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HYGIENIC-BACTERIOLOGICAL EXAMINATION OF PREPARED FOODS, PRINCIPLES AND TECHNIQUES

by

Professor Aage Jepsen
Food and Agriculture Organization Consultant*

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* Royal Veterinary and Agricultural College, Copenhagen, Denmark

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From experience, it is a well established fact that the application of hygienic-bacteriological methods of laboratory control has lent very important support to the progress in hygienic safety or water and milk, and much work has been dedicated to the standardization of the appropriate techniques.

By deduction from this successful experience, food hygienists naturally have found it expedient to apply similar methods in an effort to improve sanitary standards within other fields of food hygiene. In many countries, the food trade is rapidly changing from a business of natural raw materials or semi-manufactured food products into a highly specialized industry engaged in centralized mass-production of prepared, ready-to-eat food articles. By this development, a large new field has been opened which presents itself as a logical object for the application of hygienic-bacteriological control methods.

Generally speaking the purpose of bacteriological examination of food products is to control one or more of the following characteristics: state of freshness, keeping quality, hygienic standards of production and contents of pathogenic organisms. In order to achieve this purpose, the bacteriologist must perform a qualitative as well as a quantitave analysis of the bacterial flora of the food. His methods must include the application of selective and indicative media which will allow him as fast as possible to distinguish the important groups of organisms, and as the practical evaluation of findings of micro-organisms very often depends upon a quantitative estimation, it is essential that he adopts quantitative or semi-quantitative cultural methods.

In water and milk bacteriology, total aerobic counts and coliform tests are the universally applied methods, and no one would dispute the value of such methods in this field, as results usually show good correlation to hygienic standards of production, state of freshness and keeping quality respectively. However, whether this supposition is valid when the same methods of examination are applied to foods in general, needs clarification. Food articles manufactured from different kinds of raw materials and submitted to different types of chemical and physical treatment cannot be considered a uniform object for bacteriological analyses to be judged by uniform standards. On the contrary, selection of

appropriate methods of examination and correct interpretation of findings must be based upon a sound knowledge of the micro-biological characteristics of each type of product. The food bacteriologist must be familiar with the quantity and quality of the natural contaminating flora of the product as present under normal standards of production, before he can establish findings of undesirable numbers and types of micro-organisms. In this evaluation he must take into account.

1) The natural microbiological status of the raw materials from which the article of food has been made up.

2) The quantitative and qualitative changes of the composition of the microflora which follow from the normal procedures of manufacture.

3) The ability of the food as a medium to support growth of micro-organisms.

Following such criteria, prepared foods, broadly speaking, can be classified into four categories.

1. High-temperature heat-treated, hermetically packed products

Sterilized canned meat-fish-vegetable and milk products are examples. This type of products usually presents a high equilibrium relative humidity, chemical inhibitors are absent, and hence the product allows for unrestricted bacterial growth. The bacteriological examination is based upon the assumption that the purpose of processing is to produce sterility or a condition close to sterility of the contents of the hermetically sealed container. The bacteriological examination of apparently normal cans should therefore be performed as a sterility test. The cans should be preincubated before bacteriological examination, and the various media should be inoculated with undiluted samples that are transferred directly from the cans to the test tubes and Petri dishes. The media should provide optimal conditions of growth for aerobes as well as for anaerobes: glucose-broth tube, blood agar pour plate, Crossley anaerobic milk medium, anaerobic brain broth and deep glucose agar tube.

Required standards: Cultures sterile or scanty growth of aerobic spore bearers (Bacillus). Demonstration of viable clostridia, significant numbers of bacillus or growth of non-sporogenic organisms should always be regarded as suspicious and further detailed examination including

2. Low-temperature heat-treated, hermetically packed products

Low-temperature heat-treated canned cured meat products, (hams, shoulders, Wiltshire style bacon, sliced bacon) are examples.

This type of product usually presents a low equilibrium relative humidity, (high ratio of salt to water, $\frac{\text{salt \% x 100}}{\text{water \% + salt \%}}$) and contains curing chemicals (nitrate) which allow only restricted growth of salt-tolerant organisms. The bacteriological examination is based upon the assumption that the purpose of processing is to eliminate all organisms of low thermal resistance (Gramnegative bacteria, non-heat-resistance grampositive bacteria, vegetative cells of sporebearers, mold and yeast) and to decimate those of medium and high thermal resistance (faecal streptococci, lactobacilli and spores). The bacteriological status of this type of product in principle is similar to that of pasteurized milk. 1 ml samples of a homogenized 1 - 10 dilution are transferred to a series of media which should provide conditions of growth for aerophilic, microaerophilic and anaerobic organisms as well: glucose-broth tube, blood agar pour plate, Niven broth tube (lactobacilli and faecal streptococci), Crossley anaerobic milk medium, anaerobic brain broth and deep glucose agar tube.

Required standards: Gramnevative rods including coliforms, clostridia and hemolytic micrococci should be absent, and total aerobic counts should not exceed 10,000 per gramme (Bacillus species, non-hemolytic micrococci with streptococci and lactobacilli absent or present in insignificant numbers only). When desirable, a special test for salmonella might be included.

3. Low-temperature heat-treated, non-hermetically packed products (cooked or baked)

This type of product in contra-distinction to the hermetically heated products of categories 1 and 2 is exposed to recontamination after heat-treatment. They may appear for retail distribution either unpackaged or prepackaged in plastic films (for self-service food markets). With varying degrees of stability according to different levels of equilibrium relative humidity (salt to water ratio), contents of chemical inhibitors, these food articles are kept cool or frozen, e.g. liver paste, brawn, meat loaves, cooked

loaves, cooked luncheon sausage (sliced or in whole pieces), and Vienna sausages, cooked and smoked fish, cooked peeled shrimps, mayonnaise salads with cooked vegetables and mayonnaise from pasteurized liquid egg yolks, ice-cream, confectionery (custard and cream), sandwich fillings of cooked or fried meat, fish or vegetables (Danish smørrebrød), frozen plastic-packaged prepared meals. Some dehydrated foods such as dried milk, dried soups and egg powder also fit in with this group.

The bacteriological examination is based upon the assumption that the purpose of processing is to eliminate organisms of low thermal resistance and to decimate the medium and high resistant types. Afterwards the protective hermetic container is supposed to be replaced by hygienic precautions in handling for prevention of recontamination, while storage at controlled temperatures, eventually combined with the inhibitory effect of various preservatives (salt, sugar, smoking, acids, chemical inhibitors), is supposed to prevent excessive bacterial growth and to keep total counts low. Consequently the bacteriological examination should offer information about:

1) Whether the total load of micro-organisms is reasonable, when considering the bacteriological status of the raw materials and the lethal effect of the heat-treatment employed, and whether numbers have been kept within the limits that permit satisfactory standards of freshness and keeping quality;

2) Whether any undesirable types of micro-organisms, indicating fresh faecal pollution or contamination with pathogenic or toxinogenic organisms, are present.

Naturally there is room for different opinions and practices regarding technical details of this examination. A simple routine procedure may be as follows· From decimal dilutions of a homogenized sample, two blood agar pour plates are inoculated with dilutions 10^{-3} and 10^{-5}, while one red violet bile agar pour plate is inoculated with dilution 10^{-1}. The blood agar plates are incubated for 24 hours at 37°C and counted to estimate at the same time the total aerobic count and the contents of potentially toxinogenic hemolytic bacteria,

(Micrococcus pyogenes, Bacillus cereus). The red violet bile agar plate is incubated for 24 hours at 44°C.

Minimum standards. Total count less than 100,000 to 1,000.000, depending upon the natural bacteriological status of the product. Hemolytic bacteria should be absent in dilution 10^{-3}. Coliform organisms that will grow at 44°C (mainly E. coli I) should be absent in dilution 10^{-1}.

This method of examination and of interpretation obviously cannot satisfy all special demands which may arise from examination of a diversity of heterogeneous foods such as listed in category 3. However, all of the products have been low-temperature heat-treated and the surviving thermo-resistant flora can be expected to be rather uniform and composed of species which will grow well in blood agar at 37°C. Moreover, as all of the products have been handled after heat-treatment under conditions which do not automatically exclude recontamination, it may not be justified to accept straight-away coliform organisms of non-faecal origin as an indication of dangerous contamination. It has to be taken into account whether or not such organisms must be expected to occur universally under standard conditions of manufacture for that particular product. In meat works such infection is almost environmental, in bakeries perhaps not. So in special cases where coliform infection is not environmental or handling and packaging of the product after heat-treatment approaches complete protection, it may be feasible to adopt 37°- Coliform standards, but in most instances there is probably no better way of tracing a potentially dangerous fresh faecal contamination than by 44° E. coli counts. When desirable, special tests for salmonella (egg powder), faecal streptococci, clostridia or any other topical type of organism may be included.

4. Non-heat-treated, salted or chemically preserved products

This type of product may be packaged in hermetic containers after chemical preservation, e.g. anchovies, pickled herring fillets, or may appear unpackaged, e.g. raw salami sausage, certain types of ripened cheese, pickled fish and vegetables.

The micro-population of these products is essentially that of the fresh raw material, but due to the very specific chemico-physical environment, certain types and species will outgrow the others in the

microbiological "fermentation" which normally develops during manufacture and storage. These processes are accompanied by a rapid increase in bacterial contents followed perhaps by a decrease due to auto-sterilization. Total counts consequently are of limited value as bacterial counts bear no or little relation to the state of freshness and keeping quality of the product. Bacteriological tests designed to trace undesirable types of micro-organisms indicating fresh faecal pollution or contamination with pathogenic or toxinogenic organisms may be applicable.

Attention is drawn to the usefulness of supplementing bacteriological control with microbiological methods for the detection of preservatives (Kluyver yeast fermentation test) as well as with determination of salt to water ratio. Such information often forms the basis of correct interpretation of bacteriological results. Finally, reference is made to the valuable information with respect to numbers and types of micro-organisms yielded by direct microscopic examination. So this simple examination never should be omitted in the bacteriological analysis of foods.

For references, the following may be consulted

(1) D.A.A. Mossel. Wiener Tierärztliche Monatsschrift 1956

(2) World Health Organization. Monograph Series No. 33, Meat Hygiene, Geneva, 1957.

Summary

Principles and techniques of hygienic-bacteriological examination of prepared foods are discussed. Selection of appropriate methods of examination and correct interpretation must be based upon a sound knowledge of the microbiological characteristics of each type of product. Methods of examination and standards of interpretation are given for the following four categories of prepared foods:

1. High-temperature heat-treated, hermetically packed products.
2. Low-temperature heat-treated, hermetically packed products.
3. Low-temperature heat-treated, non-hermetically packed (cooked and baked) products.
4. Non-heat-treated, salted or chemically preserved products.

For routine control of the large group of foods in category 3 blood agar counts and 44°- *E. coli* counts in red-violet-bile agar are proposed. Attention is drawn to the importance of supplementing bacteriological control with microbiological methods for the detection of preservatives as well as with determination of salt to water ratio.