MICROBIOLOGICAL METHODS

Indicator Organisms for Safety and Quality—Uses and Methods for Detection: Minireview

MARY L. TORTORELLO
U.S. Food and Drug Administration, National Center for Food Safety and Technology, 6502 South Archer Rd, Summit-Argo, IL 60501

Indicator organisms have been used for nearly a century to assess the microbiological status of water and foods. Beginning with their use in water sanitation programs, their applications have been extended over the years to other products, and they have become important components of the microbiological testing programs of both industry and regulatory agencies. Functionally, they may be viewed as safety or quality indicators. Safety indicators suggest the presence of conditions associated with increased risk of exposure to a pathogen. Quality indicators assess conditions of importance to product manufacture or consumer acceptability. This minireview summarizes the history, use, and analytical methods for the most commonly used indicator organisms, including the aerobic plate count, yeasts and molds, the coliform groups, *Escherichia coli, Enterobacteriaceae*, and *Listeria*.

Indicator organisms are bacteria that are used as a sign of quality or hygienic status in a food, water, or environment. The definition of the word "indicator," . . . in fact, includes the concept of the indicator organism, i.e., something "so strictly associated with particular … conditions that its presence is indicative of the existence of these conditions" (1). Historically, these conditions have been related to insanitation and public health concerns. Over the years, however, the use of indicator organisms has been extended to provide evaluations of the quality, in addition to the safety, of particular commodities.

History

Indicator organisms were first used in the testing of water supplies for sanitary quality. The mid to late 1800s were marked by huge developments in the sciences of public health and microbiology. The accomplishment list included the recognition by William Budd in 1859 that typhoid fever, an important waterborne disease throughout human history, was spread by infective material in feces, and that the disease could be prevented by avoiding fecal contamination of water supplies. Later, the germ theory of disease and the development of microbiological pure culture methods set the stage for the isolation of *Salmonella typhi* and its identification as the causative agent of typhoid fever (1880, Karl Eberth). In 1885, Theodor Escherich isolated *Bacterium coli* (later, *Escherichia coli*) from feces and noted it to be a natural inhabitant of the human intestine. The nearly ubiquitous existence of *E. coli* in human feces was soon recognized, and it was not long before the idea was proposed that *E. coli* could be used to indicate that a water supply was contaminated with feces (1892, Franz Schardinger).

Methods for identification of *E. coli* were not as easy in the late 1800s as they are today. Other organisms that often existed in association with *E. coli* were similar to it in many respects, but could be distinguished by certain physiological traits. Because of their similarity, *E. coli* and these close relatives were termed "coliforms." Testing for the coliform group was simple, compared with *E. coli*. Microbiologists began to regard coliforms as a testing alternative for *E. coli* and therefore, as indicators of fecal pollution. In 1914, the U.S. Public Health Service (PHS) adopted coliform testing as a means of ensuring the sanitary nature of drinking water supplies.

At that time, nearly a quarter of all food- and waterborne illness outbreaks were caused by milk. The Pasteurized Milk Ordinance was developed by the PHS in 1924 as a measure to prevent milkborne disease. In addition to pasteurization standards and recommendations for sanitary production, the Ordinance included coliform testing of the pasteurized milk. Also in that year, a multistate outbreak of typhoid was traced to consumption of oysters harvested from sewage-polluted waters. The National Shellfish Sanitation Program swiftly followed in 1925, and among the recommendations to enhance consumer safety was coliform testing of shellfish growing areas and harvested products.

What Do Indicator Organisms Indicate?

Indicator organisms are important components of microbiological testing programs conducted both by regulatory agencies and the food industry. They may signify the potential presence of pathogens, a lapse in sanitation as required in good manufacturing practices (GMPs), or a process failure. They may reflect quality attributes that can influence con-

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Corresponding author's e-mail: mlt@cfsan.fda.gov.

sumer acceptability of a product. Sometimes the presence of an indicator organism alone is cause for concern; in other cases, it is the quantity that is significant. Many foods provide an environment conducive to microbial growth, and indicator counts in such foods may reflect the time and conditions of storage. The microbiological snapshot that is the indicator test must always be assessed in an appropriate context, taking into account the natural microbial ecology, intrinsic and extrinsic chemical and physical factors that might influence microbial growth, process history, and storage conditions of the product.

The dual goals of safety and quality often overlap in the water, food, and environmental arenas, and it is important to choose the type of indicator organism that best fits a particular system. This is not an easy task, and the question of indicator selection has generated much discussion and debate. Perhaps adding confusion to the discussion are attempts that have been made over the years to apply various terms so as to distinguish the different functions of indicators, e.g., index, marker, model, sentinel, and surrogate organisms (2). It seems reasonable to view 2 general categories of indicators, i.e., safety and quality indicators (3, 4). Safety indicators suggest that a microbial hazard may exist, and their use is intended to minimize the risk of exposure to the hazard. Quality indicators are used to assess issues important to product acceptability, e.g., shelf life, organoleptic characteristics, spoilage, etc.

The International Commission on Microbiological Specifications for Foods (ICMSF; 5) has noted that selection of an indicator must be considered carefully with an understanding of how to interpret the results of indicator testing. Indicators are a compromise, representing an analytical substitute for detection of the target hazard or concern directly. They can never be used to prove the presence or absence of the target.

The ICMSF (5) has listed the factors that should be considered when selecting an indicator organism for a particular purpose:

(**a**) Presence of the indicator should suggest a faulty process or practice or a potential for spoilage.

(**b**) Survival or stability of the indicator should be similar to or greater than the hazard or spoilage organism.

(**c**) Growth characteristics of the indicator should be similar to or faster than the hazard or spoilage organism.

(**d**) Identifiable characteristics of the indicator should be stable.

(**e**) Method for detection and/or quantitation should be easy, rapid, inexpensive, reliable, sensitive, and validated; does not risk analyst health; and is suitable for in-plant use.

(**f**) Quantitative results should show a correlation between the concentration of the indicator and the level of the hazard or spoilage organism.

(**g**) Results should be applicable to process control.

Safety Indicators

Buchanan (6) has noted that safety indicators signify exposure "to conditions that pose an increased risk … (of contamination) with a pathogen." Coliforms have had the longest history of use as indicators, having been recommended since the early 1900s for water testing. The National Primary Drinking Water Regulation (NPDWR) requires routine monitoring of all drinking water systems for total coliforms, with confirmation of positives by testing for fecal coliforms or *E. coli* (40 CFR 141.21). In this case, *E. coli* is used as a predictor of fecal contamination. By extension, its presence suggests the potential that pathogens also may be present in water systems. *E. coli* possesses the attributes of a good indicator, as suggested by the ICMSF. *E. coli* and certain enteric pathogens, e.g., *S. typhi*, have similar origins in feces. The organisms do not grow appreciably in water, and their death rates in water are similar. A number of validated methods are available for detection, differentiation, and quantitation of *E. coli* in water. Thus, as applied to water safety, *E. coli* possesses the desirable attributes of an appropriate safety indicator, and its presence suggests fecal contamination and the potential entry of pathogens into the system.

The Pasteurized Milk Ordinance provides detailed recommendations for all aspects of the sanitary production of milk, and among its requirements is coliform testing of pasteurized milk and milk products (7). In this application, coliforms are used as process integrity indicators, because pasteurization destroys coliform populations as efficiently as milkborne pathogens. Thus, as applied to milk safety, positive coliform tests indicate an inadequate pasteurization treatment or post-process contamination of the product.

A related use of safety indicators is validation of processes for pathogen control. Such indicators or "surrogates" can be used as pathogen substitutes in processes if they have been shown to have similar survivability or inactivation characteristics. The organisms may be used in laboratory challenge tests, in which they are inoculated into the food, and then subjected to the process to determine the efficacy of the treatment. When validation must be done in a manufacturing facility, the ideal indicator would be one that exists naturally in high numbers in the food and has greater resistance to the process than the pathogen. The choice of a naturally occurring indicator would preclude the need to introduce high numbers of microorganisms into the manufacturing facility. Monitoring the destruction of the indicator would verify the destruction of the pathogen during product manufacture.

Critical to any monitoring program using indicator organisms are appropriate statistical sampling procedures to ensure that lack of detection is not due to an insufficient number of samples. Underlying the design of a sampling program is the sensitivity or detection limit of the methods specified. Thus, for example, the NPDWR specifies both the type of testing to be performed and the sampling requirements, based on the size of the population served by the water system. A "maximum contaminant level" violation is triggered above a certain incidence, relative to the number of samples collected per month (40 CFR 141.63). Similarly, testing frequencies based on production volume and performance standards are specified in the regulations for meats and poultry to verify process controls (9 CFR 310.25 and 9 CFR 381.94). The ICMSF views indicator organisms as "low, indirect hazards" and recommends different sampling plans for indicator testing in

foods, depending on the conditions under which the foods normally are handled before consumption. More stringent criteria are applied to foods that are apt to be handled so as to result in greater risk, e.g., microbial growth, than those in which the risks would be expected to decrease, e.g., cooking or other inactivation techniques (8).

Regardless of the sampling procedures, negative indicator tests cannot be used to guarantee the absence of a microbial hazard. In a survey of 132 raw and 593 ready-to-eat foods, neither a coliform nor *E. coli* standard of <3 organisms/g was useful in predicting the absence of pathogens (9). Fecal coliforms were absent in a number of drinking water-linked outbreaks of giardiasis (10). Samples of the ice cream mix associated with a multistate outbreak of *S. enteritidis* infections revealed both coliform and *E. coli* counts of <1 organism/g (11). These reports also demonstrate that, for many applications, the selection and implementation of appropriate safety indicators remain elusive goals.

Quality Indicators

The microbial composition of a product significantly determines its quality. The types and number of microorganisms present influence the sensory properties (taste, aroma, texture, color) and shelf life of the product. Among these microbial populations, a particular one may be useful as an indicator to reflect quality changes in the product. Such quality indicators are often used to ensure that the product is microbiologically stable and aesthetically acceptable.

The primary attribute of a quality indicator is that its growth and numbers should be inversely related to acceptable product quality. The indicator should be present in all products whose quality is to be assessed, its growth unaffected by other microbial populations present, and there should be relatively simple methods available for detection, differentiation, and quantitation. A good example is the yeast and mold determination, which can serve as a quality indicator for cereal grains. This commodity routinely harbors diverse populations of yeasts and molds, usually in the range of 10^2–10^4 colony-forming units (CFU)/g. Above this range, an unpleasant "mildewy" smell develops (12). Growth of yeasts and molds in cereal grains is generally not influenced by the presence of many other microorganisms, which would be inhibited by the low water activity of the commodity. A number of standard methods using antibiotic-supplemented media have been validated for performing yeast and mold counts. Thus, as applied to cereal grains, the yeast and mold population possesses the desirable attributes of an appropriate quality indicator.

Other quality indicators have been adopted by various food industry sectors. Reflecting the importance of alcohol-tolerant yeasts, molds, and bacteria as spoilage agents of wine, a membrane filtration method for their detection on cork stoppers has been validated (Table 1; ISO 10718). Quality indicators may be broadly classified, such as the lactic acid bacteria in beer and wine, and the flat-sour spores in canned vegetables, or they may be quite specific in nature, such as *Acetobacter* spp. in fresh cider, and *Leuconostoc mesenteroides* in sugar refining (3).

Commonly Used Indicator Organisms

Many different types of indicators have been advocated for use in particular applications; however, this minireview is limited to the most common indicators used for foods and drinking water, i.e., the aerobic plate count; coliforms; *E. coli; Enterobacteriaceae*; *Listeria* spp.; and the yeasts and molds. Microbial groups which may have use as indicators in certain applications, e.g., enterococci, *Staphylococcus*, endospore-formers, lactic acid bacteria, and others, have not been included in this discussion, nor have cellular components, products or nonorganismal agents, e.g., ATP, phosphatase, endotoxin, coliphages.

Aerobic Plate Count

The aerobic plate count (APC) is one of the most widely used indicator tests. Although the applications of the APC are diverse, on one thing there is agreement: it cannot be used as a safety indicator, as there is generally no correlation between APCs and the presence of pathogens or their toxins. The APC may be a quality indicator, and then only when used in an appropriate context. It has no indicator value for some products, for example, vegetable sprouts, which naturally have high APCs in the range of 10^8–10^9 CFU/g, or for fermented products, such as yogurt, which yield high APCs due to the starter cultures incorporated. The APC of a product may reflect the microbial load of raw materials and ingredients, or its age and storage history. Assays for specific spoilage microorganisms may be necessary and more reliable than APCs for determining the acceptability of certain products. Nevertheless, in the appropriate context, the APC can indicate adherence to sanitation GMPs and product acceptability. In the United States, the APC is used in the regulatory testing of raw and pasteurized milk and milk products (7) and drinking water (40 CR 141.74).

Coliforms and E. coli

The coliform group is not a valid taxonomic distinction, but is defined functionally, i.e., by the fermentation of lactose in the coliform test (13). Coliforms may be defined as Gram-negative, oxidase-negative, aerobic or facultative anaerobic non-spore-forming rods, able to grow in the presence of bile salts, and which ferment lactose to produce acid and gas within 48 h at 37°C (14). Genera that fit this description are *Escherichia, Enterobacter, Klebsiella*, and *Citrobacter*. However, *Enterobacter, Klebsiella*, and *Citrobacter* include species that are normal inhabitants of plants and the environment (15); thus, a positive coliform test does not necessarily indicate fecal contamination, as originally proposed by the PHS in 1914 for evaluation of drinking water. This realization discredited the coliform test as an indicator of fecal pollution and prompted development of the fecal coliform test.

Sometimes referred to as thermotrophic, thermoduric, or thermotolerant coliforms, the fecal coliforms have the same properties as the coliform group, except that the fermentation is able to proceed at 44.5°–45.5°C (13). However, species that have this capacity also are known to be present naturally in the environment; thus the fecal coliforms are not specific indicators of fecal pollution of water, either.

E. coli is present in all mammalian feces at high concentrations; it does not multiply appreciably but can survive in water for 4–12 weeks, and so it is useful as an indicator of fecal pollution of drinking water systems (15, 16). The case for *E. coli* as an indicator in foods and the processing environment is not as clear, however. Certainly, the organism can survive, but it can also grow, in certain foods. It can become established in the food processing environment and contaminate foods in the facility (13); thus, recent fecal contamination cannot be concluded when it is detected in foods or food manufacturing plants. The coliform groups and *E. coli* are most widely applied in the food industry as sanitation and process integrity indicators and for Hazard Analysis Critical Control Point (HACCP) verification.

Regulatory uses for coliform or *E. coli* determinations in the United States include: coliform standards for pasteurized milk and milk products (7) and for bottled water (21 CFR 165.100); coliform standards, with *E. coli* or fecal coliform confirmation, for drinking water systems (40 CFR 141.21); fecal coliform standards for shellfish harvesting areas (17); and *E. coli* process control verification criteria in meats and poultry (9 CFR 310.25 and 9 CFR 381.94). Quantitative determinations, i.e., density of organisms per analytical unit, are specified for the dairy, meats and poultry, bottled water, and shellfish regulations. Presence/absence determinations are required for drinking water systems, although quantitative determinations may be performed as an option.

Enterobacteriaceae

The family *Enterobacteriaceae* encompasses approximately 20 genera, including *E. coli* and the other members of the coliform group; foodborne pathogens *Salmonella, Shigella, Yersinia*; and other related genera (18). The family was originally proposed as an indicator alternative to the coliform group, because testing for the entire family would be more inclusive for the pathogenic genera. Lactose, the carbohydrate specified in the coliform test, is not fermented by *Salmonella, Shigella*, or *Yersinia*, so their presence would not be detected by the test. But substituting glucose for the lactose in the test would allow detection of all members of the *Enterobacteriaceae*, including the pathogens, as well as variant strains that do not show the typical lactose fermentation trait.

The rationale for the use of the *Enterobacteriaceae* as indicators was advanced by reports noting low or negative coliform test results despite detection of *Salmonella* in certain foods (19, 20), by a shigellosis outbreak in a nursing home in which *Enterobacteriaceae* tests might have indicated a cause for concern (21), and by a cheese-associated outbreak caused by an enteropathogenic *E. coli* strain that was a slow lactose fermenter (22). These reports notwithstanding, the *Enterobacteriaceae* are no more indicative of fecal contamination in foods than are the coliforms, i.e., not indicative at all. Nevertheless, they are useful, like the coliforms, as process integrity indicators (23).

The *Enterobacteriaceae* may be superior to the coliforms as indicators of sanitation GMPs because they have collectively greater resistance to the environment than the coliforms, can colonize where sanitation has been inadequate, and are sensitive to sanitizers. Thus, the *Enterobacteriaceae* are useful for monitoring sanitation in food manufacturing plants (13), although they are more widely used as indicators in Europe than in the United States.

Listeria spp.

Listeria monocytogenes is an important foodborne pathogen, which exhibits characteristics quite distinct from the enteric microorganisms. Although it may be found in feces, it is ubiquitous in the environment and cannot be considered to be an indicator of fecal contamination (24). It has greater resistance to environmental stresses than *E. coli* and its relatives, being able to grow in salt concentrations up to 10% and at refrigeration temperatures (25). It is inactivated by pasteurization treatments, but it is more heat-resistant than the other enteric pathogens (26). Thus, the enteric indicator organisms are not well-suited for assessing the risk of exposure to this species.

Efforts to control *L. monocytogenes* may be evaluated by testing for the genus *Listeria*, which is widespread in the environment and commonly found in food processing facilities (26). All species belonging to the genus are included in testing for *Listeria* spp. The group is considered to be a good indicator for *L. monocytogenes,* because the various species are found more frequently in the environment and are faster and easier to detect than the pathogen. The recurring detection of *Listeria* spp. may indicate sanitation problems that might lead to niche growth and establishment of *L. monocytogenes* in the plant (27). Tompkin (28) has stated that an aggressive environmental monitoring program is key to controlling the pathogen in the food processing environment. A program that would allow many samples to be taken and is workable in industry needs to be easy, quick, and inexpensive. Rigorous sampling of the environment and testing for *Listeria* spp. or even the abbreviated protocol for "*Listeria*-like organisms" allows for a timely response to positive results, which should consist of swift corrective action and verification by follow-up sampling and testing. The strategy is to monitor the environment and respond to positive results aggressively, with the objective of making continuous improvements toward the goal of controlling the pathogen in the processing facility. Such a strategy is more likely to be achieved by the quicker turnaround and lower cost of testing for indicators rather than the pathogen.

The U.S. Department of Agriculture (USDA) requires testing of food contact surfaces for *Listeria* spp. by certain establishments that produce ready-to-eat (RTE) meat and poultry products (29), and it has directed its inspectors to test the environment and food contact surfaces in RTE establishments under its verification-testing program for *L. monocytogenes* (http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/10240.3.pdf).

Yeasts and Molds

Yeasts and molds are commonly enumerated in foods as quality indicators. They have no predictive value for the occurrence of toxigenic fungi or other pathogens. As a group, the yeasts and molds are diverse and can grow on virtually any

Table 1. Numerical listing of AOAC INTERNATIONAL Official Methods℠ and ISO validated methods for common indicator organisms

Ref. No.	Title of method
AOAC **966.23C**	Microbiological Methods/Aerobic Plate Count
AOAC **983.25**	Total Coliforms, Fecal Coliforms, and *Escherichia coli* in Foods. Hydrophobic Grid Membrane Filter Method
AOAC **986.32**	Aerobic Plate Count in Foods. Hydrophobic Grid Membrane Filter Method
AOAC **986.33**	Bacterial and Coliform Counts in Milk. Dry Rehydratable Film Methods. (Petrifilm™ Aerobic Count Plate and Petrifilm™ Coliform Count Plate) Methods
AOAC **988.18**	Aerobic Plate Count. Pectin Gel Method
AOAC **988.19**	*Escherichia coli* in Chilled or Frozen Foods. Fluorogenic Assay for Glucuronidase
AOAC **989.10**	Bacterial and Coliform Counts in Dairy Products. Dry Rehydratable Film Methods. (Petrifilm™ Aerobic Count Plate and Petrifilm™ Coliform Count Plate) Methods
AOAC **989.11**	Coliforms in Dairy Products. Pectin Gel Method
AOAC **990.11**	Total Coliform and *Escherichia coli* Counts in Foods. Hydrophobic Grid Membrane Filter/MUG (ISO-GRID™) Method
AOAC **990.12**	Aerobic Plate Count in Foods. Dry Rehydratable Film. (Petrifilm™ Aerobic Count Plate) Method
AOAC **991.14**	Coliform and *Escherichia coli* Counts in Foods. Dry Rehydratable Film (Petrifilm™ *E. coli* Count Plate and Petrifilm™ Coliform Count Plate) Methods
AOAC **991.15**	Total Coliforms and *Escherichia coli* in Water. Defined Substrate Technology (Colilert®) Method
AOAC **992.18**	*Listeria* species. Biochemical Identification Method (MICRO-ID® *Listeria*)
AOAC **992.19**	*Listeria* species. Biochemical Identification Method (Vitek® GPI and GNI?)
AOAC **992.30**	Confirmed Total Coliform and *Escherichia coli* in All Foods. Substrate Supporting Disc Method (ColiComplete)
AOAC **993.09**	*Listeria* in Dairy Products, Seafoods and Meats. Colorimetric Deoxyribonucleic Acid Hybridization Method (GENE-TRAK® *Listeria* Assay)
AOAC **994.03**	*Listeria monocytogenes* in Dairy Products, Seafoods and Meats. Colorimetric Monoclonal Enzyme-Linked Immunosorbent Assay Method (*Listeria*-Tek™)
AOAC **995.21**	Yeast and Mold Counts in Foods. Hydrophobic Grid Membrane Filter (ISO-GRID™) Method Using YM-11 Agar
AOAC **995.22**	*Listeria* in Foods. Colorimetric Polyclonal Enzyme Immunoassay Screening Method. (TECRA® *Listeria* Visual Immunoassay [TLVIA™])
AOAC **996.02**	Coliform Count in Dairy Products. High-Sensitivity Dry Rehydratable Film Method
AOAC **996.14**	*Listeria monocytogenes* and Related *Listeria* Species Detection in Selected Foods. Assurance® Polyclonal Enzyme Immunoassay Method
AOAC **997.02**	Yeast and Mold Counts in Foods. Dry Rehydratable Film Method (Petrifilm™ Method)
AOAC **997.03**	*Listeria monocytogenes* and Related *Listeria* spp. in Selected Foods. Visual Immunoprecipitate Assay (VIP™)
AOAC **998.08**	Enumeration of *Escherichia coli* Counts in Poultry, Meats and Seafood. Dry Rehydratable Film Method. Petrifilm™ *E. coli*/Coliform Count Plate
AOAC **999.06**	*Listeria* in Foods. Enzyme-Linked Immunofluorescent Assay (ELFA). VIDAS® LIS Assay Screening Method
AOAC **2002.07**	Enumeration of Total Aerobic Microorganisms in Foods, SimPlate® Total Plate Count Color Indicator Method
AOAC **2002.09**	*Listeria* in Foods. Colorimetric Polyclonal Enzyme Immunoassay Screening Method (TECRA® *Listeria* Visual Immunoassay) Using TECRA® *Listeria* Enrichment Broth
AOAC **2002.11**	Total Yeast and Mold in Foods by SimPlate® Yeast and Mold Color Indicator Method
AOAC **2003.01**	3M™ Petrifilm™ *Enterobacteriaceae* Count Plate Method for the Enumeration of *Enterobacteriaceae* in Selected Foods
ISO 4831	Microbiology. General guidance for the enumeration of coliforms. Most probable number technique
ISO 4832	Microbiology. General guidance for the enumeration of coliforms. Colony count technique
ISO 4833	Microbiology. General guidance for the enumeration of microorganisms. Colony count technique at 30°C
ISO 5541/1	Milk and milk products. Enumeration of coliforms. Part 1: Colony count technique at 30°C
ISO 7251	Microbiology. General guidance for enumeration of presumptive *Escherichia coli*. Most probable number technique
ISO 7402	Microbiology. General guidance for the enumeration of *Enterobacteriaceae* without resuscitation. MPN technique and colony-count technique
ISO 7954	Microbiology. General guidance for enumeration of yeasts and moulds. Colony count technique at 25°C
ISO 8523	Microbiology. General guidance for the detection of *Enterobacteriaceae* with pre-enrichment

Table 1. (*continued*)

Ref. No.	Title of method
ISO 9308-1	Water quality. Detection and enumeration of *Escherichia coli* and coliform bacteria in surface and wastewater. Part 1. Membrane filtration method
ISO 9308-3	Water quality. Detection and enumeration of *Escherichia coli* and coliform bacteria in surface and wastewater. Part 3. Miniaturized method (most probable number) by inoculation in liquid medium
ISO 10718	Cork Stoppers. Enumeration of colony-forming units of yeasts, moulds, and bacteria capable of growth in an alcoholic medium
ISO 11290-1	Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1: Detection method
ISO 11866-1	Milk and milk products. Enumeration of presumptive *Escherichia coli*. Part 1: Most probable number technique
ISO 11866-2	Milk and milk products. Enumeration of presumptive *Escherichia coli*. Part 2: Most probable number technique using 4-methylumbelliferyl-ß-D-glucuronide (MUG)

type of food. They survive a wide range of environmental conditions: pH 2–9; temperatures of 5°–35°C; and water activity (A_w) of 0.85 or less (30). As quality indicators, they can be used to assess ingredient acceptability, organoleptic characteristics, stability, and shelf life of a product. Osmophilic yeasts, commonly members of the genus *Zygosaccharomyces,* can grow down to A_w of 0.65 and are used as indicators in low A_w foods, e.g., jams, syrups, juice concentrates (31).

Methods for Detection and Enumeration of Commonly Used Indicator Organisms

Official methods, i.e., those that are preferred by regulatory authorities, are available for detecting and enumerating indicator organisms. In some cases, these methods have been made available via the Internet, e.g., *the Bacteriological Analytical Manual* (BAM) of the U.S. Food and Drug Administration (FDA; http://vm.cfsan.fda.gov/~ebam/bam-toc.html); *The Microbiology Laboratory Guidebook* of the USDA Food Safety and Inspection Service (http://www.fsis.usda.gov/OPHS/microlab/mlgbook.htm); *The Compendium of Analytical Methods* of the Canadian Food Inspection Agency (http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhe-dme/compendium/e_index.html). Often these methods are similar or identical to the procedures that have been validated in collaborative trials and are published by international standardization organizations, such as AOAC INTERNATIONAL and ISO (Table 1). The AOAC *Official Methods of Analysis*^{SM} is legally cited as the source of methods to be used in U.S. regulatory work if an alternate method is not otherwise prescribed in a regulation (9 CFR 310.25, 9 CFR 381.94, 21 CFR 2.19). Methods used in testing particular commodities for indicator organisms are also included in the various compendia, such as the *Standard Methods for the Examination of Dairy Products*; the *Compendium of Methods for the Microbiological Examination of Foods*; and the *Standard Methods for the Examination of Water and Wastewater* (all published by the American Public Health Association, Washington, DC). While many of the methods are well-established for the testing of various foods and water, it should be noted that few if any of them have been developed specifically for testing environmental samples, i.e., for monitoring of food processing environments for indicator organisms. Such samples usually are tested by adapting the existing methods for foods and water.

Many methods for indicator organisms are commercially marketed as rapid test kits, which have been developed to provide quick turnover of results with minimal labor. Reductions in time and effort are often achieved as a result of incorporating streamlined assay formats or molecular techniques based on specific antibody or nucleic acid binding. Some of these rapid test kits have undergone the rigorous collaborative trial process and have been validated as *Official Methods*^{SM} by AOAC INTERNATIONAL (Table 1). Others have been designated as *Performance Tested Methods*^{SM} by the AOAC Research Institute (AOAC-RI), which indicates that the manufacturer's claims regarding the performance of the product have been reviewed and tested by an independent third party. Currently recognized *Performance Tested Methods*^{SM} for the common indicator organisms are listed in Table 2. A more comprehensive listing of most of the rapid test kits currently available commercially (including foodborne pathogen test kits) and their recognition status may be obtained from the AOAC website (http://www.aoac.org/ testkits/microbiologykits.htm).

The research literature is abundant with descriptions of new developments in indicator organism methods, which often incorporate molecular techniques. These assays are numerous and promising, but generally lack validation (14, 32), so they will not be addressed in this review. AOAC Official and *Performance Tested* methods for the most commonly used indicator organisms are reviewed below.

The Aerobic Plate Count

The APC determines the viable microbial populations that are able to grow under aerobic conditions at mesophilic temperatures. Its assumption is that a single cell or chain or clump of cells (CFU) will give rise by growth and cell division to a visible colony when incubated on a solid nutrient medium.

Table 2. AOAC Research Institute *Performance Tested Methods*[SM] for common indicator organisms[a]

Microorganism or microbial group	Company	Name of test	Method No.
Total counts	Chisso Corp.	Sanita-kun Total Plate Count	011001
Coliforms	BioSys Inc./MicroFoss	BioSys/MicroFoss Coliform Test	010302
Listeria spp.	Foss Electric A/S	EIAFoss *Listeria* ELISA Kit	000401
	Matrix MicroScience Ltd.	Pathatrix *Listeria* species Test System	090201
	Neogen Corp.	GENE-TRAK® *Listeria* DLP Assay	981201
		REVEAL® for *Listeria*	960701
	Oxoid Ltd.	Oxoid Rapid Test for *Listeria*	960701
	Vicam LP	Listertest® Lift	930701B

[a] AOAC Research Institute, June 18, 2003 update, obtained from http://www.aoac.org/testkits/microbiologykits.htm.

The APC is sometimes referred to as the "total plate count," but that is an inaccurate term. The APC measures only populations that are capable of growth under the particular incubation conditions used in the assay: the aerobic and facultative anaerobic mesophiles that can grow on nonselective agar. Other physiological groups (e.g., anaerobes, psychrophiles) will be excluded. Injured cells, such as those that have been subjected to environmental or processing stresses (temperature, pH, osmotic extremes, etc.) may not grow sufficiently to be counted within the time of the assay. There is no plate count assay that will provide "total counts." Other microbial groups (e.g., thermoduric, psychrotrophic, proteolytic, lipolytic organisms, etc.) may be determined by modifying the incubation conditions, such as temperature or nutrient composition, as appropriate.

Procedural differences exist for performing the assay. The method recommended by the International Organization for Standardization (ISO 4833) calls for aerobic incubation on plate count agar at 30°C for 72 h. The FDA's BAM recommends 35°C for 48 h (33) for nondairy foods. The Standard Plate Count, which is used for estimating bacterial populations in dairy products, strictly specifies 32°C for 48 h (34). Jay (35) found 15 different plating media and 29 different temperature–time combinations in a survey of the research literature in which APCs were used for meat and poultry products.

The "pour plate" method for the APC is officially recognized (33; AOAC **966.23C**; ISO 4833). The "spread plate" technique is generally easier to perform and may have other advantages: different colony morphologies may be recognized, translucent media are not required, and microorganisms are not exposed to the heat of the molten agar (36). Other rapid methods have been officially recognized, including use of the hydrophobic grid membrane filter (HGMF; AOAC **986.32**), pectin gel (AOAC **988.18**), and dry rehydratable film (AOAC **990.12**). SimPlate® Total Plate Count, which uses colorimetric detection of growth in microwells to determine the most probable number (MPN) of the microorganisms, is the most recent method to receive official status (AOAC **2002.07**). A SimPlate assay based on detection of fluorescence in the microwells is approved by the U.S. Environmental Protection Agency for monitoring disinfection of drinking water (40 CFR 141.74). Currently, one AOAC *Performance Tested Method*[SM] is available: the Sanita-kun Aerobic Count. The method depends on a dye reduction reaction in a nutrient film for enumeration of colonies.

Total Coliforms, Fecal Coliforms, and E. coli

Both quantitative and presence/absence methods are described for determining total coliforms and fecal coliforms. Methods for coliform testing generally incorporate the distinguishing physiological characteristics of the group, i.e., lactose fermentation and resistance to bile salts (or a similar surfactant, such as sodium lauryl sulfate). Colony counts of the coliform group are obtained from violet red bile lactose (VRBL) agar (34, 37; ISO 4832; ISO 5541/1). Injured coliform populations may be recovered by first inoculating the sample onto a nonselective agar medium, incubating for several hours to allow resuscitation, followed by a VRBL agar overlay for selection (13, 37). The MPN method for enumeration of coliforms uses lauryl sulfate tryptose (LST) broth as a first step, with confirmation of positive tubes, indicated by gas production, in brilliant green bile lactose broth (BGBLB; 37). Because certain strains of *E. coli* known to exist in some foods, e.g., meat products (13), do not produce gas in LST, the ISO method 4831 recommends transfer of all turbid LST tubes, regardless of gas production, to BGBLB for confirmation. Membrane filtration, which allows analysis of a larger sample volume than other methods, is recommended for coliform counts in 100 mL water (ISO 9308-1). Appropriate enzymatic treatments for foods are necessary to allow filtration of 0.5–2.0 mL sample volumes in the application of HGMF for coliform determinations (AOAC **983.25**).

The huge number of samples that are routinely tested for coliforms spurred development of rapid methods for these determinations. Official rapid methods include use of pectin gel (AOAC **989.11**) and dry rehydratable film in various applications (AOAC **986.33**; AOAC **989.10**; AOAC **996.02**). A miniaturized MPN method using microtiter plates has been validated for enumeration of coliforms in water systems (ISO 9308-3). Coliform numbers may be determined in the BioSys/MicroFoss Coliform Test (AOAC *Performance*

Tested Method[SM]), by measuring the time to detect a pH indicator color change resulting from lactose fermentation.

Specific *E. coli* determinations usually are conducted by incorporating strategies for fecal coliform selection, and by including confirmation of colonies isolated on Levine's Eosin Methylene Blue (EMB) agar by the Indole, Methyl red, Voges-Proskauer Citrate (IMVC) tests: IMVC (13, 37). Typical *E. coli* strains (Biotype I) are IM-positive and VC-negative. However, atypical strains (Biotype II), which are I-negative due to slow indole production, have been described (13). Standard MPN methods, in which the confirmatory steps are limited to the indole reaction, may be used as presumptive determinations (ISO 7251; ISO 11866-1). Most standard and official rapid methods for *E. coli* take advantage of its unique production of the enzyme β-glucuronidase. The widely used substrate for the enzyme reaction, 4-methylumbelliferyl-β-D-glucuronide (MUG), provides a convenient detection system in which positive reactions are identified simply by their fluorescence (38). The MUG assay is especially useful in cutting down on the workload in MPN assays, because tubes showing fluorescence may be considered presumptively positive for *E. coli* (AOAC **988.19**; ISO 11866-2). MUG may also be used to identify *E. coli* colonies by their fluorescence in an HGMF method (AOAC **990.11**). The presence of coliforms and *E. coli* can be determined simultaneously by combining, in one reaction tube, assays for the lactose fermentation enzyme β-galactosidase and β-glucuronidase activity. A chromogenic substrate [o-nitrophenyl-β–D-galactopyranoside (ONPG) or X-gal] serves as a substrate for β-galactosidase and is combined with MUG for detecting positive reactions for coliforms and *E. coli* by color development and by fluorescence, respectively (AOAC **991.15**; AOAC **992.30**). The chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG) also has been adapted for *E. coli* determinations in the methods using dry rehydratable film (AOAC **991.14**; AOAC **998.08**).

Enterobacteriaceae

Like the coliforms, members of the *Enterobacteriaceae* family demonstrate bile resistance, but unlike the coliforms, they do not universally demonstrate lactose fermentation. However, they all ferment glucose. A simple switch of the carbohydrate from lactose to glucose in the coliform selective medium formulation provides a way to test for all of the members of the family, including the pathogens. The *Enterobacteriaceae* are enumerated on violet red bile glucose (VRBG) agar or by MPN determination using brilliant green bile glucose (BGBG) broth (13; ISO 7402). A Petrifilm™ method for determining *Enterobacteriaceae* counts has recently received AOAC *Official Method*[SM] status (AOAC **2003.01**). Given that the *Enterobacteriaceae* are commonly used as sanitation indicators, and as such may be subjected to various environmental stresses, a method for their recovery by pre-enrichment in nonselective buffered peptone water, followed by selective growth in BGBG broth and isolation on VRBG agar, has been validated (ISO 8523). None of the methods, however, has been validated specifically for environmental samples; they have all been validated for foods.

Listeria spp.

Listeria spp. can be difficult to detect, especially when conditions exist for overgrowth by other common environmental microorganisms (24). Bacteria in the genus exhibit resistance to many antibiotics (39), and this trait is used in enrichment media formulations, which usually include several compounds inhibitory to the growth of the background communities. Nalidixic acid, acriflavin, cycloheximide, and lithium chloride are variously used in the standard enrichment media, e.g., Enrichment Broth, M52 (40), Modified University of Vermont (UVM) Broth (41), and Fraser Broth (41; ISO 11290-1).

Analytical methods for *Listeria* spp. should detect all of the species of the genus: *L. monocytogenes, L. ivanovii, L. innocua, L. seeligeri, L. welshimeri*, and *L. grayi.* Methods developed for *L. monocytogenes* detection may be used for detecting *Listeria* spp. by omitting the tests recommended for confirmatory identification of the species, or by using molecular probes that are genus- rather than species-specific. Tests for "*Listeria*-like organisms," performed for obtaining results quickly in environmental monitoring programs (27), are based on esculin hydrolysis, which is evident by a blackening of the medium. No further steps toward confirmation of genus or species are performed.

As sanitation indicators in environmental monitoring programs, *Listeria* spp. are likely to be sampled as stressed or injured cell populations, and the selective agents may reduce their recovery. A strategy of resuscitation in nonselective enrichment broth for 4 h, followed by addition of the selective agents, has been recommended (40). Nevertheless, there is evidence that the presence of selective agents may not be the only determinant in recovery of stressed or injured populations (42, 43); therefore, other agents such as sodium pyruvate are often recommended to enhance recovery. Various times and temperatures in primary and/or secondary enrichment media are recommended (40, 41; ISO 11290-1), and it is recognized that use of more than one method improves detection success (44, 45). Isolation of colonies after enrichment may be attempted on different selective agars, including Oxford, modified Oxford, polymyxin β-acriflavin-lithium chloride-ceftazidime-aesculin-mannitol (PALCAM), and lithium chloride-phenylethanol-moxalactam (LPM). As with the enrichment schemes, use of more than one selective agar is recommended. A number of chromogenic agar media developed recently have been shown to be useful for differentiating *L. monocytogenes* and *L. ivanovii* from other *Listeria* species.

There are a number of rapid methods that have been validated, but they have all been developed for detection of *L. monocytogenes* in foods. They may or may not be adaptable to detection of *Listeria* spp., depending on the format or specificity of the reagents. An updated listing of *Listeria* rapid methods is available (40). Two methods based on rapid biochemical identification of species may be used for *Listeria* spp. detection (AOAC **992.18**; AOAC **992.19**), as may a method based on

DNA hybridization to ribosomal RNA (AOAC **993.09**). Rapid immunological methods have also been validated (AOAC **994.03**; AOAC **995.22**; AOAC **996.14**; AOAC **997.03**; AOAC **999.06**; AOAC **2002.09**); their applicability depends on the ability of the antibody reagents to react broadly with all members of the genus.

The popular use of *Listeria* spp. as indicators in sanitation monitoring programs also is reflected in the number of rapid test kits that have gained AOAC *Performance Tested*^{SM} recognition. All of them are based on some type of molecular detection of the genus, taking advantage of the specificity of antibody or nucleic acid probes. Neogen® Corp. (Lansing, MI) markets 2 AOAC *Performance Tested*^{SM} kits: GENE TRAK *Listeria* DLP, based on hybridization of an enzyme-labeled DNA probe to *Listeria* spp. ribosomal RNA and colorimetric detection of added substrate; and REVEAL® for *Listeria*, a device in which a positive reaction is visible as a colored band from the immunoprecipitation of antibodies with listerial flagellar antigens. A similar immunoprecipitation device is the Oxoid (Basingstoke, Hampshire, UK) Clearview Rapid Test for *Listeria*. The ListerTest® Lift (VICAM, Watertown, MA) incorporates immunomagnetic beads to capture *Listeria* cells, plating of the beads, and immunoblotting for identification of positive colonies. The Pathatrix (Newmarket, Cambridgeshire, UK) *Listeria* Species Test uses immunomagnetic capture in a circulating system, followed by concentration and plating for colony isolation. The EIAFoss (Hillerod, Denmark) *Listeria* system uses immunomagnetic bead capture and detection of a fluorescent signal in an autoanalyzer.

Because of the focus on detection of *L. monocytogenes* in foods, there is a lack of official methodology developed purposely for environmental monitoring of the *Listeria* spp. The processing environment may harbor microflora that behave differently than the microflora in foods with respect to competition or interference in detection methods. Thus, methods developed for foods should be validated for use in environmental monitoring. A number of AOAC *Official Methods*^{SM} are currently under study as modifications to extend their applicability for testing environmental samples for *Listeria*. More method development work needs to be done in the environmental testing area, particularly for the transport, enrichment, and recovery of stressed or injured cell populations from various types of environmental samples.

Yeasts and Molds

Although they have a diverse growth habit, yeasts and molds grow slowly in laboratory culture when compared with bacterial groups. Thus, yeasts and molds are enumerated by a plate count procedure that uses agar supplemented with agents inhibitory to bacteria. Chloramphenicol, rose bengal, and dichloran are common selective agents. Spread or pour plates, incubated at 25°C for 3–7 days, are recommended (30, 46; ISO 7954). If osmophilic types are suspected, care must be taken to decrease the A_w of both the plating media and diluent as appropriate and to allow extended incubation times (31). Rapid official methods using HGMF (AOAC **995.21**) and dry rehydratable film (AOAC **997.02**) recommend 50 h or 5-day incubation, respectively. A method using the SimPlate colorimetric format determines yeast and mold counts in 56–72 h (AOAC **2002.11**). Despite the improvements provided by the rapid methods, a relatively lengthy time of analysis still is required for yeast and mold determinations, compared with other microbial groups. Significant economic consequences can result if product release is delayed until assay results are obtained. Clearly, there is a need for more research to improve methods for determining yeasts and molds in foods.

Conclusions

Indicator organisms continue to serve important functions in microbiological testing programs. Their use has been written into regulations guiding the production and provision of our water and foods. It is difficult to imagine the evolution of these systems to their current state without the use of indicator testing. Although all products have overlapping goals of safety and quality, it is generally not the case that an indicator can be used for both. Indicators must be carefully chosen for a particular application and used in an appropriate context, taking into consideration all aspects of the nature of the product. Analytical methods have improved immensely since indicator testing was first proposed nearly a century ago. Many rapid methods based on molecular techniques have been developed, but comparatively few have undergone collaborative study for *Official Method*^{SM} status.

References

(1) Merriam-Webster Online, http://www.m-w.com/cgi-bin/dictionary

(2) Institute of Food Technologists (2001) in *Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce,* Report to the U.S. Food and Drug Administration, IFT/FDA Contract No. 223-98-2333, http://www.cfsan.fda.gov/~comm/ift3-toc.html

(3) Smoot, L.M., & Pierson, M.D. (1997) in *Food Microbiology Fundamentals and Frontiers*, M.P. Doyle, L.R. Beuchat, & T.J. Montville (Eds), American Society for Microbiology Press, Washington, DC, pp 66–80

(4) Jay, J.M. (2000) in *Modern Food Microbiology,* 6th Ed., Aspen Publishers, Inc., New York, NY, pp 387–406

(5) International Commission on Microbiological Specifications for Foods (2002) in *Microorganisms in Foods 7*, Kluwer Academic/Plenum Publishers, New York, NY, pp 108–109

(6) Buchanan, R.L. (2000) *J. Food Prot*. **63**, 832–838

(7) U.S. Food and Drug Administration (2001) Grade "A" Pasteurized Milk Ordinance, 2001 Revision, http://www.cfsan.fda.gov/~ear/pmo01toc.html

(8) International Commission on Microbiological Specifications for Foods (2002) in *Microorganisms in Foods 7*, Kluwer Academic/Plenum Publishers, New York, NY, pp 145–165

(9) Miskimin, D.K., Berkowitz, K.A., Solberg, M., Riha, Jr, W.E., Franke, W.C., Buchanan, R.L., & O'Leary, V. (1976) *J. Food Sci.* **41**, 1001–1006

(10) Centers for Disease Control and Prevention (2002) *Morbid. Mortal. Weekly Rep.* **51**, **SS-08,** 1–28

(11) Vought, K.J., & Tatini, S.R. (1998) *J. Food Prot.* **61**, 5–10

(12) Deibel, K.E., & Swanson, K.M.J. (2001) in *Compendium of Methods for the Microbiological Examination of Foods,* 4th Ed., F. Pouch Downes & K. Ito (Eds), American Public Health Association, Washington, DC, pp 549–553

(13) Kornacki, J.L., & Johnson, J.L. (2001) in *Compendium of Methods for the Microbiological Examination of Foods,* 4th Ed., F. Pouch Downes & K. Ito (Eds), American Public Health Association, Washington, DC, pp 69–82

(14) Rompre, A., Servais, P., Baudart, J., de-Roubin, M.-R., & Laurent, P. (2002) *J. Microbiol. Methods* **49**, 31–54

(15) Leclerc, H., Mossel, D.A.A., Edberg, S.C., & Struijk, C.B. (2001) *Annu. Rev. Microbiol.* **55**, 201–234

(16) Edberg, S.C., Rice, E.W., Karlin, R.J., & Allen, M.J. (2000) *J. Appl. Microbiol.* **88**, 106S–116S

(17) U.S. Food and Drug Administration (1999) *National Shellfish Sanitation Program Model Ordinance*, 1999 Revision, http://www.cfsan.fda.gov/~ear/nsspotoc.html

(18) Murray, P.R. (1998) *Pocket Guide to Clinical Microbiology*, 2nd Ed., American Society for Microbiology, Washington, DC

(19) Mossel, D.A.A., Visser, M., & Cornelissen, A.M.R. (1963) *J. Appl. Bacteriol.* **26**, 444–452

(20) Silliker, J.H., & Gabis, D.A. (1976) *Can. J. Microbiol.* **22**, 971–974

(21) Kayser, A., & Mossel, D.A.A. (1984) *Int. J. Food Microbiol.* **11**, 1–4

(22) Marier, R., Wells, J.G., Swanson, R.C., Callahan, W., & Mehlman, I.J. (1973) *Lancet* **2**, 1376–1378

(23) National Academy of Sciences (1985) in *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients*, National Academy Press, Washington, DC, pp 104–131

(24) Ryser, E.T., & Donnelly, C.W. (2001) in *Compendium of Methods for the Microbiological Examination of Foods,* 4th Ed., F. Pouch Downes & K. Ito (Eds), American Public Health Association, Washington, DC, pp 343–356

(25) Lou, Y., & Yousef, A.E. (1999) in *Listeria, Listeriosis and Food Safety*, 2nd Ed., E.T. Ryser & E.H. Marth (Eds), Marcel Dekker, New York, NY, pp 131–224

(26) Gravani, R. (1999) in *Listeria, Listeriosis and Food Safety*, 2nd Ed., E.T. Ryser & E.H. Marth (Eds), Marcel Dekker, New York, NY, pp 657–709

(27) Tompkin, R.B., Scott, V.N., Bernard, D.T., Sveum, W.H., & Gombas, K.S. (1999) *Dairy Food Environ. Sanit.* **19**, 551–562

(28) Tompkin, R.B. (2002) *J. Food Prot.* **65**, 709–725

(29) U.S. Department of Agriculture (2003) *Fed. Regist.* **68**, 34208–34254

(30) Beuchat, L.R., & Cousin, M.A. (2001) in *Compendium of Methods for the Microbiological Examination of Foods*, 4th Ed., F. Pouch Downes & K. Ito (Eds), American Public Health Association, Washington, DC, pp 209–215

(31) Baross, J.A. (2001) in *Compendium of Methods for the Microbiological Examination of Foods*, 4th Ed., F. Pouch Downes & K. Ito (Eds), American Public Health Association, Washington, DC, pp 187–193

(32) de Boer, E., & Beumer, R.R. (1999) *Int. J. Food Microbiol*. **50**, 119–130

(33) Maturin, L.J., & Peeler, J.T. (1998) in *Bacteriological Analytical Manual,* 8th Ed., Rev. A, G.J. Jackson (Ed.), AOAC INTERNATIONAL, Gaithersburg, MD, pp 3.01–3.10

(34) Marshall, R.T. (1993) *Standard Methods for the Examination of Dairy Products,* 16th Ed., American Public Health Association, Washington, DC

(35) Jay, J.M. (2002) *J. Food Prot.* **65**, 1200–1206

(36) Morton, R.D. (2001) in *Compendium of Methods for the Microbiological Examination of Foods,* 4th Ed., F. Pouch Downes & K. Ito (Eds), American Public Health Association, Washington, DC, pp 63–67

(37) Hitchins, A.D., Feng, P., Watkins, W.D., Rippey, S.R., & Chandler, L.A. (1998) in *Bacteriological Analytical Manual,* 8th Ed., Rev. A, G.J. Jackson (Ed.), AOAC INTERNATIONAL, Gaithersburg, MD, pp 4.01–4.29

(38) Feng, P.C.S., & Hartman, P.A. (1982) *Appl. Environ. Microbiol*. **43**, 1320–1329

(39) Donnelly, C.W. (1999) in *Listeria, Listeriosis and Food Safety*, 2nd Ed., E.T. Ryser & E.H. Marth (Eds), Marcel Dekker, New York, NY, pp 225–260

(40) Hitchins, A.D. (1998) in *Bacteriological Analytical Manual,* 8th Ed., Rev. A, G.J. Jackson (Ed.), AOAC INTERNATIONAL, Gaithersburg, MD, pp 10.01–10.13

(41) Johnson, J.L. (1998) in *USDA Microbiology Laboratory Guidebook*, 3rd Ed., B.P. Dey & C.P. Lattuada (Eds), U.S. Government Printing Office, Washington, DC, pp 8-1–8-18

(42) Osborne, C.M., & Bremer, P.J. (2002) *J. Food Prot.* **65**, 1122–1128

(43) Silk, T.M., Roth, T.M., & Donnelly, C.W. (2002) *J. Food Prot.* **65**, 1333–1337

(44) Donnelly, C.W. (2002) *J. AOAC Int.* **85**, 495–500

(45) Hayes, P.S., Graves, L.M., Swaminathan, B., Ajello, G.W., Malcolm, G.B., Weaver, R.E., Ransom, R., Deaver, K., Plikaytis, B.D., Schuchat, A., Wenger, J.D., Pinner, R.W., Broome, C.V., & the Listeria Study Group (1992) *J. Food Prot.* **55**, 952–959

(46) Tournas, V., Stack, M.E., Mislivec, P.B., Koch, H.A., & Bandler, R. (1998) in *Bacteriological Analytical Manual,* 8th Ed., Rev. A, G.J. Jackson (Ed.), AOAC INTERNATIONAL, Gaithersburg, MD, pp 18.01–18.11