

# AOAC INTERNATIONAL Qualitative and Quantitative Microbiology Guidelines for Methods Validation

*These guidelines appear as Appendix E in the Peer-Verified Methods Program Manual on Policies and Procedures.*

## Introduction

AOAC INTERNATIONAL harmonized 3 method validation programs: *Performance Tested Methods* (PTM), *Peer-Verified Methods* (PVM), and *Official Methods* in January 1998. As a result, definitions, statistics, and terminology are now consistent across each of the 3 programs. With the harmonization, methods from PVM or PTM can be submitted to the *Official Methods of Analysis of AOAC INTERNATIONAL* (OMA) and be subjected to a full collaborative study. As part of the harmonization, microbiology methods that have PTM status are now equivalent to a precollaborative study in the *Official Methods* program. The PVM program offers flexibility to method authors: PVMs not intended for submission into the *Official Methods* program are not required to follow the requirements for a precollaborative study. However, PVMs intended for submission into the *Official Methods* program are required to follow the requirements of a precollaborative study. Harmonization of the 3 AOAC method validation programs offers a smooth transition of methods from the PTM and PVM programs to OMA.

These qualitative and quantitative microbiology guidelines were produced by the PVM Advisory Committee as part of an initiative to develop guidelines for topic-specific areas and are included as Appendix E in the *Peer-Verified Methods Program Manual on Policies and Procedures*. AOAC INTERNATIONAL thanks Anthony D. Hitchins and Anita Mishra-Szymanski for their contributions in the development of these guidelines. As of December 1998, guidelines are under development for pharmaceuticals and environmental, food composition, and drug residues and will eventually be included as appendices in the manual.

The microbiology guidelines presented here were reviewed and endorsed in October 1998 by AOAC General Referees, the Methods Committee on Microbiology and Extraneous Materials, and the Official Methods Board. In November 1998, the AOAC Board adopted the microbiology guidelines for the *Peer-Verified Methods Program Manual on Policies and Procedures*. These guidelines are being distributed as an interpretation of current policies for the *Official Methods* and PTM programs\*. Please contact the AOAC Technical Services department with any questions about these guidelines.

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\* These guidelines reflect current AOAC policies, which may change in the future as international harmonization develops.

## Qualitative Microbiological Tests

### I. Scope

These guidelines are primarily designed for presence–absence detection methods for microbes in food matrices. However, they can form the basis of guidelines for qualitative nonfood matrix methods, that is, for drug-related and device-related microbiology methods, microbiological efficacy tests of cosmetic preservation, microbiological efficacy testing of disinfectants, and microbial identification kits. Qualitative bacterial toxin bioassays are not considered in this document at this time.

### II. Performance Characteristics

#### 1. Accuracy

It is defined as samples known to contain the microbial analyte that are correctly identified by the test method. Certified inoculated reference samples may be used as available and as applicable.

#### 2. Recovery

Recovery cannot be estimated for qualitative microbiological methods at the very low levels of most interest, because quantitation of the spike at these levels often is not precise. Thus, recovery may be estimated by comparing the proportion of positive recoveries either with those obtained in parallel with a recognized conventional method, normally the AOAC cultural method, or with those expected from the various inoculation levels according to the Poisson distribution equation. Incurred microbial analyte in the matrix may be automatically corrected for by using a genetically marked test microorganism as the spiking analyte. Sample homogeneity is crucial for proper estimates of recovery.

#### 3. Calibration curve

In qualitative microbiology, the response is typically nonlinear (sigmoidal). The Poisson response or, alternatively, the response in the optional reference culture method can be used for calibration.

#### 4. Linearity

Not applicable.

#### 5. Limits of detection

These terms are not exactly applicable. Theoretically, the detection limit will be one analyte particle in some undetermined upper limit of analytical sample size. The conventional analytical sample size is 25 g.

#### 6. Limit of quantitation and determination

Theoretically, the limit of quantitation is about one analytical particle per chosen analytical sample size (usually 25 g matrix). However, spiking at this low level is known to result in about 40% true negatives.

Interference by matrix microflora that are relatively resistant to the selective agents used in the enrichment culture, as well as other factors, can affect the values observed.

#### 7. Precision

It is defined in terms of repeatability, reproducibility, and the associated true or false (positive and negative) rates. Different matrices and different analytes are considered the overriding sources of variation.

**Table 1. Generalized categorization of test samples (McClure, F.D., 1990)**

Status of test samples <sup>a</sup>	Test result <sup>b</sup>		Total
	Positive	Negative	
Positive	$N_{11}$ <sup>c</sup>	$N_{12}$	$N_{1\_}$
Negative	$N_{21}$	$N_{22}$	$N_{2\_}$
Total	$N_{\_1}$	$N_{\_2}$	$N = N_{1\_} + N_{2\_}$ or $N_{\_1} + N_{\_2}$

Note: The categorization is used to define false negative, false positive, specificity, and sensitivity.

<sup>a</sup> Status defined by reference method or knowledge of inoculation.

<sup>b</sup> Test result is defined as the screening assay result  $\pm$  the confirmation of the positive assay results, according to usage for the specificity and sensitivity calculations.

<sup>c</sup> N = number of results in any particular cell. First subscript is the row and second subscript is the column. Examples:  $N_{11}$  = row 1, column 1;  $N_{1\_}$  = row 1 total;  $N_{2\_}$  = column 2 total;  $N_{12}$  = row 1, column 2.

#### (A) Intralaboratory repeatability

Each spiking level (at least 3 levels, but preferably 5) should be tested at least in pentuplicate and preferably with 10 replicates at each level. If a PVM study is a preliminary step to obtaining OMA status, the number of replicates per level should be increased to 20, with 3 spiking levels (low, high, and unspiked).

#### (B) Interlaboratory reproducibility

The test must be validated in a minimum of 2 laboratories, preferably with different lots of each matrix. Each spiking level (at least 3 levels, but preferably 5) should be tested at least in pentuplicate and preferably with 10 replicates at each level. Matrix lot variability is important, due to potential competitive microflora variability from lot to lot. If a PVM study is a preliminary step to obtaining OMA status, then common matrix samples should be prepared in a central laboratory and the number of replicates per level should be increased to 20, with 3 spiking levels (low, high, and unspiked).

#### (C) False negatives and false positives

Following *Official Methods* precollaborative study procedure, Table 1 is based on the allocation of the response of the test method to the known presence or absence of the analyte as determined by inoculation or reference methodology.

In *Official Methods* precollaborative studies, analyte presence is generally determined by reference methodology and the following calculation procedure is adjusted: test positives that are false relative to the reference method but are confirmed to be positives are considered as true positives. Consequently, the false-positive rate decreases and can even be zero. Test false positives that are not confirmed to be true positives are still considered as false positives.

Enter study data in Table 1 and calculate the false-positive and false-negative rates as follows:

False-negative rate =  $f^- = N_{12}/N_{1-} = 1 - \text{sensitivity rate} = P(T^-/S^+)$ , which is the probability that the test is negative for samples that contain the analyte.

False-positive rate =  $f^+ = N_{21}/N_{2-} = 1 - \text{specificity rate} = P(T^+/S^-)$ , which is the probability that the test is positive for samples that do not contain the analyte.

### 8. Sensitivity

For specified test conditions, sensitivity represents the proportion of test samples that contain the analyte and respond positively to the test. Referring to Table 1 and defining test result as the result of the completed method (screen assay with confirmation) for a positive assay, then:

$$\text{Sensitivity rate} = p^+ = N_{11}/N_{1-} = P(T^+/S^+)$$

where  $P(T^+/S^+)$  is the probability that the test is positive, given that a randomly chosen test sample is a true positive. The sensitivity rate is 1 minus the false-negative rate. This calculation should be done at each inoculation level.

### 9. Specificity

For specified test conditions, specificity is the proportion of test samples that do not contain the analyte and respond negatively to the test. Referring to Table 1 and defining test result as the result of a completed method (screen assay with confirmation) for a positive assay, then:

$$\text{Specificity rate} = p^- = N_{22}/N_{2-} = P(T^-/S^-)$$

where  $P(T^-/S^-)$  is the probability that the test is negative, given that a randomly chosen test sample is a true negative. This way, the specificity will be 100% because  $N_{21}$  will always be zero. This indicates that, when the confirmation step is used, there is no false-positive rate. If instead test result is defined as the result of the screening assay without confirmation, then when  $N_{21} > 0$ , the specificity will be less than 100%. The specificity rate is 1 minus the false-positive rate. Specificity should be calculated at each inoculation level and should be recalculated with the negative control results included.

The primary laboratory in the PVM validation should study pure cultures on nonselective media of the inclusive and exclusive microbes. Inclusive microbes are those that should be detected by the method, and exclusive microbes are those that should not be detected by the method. The identification process of the method under test should be acceptably specific, with little or no cross-reactivity or few false-positive results relative to the identifications determined by currently accepted methods. The acceptable level of cross-reactivity is left to the discretion of the general referee. The total number of strains of inclusive and exclusive microorganisms (representative of appropriate genera and species) required has not been formalized. It should probably be between 50 and 200 for inclusivity. For example, usually 100–200 *Salmonella* serovars are tested in precollaborative inclusivity studies. Generally, exclusivity test strains will not be greater than 30, but the exact number to be tested will depend on the specificity claim for the method.

Additional selective culture testing of target strains grown in a method's selective media may be necessary if there is reason to suppose that such culturing may alter the phenotypic response of a substantial number of analyte strains, that is, increase the likelihood of artifactual false negatives. This precaution is probably not so important for nonanalyte strains, because such media are designed to select against them, although no selective system is perfect.

Consistent with past practices, identification performance verification for presence–absence detection tests may be either noncollaboratively (intralaboratory) or collaboratively (interlaboratory) studied. The methods and results should be fully documented in the final report.

#### **10. Ruggedness testing**

Study of the effect on the method of small changes likely to arise in different microbiological test environments (e.g., incubation temperature) is discretionary.

#### **11. Comparison with existing reference methods**

Optionally, the results of the study method may be compared with the results obtained by a corresponding conventional culture method using analytical samples from the same portion.

Alternatively, increasing the scope of matrixes and/or lots of each type of matrix to be examined by the test method is preferable. When a particularly difficult matrix is encountered, method comparison may be more worthwhile. In *Official Methods* precollaborative studies, all test methods (conventional, as well as rapid) are compared with the corresponding existing AOAC cultural method.

#### **12. Additional parameters for qualitative microbiological methods**

##### **(A) Sample matrixes**

Refer to the Recommended Foods and Food Groups for Microbiology Methods (*see Attachment*). Effort should be made to choose matrixes where the organism has been found to cause a problem. One or more different matrix samples per claimed food category are required. In an *Official Methods* qualitative precollaborative study, 20 matrixes are required if applicability is claimed for all foods and 5 matrixes if applicability is to a single food category. An appropriate intermediate number of matrixes is required if applicability is less than all foods but more than one food category.

##### **(B) Microbial analytes**

A single representative strain is recommended for all matrixes in PVM studies, if possible. In *Official Methods* precollaborative studies, different single strains are used for different matrixes. The analyst should prescreen candidate strains, either empirically or on the basis of literature data. Generally, as in *Official Methods* precollaborative studies, spiking with mixtures is not recommended because of potential interactions between strains. Ideally a variety of strains (serotypes, etc.) of the target (analyte) microorganisms would be tested. Each matrix would be tested with at least one strain

isolated from that matrix, that is, one of its natural contaminants. It has been suggested that a wide selection of serotypes be studied.

**(C) State of stress of the microbial analyte**

The spiking strain may be used unstressed or stressed, as appropriate. Stress treatments should be described quantitatively in terms of environmental parameters, time of exposure, surviving fraction of stressed population, and fraction of survivors exhibiting an operationally defined injury level. The analyte cells in naturally contaminated (incurred) samples may be used, but the degree of stress generally will be unknown. In precollaborative *Official Methods* studies, spiked samples are stored “to condition” the spike and simulate the stresses to which the microbes may be subject in a particular matrix; dry inocula are used for dry foods and wet inocula are used for wet foods. It is recommended that, when an inoculum is enumerated on nonselective medium, a parallel enumeration must be done on an appropriate selective medium to estimate possible stress among inoculum population.

**(D) Spiking levels**

Each strain is to be used at a minimum of 3 levels per analytical sample (25 g/mL): 1–10 cfu (colony forming unit); 10–50 cfu; blank: no addition. But just as in *Official Methods* precollaborative studies, more levels may be used. Five (5) levels, spanning the 1–50 cfu range are preferred if a 50% endpoint determination with confidence limits is performed to ensure that at least one level provides fractionally positive results. As in *Official Methods* precollaborative studies, more than one level is used to increase the probability that one level will exhibit fractional positives defined as some positive and negative samples, or ideally 80% positive and 20% negative. The difference is that, although the fractional positives are still essential, data from all the levels are used to calculate a 50% endpoint and its 95% confidence limits.

The spikes should be quantitated at the time of inoculation by most probable number (MPN) or colony counts of the inoculating cell suspension or the suspension used to prepare the inoculum suspension, preferably the former suspension. A one-level MPN or colony count of low-level inoculating suspensions can be made more accurate if the replications are sufficiently high, for example, 50–100 cfu. In contrast, 3- and 5-tube MPN counts have wide confidence limits, namely, mean count  $\pm$  about a 3-fold range for the 3-tube method.

MPN enumerations of analyte in spiked matrixes on the day of analysis are currently essential in *Official Methods* collaborative studies. Although this allows for any decrease in initial populations during stabilization of the microbes in the matrix, it may be subject to recovery errors as well as MPN variability. Thus, in PVM

studies, MPN enumeration done on the day of analysis is an augmentation of, not a substitute for, knowledge about the number of microbes initially spiked into the food. Alternatively, the spike may be determined by one- or multilevel MPNs on the cultural part of a test kit methodology or on a reference culture, if one is used (Hitchins, 1998).

**(E) Interfering microflora (matrix microbiota)**

Optionally, this major lot-to-lot variable of food matrixes may be assessed with a competitor at a level that just gives a measurable adverse effect with the conventional selective culture method. The analyst could prescreen suitable candidate competitors to choose a single representative microfloral competitor. Use of a cocktail of strains, typically with uncharacterized competitive abilities, is not recommended (except perhaps in preservative challenge studies). To distinguish effects of the competitor from incurred matrix microflora effects, the competition test may be run in the absence of a food matrix or with a sterilized matrix, as long as allowances can be made for any inhibitory substances induced by the sterilization process. The natural matrix microflora may be of value in the competitive evaluation as long as the portion of the microflora that is truly competitive is estimated by plate count on a solid medium made from the proanalyte selective medium used in the test methodology. But this portion would represent only the potential competitor population because it does not take into account the growth rates of its individual constituents. In *Official Methods* precollaborative studies, the matrix studies are expected to include food types with low ( $<10^5$  cfu/g) and high ( $>10^5$  cfu/g) matrix microflora (microbiota) concentrations.

**(F) Acceptance criteria**

Criteria should be definitively stated up front for a given matrix type. At low inoculum levels ( $<2$  cfu per analytical sample size), expected or true negative rates can range from  $<10$  to 100% because of failure to inoculate or spike as defined by the Poisson distribution. Thus, when stating false-negative rates, the inoculum level must also be stated so that allowances can be made for this. For example, a method giving a value of 21% false negatives at 0.08 cfu/g of a 25 g sample of ground beef would probably not be acceptable for ground beef in a precollaborative study. However, this ground beef result would be acceptable under these guidelines because they are making allowances for statistical failure to spike at this low spiking level. This allowance is automatically done by the 50% endpoint (with 95% confidence limits) method.

Results by this method can be compared with the theoretical maximum of 1 cfu per analytical sample (usually 25 g) with a corresponding endpoint of 0.04 cfu/g. Fifty percent endpoints, calculated from published *Official Methods Listeria* studies, typically

fall in the range 0.01–0.10 cfu/g. This range, allowing for confidence limits, might be a basis for devising interim up-front acceptance criteria.

False-positive rates somewhat higher than 0% may be acceptable when a method is intended as a screen.

There are no criteria for accepting the results of OMA precollaborative studies. If the method fails the McNemar test for control/test pairs when applied to a food at some inoculum level, either the food must be removed from the applicability statement or extra testing must be performed to demonstrate that the result was a chance error. To do this, it is proposed in draft precollaborative guidelines that 5 new lots of product be tested, each at 3 levels with 20 replicates at each level. In the past, only 1 or 2 levels have been used.

### III. Background

These guidelines take into account that qualitative microbiological methods are theoretically, and often practically, capable of measuring one replicable biological particle, namely, 1 cfu, per analytical sample. Spiking at this level is statistically uncertain. Thus, at an input concentration of 1 particle per sample, it is uncertain that the inoculated (spiked) sample really is inoculated, so that true negatives, as opposed to just false negatives, can be expected to occur among the inoculated samples. The Poisson distribution adequately describes this situation. Again, it must be stressed that sample homogeneity is crucial for proper estimates of recovery. The culture methods addressed by these guidelines obviously do not include the detection of viable but nonculturable bacteria.

### References

- (1) Refer to the AOAC *Peer-Verified Methods Program Manual on Policies and Procedures* and *Performance Tested Methods Program Policies and Procedures* for definitions of the terms outlined in this document. The manuals can be obtained from: AOAC INTERNATIONAL, 481 N. Frederick Ave, Suite 500, Gaithersburg, MD 20877-2417, Tel: +1-301-924-7077, Fax: +1-301-924-7089.
- (2) McClure, F.D. (1990) Design and Analysis of Qualitative Collaborative Studies: Minimum Collaborative Program, *J. AOAC Int.* **73**, 953.
- (3) Hitchins, A.D. (1998) Retrospective Interpretation of Qualitative Collaborative Study Results: *Listeria* Methods. *Final Program 112th AOAC INTERNATIONAL Annual Meeting and Exposition*, p. 102.

## Quantitative Microbiological Tests

### I. Scope

These guidelines are for studies on microbial cell enumeration methods including cfu counts and MPN counts. The following quantitative microbiological methods are not covered by these guidelines: antibiotic microbiological assays (*see* Committee on Feeds, Fertilizers, and Agricultural Related

Products guidelines) and nutrient microbiological assays (*see* Committee on Food Nutrition guidelines). Also, logically, bacterial toxin assay guidelines will be included in the guidelines for mycotoxin and phytotoxin assay methods (Guidelines for Chemical, Veterinary Drug and Pesticide Residues, and Mycotoxins; Committee on Natural Toxins). If the deviations from the general residue and toxin guidelines turn out to be substantial for bacterial toxins, these can then be developed in a bacterial toxin sub-guideline.

## II. Performance Characteristics

### 1. Accuracy

This is usually determined by statistically comparing the novel enumeration method's results with those from an *Official Method*, or a conventional method where an *Official Method* does not exist, over the applicable enumeration range. Usually, samples with incurred analyte are often used, but quantitatively spiked and fortified samples also may be used. Certified inoculated reference samples may be used as available and applicable.

### 2. Recovery

This is the fraction of microbial analyte added to a test sample (fortified or spiked sample) prior to analysis, that is measured (recovered) by the test method. It may, in theory, be corrected for any naturally contaminating (incurred) microbial analyte present in the unfortified sample. With incurred microbial analyte, recovery is the fraction of microbial analyte enumerated by a standard method, such as the comparable AOAC method, that is enumerable by the test method.

### 3. Calibration curve

Not generally applicable.

### 4. Linearity

In general, microbial enumeration methods yield results proportional to the microbial analyte's concentration as long as the samples are quantitatively diluted to an appropriate concentration range. This range is such as to give from 20–30 to 200–300 cfu per culture plate in solid-culture-media methods. Subject to the wide statistical bounds of the method, linearity applies in the MPN method.

### 5. Limit of detection

The lowest content that can be measured is 10–100 cfu/g in plating methods for food matrixes. Sometimes food particles can interfere with colony visibility. In the MPN method, it is 0.03 cfu/g or less, depending on the number of tubes and the largest sample size cultured, but the confidence limits are broad for the 3–5 tube methods.

### 6. Limits of quantitation and determination

The lowest amount of analyte in the sample that can be determined with suitable precision and accuracy in the conventional plating method is 2000–3000 cfu/g food, because only about 0.01 g food can be actually plated without making colony detection difficult.

The limit of determination or decision—the lowest analyte content that, if actually present, will be detected and can be identified—is about 100 cfu/g in food plating methodology and as low as 0.01 cfu/g in MPN food methodology.

**7. Precision****(A) Intralaboratory repeatability**

Tests must be performed on at least duplicate lots of specific product types, with a minimum of 5 replicates per lot per microbial level. For *Official Methods* precollaborative studies, 4 levels (control, low, medium, and high) are required. Calculate the mean of the logarithm (base 10) of the counts and its  $s_r$  for each matrix lot tested. Calculate  $RSD_r$  ( $CV_r$ ) and  $r = 2.8 \times s_r$ .

**(B) Interlaboratory reproducibility**

A minimum of 2 laboratories is required. The samples studied by different laboratories need not be prepared centrally or even be from the same lot unless true reproducibility statistics are required as in an *Official Methods* study. Compare logarithmic (base 10) count means and  $s_r$  values obtained by each laboratory for each matrix lot tested. Compare respective  $RSD_r$  ( $CV_r$ ) values. If common samples have been tested by more than 2 laboratories, calculate the mean logarithmic (base 10) counts and  $s_R$  for each matrix lot tested and calculate the  $RSD_R$  ( $CV_R$ ). Calculate  $R = 2.8 \times s_R$ .

**(C) False positives and false negatives**

The presence of interfering substances in a food has to be allowed for by removing the particular food from the matrix applicability list or by correcting for it (*see* 12D, Interfering microorganisms).

**8. Sensitivity**

This is usually measured in terms of previously defined parameters such as recovery and accuracy.

**9. Specificity**

This is the ability of a method to measure only what it is intended to measure.

**10. Ruggedness testing**

The inherent variability of microbiological enumeration methods generally overrides the effect of small changes in operating conditions and environment, such as incubation temperature, on the methods. Study of such changes is left to the expertise and discretion of the primary laboratory.

**11. Comparison with existing reference methods**

Comparison with existing *Official Methods* is mandatory, pending availability and acceptance of quantitated standard microbial samples.

**12. Additional parameters for quantitative microbiological methods****(A) Matrixes**

One representative matrix is required for each of the food categories claimed for the method. In an *Official Methods* quantitative precollaborative study, 20 matrixes are required if applicability is claimed for all foods and 5 matrixes if applicability is to a single food category such as meats, dairy products, or vegetables. An appro-

appropriate intermediate number of matrixes is required if applicability is less than all foods but more than one food category.

**(B) Analyte microbial strains**

Testing may be performed with a viable cell inoculum (spike or fortification) and/or with incurred (natural contaminant) viable cells. It has been recommended that 10 different strains of relevant microorganisms be studied as spikes or as incurred analytes. However, if only one particular strain or serotype is the analyte of interest, fewer strains (e.g., 5) might be sufficient. But it is preferable if the lead laboratory screens appropriate strains for the matrixes involved so that as few as possible are selected to be studied in depth and preferably only one strain per matrix is used. Mixed spikes should be avoided unless a broad-category method is involved, such as for enumeration of molds and yeasts, coliform bacteria, or gram negative bacteria. When inclusivity/exclusivity segments are to be performed in *Official Methods* precollaborative studies, 30–40 analyte strains have been recommended and 10–20 nonanalyte strains have been used.

**(C) Levels**

Three or more spiked or naturally incurred levels per matrix are required to represent the stated range of the method. Depending on the organism and the enumeration technique, a 0 to 5 log range may be appropriate. Each level (unspiked, low, middle, high) should be analyzed at least 5 times per matrix. If the analyte is incurred, at least 15 samples per matrix covering the claimed range should be used. If spiked and incurred samples are studied, a minimum of 15 samples per matrix should be tested. In *Official Methods* quantitative precollaborative studies, 20–30 different matrixes are generally needed. Applicable statistical tests are regression, correlation, and paired *t*-test.

**(D) Interfering microorganisms**

Matrixes generally have a natural microflora (microbiota). If a matrix microflora interferes with the method, the matrix must be removed from the matrix applicability list. Use matrixes with high and low levels of incurred microflora. Ideally the microflora should exceed the indicator microorganisms by at least 2 to 3 logarithms in the samples with high level of microflora.

**(E) Acceptance criteria**

Agreement between test and conventional methods must be established at the  $p = 0.05$  level. There are currently no printed criteria for acceptance of a method on the basis of types of statistical data (regression, correlation, and paired *t*-test) that are developed in *Official Methods* precollaborative studies.

### **III. Background**

These guidelines are designed to be harmonizable with the current guidelines for microbiological precollaborative studies to facilitate processing from peer verification to interlaboratory collaborative verification when desired. The culture methods addressed by these guidelines obviously do not include detection of viable but nonculturable bacteria.

### **References**

Refer to the AOAC *Peer-Verified Methods Program Manual on Policies and Procedures* and *Performance Tested Methods Program Policies and Procedures* for definitions of the terms outlined in this document. These manuals can be obtained from AOAC INTERNATIONAL, 481 N. Frederick Ave, Suite 500, Gaithersburg, MD 20877-2417, Tel: +1-301-924-7077, Fax: +1-301-924-7089.

**ATTACHMENT:  
Recommended Foods and Food Groups  
for Microbiology Methods**

**General Recommendations**

Foods and/or food groups that one would like to include in a validation study but that are not found on this list should be reviewed by the appropriate General Referee and the Methods Committee on Microbiology and Extraneous Materials.

AOAC INTERNATIONAL has recommended *Salmonella* methods for "ALL FOODS" and has been recommending *E. coli* O157, *Listeria*, and *Campylobacter* methods for only specific food types or groups/categories.

AOAC INTERNATIONAL recommends the following food groups for *Listeria*, generic *E. coli*, *Salmonella*, and *Campylobacter*:

*Salmonella*: groups 1–14

generic *E. coli*: groups 1–14

*Listeria*: groups 1–6, and 9

*Campylobacter*: groups 1, and 3–6. Optional 2 and 9.

**Group 1: Raw meats**

Ground beef, ground chicken, ground turkey, ground pork, meat by-products, animal substances, glandular products, frog legs, rabbit carcasses

**Group 2: Fresh produce/vegetables**

Lettuce, spinach, kale, collard greens, cabbage, bean sprouts, peas, mushrooms, green beans

**Group 3: Processed meats**

Sausage, frankfurters, lunch meat, beef jerkies, meat substitutes

**Group 4: Seafood**

Raw shrimp, fish sticks, surimi, raw fish filet, raw oysters, raw mussels, raw clams, cooked lobster, cooked crawfish, smoked fish, pasteurized crab meat, cooked langostinos

**Group 5: Dairy cultured/noncultured**

Yogurt, cottage cheese, hard and soft cheeses, raw/pasteurized liquid milk (skim, 2% fat, whole, buttermilk), infant formula, coffee creamer, ice cream, nonfat dry milk/dry whole milk, dried buttermilk, dried cheese spray

**Group 6: Egg and egg products**

Shell eggs, liquid whole eggs, oral or tube feedings containing egg, dried whole egg or dried egg yolk, dried egg whites

**Group 7: Spices**

Oregano, pepper, paprika, black pepper, white pepper, celery seed or flakes, chili powder, cumin, parsley flakes, rosemary, sesame seed, thyme, vegetable flakes, onion flakes, onion powder, garlic flakes, allspice, cloves

**Group 8: Dry foods**

Wheat flour, casein, cake mixes, whey, nonfat dry milk/dry whole milk, corn meal, dried whole egg or dried egg yolk, dried egg whites, soy flour, dried yeast, cereals, dried buttermilk, dry cheese spray

**Group 9: Fruit/juices**

Fresh/frozen fruits or dried fruits, orange juice, apple juice, apple cider, tomato juice, melon cubes, berries

**Group 10: Uncooked pasta**

Noodles, macaroni, spaghetti

**Group 11: Nuts**

Pecans, walnuts, peanut butter, coconut, almonds

**Group 12: Confectionery (candies/chocolate)**

Frosting and topping mixes, candy and candy coating, milk chocolate

**Group 13: Pet food**

Dry pet food

**Group 14: Food dyes and food coloring****Group 15: Miscellaneous**

Dough, refrigerated or frozen; pizza, refrigerated or frozen

**Recommended food types for *E. coli* O157 and *E. coli* O157:H7 (all foods cannot be claimed)**

Cooked ground turkey, frozen; cooked ground beef, frozen; ground beef, frozen; ground beef, refrigerated; raw ground turkey, frozen; raw ground turkey, refrigerated; raw ground pork, frozen; raw ground pork, refrigerated; raw ground lamb, frozen; raw ground lamb, refrigerated; raw fermented sausage, refrigerated

Raw milk, refrigerated; pasteurized milk, refrigerated; ice cream, frozen; cottage cheese, refrigerated; cheddar cheese, refrigerated; cream cheese, refrigerated; soft cheese, refrigerated; liquid infant formula, refrigerated; dry infant formula, room temperature

Pasteurized whole egg, frozen; liquid egg, refrigerated; mayonnaise, refrigerated

Pecans; walnuts; apple cider, refrigerated; apples, fresh; orange juice, refrigerated

Crab meat, pasteurized; shrimp, raw, frozen, peeled, or unpeeled; lobster meat, cooked

Broccoli, fresh; mushrooms, fresh; cauliflower, fresh; carrots, fresh

