microbial counts do not guarantee the safety of the food, foods that are consistently within established microbiological standards are more likely to be safe.

Defects in a food processing plant that are missed in a physical inspection may be brought to light as a result of microbiological examinations of the raw or finished products or of the food handling equipment. Even when foods are examined after some has been consumed, general plant defects can frequently be uncovered and further output and consumption of low-quality food prevented.

Standards are especially useful in controlling the quality of foods that are consumed far away from the processing plant. In this situation, the regulatory authority has no opportunity to inspect the plant.

The problem of microbiological standardization was discussed by the WHO Expert Committee on Microbiological Aspects of Food Hygiene with the participation of FAO² in 1967 and two types of microbiological standards were recognized: (1) standards for specific types of pathogen, and (2) standards based on indicator organisms (e.g., *Escherichia coli*) or on total counts of microorganisms.

That Expert Committee strongly supported microbiological testing of food as a valuable means of improving the hygienic quality and safety of foods. Because of the complexity of the matter the Expert Committee recommended that microbiological food standards should be formulated mainly for administrative or advisory use. The Committee recommended that implementation of legal microbiological standards for foods should

¹ See definition on p. 10.

² *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 399.

be restricted to carefully selected cases where such standards serve a clearly defined purpose. The present Study Group endorses these views.

When it is decided to establish a microbiological standard for a food or class of foods, the following technical and administrative aspects must be considered :

(1) The standard should be based on factual studies and serve one or more of the following objectives :

(a) to determine the conditions of hygiene under which the food should be manufactured ;

(b) to minimize the hazards to public health ;

(c) to measure the keeping quality and storage potential of the food.

(2) The standard should be attainable under practicable operating and commercial conditions and should not entail the use of excessive heat treatment or the addition of extra preservatives.

(3) The standard should be determined after investigation of the processing operation.

(4) The standard should be as simple and inexpensive to administer as possible, the number of tests being kept to a minimum.

(5) Details of methods to be used for sampling, examining, and reporting should accompany all published microbiological standards.

(6) In establishing tolerance levels for the permissible number of defective samples, allowance should be made for sampling and other variations due to differences in the laboratory methods.

The following additional points should be kept in mind :

(1) It is not satisfactory to establish one set of microbiological standards for a miscellaneous group of foods, such as "frozen foods" or "precooked foods".

(2) Microbiological standards should be applied first to the more hazardous types of food on the basis of experience of expected microbiological levels, taking into account variations in composition, processing procedures, and storage.

(3) When a standard is established, there should be a definite relationship between the standard and the hazard against which it is meant to protect the public.

(4) The sensitivity, reliability, and reproducibility of the sampling and analytical methods should be compared in different laboratories and the methods to be used should be specified in detail as part of the standard.

(5) Tolerances should be included in the standard to account for inaccuracies of sampling and analysis.

(6) Standards should be applied on a voluntary basis before compliance is made mandatory.

2. RATIONALE FOR STANDARDIZING THE MICROBIOLOGICAL ANALYSIS OF FOODS (WITH PARTICULAR REFERENCE TO BACTERIA AND BACTERIAL TOXINS)

2.1 Objectives

The primary objective of microbiological analysis of foods is protection of the consumer. The microbiological criteria used relate to the different points of interest to the consumer including (a) the actual presence of pathogens or toxins, (b) the possibility that they may be present, and (c) the storage properties of the food itself.

The application of microbiological criteria at all stages of food production, processing, and handling, is becoming increasingly important with the increasing use of processed foods.

Microbiological analysis may be used for the purposes of routine control or for the requirements of a specific investigation. Routine control may be used in several different contexts: (a) for in-plant control, (b) by a national, provincial, or local regulatory agency, and (c) for the purposes of international trade. Difficulties arise if different criteria and/or methods are used in these different contexts. For example, if the in-plant standards used during manufacture are incompatible with those of the regulatory agency, the products may be rejected. Similarly, a country wishing to export food must use the criteria of the recipient country if its products are to be acceptable there. For the purposes of routine control there is a need to standardize criteria and methods as widely as possible.

Epidemiological investigations may be of two kinds. First, they may attempt to relate an undesirable effect (e.g., a particular kind of food poisoning) to a particular kind of food and the way it is used. In order to establish a complete picture, it is usually necessary to compile and evaluate all obtainable data. This evaluation would be of doubtful validity if the methods used had not been standardized. Secondly, investigations are often concerned with identifying the source of a specific outbreak of food-borne disease. For such work, standardized methods are not essential but interpretation of the results is simpler if they are used.

The application of standards was thoroughly discussed in the report of a WHO Expert Committee on Microbiological Aspects of Food Hygiene¹

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 399.

in 1968 and it was concluded that, in general, standards for raw foods offer little or no assurance of their safety for consumption. However, the Committee considered that standards are useful for specific pathogens (e.g., *Salmonella*) when certain foods were identified as important sources of that pathogen. The Study Group endorses these conclusions.

In connexion with the many processed but not sterilized foods, the degree of safety offered by the establishment and use of microbiological standards will vary with the type of food and the method of processing involved. Excellent work has been done on standards for precooked frozen foods. Enzyme tests may be used in addition to microbiological tests to measure processing efficiency, for example in milk and egg pasteurization.

Comparative practical studies are essential if standardized criteria are to be introduced successfully, as the outcome of a microbiological examination depends to a high degree on details of technique. The prescribed standard methods must be followed universally and the reagents, culture media, reference strains, specific toxins, antigens, and antisera must be strictly specified and standardized. Moreover, since the same method may yield different results when used in different laboratories, the training of laboratory workers should be coordinated.

2.2 Application of microbiological criteria

2.2.1 General

In order to standardize the terminology used in connexion with microbiological criteria the International Commission on Microbiological Specifications for Foods has tentatively agreed upon the following definitions:¹

(1) *Microbiological criterion* — a microbiological value (e.g., number of organisms per gram of food) established by use of defined procedures and applied in acceptance sampling of food.

(2) *Microbiological purchasing specification* — the microbiological criterion or criteria that form the conditions of acceptance of a specific food or food ingredient by a food manufacturer or private or public purchasing agency.

(3) *Microbiological limit* — a microbiological criterion recommended by an authoritative body for adoption in specific regions but *not* incorporated into law.

¹ International Commission on Microbiological Specifications for Foods (in preparation) *Microorganisms in foods*. 2. *Sampling for microbiological analysis: principles and specific applications*, Toronto, University of Toronto Press. The definitions are reproduced with slight editorial changes.

(4) *Microbiological standard* — a microbiological criterion, incorporated in a law or regulation, controlling foods produced, processed, or stored in, or imported into, the area of jurisdiction of the regulatory agency.

The above usage is followed in this document.

Microbiological analysis of a particular food might include determination of the total count of indicator organisms, detection of specific pathogens, or more specific examinations, e.g., serological tests.

Such criteria are used to judge the safety of food, and they may differ in different foods and with the age, the physiological and health status of the consumer, and according to the local ecological situation. Microbiological criteria to check the presence or absence of certain microorganisms can be applied easily to processed foods. For heat-treated products standard methods are of the utmost importance. Microbiological criteria are more difficult to apply in relation to raw materials because of the great variety of microorganisms involved. It is nevertheless common practice for manufacturing concerns and certain national agencies to apply purchasing specifications to incoming raw materials across national boundaries.

Standardized methods may be used for “ultimate” or “routine” purposes. An “ultimate” method, in this context, is the method used to compare the sensitivity of the methods proposed for routine use. Such an ultimate method may be relatively laborious and expensive, and hence initially may be unsuitable for routine testing. However, wide usage of such a method may lead to its adoption for routine use. It is desirable that routine methods should be relatively simple and cheap.

2.2.2 *Considerations related to the nature of the food*

In choosing and establishing microbiological criteria for a food, the following factors regarding the nature of the food should be considered:

- (1) The microbiological status of the basic raw ingredients.
- (2) The possibility of the food providing conditions for the multiplication of hazardous microorganisms or the production of their toxins during handling or processing.
- (3) The methods of processing, handling, storage, packaging, and distribution; the time-temperature relationships from production to consumption; and the consequent quantitative and qualitative effects on the microbial flora.
- (4) The epidemiological history of the food as a vehicle of food-borne disease, the potential of the food as a vehicle of food-borne disease, and the sanitation record of the food industry.
- (5) Spoilage problems presented by the food.

2.2.3 Considerations related to local circumstances

The indiscriminate application of the most stringent criteria, which have been designed for the circumstances of greatest hazard, to less hazardous circumstances will lead to rejection of food that would otherwise be satisfactory in the given circumstances. On the other hand, to apply the least stringent criteria, designed for circumstances of lesser hazard, to circumstances of greater hazard would involve an avoidable risk of food poisoning. In any set of conditions, the most appropriate choice of a criterion represents a balance between the risks for the consumers and the producers. The best choice will vary with the local circumstances and will depend on the relative importance attached to the many factors involved. Hence there is a need to establish guidelines for estimating and classifying the degree of hazard.^{1, 2}

In principle, microbiological criteria to protect the consumer's health should be the same all over the world, but, in practice, criteria often have to be related to the local circumstances. Circumstances of food shortage introduce the need for administrative judgments beyond those of normal food control. For example, condemnation of a food that fails to meet international standards might well cause nutritional deficiency or even starvation and create a health hazard greater than a limited risk of food poisoning. In certain regions, some animal infections may be so common that it would be unrealistic to set up criteria requiring the absence of certain microorganisms or parasites. In such cases, it is of great importance that both consumers and kitchen staff should be instructed on the appropriate methods of handling and preparing the foodstuffs concerned.

It is to be expected that food production will increase in some developing countries in the coming decades, and that they will wish to export food. The microbiological methods used in the food industries in the developing countries should be the same as those in the importing countries in order to facilitate international trade. This objective will be facilitated if these methods are standardized and accepted at an international level, i.e., within the FAO/WHO Food Standards Programme. The acceptance of such generally approved methods can also be valuable in protecting consumers at the local and national levels.

2.2.4 Practicability of methods

Often a choice has to be made between analysing a relatively small proportion of food lots by a comparatively sensitive, expensive method and

¹ Committee on Salmonella, National Research Council (1969) *An evaluation of the Salmonella problem*, Washington, D.C., National Academy of Sciences.

² International Commission on Microbiological Specifications for Foods (in preparation) *Microorganisms in foods. 2. Sampling for microbiological analysis: principles and specific applications*, Toronto, University of Toronto Press.

analysing more lots by a cheaper, less sensitive method. The latter choice might well provide greater protection for the consumer.

The application of standard methods on an international scale will increase the number of analyses required and will thus require more analysts, laboratories, and equipment. In countries that export food, it is particularly important that there should be a central laboratory and adequate trained staff. Additional supporting laboratories are required close to the site of production. In connexion with the establishment of laboratories and staff training, the guidance and assistance of international organizations such as FAO and WHO could be of great value. Other consequences of such an increased demand for analysis are discussed in section 2.5.

The developing countries need help from experienced specialists not only in the public health aspects of food control but also in plant sanitation and in plant control procedures. A recommended approach is to concentrate initially on a few food products, with a single plant for each product as a focus for educational efforts and to offer on-site experience. To be successful, such plants must have competent staff and must meet the requirements of design, equipment, and sanitation that are necessary for the production of safe food.

2.3 Choice of methods

2.3.1 General

Microbiological analysis is undertaken to determine the acceptability of a consignment of food. Acceptability is based on the results of different laboratory analyses believed to indicate : (a) effective processing ; (b) manufacture under acceptable conditions of factory sanitation and hygiene ; (c) satisfactory handling and storage after processing ; (d) absence of pathogens under the conditions of the test, absence of pathogens lacking capacity for further extensive spread, or the presence of such pathogens below specified limits ; and (e) absence of toxins to the limit of the analytical method, as in the case of staphylococcal enterotoxins, for example, or the presence of toxins within specified " acceptable " limits, as in the case of mycotoxins.

2.3.2 Sampling

The sampling methods to be used will depend on the nature of the contaminating organisms, the methods of detecting them and the methods of sample preparation required.

The International Commission on Microbiological Specifications for Foods (ICMSF) has recently been preparing a handbook¹ on statistically

¹ International Commission on Microbiological Specifications for Foods (in preparation) *Microorganisms in foods. 2. Sampling for microbiological analysis : principles and specific applications*, Toronto, University of Toronto Press.

based sampling procedures designed mainly for the routine microbiological analysis of foods. Its proposals are recommended as guidelines for international use. It is explained in the book that the application of microbiological analysis for the purpose of determining acceptance or rejection of a consignment of food is a specialized aspect of acceptance sampling. An explicit statement of the "sampling plan" is required. This statement should include the number of sample units¹ to be examined, the quantity in each sample unit, the contaminating organism(s), the method of analysis, and the criteria of acceptance ("standards"). The procedures for obtaining a random sample (and the corollaries of this procedure, stratification, sampling a "frame",² etc.) must also be known to the analyst. The associated probabilities of acceptance associated with the plan chosen and hence a statement of "consumer's risk" and "producer's risk" must also be understood and specified.

Several other considerations are relevant in connexion with acceptance sampling in addition to the presence of microorganisms in a food. The organisms present in food may induce spoilage, may be useful as indicator organisms, or may be infectious or toxinogenic pathogens. It is also necessary to take into account the nature of the food, the way it is usually handled before consumption (raw, processed, eaten with or without additional cooking, etc.) and also the "vulnerability" of the probable consumers (normal, infants, the aged, those needing special dietetic foods, and malnourished, ill, or otherwise "stressed" persons). The ICMSF has recognized 15 sets of conditions, or "cases", that warrant increasingly stringent analyses and hence require plans embodying relatively lower probabilities of acceptance and a progressively reduced risk to the consumer. In general, plan stringency should increase progressively from microorganisms measured by means of total plate-count, to "indicator" bacteria, to organisms of direct hazard (moderate to severe).

It is clear that acceptance sampling must be based on "attributes plans"³ for some time to come because of the great variability in the microbial content of foods, the many different processes involved, the widely divergent conditions of production, manufacture, and control, and the very dissimilar regional conditions of hygiene, food surveillance, and frequency of disease.

¹ A sample unit is the individual portion or container of food selected at random. The analytical test will be applied to each sample unit selected.

² Defined by the ICMSF as: "That portion of the consignment from which the sample units are drawn. Ideally it should be the whole lot, in practice it is the accessible portion of the lot."

³ Defined by the ICMSF as: "A sampling plan in which each selected sample unit is classified according to quality characteristics of the product and in which there are only two or three grades of quality, e.g., acceptable, defective; absent, present; acceptable, marginally acceptable, defective; low count, medium count, high count."