AOAC INTERNATIONAL

The Scientific Association Dedicated to Analytical Excellence®

AOAC INTERNATIONAL Committee on Statistics

June 20, 2017

Meeting Book

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AOAC INTERNATIONAL
BYLAWS

As Amended September 26, 2010

ARTICLE I
Name

The name by which this Association shall be known is "AOAC INTERNATIONAL" (hereinafter referred to as the "Association").[1]

ARTICLE II
Purpose

The primary purpose of the Association is to promote methods validation and quality measurements in the analytical sciences.

ARTICLE III
Membership

Section 1. Types of Membership

There shall be three (3) types of membership in the Association: Individual Members, Sustaining Member Organizations, and Organizational Affiliates.

A. Individual Members

There shall be four (4) categories of Individual Members in the Association: Members, Retired Members, Student Members, and Honorary Members.

B. Sustaining Member Organizations

There shall be one (1) category of Sustaining Member Organizations.

C. Organizational Affiliate

There shall be one (1) category of Organizational Affiliate.

Section 2. Qualifications for Membership

A. Individual Members

[1] Members

Qualifications for Members shall be a degree in science, or equivalent as approved by the Board of Directors, and interest in supporting and furthering the purpose and goals of the Association. Such scientists shall be eligible for membership provided they are engaged, or have been engaged, directly or indirectly, in a field relevant to the purpose of the Association.

[2] Retired Members

[1] AOAC INTERNATIONAL was incorporated in the District of Columbia on January 20, 1932, as the Association of Official Agricultural Chemists. On November 10, 1965, the name of the corporation was changed to the Association of Official Analytical Chemists, and on September 12, 1991, the current name was adopted.

A current Member who is no longer actively engaged, directly or indirectly, in a field relevant to the purpose of the Association but who has served the Association as a Member for at least ten (10) years shall be eligible for Retired Member status upon written request and payment of the annual Retired Member dues. Any special benefits accorded Retired Members shall be determined by the Executive Director.

[3] Student Members

Any full-time student working toward an undergraduate or graduate degree in the areas of chemistry, microbiology, food science or other related science shall be eligible for Student Membership in AOAC INTERNATIONAL.

[4] Honorary Members

Honorary Members shall be persons recognized for their substantial contribution toward the achievement of the objectives of the Association. They shall be nominated by the Board of Directors and may be elected by a two-thirds vote of the Individual Members voting.

B. Sustaining Member Organizations

A Sustaining Member Organization shall be any agency of a local, state, provincial, national, or international government; a university, college, or academic department; or any firm, business, or organization with an interest in supporting and furthering the purpose of the Association. Every Sustaining Member Organization must have a designated representative(s). All such Sustaining Member Organization representatives must meet the qualifications for Members and become Individual Members with all the rights and privileges thereof.

C. Organizational Affiliate

An Organizational Affiliate Organization shall be any agency of a local, state, provincial, national, or international government; a university, college, or academic department; or any firm, business, or organization with an interest in supporting and furthering the purpose of the Association. Every Organizational Affiliate must have a designated representative(s). All such Organizational Affiliate representatives must meet the qualifications for Members and become Individual Members with all the rights and privileges thereof.

Section 3. Application for Membership

Applications or requests for membership shall be submitted to the Association's headquarters office. Membership shall become effective upon approval of the application or request, payment of any required membership dues, entry on the membership rolls, and assignment of a member number.

Section 4. Expulsion

The Board of Directors, at any duly called meeting of the Board, by a two-thirds vote of those holding office, may terminate the membership of any member who in its judgment has violated the Bylaws or has been guilty of conduct detrimental to the best interests of the Association. Any member convicted of a felony is subject to immediate expulsion from the Association. Expulsion of a member by the Board of Directors shall be final and shall cancel all rights, interest, or privileges of such member in the services or resources of the Association. Any member, for whom expulsion is proposed, for reasons other than conviction of a felony, shall be entitled to not less than 60 days advance notice of the charges, the date upon which a hearing will be scheduled, and the right to present evidence in defense. The date and place of any such hearing, if held other than at the headquarters or annual meeting site of the Association, must be reasonable with respect to the location of any individual so charged.

Section 5. Dues, Membership Year, and Waivers

A. Annual dues for membership in the Association shall be fixed by the Board of Directors, subject to approval by the majority of the Individual Members voting by ballot by any of the following means (whichever is deemed appropriate by the Board at the time): mail, telephone call, telegram, cablegram, electronic mail or other means of electronic or telephonic transmission.

B. Honorary Members of the Association shall be exempt from payment of dues and annual meeting registration fees.

C. The membership year and the delinquency date shall be determined by the Board of Directors.

D. The authority to grant waivers of membership dues rests with Executive Director.

E. Student Member dues shall be one-third of regular Member dues, rounded up to the nearest $5.00 increment.

Section 6. Members in Good Standing; Rights and Privileges

All Individual Members who maintain their membership by payment of dues as required under these Bylaws and who otherwise qualify shall be considered in good standing and entitled to full privileges of membership.

ARTICLE IV
Officers

Section 1. Elected Officers

The elected officers of the Association shall be Individual Members and shall consist of a President, President-Elect, Secretary, Treasurer, and Immediate Past President.

A. President

The President shall be the principal elected officer of the Association, shall preside at meetings of the Association and of the Board of Directors and of the Executive Committee, and shall be a member ex-officio, with right to vote, of all committees except the Nominating Committee. He or she shall also, at the annual meeting of the Association and at such other times as he or she shall deem proper, communicate to the Association or the Board of Directors such matters and make such suggestions as may in his or her opinion tend to promote the welfare and further the purpose of the Association and shall perform such other

duties as are necessarily incident to the office of President or as may be prescribed by the Board of Directors.

B. President-Elect

In the absence of the President, or in the event of the President's inability or refusal to act, the President-Elect shall perform the duties of the President, and, when so acting, shall have all the powers of and be subject to all the restrictions upon the President. The President-Elect shall perform such other duties as from time to time may be assigned to him or her by the President or by the Board of Directors.

C. Secretary

The Secretary shall give notice of all meetings of the Association, keep a record of all proceedings, attest documents, and, in general, perform such other duties as are usual of the office of Secretary and such other duties as may be assigned by the President or by the Board of Directors.

D. Treasurer

The Treasurer shall be responsible for the funds and securities of the Association; serve as financial officer of the organization and as Chairperson of the Finance Committee; manage the Board of Director's review of and action related to the Board of Director's financial responsibilities; serve as the chief Board liaison in overseeing and reviewing the annual audit, and in general, perform such other duties as are usual of the office of Treasurer and such other duties as may be assigned by the President or by the Board of Directors.

E. Immediate Past President

The Immediate Past President shall serve as advisor to the President and Directors and perform such other duties as may be assigned from time to time by the President or by the Board of Directors.

Section 2. Appointed Officers

The appointed officers shall include the Executive Director and such other appointed officers as may be designated by the Board of Directors from time to time.

A. Executive Director

The day-to-day administration and management of the Association's offices shall be vested in a salaried manager employed or appointed by, and directly responsible to, the Board of Directors. This manager shall have the title of Executive Director with responsibility for the management and direction of all operations, programs, activities, and affairs of the Association, as approved or delegated by the Board of Directors. The Executive Director shall have direct responsibility for employment and termination of employment and the determination of compensation for staff members within the budgetary framework determined by the Board of Directors. The Executive Director functions as the chief operating officer of the Association within the guidelines established by the policies and procedures of the Board of Directors and, as necessary, with the concurrence of the President. The Executive Director shall have such other duties as may be prescribed by the Board.

B. Other Appointed Officers

Other appointed officers shall have such duties as may be prescribed by the Board.

ARTICLE V
Nominations, Elections, Terms, and Appointments to the Board of Directors

Section 1. Nominating Committee

The Nominating Committee shall annually recommend to the Board of Directors a slate of Individual Members as potential nominees for the elected positions where vacancies will occur. The Nominating Committee shall consist of five (5) members who shall be three (3) immediate Past Presidents, as available, and two (2) Individual Members-at-Large of the Association. If three Past Presidents are not available to serve, other Individual Members-at-Large shall be appointed by the President to the extent necessary to form the five (5)-member committee.

Section 2. Elections and Terms of Office

The President-Elect, the Secretary, Treasurer, and the Directors of the Board of Directors shall be elected by a majority of Individual Members voting, from a slate of nominees recommended annually by the Board of Directors.

Terms of office for all Officers and Directors shall begin with the adjournment of the annual meeting following their election and shall end with the adjournment of the annual meeting occurring nearest the expiration of their term. The six (6) Directors shall be elected to staggered three-year terms with two Directors elected to full three-year terms each year, but not to more than two (2), consecutive, three-year terms. Appointment or election to fill an unexpired term shall not affect the eligibility of a person to subsequently be elected to two (2) full terms. The Secretary shall be elected to a one-year term and may be re-elected to successive one-year terms. The Treasurer shall be elected for a one-year term and may be re-elected to successive one-year terms. The President-Elect shall be elected to a one-year term; whereupon the current President-Elect shall become President and the current President shall become the Immediate Past President, each serving a one-year term.

Section 3. Appointments

Directors-at-Large are appointed by the Board in accordance with Article VI, Section 2. Directors-at-Large are appointed for one (1) year terms, renewable at the discretion of the elected Board.

ARTICLE VI
Board of Directors

Section 1. Composition

The Board of Directors shall consist of eleven (11) elected members to include the President, President-Elect, Secretary, Treasurer, Immediate Past President, six (6) Directors, and up to three (3) appointed Directors-at-Large, all of whom shall be Individual Members of the Association. The elected Board shall reflect the makeup of the Association membership and shall not be dominated by any single interest.

Section 2. Powers and Duties

The Board of Directors shall provide supervision, control, and direction of the affairs of the Association, shall determine the Association's policies or changes therein within the limits of the Bylaws, shall actively prosecute

its purpose, and shall have discretion in the disbursement of its funds. It may adopt such rules and procedures for the conduct of its business as shall be deemed advisable, and may, in the execution of the powers granted, appoint such agents as it may consider necessary. The Board of Directors may appoint up to three (3) Directors-at-Large, if, in their opinion, such appointments advance the purpose of the Association. Directors-at-Large shall be accorded the same voting privileges as elected Directors.

Section 3. Meetings

Except that the Board shall have a regular meeting at the time and place of the annual meeting, the Board shall meet, in person or via telephone conference call, upon call of the President at such times and places as he or she may designate within the policies adopted by the Board, and shall be called to meet upon demand of a majority of its members. Notice of all meetings of the Board of Directors shall be sent by any of the following means (whichever is deemed appropriate by the President at the time): mail, telephone call, telegram, cablegram, electronic mail or other means of electronic or telephonic transmission to each member of the Board at his or her last recorded address or number at least fourteen (14) days in advance of in-person meetings or forty-eight (48) hours in advance of conference call meetings.

Section 4. Quorum

A quorum for any meeting of the Board is six (6) Board members elected in accordance with Article V (1). Any less number may: (1) set a time to adjourn, (2) adjourn, (3) recess, or (4) take measures to obtain a quorum.

Section 5. Absence

Any member of the Board of Directors unable to attend a meeting of the Board shall notify the President and state the reason for his or her absence. If a member of the Board is absent from two (2) consecutive meetings, he or she may be removed by a two-thirds vote of the Board Members then in office.

Section 6. Compensation

Members of the Board of Directors, as such, shall not receive any compensation for their services as Board members, but the Board may, by resolution under policies it may adopt, authorize reimbursement of expenses incurred in the performance of members' duties. Such authorization may prescribe conditions and procedures for approval and payment of such expenses. Nothing herein shall preclude a Board member from serving the Association in any other capacity and receiving compensation for such services, if compensation is customarily paid for such services.

Section 7. Resignation or Removal

Any member of the Board may resign at any time by giving written notice to the President, Secretary, Treasurer, or to the Board of Directors. Such resignation shall take effect at the time specified therein, or, if no time is specified, at the time of acceptance thereof as determined by the President or the Board.

Any member of the Board may be removed by a three-fourths vote of the Board members then in office and present at any regular or special meeting of the Board.

Section 8. Vacancies: Members of the Board

If a vacancy should occur in the membership of the elected Board of Directors, any Past President may be appointed by action of the remaining members of the Board to temporarily fill such vacancy until the next

regularly scheduled election. At the next regularly scheduled election nominations will be presented to fill the vacancy for the unexpired portion of the term remaining.

Section 9. Vacancies: President and Other Officers

If the office of the President shall become vacant, the President-Elect shall thereupon become President of the Association for the unexpired term, followed by his or her duly elected term. In the event the office of President becomes vacant at a time when the office of President-Elect is also vacant, the Presidency shall be filled for the remainder of the term by the action of the Board of Directors. If any other officer position shall become vacant, the office may be filled for the remainder of the term by action of the Board.

ARTICLE VII
Committees

Section 1. Committee Formation

The Board of Directors shall form and adopt terms of reference for such standing or special boards, committees, subcommittees, task forces, or task groups as may be required by these Bylaws or as the Board may determine necessary to carry out the affairs of the Association.

Section 2. Committee Appointments

Subject to the requirements of these Bylaws and the specific terms of reference adopted by the Board, the President shall make the appointments to fill the vacancies occurring in the Association's standing or special boards, committees, subcommittees, task forces, or task groups.

ARTICLE VIII
Official Methods of Analysis

The Board of Directors (BoD) is empowered to develop written policies and procedures for the study, adoption, and change in status of the Official Methods of Analysis of AOAC INTERNATIONAL. Implementation of the policies and procedures shall be delegated to an Official Methods Board (OMB).

Section 1. Composition of the Official Methods Board

The Official Methods Board shall consist of a chair and a vice chair, and members who are recommended by the chair. The chair, vice chair and members are appointed by the President of AOAC INTERNATIONAL. The OMB shall be composed of members representing a balance of government, industry, and academia as appropriate to the scope of the group and shall not be dominated by any single interest.

Section 2. Purpose of the Official Methods Board

The OMB shall serve the Association in a scientific and advisory capacity on methods and the process of their adoption. The OMB shall be responsible for implementation of procedures adopted by the BoD, according to the principles in section 3 below.

Section 3. Principles of the Official Methods Program

A. Adequate records of technical data, discussions, and decisions on the study, adoption, and change of status of Official Methods of Analysis shall be maintained for a reasonable time.

B. Timely notice of proposed method studies, adoption, or change in status shall be published in an Association publication that is circulated to the members.

C. Opportunity shall be provided for materially interested parties to submit input during method study and adoption procedures and to submit comments on the adoption, use of, or change in status of specific methods.

D. Methods submitted to the OMB for inclusion in the OMA shall be thoroughly studied, scientifically reviewed, and available in published form prior to adoption as Final Action by the OMB.

E. The OMB shall adopt methods as Final Action.

ARTICLE IX
Meetings

Section 1. Annual Meeting

The annual business meeting of the Association shall be held at the time and place decided by the Board of Directors. A special meeting of the entire Association may be called by the Board of Directors; announcement thereof shall be made at least thirty (30) days prior to the time of said meeting.

Section 2. Quorum

One hundred Individual Members who are present in person or by proxy and entitled to vote shall constitute a quorum at any meeting of the Association which is duly called pursuant to the provisions of these Bylaws.

ARTICLE X
Voting

Section 1. Voting by Ballot

By direction of the Board of Directors, unless otherwise required by these Bylaws or conducted under alternative procedures established under these Bylaws, voting on any matter, including the election of officers and directors, the election of Honorary Members, amendment of the Bylaws, and the approval of dues, may be conducted by ballot of the voting membership by any of the following means (whichever is deemed appropriate at the time): mail, telephone call, telegram, cablegram, electronic mail or other means of electronic or telephonic transmission, and the question(s) thus presented shall be determined according to the votes received, provided in each case votes of at least five (5) percent of the voting membership shall be received. Any and all action taken in pursuance of a vote by any of the means indicated above (whichever the Board deemed appropriate at the time)

in each case shall be binding upon the Association in the same manner as would be action taken at a duly called meeting and shall become effective, unless otherwise provided for in these Bylaws or otherwise stated in the ballot, on the day following certification of the vote.

Section 2. Voting by Proxy

At any duly called meeting of Individual Members, a member-of-record, as determined thirty (30) days prior to any meeting and who is entitled to vote, may vote by proxy executed in writing by the Individual Member or his or her duly authorized attorney-in-fact. No proxy shall be valid for more than eleven (11) months after the date of its execution unless otherwise provided in the proxy.

ARTICLE XI
Earnings and Assets

Section 1. Non-Profit Status

A. Regardless of any provision of the Bylaws which may be construed otherwise:

[1] No part of the net earnings of the Association shall under any circumstances inure to the benefit of any member or individual.

[2] The Association shall not be operated for a private profit.

B. On lawful dissolution of the Association and after settlement of all just obligations of the Association, the Board of Directors shall distribute all remaining assets of the Association to one (1) or more organizations selected by the Board of Directors which have been held exempt from Federal Income Tax as organizations described in section 501(c)(3) of the Internal Revenue Code of 1954.

Section 2. Political Activities

A. No substantial part of the Association's activities shall consist of carrying on propaganda or otherwise attempting to influence local, state, or national legislation. All activities of the Association shall be determined by the Board of Directors.

B. The Association shall not participate or intervene in any manner in any campaign on behalf of any candidate for a political office.

ARTICLE XII
Sections

Section 1. Sections

The Board of Directors shall set geographic limits and grant authority to groups of Individual Members of the Association residing or working in the same geographical areas for the establishment of Sections.

Section 2. Purpose of Sections

The purpose of Sections shall be to promote and further the purpose of the Association.

Section 3. Membership in Sections

Individuals interested in the purpose of the Section shall be eligible for Section membership. Only Individual Members of the Association shall be eligible for election to the Executive Committee of the Section.

Section 4. Bylaws of Sections

Subject to approval of the Board of Directors, each Section shall adopt, for its own governance, bylaws not inconsistent with these Bylaws.

Section 5. Dissolution of Sections

When any Section shall cease to function as a Section for a period of more than one year, or if its membership shall be less than ten (10) Individual Members of the Association for a period of one (1) year, the Board of Directors may terminate the existence of such Section.

Section 6. Actions of Sections

No act of a Section or its members shall be considered an act of the Association unless expressly authorized, ratified, or affirmed by the Board of Directors.

ARTICLE XIII
Technical Divisions

Section 1. Purpose

Technical Divisions shall represent communities of interest within the Association which have the purpose of furthering the purpose of the Association through the development of the analytical sciences either in a commodity-based or scientific discipline-based field. Their activities shall not duplicate the organizational structure nor conflict with the policies or procedures for the adoption of official methods of analysis by the Association.

Section 2. Creation, Combination, Discontinuance, or Change

Technical Divisions may be created, existing Technical Divisions may be combined or discontinued, or the name of a Technical Division may be changed under policies and procedures adopted by the Board of Directors. Each Technical Division shall adopt bylaws not inconsistent with these Bylaws. The jurisdiction of each Technical Division shall be described in its bylaws. No act of any Technical Division or its members shall be considered an act of the Association unless expressly authorized, ratified, or affirmed by the Board of Directors.

ARTICLE XIV
Indemnification

The Association shall have the power to pay, by indemnity, reimbursement, or otherwise, to or for the use of any person designated by resolution of the Board of Directors who was or is a party or is threatened to be made a party to any threatened, pending, or completed action, suit, or proceeding, whether civil, criminal, administrative, or investigative (other than an action by or on behalf of the Association), by reason of the fact he or she is or was a director, officer, committee member, employee or agent of the Association, or was serving as such for another at the request of the Association, against expenses (including legal, accounting, witness and other), judgments, fines, and amounts paid in settlement so long as such person was not found by a court of competent jurisdiction to have been willfully negligent of the interests of the Association or such person had reasonable cause to believe that his or her conduct was lawful.

ARTICLE XV
Parliamentary Authority

The rules contained in the current edition of *Robert's Rules of Order Newly Revised* shall govern the Association in all cases in which they are applicable and in which they are not inconsistent with these Bylaws or any special rules of order the Association may adopt.

ARTICLE XVI
Amendments to the Bylaws

These Bylaws may be amended, repealed, or altered, in whole or in part, by a three-fourths vote: (a) of the Individual Members at any annual business or duly called special meeting of the Association, provided notice of any amendment proposed for consideration shall be sent by any of the following means (whichever may be deemed appropriate at the time): mail, telephone call, telegram, cablegram, electronic mail or other means of electronic or telephonic transmission to the last recorded address or number of each Individual Member at least thirty (30) days prior to the date of the meeting; or (b) by approval of the Individual Members through ballot sent by any means indicated above in accordance with the provisions of Article X, Voting.

All proposed amendments of these Bylaws shall be presented in writing to the Board of Directors. The Board shall present the proposals to the Association membership, with recommendations. All amendments to the Bylaws, unless otherwise stated, will become effective at the adjournment of the meeting where action is taken or on the day following the certification of a vote by mail ballot.

AOAC INTERNATIONAL
POLICY ON THE USE OF THE
ASSOCIATION NAME, INITIALS,
IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS

Introduction

The following policy and guidelines for the use of the name, initials, and other identifying insignia of AOAC INTERNATIONAL have been developed in order to protect the reputation, image, legal integrity and property of the Association.

The name of the Association, as stated in its bylaws, is "AOAC INTERNATIONAL". The Association is also known by its initials, AOAC, and by its logo, illustrated below, which incorporates the Association name and a representation of a microscope, book, and flask. The AOAC logo is owned by the Association and is registered with the U.S. Patent and Trademark Office.

The full Association insignia, illustrated below, is comprised of the logo and the tagline, "The Scientific Association Dedicated to Analytical Excellence," shown below. The typeface used is Largo. The AOAC tagline is owned by the Association and is registered with the U.S. Patent and Trademark office.

The Scientific Association Dedicated to Analytical Excellence®

Policy

Policy on the use of the Association's name and logo is established by the AOAC Board of Directors as follows:

> "The Board approves and encourages reference to the Association by name, either as AOAC INTERNATIONAL or as AOAC; or reference to our registered trademark, AOAC®, in appropriate settings to describe our programs, products, etc., in scientific literature and other instances so long as the reference is fair, accurate, complete and truthful and does not indicate or imply unauthorized endorsement of any kind.
>
> The insignia (logo) of AOAC INTERNATIONAL is a registered trade and service mark and shall not be reproduced or used by any person or organization other than the Association, its elected and appointed officers, sections, or committees, without the prior written permission of the Association. Those authorized to use the AOAC INTERNATIONAL insignia shall use it only for the purposes for which permission has been specifically granted.
>
> The name and insignia of the Association shall not be used by any person or organization in any way which indicates, tends to indicate, or implies AOAC official endorsement of any product, service, program, company, organization, event or person, endorsement of which, has not been authorized by the Association, or which suggests that membership in the Association is available to any organization."

The Executive Director, in accordance with the above stated policy, is authorized to process, approve, fix rules, and make available materials containing the Association name and insignia.

It should be noted that neither the Association's name nor its insignia nor part of its insignia may be incorporated into any personal, company, organization, or any other stationery other than that of the Association; nor may any statement be included in the printed portion of such stationery which states or implies that an individual, company, or other organization is a Member of the Association.

Instructions

1. Reproduction or use of the Association name or insignia requires prior approval by the Executive Director or his designate.

2. Association insignia should not be altered in any manner without approval of the Executive Director or his designate, except to be enlarged or reduced in their entirety.

3. Artwork for reproducing the Association name or insignia, including those incorporating approved alterations, will be provided on request to those authorized to use them (make such requests to the AOAC Marketing Department). Examples of the types of alterations that would be approved are inclusion of a section name in or the addition of an officer's name and address to the letterhead insignia.

4. When the Association name is used without other text as a heading, it should, when possible, be set in the Largo typeface.

5. Although other colors may be used, AOAC blue, PMS 287, is the preferred color when printing the AOAC insignia, especially in formal and official documents. It is, of course, often necessary and acceptable to reproduce the insignia in black.

6. Do not print one part of the logo or insignia in one color and other parts in another color.

7. The letterhead of AOAC INTERNATIONAL shall not be used by any person or organization other than the Association, its elected and appointed officers, staff, sections, or committees; except by special permission.

   Correspondence of AOAC official business should be conducted using AOAC letterhead. However, those authorized to use AOAC letterhead shall use it for official AOAC business only.

   Copies of all correspondence using AOAC letterhead or conducting AOAC official business, whether on AOAC letterhead or not, must be sent to the appropriate office at AOAC headquarters.

8. AOAC INTERNATIONAL business cards shall not be used by any person or organization other than the Association, its staff, and elected officials, except by special permission.

   Those authorized to use AOAC business cards shall use them for official AOAC business only and shall not represent themselves as having authority to bind the Association beyond that authorized.

Sanctions

1. Upon learning of any violation of the above policy, the Executive Director or a designate will notify the individual or organization that they are in violation of AOAC policy and will ask them to refrain from further misuse of the AOAC name or insignia.

2. If the misuse is by an Individual Member or Sustaining Member of the Association, and the misuse continues after notification, the Board of Directors will take appropriate action.

3. If continued misuse is by a nonmember of the Association or if a member continues misuse in spite of notification and Board action, ultimately, the Association will take legal action to protect its property, legal integrity, reputation, and image.

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Adopted by the AOAC Board of Directors: September 24, 1989
Revised: June 13, 1991; February 26, 1992; March 21, 1995; October 1996

AOAC INTERNATIONAL
ANTITRUST POLICY
STATEMENT AND GUIDELINES

Introduction

It is the policy of AOAC INTERNATIONAL (AOAC) and its members to comply strictly with all laws applicable to AOAC activities. Because AOAC activities frequently involve cooperative undertakings and meetings where competitors may be present, it is important to emphasize the on_going commitment of our members and the Association to full compliance with national and other antitrust laws. This statement is a reminder of that commitment and should be used as a general guide for AOAC and related individual activities and meetings.

Responsibility for Antitrust Compliance

The Association's structure is fashioned and its programs are carried out in conformance with antitrust standards. However, an equal responsibility for antitrust compliance __ which includes avoidance of even an appearance of improper activity __ belongs to the individual. Even the appearance of improper activity must be avoided because the courts have taken the position that actual proof of misconduct is not required under the law. All that is required is whether misconduct can be inferred from the individual's activities.

Employers and AOAC depend on individual good judgment to avoid all discussions and activities which may involve improper subject matter and improper procedures. AOAC staff members work conscientiously to avoid subject matter or discussion which may have unintended implications, and counsel for the Association can provide guidance with regard to these matters. It is important for the individual to realize, however, that the competitive significance of a particular conduct or communication probably is evident only to the individual who is directly involved in such matters.

Antitrust Guidelines

In general, the U.S. antitrust laws seek to preserve a free, competitive economy and trade in the United States and in commerce with foreign countries. Laws in other countries have similar objectives. Competitors (including individuals) may not restrain competition among themselves with reference to the price, quality, or distribution of their products, and they may not act in concert to restrict the competitive capabilities or opportunities of competitors, suppliers, or customers.

Although the Justice Department and Federal Trade Commission generally enforce the U.S. antitrust laws, private parties can bring their own lawsuits.

Penalties for violating the U.S. and other antitrust laws are severe: corporations are subject to heavy fines and injunctive decrees, and may have to pay substantial damage judgments to injured competitors, suppliers, or customers. Individuals are subject to criminal prosecution, and will be punished by fines and imprisonment.
Under current U.S. federal sentencing guidelines, individuals found guilty of bid rigging, price fixing, or market allocation must be sent to jail for at least 4 to 10 months and must pay substantial minimum fines.

Since the individual has an important responsibility in ensuring antitrust compliance in AOAC activities, everyone should read and heed the following guidelines.

1. Don't make any effort to bring about or prevent the standardization of any method or product for the purpose or intent of preventing the manufacture or sale of any method or product not conforming to a specified standard.

2. Don't discuss with competitors your own or the competitors' prices, or anything that might affect prices such as costs, discounts, terms of sale, distribution, volume of production, profit margins, territories, or customers.

3. Don't make announcements or statements at AOAC functions, outside leased exhibit space, about your own prices or those of competitors.

4. Don't disclose to others at meetings or otherwise any competitively sensitive information.

5. Don't attempt to use the Association to restrict the economic activities of any firm or any individual.

6. Don't stay at a meeting where any such price or anti_competitive talk occurs.

7. Do conduct all AOAC business meetings in accordance with AOAC rules. These rules require that an AOAC staff member be present or available, the meeting be conducted by a knowledgeable chair, the agenda be followed, and minutes be kept.

8. Do confer with counsel before raising any topic or making any statement with competitive ramifications.

9. Do send copies of meeting minutes and all AOAC_related correspondence to the staff member involved in the activity.

10. Do alert the AOAC staff to any inaccuracies in proposed or existing methods and statements issued, or to be issued, by AOAC and to any conduct not in conformance with these guidelines.

Conclusion

Compliance with these guidelines involves not only avoidance of antitrust violations, but avoidance of any behavior which might be so construed. Bear in mind, however, that the above antitrust laws are stated in general terms, and that this statement is not a summary of applicable laws. It is intended only to highlight and emphasize the principal antitrust standards which are relevant to AOAC programs. You must, therefore, seek the guidance of either AOAC counsel or your own counsel if antitrust questions arise.

* * * * *

Adopted by the AOAC Board of Directors: September 24, 1989
Revised: March 11, 1991
Revised October 1996

AOAC INTERNATIONAL

POLICY AND PROCEDURES ON

VOLUNTEER CONFLICT OF INTEREST

Statement of Policy

While it is not the intention of AOAC INTERNATIONAL (AOAC) to restrict the personal, professional, or proprietary activities of AOAC members nor to preclude or restrict participation in Association affairs solely by reason of such activities, it is the sense of AOAC that conflicts of interest or even the appearance of conflicts of interest on the part of AOAC volunteers should be avoided. Where this is not possible or practical under the circumstances, there shall be written disclosure by the volunteers of actual or potential conflicts of interest in order to ensure the credibility and integrity of AOAC. Such written disclosure shall be made to any individual or group within the Association which is reviewing a recommendation which the volunteer had a part in formulating and in which the volunteer has a material interest causing an actual or potential conflict of interest.

AOAC requires disclosure of actual or potential conflicts of interest as a condition of active participation in the business of the Association. The burden of disclosure of conflicts of interest or the appearance of conflicts of interest falls upon the volunteer.

A disclosed conflict of interest will not in itself bar an AOAC member from participation in Association activities, but a three-fourths majority of the AOAC group reviewing the issue presenting the conflict must concur by secret ballot that the volunteer's continued participation is necessary and will not unreasonably jeopardize the integrity of the decision-making process.

Employees of AOAC are governed by the provision of the AOAC policy on conflict of interest by staff. If that policy is in disagreement with or mute on matters covered by this policy, the provisions of this policy shall prevail and apply to staff as well.

Illustrations of Conflicts of Interest

1. A volunteer who is serving as a committee member or referee engaged in the evaluation of a method or device; who is also an employee of or receiving a fee from the firm which is manufacturing or distributing the method or device or is an employee of or receiving a fee from a competing firm.

2. A volunteer who is requested to evaluate a proposed method or a related collaborative study in which data are presented that appear detrimental (or favorable) to a product distributed or a position supported by the volunteer's employer.

3. A referee who is conducting a study and evaluating the results of an instrument, a kit, or a piece of equipment which will be provided gratis by the manufacturer or distributor to one or more of the participating laboratories, including his or her own laboratory, at the conclusion of the study.

4. Sponsorship of a collaborative study by an interest (which may include the referee) which stands to profit from the results; such sponsorship usually involving the privilege granted by the investigator to permit the sponsor to review and comment upon the results prior to AOAC evaluation.

5. A volunteer asked to review a manuscript submitted for publication when the manuscript contains information which is critical of a proprietary or other interest of the reviewer.

The foregoing are intended as illustrative and should not be interpreted to be all-inclusive examples of conflicts of interest AOAC volunteers may find themselves involved in.

Do's and Don'ts

Do avoid the appearance as well as the fact of a conflict of interest.

Do make written disclosure of any material interest which may constitute a conflict of interest or the appearance of a conflict of interest.

Do not accept payment or gifts for services rendered as a volunteer of the Association without disclosing such payment or gifts.

Do not vote on any issue before an AOAC decision-making body where you have the appearance of or an actual conflict of interest regarding the recommendation or decision before that body.

Do not participate in an AOAC decision-making body without written disclosure of actual or potential conflicts of interest in the issues before that body.

Do not accept a position of responsibility as an AOAC volunteer, without disclosure, where the discharge of the accepted responsibility will be or may appear to be influenced by proprietary or other conflicting interests.

Procedures

Each volunteer elected or appointed to an AOAC position of responsibility shall be sent, at the time of election or appointment, a copy of this policy and shall be advised of the requirement to adhere to the provisions herein as a condition for active participation in the business of the Association. Each volunteer, at the time of his or her election or appointment, shall indicate, in writing, on a form provided for this purpose by AOAC, that he or she has read and accepts this policy.

Each year, at the spring meeting of the AOAC Board of Directors, the Executive Director shall submit a report certifying the requirements of this policy have been met; including the names and positions of any elected or appointed volunteers who have not at that time indicated in writing that they have accepted the policy.

Anyone with knowledge of specific instances in which the provisions of this policy have not been complied with shall report these instances to the Board of Directors, via the Office of the Executive Director, as soon as discovered.

* * * * * *

Adopted: March 2, 1989
Revised: March 28, 1990
Revised: October 1996

Statistics Committee As of: October 21, 2016

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Term: October 1, 2012 - September 30, 2018

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Term: September 20, 2008 - December 31, 2016

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Term: January 10, 2011 - September 30, 2017

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Term: March 13, 2013 - September 30, 2017

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AOAC INTERNATIONAL

COMMITTEE ON STATISTICS

Draft Meeting Agenda

Tuesday, June 20, 2017

Meeting Start Time: 11:00AM (Eastern US)

Chair: Sidney Sudberg

(Alkemists Pharmaceuticals)

(***Need member to volunteer to take the minutes***)

I. WELCOME & INTRODUCTION (Sudberg – 11:00AM-11:10AM) *

Sidney Sudberg will call the meeting to order, welcome and introduce the members of Statistics Committee. Members will review the AOAC policy documents and vote on the draft meeting agenda.

II. REVIEW OF MEETING AGENDA & MINUTES (All – 11:10AM-11:15AM) *

The members of the Statistics Committee will review and vote on the May 17, 2017 meeting minutes.

III. CURRENT METHOD CONFORMITY ASSESSMENT PROJECTS (Wehling/LaBudde/Sudberg/Thompson/Graves – 11:15AM-11:35AM)

Progress updates

a. Statistics for Microbiology Methods Validation - Paul Wehling & Robert LaBudde (Leads)

1. Progress for Internal Validation Plan
2. Next Subcommittee Meeting?

Volunteers:

1. Caryn Thompson
2. Qian Graves
3. Jane Weitzel
4. Mike Zapf
5. Hilde Skår Norli
6. Sidney Sudberg

b. Changes to Appendix J - Paul Wehling & Robert LaBudde (Leads)

1. Quantitative methods need changes (performed during &/or after validation)
   a. Candidate minus Reference
   b. New method of calculating the CI for the paired DPod
   c. Use of Z (n) vs. t (n-1)
   d. Removal of reference to Welch/Satterthwaite t-test?
   e. Chi-Square Test Calculator (Robert/Paul)
      http://lcfltd.com/AOAC/aoac-binary-v2-5.xlsx
      i. To be handled with Appendix J changes

c. Statistics Committee's Terms of Reference (ToR)

1. Latest version in progress – v.6
   a. Review of Submitted Comments
   b. Staff to compile the changes as v.6

c. Committee to decide what final changes should be accepted & then submit to the OMB meeting in Chicago this week

d. Appendix K - Intermediate Precision (IP) Working Group - Paul Wehling /Robert LaBudde/Qian Graves/Jane Weitzel/Anli Gao/Sidney Sudberg (Leads)

1. Completed its task for Scott Coates purposes
2. Terms, Definitions & Measurement need to be defined
   a. To be reviewed with Appendix K revision
   b. Mike Zapf volunteered to be Project Manager
   c. Schedule first meeting
      i. Volunteers needed?

IV. OTHER PROJECTS & DISCUSSIONS (All – 11:35AM-11:50AM)

(Other projects and discussions)

a. Committee on Statistics to produce a separate appendix &/or glossary, i.e. remove statistical details from various appendices.
   1. Appendices J, K & others

b. Possible new committee members Tom Phillips (MD Dept. of Agriculture), Dan Morse (3M), Jim Harnly (USDA)
   1. Once ToR completed, start process of recruiting New Members?

c. Scientific Session for the 2017 AOAC Annual Meeting is scheduled for Wednesday, September 27, 2017 at 8:15am – 10:00am
   1. Current presenters are Paul, Jane, Sidney

d. The AOAC Cannabis working group: Ongoing
   1. There will be a need for statistical evaluation of data, as needed

V. NEW BUSINESS (All – 11:50AM-11:55AM)

a. Quarterly article published in ILM of Statistical interest, to General Membership
   1. Stat Chat
   2. Any volunteers to write something?

b. Method Format & Performance Characteristics
   1. To be discussed after IP & Appendix edits

c. Any other New Business?

VI. SCHEDULE NEXT STATISTICS COMMITTEE MEETING (All – 11:55AM-12:00PM)

Next Statistics Committee meeting dates (11:00am–12:00pm ET)

a. Tuesday, July 18, 2017 or other options, if necessary

VII. ADJOURN MEETING (All – 12:00PM)

Conference number/code:

1-877-647-3411 (US/Canada)
800-56094 (Norway)

To view a list of toll-free international dial-in numbers **Click Here**

Pass code: 3735235702#

AOAC INTERNATIONAL
Committee on Statistics
Monthly Teleconference

Wednesday, May 17, 2017
11:00AM EST to 12:00 EST

Name	Present	Absent	Guest / Other
Sidney Sudberg (Alkemist Labs), Chair	X		
Qian F. Graves, (FDA)	X		
Robert A. LaBudde (Least Cost Solutions)	X		
Hilde Skaar Norli (NMKL)		X	
Anli Gao (University of Guelph)	X		
Caryn M. Thompson (Elanco Animal Health)		X	
Paul Wehling (Medallion Labs)	X		
M.L. Jane Weitzel (Independent Consultant)	X		
Charles M. Zapf (Independent Consultant)	X		
Jim Yuk (Waters)		X	
Wolfhard Wegscheider (Montanuniversität Leoben)		X	
Mei-Ling Ting Lee (University of Maryland)		X	
Deborah McKenzie, AOAC Staff Liaison		X	
Delia A. Boyd, AOAC Staff Liaison	X		
Scott Coates	X (attended part of meeting)		

Minutes Taken By: Jane Weitzel

Minutes

ITEM	Responsible
1. Welcome and introduction. Sidney opened the meeting. Roll call indicated a quorum was not achieved. The meeting proceeded with discussion of the items.	
2. The agenda was accepted by the people on the call. a. Information for this call is in the *Statistics Meeting Book (5-17-17)*. These minutes reference this book and does not repeat information in the book.	
3. The minutes and agenda were accepted by the people on the call.	
4. Intermediate Precision Working Group (Item IIIe item on agenda) This item was discussed first in the agenda. Scott Coates provided an update on the intermediate precision working group's activity. A third revision of the "Intermediate Precision Memo" had been distributed before the meeting. It had been decided to not provide a criteria for the intermediate	

ITEM	Responsible
precision (s_i) because *si* is dependent upon the method itself and upon the conditions varied in the intermediate precision study. The working group is developing a guide on how to perform intermediate precision studies. Scott had distributed the initial draft before this call. The working group will meet May 25. If you do not have an invitation and would like to participate contact Delia Boyd.	
5. Current Method Conformity Projects - Statistics for microbiology. (Item IIIa & b &c on agenda) Paul reported that all the spreadsheets are done. Paul will compile list of needed changes. Paul will arrange a call of the subteam in the next month to review what hs been done. The date is June 8 at 11am EDT. Delia will schedule the call. Sidney will brief the OMB that the appendix will be revised. The statistics meeting documents includes a plan that Paul had put together: *Qualitative Micro Validation Proposed Plan*. Paul is planning to have the volunteers build their section/software and compare that to Caryn's spreadsheet. Then compare the two spreadsheets to validate them. Qian will build her spreadsheet next week. Changes to Appendix J are included in page 33 of the meeting briefing book. For example *Item 3 QUAL SLV PAIRED* lists a needed change to appendix J.	Paul Delia Sidney Qian Paul
6. Terms of Reference (Item IIId on agenda) Version 5 of the file was emailed and included in the meeting book. The following revision was discussed: *If a Full Member fails to participate in 3 or more consecutive meetings of the Committee, the Chair may petition to transfer that member to Associate status only due to non-participation in Committee Meetings.* All comments given till now have been addressed. For terminology, use full member or associate member. Do not use term voting member. Delia will distribute a form for people to use to comment. Then she will compile the comments. Then the document can be changed. Everyone – review TOR and supply comments on the form.	 Delia Everyone

ITEM	Responsible
7. (Item IVa on the agenda) glossary and appendices revision Appendix K may not have been vetted by the AOAC statistics committee. It was created by a community. Discussion of items such as repeatability and intermediate precision (which is not discussed in Appendix K) need to be improved. It was agreed that there is a need for a guide on single laboratory method validation. The AOAC statistics committee proposes to take on revising Appendix K after the TORs are revised and present Appendix revisions complete.	
8. Possible new members will be addressed after the TORs have been revised. Sidney will send a note again to address meeting attendance because we need a quorum to make decisions.	Sidney
9. New Business a. None	
10. Next Meeting: June 20, 2017 at 11am EDT.	All
11. Meeting adjourned at noon EDT.	

Qualitative Micro Validation Proposed Plan

Then for the other people, just assign them a page or 2 to do, and have them build the sheet from scratch in their favorite software – WITHOUT LOOKING AT THE CODES IN THE EXCAL SHEET UNDER TEST. It is very critical that they do it from the Appendix. Have them call me or email if they get stuck. The sheets were:

1. QUAL SLV – No Ref method comparison
2. QUAL SLV UNPAIRED
3. QUAL SLV PAIRED
4. QUAL COLLAB UNPAIRED
5. QUAL COLLAB PAIRED

The calculations for confidence intervals are the tricky parts, although we also need to check all the calcs for mean PODs and dPODs. In addition, people may have difficulty finding the correct formulae for these confidence intervals, due to the fact that there are several, and also due to the fact that we have added some since publishing the Appendix. Here's my best shot at identifying where the formula are:

1. QUAL SLV – No Ref method comparison

   CI calcs are given in Appx J, Annex C, very first section on the left of the page.

2. QUAL SLV UNPAIRED

   Calcs are Appx J, Annex C, immediately following the above section, starting at lower left, it gives calcs for dPODs then on the upper right, gives the calcs for the CIs for dPODs. For this sheet, you have 2 methods, so you calc POD values (and CIs) for both methods as done in sheet 1, then calculate dPOD and CIs on the dPOD as given here in this section of Annex C.

3. QUAL SLV PAIRED

   This one is not in the annex and is discussed in the ppt file we presented at the meeting in Dallas. The section of Annex C called "dPOD for Paired Studies" will need to be changed to incorporate the info in the ppt file.

4. QUAL COLLAB UNPAIRED

   This one is in the Annex F – we have modified it slightly though in 2 areas.

   We have found in our work in the ISO group that the very complicated Welch-Satterthwaite estimation for df for the t-based interval is not necessary and the formula df = L-1 works just as well for the collab sizes we are using. So p. 17 of the Appendix J, Annex F, that df calculation can be simplified to just df = L-1, or degrees of freedom is number of labs minus one.

   The second thing we found was better was to change how we switch from the t-based CI to the binomial-based CI calculator. Originally, we decided to do this based on the POD value, but in simulations, it seems to provide a more seamless transition if it transitions not on POD, but on x, the number of positive hits. For this new version, the Appendix will need to be changed. See ISO Draft standard or call Paul on this.

   Then in addition, you will need to calculate s(R) and s(r) and s(L) for the data. So this will be

probably a big task for someone to tackle.

5. QUAL COLLAB PAIRED

This one again, is not in the Appendix J and comes off the ppt slides. But basically, once you have sheet 3, all you have to do is replicate it for multiple labs, then be sure the CIs are good and calculate standard deviations as well.

I would say assign them this way:

Qian – Sheets 1 and 2

Mike and Hilde – work together on sheets 3 and 5 – they kinda go together.

Jane – Take a stab at Sheet 4

Caryn – let her try to do them all. I would work in this order – 1,2,3,5,4 as easiest to hardest.

The quantitative sheets we can do later.

Paul

AOAC INTERNATIONAL

TERMS OF REFERENCE

I. NAME:

COMMITTEE ON STATISTICS

II. MISSION:

To develop and recommend harmonized statistical guidelines for AOAC interlaboratory collaborative studies, and to encourage greater use of standardized statistical techniques.

III. RESPONSIBILITIES:

To advise the Official Methods Board on statistical matters concerning the methods approval process; to educate AOAC volunteers in proper application of statistical techniques.

All Committee members, other than those holding *ex officio* appointments, serve at the pleasure of the President. All Committee members are expected to actively participate in the work of the Committee; including, but not limited to, promptly responding to communications, attending and actively participating in meetings, reviewing meeting background materials and agendas prior to meetings, and accepting and following through on assignments. Persons who do not actively participate will be removed from the committee by the President at the request of the chair.

IV. COMPOSITION AND ORGANIZATION:

There shall be a minimum of 5 and a maximum of 12 members, including the chair and past chair. A member shall be appointed for a three-year term, with no maximum term limitation. The chair is appointed for one three-year term and may serve one additional year on the Committee as immediate past chair.

Methods Committee statisticians are appointed as ex-officio members of the Committee, serving in an advisory capacity.

All members of the Committee are appointed by the President Elect and assume office immediately following the Annual Meeting of the appointment year.

The Committee shall be composed of members representing a balance of government, industry, and academia as appropriate to the scope of the

Committee. No more than one-half of the members may be from a single agency. Less than one-half of the members must be from industry.

Committee members should be experienced in designing and evaluating laboratory studies, as well as understanding statistics. Subcommittees, task forces, and other appropriate subgroups shall be appointed as the needs arise.

V. STAFF LIAISON:

The Executive Director shall assign a member of the staff to serve as staff liaison.

VI. REVIEW SCHEDULE:

Every three years.

VII. DATE ESTABLISHED:

1962

VIII. DATES REVISED:

8/91; 3/99; 9/99

AOAC INTERNATIONAL
TERMS OF REFERENCE

Committee on Statistics

I. SCOPE:

The Committee on Statistics provides advice and consent on all usage or changes to statistical methodology used in AOAC studies or in the interpretation of results. The Committee reports to the AOAC Official Method Board ('OMB), and the committee chair serves as a member of the OMB.

II. COMMITTEE:

a. **Composition:** The committee shall be comprised of a maximum of 18 members including the Chair, of which 9 are voting (Full Members) the rest non-voting (Associate Members). The President of AOAC on the recommendation of the OMB shall appoint all members to the Committee (Full and Associate) for a three-year term. The term begins and ends with the AOAC Annual Business Meeting. All Members may serve a maximum of two consecutive three-year terms as a Member, unless the Official Methods Board approves reappointment with rationale. The chair is initially appointed for one three-year term and may serve one additional three-year term if reappointment is approved with rationale by the Official Methods Board and the President of AOAC.

b. **Full Members:** The Chair appoints at its pleasure, members to be Full (voting) Members of the Committee if approved with rationale by the Official Methods Board. If a Full Member is unable to attend a Committee Meeting, he/she may ask an Associate Member to serve as a Proxy in her/his absence. If a Full Member fails to participate in 3 or more consecutive meetings of the Committee, the Chair may petition to transfer that member to Associate status only due to non-participation in Committee Meetings. Once a Full Member is removed as a voting member due to failure to participate in 3 or more consecutive meetings of the Committee, the Chair will promptly appoint a replacement from the pool of Associate Members if approved with rationale by the Official Methods Board.

c. **Associate Members:** Associate Members will have all the same duties and responsibilities of a Full Member except for Voting. New members will ordinarily serve a minimum of one year as an Associate member before becoming eligible for appointment as a Full member of the Committee.

d. **Quorum:** A quorum of the Committee consists of 6 or more Full Members.

III. SUMMARY OF DUTIES AND RESPONSIBILITIES FOR MEMBERS OF THE COMMITTEE ON STATISTICS:

a. Guide and advise and consent on appropriate application and use of statistical techniques and tools and the interpretation of results;
b. Statistically evaluate and advise on study designs, numerical results, and address questions in a timely manner as requested by staff and Official Methods Board;
c. Advise AOAC stakeholder panels, working groups, and expert review panels as requested by Official Methods Board;
d. Advise Official Methods Board on changes to statistical tools, guidance, and approaches as it applies to standards development and conformity assessment activities;
e. Evaluate and/or provide guidance and advice on designing multi-laboratory studies, assigned method manuscripts, reporting work sheets, and use of appropriate statistical procedures; and
f. Other projects as assigned through AOAC staff or Official Methods Board.

IV. SUMMARY OF DUTIES AND RESPONSIBILITIES FOR THE CHAIR OF THE COMMITTEE ON STATISTICS:

a. Serve as primary representative of the Committee;
b. Must be a member of AOAC INTERNATIONAL in good standing
c. Facilitate and moderate discussions of the Committee Meetings;
d. Present and report on Committee activities and/or recommendations to AOAC Official Methods Board (OMB) and actively serve as primary Committee representative and participate on the OMB;
e. Oversee the implementation of AOAC policies and procedures in the Committee;
f. Work with staff on assigning Committee members for method reviews;
g. Work with staff and Official Methods Board on priorities to be addressed by the Committee; and
h. Work with staff and OMB on identification and approval of Committee membership.

V. CRITERIA FOR SERVING AS A COMMITTEE ON STATISTICS MEMBER:

a. Must be a trained statistician, or have documented formal training in statistics, applied statistics, or other sufficient relevant expertise;
b. Must be willing to carry out the duties and responsibilities described in the Section II;
c. Must be able to work cooperatively with other volunteers, stakeholders, experts, and staff;
d. Must submit a CV and a statement of expertise;
e. Must have a letter of support from the sponsoring organization; and
f. Must have an executed AOAC Volunteer Acceptance Form.

VI. COMMITTEE MEMBER APPOINTMENT:

If members are needed on the Committee on Statistics, AOAC will issue a Call for Experts. Interested candidates will complete an application that will include the submission of letters of interest addressing the criteria, resumes/CVs, and a letter of support from their sponsoring organization when applicable, to the AOAC staff. These letters are forwarded to the chair of the Committee on Statistics.

The Committee on Statistics chair recommends a draft of the revised Committee roster along with appropriate documentation to the OMB. A two-thirds vote in favor of approving the revised draft roster by OMB approves the addition of a new Committee member or an additional term of a current Committee member. Upon approval by the OMB, the revised roster is sent to the President of AOAC INTERNATIONAL with a request for volunteer appointments. A letter confirming the appointment is sent to all newly approved members. Copies of the letter and a final version of the revised Committee roster will be sent to Committee on Statistics chair. The appointment is generally for a three-year term unless otherwise recommended and approved by OMB.

At the conclusion of a Committee member's term, a thank you letter from the President is sent along with Certificate of Appreciation to the Committee member. A copy of the letter is sent to the Committee chair and OMB chair.

VII. COMMITTEE MEMBER REMOVAL OR RESIGNATION:

If a member of the Committee is not performing the duties appropriately, the Committee chair will submit a revised draft roster that recommends removal of the Committee member to the OMB for its consideration. A two-thirds vote in favor of approving the revised draft roster by OMB approves the removal of a Committee member.

If a member is not able or is no longer able to serve because of retirement, changes in employment responsibilities, removal, or if he/she becomes unable to carry out the duties of a Committee member, the member must notify AOAC staff and the Committee chair in writing regarding their need to resign from the Committee. The Committee chair will submit a revised draft roster that recommends removal of the Committee member to the OMB for its consideration. A two-thirds vote in favor of approving the revised draft roster by OMB approves the removal of a Committee member.

Upon removal, a thank you letter from the President is sent to the Committee member, with copies sent to the Committee chair and the Official Methods Board Chair. A thank you letter is sent if a Committee member resigns his/her appointment.

AOAC INTERNATIONAL

Committee on Statistics

(Terms of Reference Comments)

June 14, 2017

	Name	Section	Sub-Section	Line Number(s)	Type of Comment	Proposed Change by Submitter	Comment(s) - Justification for change
1	Mike Zapf	I. Scope	a. Composition	13-33	General Comment(s)	under comments	I have benefited from Paul & Qian's comments, and see there points to favor their consideration. I am surprised that the committee might be 18 rather than 12, it seems a little large and suggest it might consist of 9 full members + 3 associates, "in training". I think at 18 there could be a lot of disinterested bystanders who become bored as the amount of tasks in StatCom to disperse is small.
2	Sidney Sudberg	II. Committee	a. Composition	17, 18, 19	Editorial	All Members may serve a maximum of two consecutive three-year terms as a Full Member, after which they could choose to continue to serve as an Associate Member. The Chair, at its pleasure, can suggest removal of non-participating members if removal is approved with rationale by the Official Methods Board.	To disrupt the committee's activities by having to wait for OMB to approve a reappointment, it would seem self-defeating for a successful committee to continue to maintain its' success by 'constantly' changing that synergy? I am not sure why there would have to be term limits for members, especially now that the new TOR will address non-performing members, for whatever reasons, with OMB approval. Is there a vehicle for a Full Member, after serving 2 3-year terms to ever be a Full Member again? Maybe after 1 3-year term as an Associate Member, they could be reconsidered for Full Membership, again. Good Statisticians are hard to find & it seems like the committee & AOAC would be compromising itself by 'forcefully' removing good members. There should just be a good way of circulating all members, if nothing else, to allow for more good Talent to enter the mix.
3	Qian Graves	II. Committee	a. Composition	18	General Comment(s)	Current ToR: "With no maximum term limitation.' Newer Version:"Section II, line 18: Proposed change: "Maximum 6 years."	Comment: Not recommending this change. Reason: Due to the volunteer nature of the committee, and the fact that it takes years for a member to learn and to become productive; this change will interrupt the smoothness of running and operation of the committee. Right now about 70%-80% current committee members are on the committee for over 6 years so if we enforce this change, we will lose majority of the valuable and experienced committee members. Suggestions: a). keep the "With no maximum term limitation" Or: b). effective after annual meeting in 2017, counting 6 years after the date (not retrospective), allow members serving up to 6 years forward starting Sept 29, 2017.
4	Sidney Sudberg	II. Committee	d. Quorum	38 - ?, but could be applied to another section?	Editorial	1. A provision for the Chair &/or a Majority of Quorum to evoke a 'Roll Call Vote' for important votes to be voted on by 'entire' Committee, Associate & Full Members in a timely manner, should be included. 2. This way, the Chair or a Quorum of Full Voting Members could ask for a Roll Call Vote at any time, allowing only a Majority of 'yeas' or 'nays' to move forward & abstentions are not counted.	This would potentially allow for complex issues or 'major' changes, etc. to be 'Democratically' voted on by the entire Committee to give the chosen subject matter the consideration it is due, by all members! This would also potentially prevent a small handful of members from controlling the decision making process, if there was such a case. This would more than likely be a rare occurrence but it would serve as a safeguard against these rare occurrences.
5	Mike Zapf	V. Criteria for Serving as a Committee Member		74	General Comment(s)	...states "II", I believe it refers to section III	error

AOAC QUALITATIVE STUDY STATISTICAL REVIEW CHECKLIST

DOCUMENT ID: _____

Approve __ Changes needed, no review __ Changes needed, new review __ Reject __

(Place 'N' beside any of the below where a defect is found.)

TITLE:

1. __ Is the analyte stated?
2. __ Is the AOAC method identifier stated?
3. __ Is the type of study described (PTM vs. SLV vs. MLV, etc.)?

ABSTRACT:

4. __ Are required measures of effect reported, along with 95% confidence intervals?
5. __ Are results reported separately for each matrix?
6. __ Are results reported for repeatability and reproducibility with 95% confidence intervals?
7. __ Is the use of unjustified compositing across matrices or collaborators avoided?
8. __ Are any hypothesis test results reported in proper form and is the observed confidence interval on measure of effect used to assess any conclusion drawn?
9. __ Is the study identified as matched (paired) or unmatched (unpaired) in methods to replicates?
10. __ Are the reported results free of improper generalization?

INTRODUCTION:

11. __ Is the current state of the art for the same analyte and matrices properly reviewed?
12. __ Is the previous history of the candidate method reviewed?

METHOD & MATERIALS:

13. __ Are the matrices unambiguously defined sufficiently to allow reproduction of the study?
14. __ Are the sampling and method of preparation described adequately?
15. __ Is the mixing of the samples homogeneous, and is there validation given for this?
16. __ Is the method of removal of test portions precisely defined so as to indicate preclusion of possible correlation of results?
17. __ Are outliers excluded solely for physical cause, which has been identified and supported by objective proof?
18. __ Are the required minimum number of replicates performed?
19. __ Are the required minimum number of collaborators used?
20. __ Is the study identified as matched (paired) or unmatched (unpaired) in methods to replicates?
21. __ Are the test portion replicates adequately randomized and masked with respect to preparation and distribution to collaborators?

RESULTS & DISCUSSION:

22. __ Is the statistical analysis proper for the design?
23. __ Are the required measures of effects obtained with 95% confidence intervals?
24. __ Are repeatability and reproducibility properly obtained with 95% confidence intervals?
25. __ If a hypothesis test is performed, is it based on detection of a pre-specified size of effect, and does any stated conclusion reflect this?
26. __ Has the size of inhomogeneity across collaborators been properly assessed?
27. __ Have all observed phenomena, including surprising results, been discussed and resolved?
28. __ Are all methods of statistical calculations documented and reproducible?

TABLES:

29. __ Are tables in a format which contains all necessary information, including measures of effect and confidence intervals?
30. __ Are all statistical methods supported by references as to proper method of calculation?
31. __ Are all descriptors and symbols properly defined?

DATA:

32. __ Is a table of original data (before any outlier removal) provided in the required format?

FIGURES:

33. __ Are the data and any added curves or intervals correctly shown?

OTHER:

34. __ Are all other issues properly resolved?

(Attached any explanatory comments, indexed by the question numbers above.

Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces

Table of Contents **Page**

This document contains technical guidelines for the data collection requirements for microbiology methods. The document also contains specific programmatic requirements and information related to the AOAC *Official Methods*^{SM} and *Performance Tested Methods*^{SM} programs. Actual programmatic requirements and information may differ from the information stipulated in this guidance document. For the most current programmatic requirements, contact AOAC INTERNATIONAL staff at aoac@aoac.org.

The guidelines were approved by the AOAC Methods Committee on Microbiology and Official Methods Board in September 2011.

1 Scope

The purpose of this document is to provide comprehensive AOAC INTERNATIONAL (AOAC) technical guidelines for conducting microbiological validation studies of food and environmental analysis methods submitted for AOAC® *Official Methods of Analysis*℠ (OMA) status and/or *Performance Tested Methods*℠ (PTM) certification.

2 Applicability

These guidelines are applicable to the validation of any candidate method, whether proprietary or nonproprietary, that is submitted to AOAC for OMA status or PTM certification. Circumstances, unforeseen by AOAC, may necessitate divergence from the guidelines in certain cases. The PTM Program requires a Method Developer Study and an Independent Laboratory Study. The OMA Program requires a Single-Laboratory Validation (SLV) Study (also known as the Precollaborative Study), an Independent Validation Study, and a Collaborative Study. A harmonized PTM-OMA program can be followed in which PTM certification is sought and, if successful, serves as the SLV and Independent Validation phase of the OMA program. This approach provides a certification while working toward OMA status. *See* Table 1 for more detail.

3 Terms and Definitions

3.1 Analyte

Microorganism or associated biochemicals (e.g., DNA, proteins, or lipopolysaccharides) measured or detected by the method of analysis.

3.2 Candidate Method

The method submitted for validation.

3.3 Candidate Method Result

The final result of the qualitative or quantitative analysis for the candidate method. For methods with a confirmation phase, only presumptive positive results that confirm positive are considered as positive for the candidate method. All other results are considered as negative for the candidate method.

Table 1

AOAC Program	Study Requirements	Relevant Guideline Sections		
		Qualitative	Quantitative	Confirmatory Identification
PTM	Method Developer Validation Study	4.1	5.1	6.1
OMA	SLV (Precollaborative Validation) Study	4.1.2 and 4.1.3	5.1.2 and 5.1.3	6.1.2
	Independent Validation Study	4.2	5.2	6.2
	Collaborative Validation Study	4.3	5.3	6.3
Harmonized PTM-OMA	Method Developer Validation Study	4.1	5.1	6.1
	Independent Validation Study	4.2	5.2	6.2
	Collaborative Validation Study	4.3	5.3	6.3

3.4 Collaborative Study (CS)

A validation study performed by multiple laboratories to estimate critical candidate method performance parameters.

3.5 Composite Test Portion

Test portions taken from multiple samples of the same matrix combined together.

3.6 Confirmatory Identification Method

Method of analysis whose purpose is to determine the identity of an analyte. (Biological Threat Agent Method; BTAM)

3.7 Confirmatory Phase

A procedure specified in some qualitative assays whereby a preliminary presumptive result is confirmed by a subsequent and different method.

3.8 Confirmed Result

The qualitative response from the confirmatory phase of a candidate method.

3.9 Enrichment Pool

A pool comprised of aliquots from multiple test portion enrichments.

3.10 Exclusivity

The nontarget strains, which are potentially cross-reactive, that are not detected by the method.

3.11 Fractional Recovery

Validation criterion that is satisfied when an unknown sample yields both positive and negative responses within a set of replicate analyses. The proportion of positive responses should fall within 25 and 75% and should ideally approximate 50% of the total number of replicates in the set. A set of replicate analyses are those replicates analyzed by one method (either candidate or reference). Only one set of replicates per matrix is required to satisfy this criterion.

An alternate plan acceptable to the Statistics Committee can be used.

3.12 Inclusivity

The strains or isolates of the target analyte(s) that the method can detect. (BTAM)

3.13 Limit of Detection$_{50}$ (LOD_{50})

The analyte concentration at which the probability of detection (POD) is equal to 50%.

3.14 Matched Analyses

Two or more analyses or analytical results on the same unknown sample, which can be traced to the same test portion.

3.15 Matrix

The food, beverage, or environmental surface material to be included in the validation as per the intended use of the method.

3.16 Method Developer Validation Study or Single-Laboratory Validation (SLV or Precollaborative) Study

A validation study performed by a single laboratory in order to systematically estimate critical candidate method performance parameters. The method developer study is usually performed by the organizing laboratory or Study Director.

3.17 Precision

The closeness of agreement between independent test results under stipulated conditions. (ISO 5725-1)

3.18 Presumptive Phase

The initial qualitative determination of the analyte in a test portion. In some qualitative microbiological assays, confirmation of results is required as specified in the method.

3.19 Presumptive Result

The qualitative response from the presumptive phase of a candidate method that includes a confirmatory phase.

3.20 Probability of Detection (POD)

The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. Several POD measures can be calculated, e.g., POD_R (reference method POD), POD_C (confirmed candidate method POD), POD_{CP} (candidate method presumptive result POD) and POD_{CC} (candidate method confirmation result POD). Other POD estimates include:

dPOD – the difference between any two POD values

LPOD – the POD value obtained from combining all valid collaborator data sets for a method for a given matrix at a given analyte level or concentration

dLPOD – the difference between any two LPOD values

3.21 Qualitative Method

Method of analysis whose response is either the presence or absence of the analyte detected either directly or indirectly in a specified test portion.

3.22 Quantitative Method

Method of analysis whose response is the amount (count or mass) of the analyte measured either directly (e.g., enumeration in a mass or a volume), or indirectly (e.g., color absorbance, impedance, etc.) in a specified test portion.

3.23 Reference Method

Preexisting recognized analytical method against which the candidate method will be compared. (BTAM)

3.24 Repeatability

Precision under repeatability conditions. (ISO 5725-1)

3.25 Repeatability Conditions

Conditions where independent test results are obtained with the same method on equivalent test items in the same laboratory by the same operator using the same equipment within short intervals of time.

3.26 Reproducibility

Precision under reproducibility conditions. (ISO 5725-1)

3.27 Reproducibility Conditions

Conditions where independent test results are obtained with the same methods on equivalent test items in different laboratories with different operators using separate instruments.

3.28 Robustness Study

A study which tests the capacity of a method to remain unaffected by small but deliberate variations in method parameters and which provides an indication of its reliability during normal usage. (USP 31)

3.29 Sample

The batch of matrix from which replicate test portions are removed for analysis. The sample (naturally contaminated, uncontaminated, or inoculated) contains analyte, if present, at one homogeneous concentration.

3.30 Test Portion

A specified quantity of the sample that is taken for analysis by the method.

3.31 Unmatched Analyses

Two or more analyses or analytical results on the same unknown sample, which cannot be traced to the same test portion.

4 Qualitative Methods—Technical Protocol for Validation

4.1 Method Developer Validation Study or Single-Laboratory Validation (SLV or Precollaborative) Study

4.1.1 Scope

The Method Developer Validation Study is intended to determine the performance characteristics of the candidate method. The study is designed to evaluate performance parameters including inclusivity, exclusivity, and probability of detection (POD). For PTM submissions, robustness is also included. The Method Developer Study is normally conducted in a single laboratory, usually the method developer's laboratory. Alternatively, the method developer can contract the work to an independent site.

The SLV or Precollaborative Study is a formal submission requirement for OMA microbiology methods and is normally conducted in the method developer laboratory. It precedes the Collaborative Study. The purpose of an SLV Study is to define the applicability claims of a proposed OMA method by demonstrating the applicability of the method to various foods and/or environmental samples. For OMA methods, the applicability statement immediately follows the method title. The applicability statement for microbiological methods is generally concerned with target analyte and matrix coverage.

4.1.2 Inclusivity/Exclusivity Study

4.1.2.1 Species/Strain Selection

The choice of inclusivity strains should reflect the genetic and/or serological and/or biochemical diversity of the organisms involved, as well as other factors such as virulence, frequency of occurrence and availability. Select at least 50 pure strains of the target organism(s) to be analyzed as pure culture preparations. For *Salmonella* methods, the number of target organisms is increased to at least 100 serovars that are selected to represent the majority of known somatic groups and subtypes of *Salmonella*.

The choice of exclusivity strains should reflect closely related, potentially cross-reactive organisms. Other factors such as virulence, frequency of occurrence and availability should be considered. Select at least 30 strains of potentially competitive organisms.

Species/strains specified for use must be traceable to the source. The source and origin of each species/strain should be documented.

4.1.2.2 Study Design

Inclusivity strains are cultured by the candidate method enrichment procedure. The target concentration for testing is 100 times the LOD_{50} of the candidate method. Test one replicate per strain. Exclusivity strains are cultured in nonselective media. The target level is the growth limit of the organism. Test one replicate per strain. If the cross reactive strain is detected repeat the analysis using the enrichment conditions prescribed in the candidate method. Report all results.

Inclusivity and exclusivity evaluations shall be performed together as one study. Inclusivity and exclusivity test samples must be blind coded, randomized and intermingled so the analysts cannot know the identity, sequence or concentration of the test samples.

4.1.2.3 Data Reporting

Report inclusivity data as determined in *4.1.2.2* as number of strains detected. For example, "Of the 50 specific inclusivity strains tested, 47 were detected and 3 were not detected. Those strains not detected were the following: …"

Report exclusivity data as determined in *4.1.2.2* as number of strains not detected. For example, "Of the 30 specific exclusivity strains tested, 28 were not detected and 2 were detected. Those detected were the following: …"

The study report should include a table titled "Inclusivity/Exclusivity Panel Results," which lists all strains tested, their source, origin and essential characteristics plus testing outcome. Any unexpected results must be discussed.

4.1.3 Matrix Study

4.1.3.1 Reference Method

Candidate methods are compared to a cultural reference method where applicable. The following methods are examples of acceptable reference methods: AOAC OMA, U.S. Food and Drug Administration *Bacteriological Analytical Manual* (BAM), U.S. Department of Agriculture–Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (MLG) (for meat and poultry products), International Organization for Standardization (ISO) and Health Canada *Compendium of Analytical Methods*.

4.1.3.2 Food Categories

AOAC INTERNATIONAL recognizes claims for the range of specific food matrices successfully validated in the Method Developer Study, or the PCS and CS. The number of different matrices required for testing depends on the applicability of the method. All claimed matrices must be included in the Method Developer Study and the PCS.

4.1.3.3 Environmental Surfaces

The number of different surface types required for testing depends on the applicability of the method. The Study Director may choose from the following surfaces: stainless steel, plastic (polyethylene, polypropylene, or polycarbonate), ceramic (glazed earthen material or glass), rubber, sealed concrete (a commercially available product that "seals concrete pores"), cast iron (coated to prevent rusting), and air filter material. Alternatively, the method claim may be limited to one or more specific surfaces. All claimed surface types must be included in the Method Developer Study or the PCS.

For surfaces to be sampled with a swab, each test area should measure 1″ × 1″. For surfaces to be sampled with a sponge, each test area should measure 4″ × 4″.

4.1.3.4 Levels of Contamination

Each matrix (food, beverage, or surface material) is divided into at least three samples. One sample serves as the uncontaminated level (for naturally contaminated matrices, an uncontaminated level is not required), one or more samples are contaminated at levels that will produce at least one reference method POD (POD_R) or candidate method POD (POD_C) in the range of 0.25–0.75. Finally, one sample should be contaminated at such a level to assure a POD_C of nearly 1.00, with as high a degree of confidence as possible. Depending on the laboratory's confidence in satisfying this validation criterion, it may be advisable to prepare a fourth sample targeting the fractional POD range. All outcomes for each contamination level tested, whether fulfilling the POD requirement or not must be reported.

The target concentration for the fractional POD range is typically 0.2–2 CFU/test portion for foods and beverages, depending on the matrix. The target concentration for POD = 1.00 is approximately 5 CFU/test portion for foods and beverages. Target concentrations for fractional PODs on environmental surfaces can be in the range 10^4–10^6 CFU/surface area, depending on the surface, organism, and environmental conditions of the testing area.

A 5-tube 3-level Most Probable Number (MPN) estimation of contamination levels (1) must be conducted on the day that the analysis of test samples is initiated. The MPN analysis scheme may also make use of the reference method replicates. *See Annex A* for details.

For environmental surface studies, an MPN analysis is not applicable.

If the method is intended to detect more than one target organism simultaneously from the same test portion, the validation study should be designed so that target organisms are inoculated into a common sample and the validation tests are performed in a simultaneous manner.

4.1.3.5 Number of Test Portions

The number of replicate test portions method per level is 5 for the high inoculation level, 20 for the fractional positive level and 5 for the uncontaminated level.

4.1.3.6 Test Portion Size, Compositing and Pooling

Sample sizes required are as written in each method.

Test portion compositing is the combining of test portions prior to enrichment and can be validated alongside the standard test portion size if desired. The standard test portion size is utilized for the reference method and the standard test portion size is mixed with X uncontaminated test portions to create composite test portions for validation by the candidate method. For example, if a candidate method is to be validated for 375 g composites (15 × 25 g analytical units), then, for each level, one set of 20 composited test portions are made by combining twenty single 25 g inoculated test portions with twenty 350 g uninoculated test portions to form the twenty 375 g composited test portions. These 375 g candidate method composites are then compared to the 25 g reference method test portions. MPNs are performed only on the batch samples from which the reference method test portions are taken. Acceptance criteria for composited test portions are the same as for the standard test portion size.

Pooling is the post-enrichment combining of aliquots from more than one enriched test portion. This is validated by preparing replicate test portions for the candidate method and replicate test portions for the reference method, either as matched or unmatched test portions. At the conclusion of the enrichment procedure, test each enriched test portion by the candidate and/or reference method as appropriate. In addition, pool (dilute) an aliquot of each test portion with X aliquots, as specified by the candidate method, of known negative enriched test portions. Acceptance criteria for pooled enriched test portions are the same as for the standard test portion analyses.

4.1.3.7 Source of Contamination

Naturally contaminated matrix is preferred as a source of inoculum, if available. An effort should be made to obtain naturally contaminated matrix as it is most representative of the method usage environment. If naturally contaminated matrix cannot be found, then pure culture preparations may be used for artificial inoculation.

Numerous strains representing different serotypes or genotypes are required, if applicable. Typically a different isolate, strain, biovar or species is used for each matrix. The product inoculation should be conducted with a pure culture of one strain per target analyte. Mixed cultures are used only for multianalyte methods.

4.1.3.8 Preparation of Artificially Contaminated Samples

4.1.3.8.1 Food

Microorganisms in processed foods are typically stressed, thus the contaminating microorganisms are also stressed for these types

of foods. Microorganism stress may occur at the time of inoculation or during preparation of the food. Raw and cold-processed foods should be inoculated with unstressed organisms, heat-processed foods with heat-stressed organisms (e.g., heat culture at 50°C for 10 min), and dry foods with lyophilized culture. Mix well by kneading, stirring or shaking as appropriate. Frozen foods should be thawed, inoculated, mixed and refrozen.

The degree of injury caused by heat stressing should be demonstrated, for nonspore-formers, by plating the inoculum in triplicate on selective and nonselective agars. The degree of injury is calculated as follows:

$$(1 - \frac{n_{select}}{n_{nonselect}}) \times 100$$

where n_{select} = mean number of colonies on selective agar and $n_{nonselect}$ = mean number of colonies on nonselective agar. The heat stress must achieve 50–80% injury of the inoculum. The inoculum should be added to the sample, mixed well and allowed to equilibrate in the matrix for 48–72 h at 4°C for refrigerated foods, for a minimum of 2 weeks at –20°C for frozen foods or for a minimum of 2 weeks at room temperature for dried foods prior to analysis.

4.1.3.8.2 Environmental Surfaces

Strains should be grown in conditions suitable for target organism to achieve stationary phase cells. The selected surface types will receive an inoculum of cells sufficient to provide fractional recovery by either the candidate method or reference method, if applicable. Inoculation levels may need to be adjusted depending on the strain/surface being used to achieve fractional recovery. The initial culture should be diluted into an appropriate stabilizing medium for inoculation onto test surface. The stock culture should also be diluted to a volume that will allow for even distribution of inoculum over entire test surface area, but without producing excessive accumulation of liquid that may dry unevenly. The surface is allowed to dry for 16–24 h at room temperature (20–25°C). The surface must be visually dry at the time of test portion collection.

4.1.3.9 Preparation of Naturally Contaminated Samples

Naturally contaminated matrix may be mixed with uncontaminated matrix of the same food or incubated to achieve a level yielding fractionally positive results. Naturally contaminated surface materials may be used as is, as long as the requirement for yielding fractionally positive results is achieved.

4.1.3.10 Need for Competitive Microflora

It is more realistic and challenging to include microorganisms that act as competitors to the analyte microorganisms. The purpose of including these organisms is to more closely simulate conditions found in nature. It is sufficient to demonstrate this recovery in one matrix. This requirement may be satisfied in the SLV (Precollaborative) Study. The competitor contamination levels, which may be naturally occurring or artificially introduced, should be 10 times higher than the target microorganism.

4.1.3.11 Environmental Surface Sampling

The candidate method submitter will determine which surface will be sampled by sponge or swab. An environmental sampling sponge is a porous moisture absorbing matrix, approximately 2″ (5 cm) × 3″ (7.5 cm) often contained in a presterilized sample bag. An environmental swab is a sampling device comprised of synthetic (e.g., dacron) or cotton tips affixed to a wood or polymeric stick, delivered in a presterilized package.

Sponges and swabs are premoistened with a neutralizing broth, such as Dey-Engley (2), prior to sampling. The entire sampling area is sponged or swabbed in both a horizontal and vertical motion. Use the sponges to sample a 100 cm^2 (4″ × 4″) area and swabs to sample a 5 cm^2 (1″ × 1″) area. Sponges/swabs containing samples are placed back into their individual respective bag or tube and held at room temperature for 2 hours prior to initiation of testing.

4.1.3.12 Confirmation of Test Portions

Follow the reference method as written for isolation and confirmation of typical colonies from all candidate method test portions regardless of presumptive result. The method developer can perform their own confirmation procedure in addition to the reference method confirmation procedure.

4.1.3.13 Data Analysis and Reporting

Each level of each matrix must be analyzed and reported separately. The following section describes the data analysis to be performed according to the POD model. It is acceptable to analyze data according to the Chi Square statistical methodology for paired studies, and the Relative Limit of Detection (RLOD) for unpaired studies, as defined in the current revision of ISO 16140. Refer to ISO 16140 for detailed descriptions of Chi Square and RLOD.

4.1.3.13.1 Raw Data Tables

For each matrix and level, report each result from each test portion separately. *See Annex B* for raw data table format.

4.1.3.13.2 Probability of Detection (POD)

POD is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent.

The POD estimate is calculated as the number of positive outcomes divided by the total number of trials.

Estimate the POD with a 95% confidence interval for the candidate method, the reference method and, if included, the presumptive and confirmed results. *See Annex C* for details.

4.1.3.13.3 Difference of Probabilities of Detection (dPOD)

Difference of probabilities of detection is the difference between any two POD values.

Estimate the $dPOD_C$ as the difference between the candidate method and reference method POD values. Calculate the 95% confidence interval on the $dPOD_C$.

$$dPOD_C = POD_C - POD_R$$

Estimate the $dPOD_{CP}$ as the difference between the candidate presumptive result POD (POD_{CP}) and the candidate confirmed result POD (POD_{CC}) values. Calculate the 95% confidence interval on the $dPOD_{CP}$. *See Annex C* for details.

$$dPOD_{CP} = POD_{CP} - POD_{CC}$$

If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

4.1.3.13.4 Summary Data Tables

For all matrices and levels, use the summary table from *Annex D*.

4.1.3.13.5 Graph of Data

For each matrix, graph POD_R, POD_C, and dPOD by level with 95% confidence intervals. *See* example in *Annex E*.

4.1.3.13.6 Data Analysis and Reporting in the Absence of a Reference Method

If no appropriate reference method is available for the target analyte, indicate "Not Applicable" (NA) where appropriate in the summary tables.

4.1.4 Robustness Study [*Performance Tested Methods*℠ (PTM) submissions only]

4.1.4.1 Strain Selection

Robustness strains are prepared and analyzed as vegetative cells, spores or components thereof as applicable to the candidate method. One material is tested at a level that yields fractional recovery and one nontarget material is analyzed at the growth level achieved in a nonselective broth or at a high inoculation level.

4.1.4.2 Study Design

Minor, reasonable variations in a method of a magnitude that might well be expected to occur when the method is used are deliberately introduced and tested. Variations in method parameters that can be influenced by the end user should be tested. Use a screening factorial experimental design.

The method developer is expected to make a good faith effort to choose parameters that are most likely to affect the analytical performance and determine the range of variations that can occur without adversely affecting analytical results.

Ten replicates of each material are tested for each treatment combination.

4.1.4.3 Data Analysis and Reporting

The results are analyzed for variable detection due to changes in parameter settings. Report the appropriate statistical measures of the measured variable(s) (e.g., Ct, absorbance, POD value, etc.) for each set of replicates for each treatment combination. This should include at least means, standard deviations, and confidence intervals where appropriate.

4.2 Independent Validation Study

4.2.1 Scope

A validation study to corroborate the analytical results obtained by the method developer and to provide additional single laboratory data. The independent validation study traditionally verifies POD in the hands of an independent trained user and is required for PTM certification and OMA approval.

4.2.2 Reference Method

If there is a reference method, then the candidate method is compared to a reference method. The reference method should be the same as that used in the Method Developer Study.

4.2.3 Matrices

The independent laboratory must test at least one matrix that was tested in the Method Developer Study. The total number of matrices to be evaluated by the independent laboratory is dependent on the claim of the candidate method. For every five foods claimed, one food matrix shall be included in the independent study and for every five environmental surfaces claimed, one surface shall be included in the independent study. The choice of matrices for the Independent Study is made by the appropriate method volunteer(s) in consultation with the Study Director.

4.2.4 Study Design

The study design for validation of qualitative methods in the independent study follows the Method Developer Validation Study design. Contamination levels, number of test portions, test portion size, source of contamination, preparation of samples, confirmation of test portions, and data analysis and reporting are found in Section *4.1.3*. If composite test portions or pooling was validated in the Method Developer Validation Study, include it also in the Independent Validation Study.

4.3 Collaborative Study (CS)

4.3.1 Scope

The Collaborative Study (CS) report is a formal submission requirement for OMA methods only. The purpose of the Collaborative Study is to estimate the reproducibility and determine the performance of the candidate method among collaborators.

4.3.2 Number of Laboratories

At least 12 laboratories per matrix should be included due to potential failure to follow protocol. A minimum of 10 valid laboratory data sets per matrix are required.

4.3.3 Reference Method

The reference method used in the Collaborative Study must be the same as that used in the Method Developer Study or SLV (PCS). The reference method should be carried out by the organizing laboratory.

4.3.4 Matrix Selection

At least one matrix from those studied in the PTM or PCS shall be chosen by the appropriate method volunteer(s) in consultation with the Study Director for collaborative study. For methods with more than one sample preparation/enrichment, one matrix per procedure may be required in the collaborative study. The determination if the procedures differ significantly to warrant expanding the collaborative study is made by the appropriate method volunteer(s) in consultation with the Study Director. The Statistical Advisor and reviewers can be consulted during this determination. Examples of what constitutes a different sample preparation procedure would include different test portion size, different enrichment media or conditions, different dilution volume and different homogenization equipment. The AOAC appropriate method volunteer, Statistical Advisor and collaborative study protocol reviewers shall make the final selection of the matrix(es) with consideration of the PTM or PCS data and the relative importance of the matrices to food safety. The data from both the PCS and CS studies form the basis for defining the method applicability statement.

4.3.5 Analyte Level Estimation

Refer to Section *4.1.3.4*. Use the reference method (or candidate method if there is no reference method) test portions with additional levels to estimate the MPN using the formula in *Annex A*. The levels of contamination are one high level, one level where fractional recovery is expected, and one uninoculated level.

4.3.6 Number of Test Portions

The number of test portions is 12 at the high level, 12 at the fractional level, and 12 uncontaminated per method per laboratory. Test portions are to be randomized and blind-coded when sent to participating laboratories for analysis.

4.3.7 Test Portion Size, Compositing and Pooling

Sample sizes required are as written in each method.

Test portion compositing is the combining of test portions prior to enrichment and can be validated alongside the standard test portion size if desired. The standard test portion size is utilized for the reference method and the standard test portion size is mixed with X uncontaminated test portions to create composite test portions for validation by the candidate method. For example, if a candidate method is to be validated for 375 g composites (15 × 25 g analytical units), then, for each level, one set of 20 composited test portions are made by combining twenty single 25 g inoculated test portions with twenty 350 g uninoculated test portions to form the twenty 375 g composited test portions. These 375 g candidate method composites are then compared to the 25 g reference method test portions. MPNs are performed only on the batch samples from which the reference method test portions are taken. Acceptance criteria for composited test portions are the same as for the standard test portion size.

Pooling is the post-enrichment combining of aliquots from more than one enriched test portion. This is validated by preparing replicate test portions for the candidate method and replicate test portions for the reference method, either as matched or unmatched test portions. At the conclusion of the enrichment procedure, test each enriched test portion by the candidate and/or reference method as appropriate. In addition, pool (dilute) an aliquot of each test portion with X aliquots, as specified by the candidate method, of known negative enriched test portions. Acceptance criteria for pooled enriched test portions are the same as for the standard test portion analyses.

4.3.8 Source of Contamination

Refer to *4.1.3.7*.

4.3.9 Preparation of Artificially Contaminated Samples

Refer to *4.1.3.8*.

4.3.10 Preparation of Naturally Contaminated Samples

Refer to *4.1.3.9*.

4.3.11 Confirmation of Test Portions

Follow the reference method as written for isolation and confirmation of typical colonies from all candidate method test portions regardless of presumptive result.

4.3.12 Data Analysis and Reporting

Each concentration level of each matrix must be analyzed and reported separately. Data may be excluded due to an assignable cause if sufficient justification is provided. Excluded data must be reported, but should not be included in the statistical analysis. The following section describes the data analysis to be performed according to the POD model. It is acceptable to analyze data according to the Chi Square statistical methodology for paired studies, and the RLOD for unpaired studies, as defined in the current revision of ISO 16140. Refer to ISO 16140 for detailed descriptions of Chi Square and RLOD.

4.3.12.1 Raw Data Tables

For each matrix and concentration level, report each result from each test portion separately. *See Annex B* for raw data table format.

4.3.12.2 Estimate of Repeatability

Estimate the repeatability standard deviation (s_r) for qualitative methods according to *Annex F*.

4.3.12.3 Estimate of Reproducibility

Cross-laboratory estimates of probabilities of detection and their differences depend upon an assumption that the same performance is achieved in each laboratory. This assumption must be tested and the laboratory effect estimated. If the effect is large, method performance cannot be expected to be the same in two different laboratories.

For each matrix and level, calculate the standard deviation of the laboratory POD values (s_{POD}) and associated 95% confidence interval to estimate the reproducibility. *See Annex F* for details.

4.3.12.4 Cross-Laboratory Probability of Detection (LPOD)

Report the LPOD estimates by matrix and concentration with 95% confidence intervals for the candidate method and, if included, the presumptive and confirmed results. *See Annex F* for details.

4.3.12.5 Difference of Cross-Laboratory Probability of Detection (dLPOD)

Difference probability of detection is the difference between any two LPOD values.

Estimate the $dLPOD_C$ as the difference between the candidate and reference LPOD values. Calculate the 95% confidence interval on the $dLPOD_C$.

Estimate the $dLPOD_{CP}$ as the difference between the presumptive and confirmed LPOD values. Calculate the 95% confidence interval on the $dLPOD_{CP}$. *See Annex F* for details.

If the confidence interval of a dLPOD does not contain zero, then the difference is statistically significant.

4.3.12.6 Summary Data Tables

For all matrices and levels, use the summary table from *Annex G*.

4.3.12.7 Graph of Data

For each matrix, graph POD_R, $LPOD_C$, and $dLPOD_C$ by level with 95% confidence intervals. *See* example in *Annex E*.

4.3.12.8 Data Analysis and Reporting in the Absence of a Reference Method

If no appropriate reference method is available for the target analyte, indicate "Not Applicable" where appropriate in the summary tables.

5 Quantitative Methods—Technical Protocol for Validation

5.1 Method Developer Validation Study or SLV (Precollaborative) Study

5.1.1 Scope

The Method Developer Validation Study is intended to determine the performance of the candidate method. The study is designed to

evaluate performance parameters including inclusivity, exclusivity, repeatability, bias, and robustness. The Method Developer Study is normally conducted in a single laboratory, usually the method developer's laboratory. Alternatively, the method developer can contract the work to an independent site.

The SLV (Precollaborative) Study is a formal submission requirement for OMA microbiology methods and is normally conducted in the method developer laboratory. It precedes the Collaborative Study. The purpose of an SLV (Precollaborative) Study is to define the applicability claims of a proposed OMA microbiology method by demonstrating the applicability of the method to various food categories. For OMA methods, the applicability statement immediately follows the method title. The applicability statement for microbiological methods is generally concerned with target analyte and food type coverage.

5.1.2 Inclusivity/ Exclusivity

This requirement is not applicable to total viable count, yeast & mold count, or similar total enumeration methods that are not directed at specific microorganisms. The requirement applies to selective or differential quantitative methods.

5.1.2.1 Strain Selection

The choice of inclusivity strains should reflect the genetic and/or serological and/or biochemical diversity of the target organism(s). Select at least 50 pure strains of the target organism(s) to be analyzed as pure culture preparations. For *Salmonella* methods, the number of target organisms is increased to at least 100 serovars that are selected to represent the majority of known somatic groups and subtypes of *Salmonella*.

The choice of exclusivity strains should reflect closely related, potentially cross-reactive organisms. Other factors such as virulence, frequency of occurrence and availability should be considered. Select at least 30 pure strains of potentially competitive organisms.

Species/strains specified for use must be traceable to the source. The source and origin of each species/strain should be documented.

5.1.2.3 Study Design

Inclusivity strains are cultured in nonselective media. The target concentration for testing is 100 times the LOD_{50} of the method. Test one replicate per strain.

Exclusivity strains are cultured in nonselective media. The target level is the growth limit of the organism. Test one replicate per strain.

Inclusivity and exclusivity evaluations shall be performed together as one study. Inclusivity and exclusivity test samples must be blind coded and intermingled so the analysts cannot know the identity or concentration of the test samples.

5.1.2.4 Data Reporting

Report inclusivity data as number of strains detected. For example, "Of the 50 specific inclusivity strains tested, 47 were detected and 3 were not detected. Those strains not detected were the following: …"

Report exclusivity data as number of strains not detected. For example, "Of the 30 specific exclusivity strains tested, 28 were not detected and 2 were detected. Those detected were the following: …"

The study report should include a table titled "Inclusivity/ Exclusivity Panel Results," which lists all strains tested, their source, origin and essential characteristics plus testing outcome.

5.1.3 Matrix Study

5.1.3.1 Reference Method

Candidate methods are compared to a reference method where applicable. The following methods are examples of acceptable reference methods: AOAC OMA, FDA BAM, FSIS MLG (for meat and poultry products), ISO and Health Canada *Compendium of Analytical Methods*.

5.1.3.2 Food Categories

AOAC INTERNATIONAL recognizes claims for only the range of food categories or specific food types successfully validated in the Method Developer Study or the PCS and CS. The number of different matrices depends on the applicability of the method. All claimed matrices must be included in the Method Developer Study and the PCS.

5.1.3.3 Levels of Contamination

For the artificially contaminated food types, three inoculated levels (high, medium, and low) and one uninoculated level are required. For naturally contaminated food, three contamination levels (high, medium, and low) are required, and no uninoculated level. The low level should be near the limit of detection, and the medium and high levels should cover the analytical range of the candidate method. If the claimed range of the method is greater than 4 logs, intermediate levels may be required at the discretion of the appropriate method volunteer(s) in consultation with the Study Director.

If the method is intended to detect more than one target organism simultaneously from the same test portion, the validation study should be designed so that target organisms are inoculated into a common sample and the validation tests are performed in a simultaneous manner.

5.1.3.4 Number of Test Portions

For each level, analyze five test portions by the candidate method and five test portions by the reference method.

5.1.3.5 Source of Contamination

Naturally contaminated matrix is preferred as a source of inoculum, if available. Inoculating cultures are used only if the method is for a specific target analyte which may not routinely be found in all food types (e.g., enumeration of *Listeria* spp.) or a certain type has been referenced and the subject flora (e.g., yeast) has not been found in measurable levels.

5.1.3.6 Preparation of Artificially Contaminated Samples

Microorganisms in processed foods are typically stressed, thus the contaminating microorganisms are also stressed for these types of foods. Microorganism stress may occur at the time of inoculation or during preparation of the food. Raw and cold-processed foods should be inoculated with unstressed organisms, heat-processed foods with heat-stressed organisms (e.g., heat culture at 50°C for 10 min), and dry foods with lyophilized culture. Mix well by kneading, stirring or shaking as appropriate. Frozen foods should be thawed, inoculated, mixed and refrozen.

The degree of injury caused by heat stressing should be demonstrated, for nonspore-formers, by plating the inoculum in triplicate on selective and nonselective agars. The degree of injury is calculated as follows:

$$(1 - \frac{n_{select}}{n_{nonselect}}) \times 100$$

where n_{select} = mean number of colonies on selective agar and $n_{nonselect}$ = mean number of colonies on nonselective agar. The heat stress must achieve 50–80% injury of the inoculum. The inoculum should be added to the sample, mixed well and allowed to equilibrate in the matrix for 48–72 h at 4°C for refrigerated foods, for a minimum of 2 weeks at –20°C for frozen foods or for a minimum of 2 weeks at room temperature for dried foods prior to analysis.

5.1.3.7 Use of Artificially and Naturally Contaminated Test Samples

Approximately 50% of the food types should be naturally contaminated unless the method is for a specific microorganism that may not be naturally occurring in that number of food types. For the food types that are naturally contaminated, three different lots are required per food type. There are no uncontaminated levels required for the food types that are naturally contaminated.

The balance of the food types may be either naturally contaminated or artificially contaminated.

5.1.3.8 Need for Competitive Flora

For those candidate methods that are specific for target organisms, it is more realistic and challenging to include microorganisms that act as competitors to the analyte microorganisms. The purpose of including these organisms is to more closely simulate conditions found in nature. It is sufficient to demonstrate this recovery in one food type. This requirement may be satisfied in the Matrix Study. The competitor contamination levels, which may be naturally occurring or artificially introduced, should be at least 10 times higher than the target microorganism.

5.1.3.9 Confirmation of Test Portions

Follow the reference method as written for isolation and confirmation of typical colonies from all candidate method test portions.

5.1.3.10 Data Analysis and Reporting

5.1.3.10.1 General Considerations

Data often do not show a statistically normal distribution. In order to normalize the data, perform a logarithmic transformation on the reported CFU/unit (including any zero results) as follows:

$$\text{Log}_{10}\,[\text{CFU/unit} + (0.1)\text{f}]$$

where f is the reported CFU/unit corresponding to the smallest reportable result, and unit is the reported unit of measure (e.g., g, mL, filter). For details, *see Annex H*.

5.1.3.10.2 Initial Review of Data

If there is a reference method, plot the candidate method result versus the reference method result. The vertical *y*-axis (dependent variable) is used for the candidate method and the horizontal *x*-axis (independent variable) for the reference method. This independent variable *x* is considered to be accurate and have known values. Usually major discrepancies will be apparent.

5.1.3.10.3 Outliers

It is often difficult to make reliable estimations (average, standard deviation, etc.) with a small bias in presence of outliers. Data should be examined to determine whether there exists an occasional result that differs from the rest of the data by a greater amount than could be reasonably expected or found by chance alone. Perform outlier tests (Cochran and Grubbs) in order to discard significantly outlying values (3). There must be an explanation for every excluded result; no results can be excluded on a statistical basis only. To view the data adequately, construct a stem-leaf display, a letter-value display, and a boxplot (4).

Results excluded for justifiable cause must be reported, but should not be included in the statistical analysis.

5.1.3.10.4 Repeatability (s_r)

Calculate repeatability as the standard deviation of replicates at each concentration of each matrix for each method.

5.1.3.10.5 Mean Difference Between Candidate and Reference Where Applicable

Report the mean difference between the candidate and reference method transformed results and its 95% confidence interval. In addition, report the reverse transformed mean difference and confidence interval in CFU/unit or spores/mL.

5.1.4 Robustness Study (PTM submissions only)

5.1.4.1 Strain Selection

Robustness strains are prepared and analyzed as vegetative cells, spores or components thereof as applicable to the candidate method. One target strain is tested using the candidate method enrichment at a high and low level within the quantitative range of the candidate method. One nontarget strain is enriched in a nonselective broth and tested at the high level.

5.1.4.2 Study Design

Minor, reasonable variations in a method of a magnitude that might well be expected to occur when the method is used are deliberately introduced and tested. Variations in method parameters that can be influenced by the end user should be tested. Use a screening factorial experimental design.

The method developer is expected to make a good faith effort to choose parameters that are most likely to affect the analytical performance and determine the range of variations that can occur without adversely affecting analytical results.

Five replicates at each target concentration and five replicates of the nontarget are tested for each factorial pattern.

5.1.4.3 Data Analysis and Reporting

The results are analyzed for effects on bias and repeatability. Standard deviations (s_r) at each concentration are compared to determine if any robustness parameter value causes more than a 3-fold increase in s_r.

5.2 Independent Validation Study

5.2.1 Scope

A validation study to corroborate the analytical results obtained by the method developer and to provide additional single laboratory

data. The independent validation study traditionally verifies repeatability in the hands of an independent trained user.

5.2.2 Reference Method

If there is a reference method, then the candidate method is compared to a reference method. The reference method should be the same as that used in the method developer study.

5.2.3 Matrices

The independent laboratory must test at least one matrix that was tested in the Method Developer Study. The total number of matrices to be evaluated by the independent laboratory is dependent on the claim of the candidate method. For every five foods claimed, one food matrix shall be included in the independent study and for every five environmental surfaces claimed, one surface shall be included in the Independent Study. The choice of matrices for the Independent Study is made by the appropriate method volunteer(s) in consultation with the Study Director.

5.2.4 Study Design

The study design for validation of quantitative methods in the independent study follows the Method Developer Validation Study design. Contamination levels, number of test portions, source of contamination, preparation of samples, confirmation of test portions, and data analysis and reporting are found in Section *5.1.3*.

5.3 Collaborative Study (CS)

5.3.1 Scope

The Collaborative Study (CS) is a formal submission requirement for OMA methods and succeeds the SLV (Precollaborative) Study. The purpose of the Collaborative Study is to estimate the reproducibility and determine the performance of the candidate method among collaborators.

5.3.2 Number of Laboratories

A minimum of eight laboratories reporting valid data for each food type is required. It is suggested that at least 10–12 laboratories begin the analysis.

5.3.3 Reference Method

Candidate methods are compared to a reference method where applicable. The reference method(s) used in the collaborative study must be the same as those used in the SLV (Precollaborative) Study.

5.3.4 Matrix Selection

At least one matrix from those studied in the PTM or PCS shall be chosen by the appropriate method volunteer(s) in consultation with the Study Director for collaborative study. For methods with more than one sample preparation/enrichment, one matrix per procedure may be required in the collaborative study. The determination if the procedures differ significantly to warrant expanding the collaborative study is made by the appropriate method volunteer(s) in consultation with the Study Director. The Statistical Advisor and reviewers can be consulted during this determination. Examples of what constitutes a different sample preparation procedure would include different test portion size, different enrichment media or conditions, different dilution volume and different homogenization equipment. The appropriate AOAC method volunteer(s) shall make the final selection of the matrix(es) with consideration of the PTM or PCS data and the relative importance of the matrices to food safety. The data from both the PCS and CS studies form the basis for defining the method applicability statement.

5.3.5 Levels of Contamination

For the artificially contaminated food types, three inoculated levels (high, medium, and low) and one uninoculated level are required. For naturally contaminated food, three contamination levels (high, medium, and low) are required, and no uninoculated level. The low level should be near the limit of detection, and the medium and high levels should cover the analytical range of the candidate method. If the claimed range of the method is greater than 4 logs, intermediate levels may be required at the discretion of the appropriate method volunteer(s) in consultation with the Study Director.

If the method is intended to detect more than one target organism simultaneously from the same test portion, the validation study should be designed so that target organisms are inoculated into a common sample and the validation tests are performed in a simultaneous manner.

5.3.6 Number of Test Portions

For each contamination level, two test portions are analyzed by the candidate method and two test portions are analyzed by the reference method in each laboratory.

5.3.7 Enumeration of Specific Microorganisms

If the candidate method is for quantitation of a specific microorganism, it may be necessary to include certain food types known to support the growth of such analytes. The inoculating microorganisms must represent different genera, species and/or toxin-producing microorganisms that are intended to be included in the method applicability statement. The choice of strains should be broad enough to represent the inherent variation in the microorganisms of interest.

5.3.8 Source of Contamination

Refer to section *5.1.3.5*.

5.3.9 Preparation of Artificially Contaminated Samples

Refer to section *5.1.3.6*.

5.3.10 Use of Artificially and Naturally Contaminated Test Samples

The use of both naturally and artificially contaminated test samples is strongly encouraged. Because naturally contaminated foods are not always available particularly for methods applicable to specific microorganisms, artificially contaminated test samples may be used.

5.3.11 Confirmation of Test Portions

Follow the reference method as written for isolation and confirmation of typical colonies from all candidate method test portions.

5.3.12 Data Analysis and Reporting

For a detailed explanation of the quantitative method calculations to be performed, refer to *Appendix D* (3).

5.3.12.1 General Considerations

Data often do not show a statistically normal distribution. In order to normalize the data, perform a logarithmic transformation on the reported CFU/unit (including any zero results) as follows:

$$Log_{10} [CFU/unit + (0.1)f]$$

where f is the reported CFU/unit corresponding to the smallest reportable result, and unit is the reported unit of measure (e.g., g, mL, 25 g). For details, *see Annex H*.

5.3.12.2 Initial Review of Data

Plot the candidate method result versus the reference method result. The vertical *y*-axis (dependent variable) is used for the candidate method and the horizontal *x*-axis (independent variable) for the reference method. This independent variable *x* is considered to be accurate and have known values. Usually major discrepancies will be apparent.

Construct a Youden plot. For a given matrix–level combination, plot replicate pairs as first replicate versus second replicate. Usually major discrepancies will be apparent: displaced means, unduly spread replicates, outlying values, differences between methods, consistently high or low laboratory rankings, etc.

Only valid data should be included in the statistical analysis.

5.3.12.3 Outliers

It is often difficult to make reliable estimations (average, standard deviation, etc.) with a small bias and in presence of outliers. Data should be examined to determine whether any laboratory shows consistently high or low values or an occasional result that differs from the rest of the data by a greater amount than could be reasonably expected or found by chance alone. Perform outlier tests (Cochran and Grubbs) in order to discard the outlying values and to obtain a better estimate (3). There must be an explanation for every excluded data set; no data sets can be excluded on a statistical basis only. To view the data adequately, construct a stem-leaf display, a letter-value display, and a boxplot (4).

5.3.12.4 Performance Indicators

Performance indicators for quantitative methods include repeatability and reproducibility standard deviations of the transformed data.

5.3.12.4.1 Repeatability (s_r)

Calculate repeatability as the standard deviation of replicates at each concentration of each matrix for each laboratory.

5.3.12.4.2 Reproducibility (s_R)

Calculate reproducibility as the standard deviation of replicates at each concentration for each matrix across all laboratories.

5.3.12.5 Mean Difference between Candidate and Reference Methods Where Applicable

Report the mean difference between the candidate and reference method transformed results and its 95% confidence interval. In addition, report the reverse transformed mean difference and confidence interval in CFU/unit.

5.3.12.6 Calculations

For details, refer to *Appendix D* (3).

6 Confirmatory Identification Methods

6.1 Method Developer Validation Study or SLV (Precollaborative) Study

6.1.1 Scope

The Method Developer Study is intended to determine the performance of a microbiological confirmatory identification method. The study is designed to evaluate performance parameters including inclusivity, exclusivity, and robustness. The Method Developer Study is normally conducted in a single laboratory, usually the method developer's laboratory. Alternatively, the method developer can contract the work to an independent site.

The SLV (Precollaborative) Study is a formal submission requirement for OMA microbiology methods and is normally conducted in the method developer laboratory. It precedes the Collaborative Study. The purpose of an SLV (Precollaborative) Study is to define the applicability claims of a proposed OMA microbiology method. For OMA methods, the applicability statement immediately follows the method title.

6.1.2 Inclusivity/Exclusivity Study

6.1.2.1 Species/Strain Selection

The choice of inclusivity strains should cover the genetic, serological, biochemical or physical diversity of the target agent group(s) as appropriate for the method. The number of organisms required for validation will be determined by the diversity of the target agent group(s) and the intended use claim. The number of strains tested should be no less than 50 for each target species claimed, if available. For *Salmonella* methods, the number of target organisms is increased to at least 100 serovars that are selected to represent the majority of known somatic groups of *Salmonella*.

The choice of exclusivity strains should include organisms not claimed by the confirmatory identification method. The choice of exclusivity strains should reflect closely related, potentially competitive organisms. Other factors such as virulence, frequency of occurrence and availability should be considered. The number of species/strains tested should be no less than 30.

Species/strains selected for testing must be different than those used to develop the method if possible. Species/strains specified for use must be traceable to the source. The source and origin of each species/strain should be reported. Species/strains must have Certificate of Analysis from the source documenting the identity and method(s) used to determine the identity or be well characterized before use with documentation on file.

The study designs presented are intended to be a suggested guideline. Specific study designs and numbers of strains will be determined by the Methods Committee on Microbiology on a case by case basis.

6.1.2.2 Study Design

Inclusivity strains are prepared and analyzed as vegetative cells on the media designated in the candidate method. All media recommended for use with the candidate method must be validated. Test one replicate per strain per medium using the candidate method.

Exclusivity strains are prepared and analyzed as vegetative cells on the media designated in the candidate method. All media recommended for use with the candidate method must be validated. Test one replicate per strain per medium using the candidate method.

Inclusivity and exclusivity evaluations shall be performed together as one study. Inclusivity and exclusivity test samples must be blind coded and intermingled so the analysts cannot know the identity of the test samples.

6.1.2.3 Data Analysis and Reporting

Analyze the data for correct identification, misidentification or unidentified organism. The data is reported as number of species/strains correctly identified.

The data is reported as number of species/strains correctly identified. For example, "Of the 50 specific inclusivity strains tested, 48 were correctly identified and 2 were misidentified. Those strains misidentified were the following: …" or "Of the 30 specific exclusivity strains tested, 27 were correctly unidentified and 3 were misidentified. Those misidentified by the method were the following: …"

The study report should include a table titled "Inclusivity/Exclusivity Panel Results," which lists all species/strains tested their source, origin and essential characteristics plus testing outcome.

6.1.3 Robustness Study (PTM submissions only)

6.1.3.1 Strain Selection

Robustness strains are prepared and analyzed as vegetative cells on agar(s) recommended by the candidate method. Prepare 10 inclusivity strains and five exclusivity strains for testing.

6.1.3.2 Study Design

Minor, reasonable variations in a method of a magnitude that might well be expected to occur when the method is used are deliberately introduced and tested. Variations in method parameters that can be influenced by the end user should be tested. Use a screening factorial experimental design.

The method developer is expected to make a good faith effort to choose parameters that are most likely to affect the analytical performance and determine the range of variations that can occur without adversely affecting analytical results.

Test one replicate of each inclusivity and exclusivity organism for each factorial pattern.

6.1.3.3 Data Analysis and Reporting

The results are analyzed for the number of misidentifications when method parameters are altered. Report the identification results for each factorial pattern.

6.2 Independent Validation Study

6.2.1 Scope

A validation study to corroborate the analytical results obtained by the method developer and to provide additional single laboratory data. The independent validation study verifies the inclusivity and exclusivity in the hands of an independent trained user.

6.2.2 Study Design

Inclusivity and exclusivity strains are prepared and analyzed as vegetative cells on the media designated in the candidate method. All media recommended for use with the candidate method must be tested by the Independent laboratory. Test one replicate per strain per medium using the candidate method. For inclusivity, the independent laboratory must test at least 10 strains randomly selected from the ≥30 selected earlier per pathogenic species claimed and at least one strain per nonpathogenic species claimed. For exclusivity, the independent laboratory must test at least 10 strains not claimed by the method. The strains selected should be different from those used to develop the method where possible.

Inclusivity and exclusivity evaluations shall be performed together as one study. Inclusivity and exclusivity test samples must be blind coded and intermingled so the analysts cannot know the identity of the test samples.

The study designs presented are intended to be a suggested guideline. Specific study designs and numbers of strains will be determined by the Methods Committee on Microbiology on a case by case basis.

Species/strains selected for testing must be different than those used to develop the method if possible. Species/strains specified for use must be traceable to the source. The source and origin of each species/strain should be reported. Species/strains must have Certificate of Analysis from the source documenting the identity and method(s) used to determine the identity or be well characterized before use with documentation on file.

6.2.3 Data Analysis and Reporting

Analyze the inclusivity data for correct identification, misidentification and unidentified organisms.

Species/strains selected for testing must be different than those used to develop the method if possible. Species/strains specified for use must be traceable to the source. The source and origin of each species/strain should be reported. Species/strains must have Certificate of Analysis from the source documenting the identity and method(s) used to determine the identity or be well characterized before use with documentation on file.

The data are reported as number of species/strains correctly identified. For example, "Of the 10 specific inclusivity strains tested, 9 were correctly identified and 1 was misidentified. The strain misidentified was the following: …"

The study report should include a table titled "Inclusivity Panel Results," which lists all species/strains tested, their source, origin and essential characteristics plus testing outcome.

Analyze the exclusivity data for misidentifications and unidentified organisms. The data is reported as number of strains correctly unidentified. For example, "Of the 10 specific exclusivity strains tested, 7 were correctly unidentified and 3 were misidentified. Those misidentified by the method were the following: …"

The study report should include a table titled "Exclusivity Panel Results," which lists all strains tested, their source, origin and essential characteristics plus testing outcome.

6.3 Collaborative Study

6.3.1 Scope

The Collaborative Study is a requirement for OMA methods and succeeds the SLV (Precollaborative) Study. The purpose of the Collaborative Study is to estimate the reproducibility and determine the performance of the candidate method among collaborators.

6.3.2 Number of Collaborators

A minimum of 10 laboratories reporting valid data are required. The Study Director should plan on including additional laboratories due to potential invalid data sets, so it is recommended that at least 12 collaborators be included in the collaborative study.

6.3.3 Number of Tests

Each collaborator receives a minimum of 12 organisms recommended by the Methods Committee on Microbiology. Data collection at all test sites must begin on the same day to control for the age of the cultures.

Species/strains selected for testing must be different than those used to develop the method if possible. Species/strains specified for use must be traceable to the source. The source and origin of each species/strain should be reported. Species/strains must have Certificate of Analysis from the source documenting the identity and method(s) used to determine the identity or be well characterized before use with documentation on file.

6.3.4 Data Analysis and Reporting

Analyze the inclusivity data for correct identification, misidentification and unidentified organisms by laboratory. The data are reported as number of species/strains correctly identified by laboratory. For example, "Of the N specific inclusivity strains tested, N-2 were correctly identified and 2 were misidentified in Laboratory 1. Those strains misidentified were the following: …"

The study report should include a table titled "Inclusivity Panel Results," which lists all species/strains tested, their source, origin and essential characteristics plus testing outcome by laboratory.

Analyze the exclusivity data for misidentifications and unidentified organisms. The data are reported as number of strains correctly unidentified. For example, "Of the M specific exclusivity strains tested, M-3 were correctly unidentified and 3 were misidentified in Laboratory 1. Those misidentified by the method were the following: …"

The study report should include a table titled "Exclusivity Panel Results," which lists all strains tested, their source, origin and essential characteristics plus testing outcome by each laboratory.

7 Safety

Personnel should be aware of safety issues in the laboratory and have the appropriate training to carry out microbiological procedures dealing with the growth and safe disposal of microorganisms and biochemicals, particularly where pathogens are under test. The appropriate biohazard containment facilities and protective clothing should be available.

8 References

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(11) International Organization for Standardization (ISO) Standard 16140, *Microbiology of Food and Animal Feeding Stuffs — Protocol for the Validation of Alternative Methods*, http://www.iso.org/iso/search.htm?qt=16140&searchSubmit=Search&sort=rel&type=simple&published=true

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(14) LaBudde, R.A. (2009) *Statistical Analysis of Interlaboratory Studies, XXII. Statistical Analysis of a Qualitative Multicollaborator Study as a Quantitative Study Under the Large Sample Approximation*, TR296, Least Cost Formulations, Ltd, Virginia Beach, VA

ANNEX A
MPN Analysis of Contaminated Matrix

The most probable number ("MPN"), also known as the maximum likelihood estimate, is obtained as the root of the following equation:

$$\sum_{k=1}^{K}\left[\frac{d_k m_k}{\exp(d_k\,\mathrm{MPN})-1}-d_k\left(n_k-m_k\right)\right]=0$$

where the summation over k = 1, 2, … , K ranges over the serial dilution sets, and d_k = the amount of sample used in the k-th dilution set; m_k = the number of replicates in the k-th dilution set; n_k = the number of positive results in the k-th dilution set; MPN = the most probable number estimate.

A 95% confidence interval for the MPN estimate can be obtained as the 2.5 and 97.5% quantiles of sampling distribution of MPN generated by bootstrap resampling with 10 000 realizations. For bootstrap resampling to be acceptable, at least one dilution set with fractional response must have five replicates or more.

Approximate confidence intervals may also be found from one of the following formulas:

$$I=\sum_{k=1}^{K}\left[\frac{d_k^2 m_k \exp(d_k\,\mathrm{MPN})}{\left(\exp(d_k\,\mathrm{MPN})-1\right)^2}\right]$$

$$\mathrm{UCL,\ LCL}\ =\mathrm{MPN}\pm\frac{1.9600}{\sqrt{I}}$$

directly on MPN.

$$\mathrm{UCL,\ LCL}\ =\exp\left[\ln(\mathrm{MPN})\pm\frac{1.9600}{\mathrm{MPN}\sqrt{I}}\right]$$

for intervals on ln(MPN).

When an equal number of replicates in each set and a constant dilution ratio between sets are used, tables, such as those in the FDA *Bacteriological Analytical Manual* Appendix 2, may be used to supply estimates of MPN with 95% confidence intervals.

It is strongly recommended that no less than five replicates be used in each dilution set, and that the replicates tested in the reference laboratory be included as one of the dilutions for each concentration level. Dilution sets with fewer replicates supply unreliable estimates. For fractional detection concentration levels, a dilution ratio of 1/2 or 1/3 is recommended instead of the customary 1/10.

Example: A candidate test method is evaluated at an expected 50% fractional detection concentration level. Twenty replicates are analyzed in the reference laboratory. During test portion preparation, an additional five replicates are made each of 3 and 1/3 times the desired concentration level. All 30 test portions are tested by the reference method in the reference laboratory, with presence or absence results (*see* Table A1).

"The MPN estimate is 0.053 MPN/g (1.3 MPN/25 g) with a 95% confidence interval from bootstrap resampling of 0.034 MPN/g (0.85 MPN/25 g) to 0.086 MPN/g (2.2 MPN/25 g)."

Table A1

	Initial	Estimate	Bootstrap LCL	Bootstrap UCL
MPN	0.055	0.053	0.034	0.086
Direct			0.027	0.079
ln based			0.032	0.087
Series	Dilution factor	No. tubes	No. positive	Dilution estimate
1	3.00000	5	5	0.333
2	1.00000	20	15	0.024
3	0.33333	5	1	0.012

ANNEX B
Raw Format Data Table Template and Example for Qualitative Method Single Laboratory and Collaborative Studies

The purpose of the Raw Format Data Table is to document in a software-friendly format all of the factors, variables, and measurements in the experiment. By matrix and concentration level, report each result from each method for each test portion separately.

Each row (record) in the Raw Format Data Table should contain the following columns (fields):

(*1*) *Matrix type*.—An identifier indicating the matrix involved, such as "EGGS." The same exact identifier must be used for the same matrix.

(*2*) *Concentration level*.—The MPN/test portion for the level. (The MPN/test portion, and not MPN/g or MPN/mL, is the relevant measure for statistical analysis of the data.)

(*3*) *Laboratory*.—An identifier indicating the laboratory involved, such as "01."

(*4*) *Method*.—An identifier indicating the test method used, such as "REF" for the reference method, "C-P" for the candidate presumptive method, or "C-C" for the candidate confirmation method.

(*5*) *Replicate*.—A unique identifier for the test portion involved. If this identifier is common to two rows in the table, this implies the results are matched by test portion. Example identifiers might be "01," "001," or "A1."

(*6*) *Result*.—"0" for absence or "1" for presence (detection).

In computer format, the Raw Format Data Table should be given either as: (*1*) a "fixed-format" file with fixed column widths and blanks or tabs as separators and a file extension of ".txt" or ".xls"; or (*2*) a "comma-separated value" file with commas as separators between columns and identifiers within quotes, and a file extension of ".csv".

It is desirable to include a "header" record as the first record in the file with identifiers for each column.

An example file named "ecoli.csv" might be:

"matrix", "level", "lab", "method", "replicate", "result"
"spinach", "2.20", "01", "cpres", "001", 0
"spinach", "2.20", "01", "cconf", "002", 1
"spinach", "2.20", "01", "ref", "003", 1
"spinach", "2.20", "01", "cpres", "004", 1
"spinach", "2.20", "01", "cconf", "005", 1
"spinach", "2.20", "01", "ref", "006", 1
etc.

ANNEX C
Calculation of POD and dPOD Values from Qualitative Method Single Laboratory Data

In general, four different probabilities detected (PODs) are to be calculated: POD_R (for the reference method), POD_C (for the confirmed candidate method), POD_{CP} (for the candidate presumptive method), and POD_{CC} (for the candidate confirmation method).

For each of these four cases, calculate the POD as the ratio of the number positive (x) to total number tested (N):

$$\mathrm{POD} = \frac{x}{N}$$

where POD is POD_C, POD_R, etc.

The POD estimates and 95% confidence interval (LCL, UCL) estimates are given by:

(*1*) *For the case where x = 0*.

$$\mathrm{POD} = 0$$

$$\mathrm{LCL} = 0$$

$$\mathrm{UCL} = 3.8415/(N + 3.8415)$$

(*2*) *For the case where x = N*.

$$\mathrm{POD} = 1$$

$$\mathrm{LCL} = N/(N + 3.8415)$$

$$\mathrm{UCL} = 1$$

(*3*) *For the case where 0 < x < N*.

$$\mathrm{POD} = x/N$$

$$\mathrm{LCL} = \frac{x + 1.9207 - 1.9600\sqrt{x - \frac{x^2}{N} + 0.9604}}{N + 3.8415}$$

$$\mathrm{UCL} = \frac{x + 1.9207 + 1.9600\sqrt{x - \frac{x^2}{N} + 0.9604}}{N + 3.8415}$$

where 1.9600 = z, the Gaussian quantile for probability 0.975, $1.9207 = z^2/2$, $0.9604 = z^2/4$ and $3.8415 = z^2$.

Finally, if $x \leq 1$, set LCL = 0. If $x \geq N\text{-}1$, set UCL = 1.

The confidence interval corresponds to the uncorrected Wilson-score method, modified for x = 1 and x = N–1 to improve coverage accuracy on the boundary.

dPOD for Unpaired Studies

The differences in proportions detected are estimated by:

$$\mathrm{dPOD_C} = \mathrm{POD_C} - \mathrm{POD_R}$$

$$\mathrm{dPOD_{CP}} = \mathrm{POD_{CP}} - \mathrm{POD_{CC}}$$

If the replicates tested by the candidate and reference methods are unpaired (i.e., the enrichment conditions differ between the methods, thus the methods require analysis of distinct test portions), the associated 95% confidence interval (LCL, UCL) for the expected value of dPOD = $POD_1 - POD_2$ is estimated by:

$$\mathrm{LCL} = \mathrm{dPOD} - \sqrt{(\mathrm{POD_1} - \mathrm{LCL_1})^2 + (\mathrm{POD_2} - \mathrm{UCL_2})^2}$$

$$\mathrm{UCL} = \mathrm{dPOD} + \sqrt{(\mathrm{POD_1} - \mathrm{UCL_1})^2 + (\mathrm{POD_2} - \mathrm{LCL_2})^2}$$

where (LCL_1, UCL_1) is a 95% confidence interval for POD_1 and (LCL_2, UCL_2) is a 95% confidence interval for POD_2, as determined above.

dPOD for Paired Studies

If the replicates tested by the candidate and reference methods are paired (i.e., the enrichment conditions are the same, thus common test portions are analyzed by both methods), the associated 95% confidence interval (LCL, UCL) for the expected value of dPOD = $POD_1 - POD_2$ is estimated by the following:

Let

$$d_i = x_{1i} - x_{2i}$$

denote the numerical difference of the two method results on test portion i. Note that d_i must take on only the values –1, 0, or +1.

The recommended method for estimating dPOD is the mean of differences d_i:

$$d\mathrm{POD} = \frac{\sum_{i=1}^{N} d_i}{N}$$

where N is the number of test portions.

The recommended approximate 95% confidence interval is the usual Student-*t* based interval, with the standard error of dPOD computed in the usual manner from the replicate differences:

$$s_d = \sqrt{\frac{\sum_{i=1}^{N} (d_i - d\mathrm{POD})^2}{N - 1}}$$

$$\mathrm{SE}_{d\mathrm{POD}} = \frac{s_d}{\sqrt{N}}$$

and

$$LCL = d\mathrm{POD} - t_c \cdot \mathrm{SE}_{d\mathrm{POD}}$$

$$UCL = d\mathrm{POD} + t_c \cdot \mathrm{SE}_{d\mathrm{POD}}$$

where t_c is the 97.5% quantile of the Student-*t* distribution for N-1 degrees of freedom, and the 95% confidence interval is (LCL, UCL).

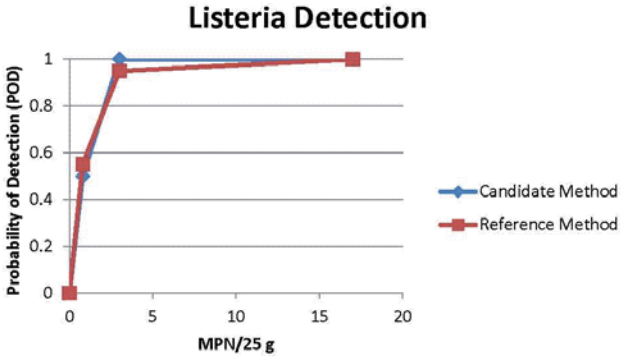
The degree of coverage accuracy for this approximate confidence interval will improve as N increases and the Central Limit Theorem forces the distribution of dPOD to become normal. Given the finite range of the d_i's, this will happen quickly, even for small N.

ANNEX D
Summary Data Table for Qualitative Method Single Laboratory Studies

Table D1. Comparative results for the detection of *Listeria innocua* in raw shrimp

Statistic	Concentration	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate method (C)			Reference method (R)			C vs R	CP vs CC
	MPN/25 g	N	x	POD(CP)	N	x	POD(CC)	N	x	POD(C)	N	x	POD(R)	dPOD(C,R)	dPOD(CP,CC)
Estimate	0.00	20	0	0.00	20	0	0.00	20	0	0.00	20	0	0.00	0.00	0.00
LCL	0.00			0.00			0.00			0.00			0.00	–0.16	–0.16
UCL	1.50			0.16			0.16			0.16			0.16	0.16	0.16
Estimate	0.80	20	12	0.60	20	10	0.50	20	10	0.50	20	11	0.55	–0.05	0.10
LCL	0.43			0.39			0.30			0.30			0.34	–0.33	–0.19
UCL	1.39			0.78			0.70			0.70			0.74	0.24	0.37
Estimate	3.00	20	20	1.00	20	20	1.00	20	20	1.00	20	19	0.95	0.05	0.00
LCL	1.58			0.84			0.84			0.84			0.76	–0.12	–0.16
UCL	5.68			1.00			1.00			1.00			1.00	0.24	0.16
Estimate	17.00	20	20	1.00	20	20	1.00	20	20	1.00	20	20	1.00	0.00	0.00
LCL	0.27			0.84			0.84			0.84			0.84	–0.16	–0.16
UCL	1060			1.00			1.00			1.00			1.00	0.16	0.16

ANNEX E
Example of Graph of POD Values from Qualitative Method Single Laboratory Data



Notes:
(*1*) The concentration plotted should be MPN/test portion.
(*2*) Confidence intervals may also be plotted.
(*3*) Collaborative data should be plotted analogously.

ANNEX F
Calculation of LPOD and dLPOD Values from Qualitative Method Collaborative Study Data

For a multilaboratory trial where L = number of laboratories, R = replicates per laboratory, N = LR = total replicates, LPOD estimate is given by

$$\text{LPOD} = \frac{x}{N}$$

where x is the number of positive results.

Method for estimating LPOD 95% confidence intervals:

Step 1.—Enter data into AOAC spreadsheet with 1 for positive response and 0 for negative response. Record the mean LPOD, s(R), and s(r).

Step 2.—Calculate s(L), standard deviation due to laboratory effect as:

$$s(L) = \sqrt{s(R)^2 - s(r)^2}$$

Step 3.—Calculate s(POD) as the standard deviation of the individual laboratory POD estimates.

$$s(POD) = \sqrt{\frac{\sum (POD_i - LPOD)^2}{L-1}}$$

Step 4.—Calculate degrees of freedom, df for s(POD) as follows:

$$df = \frac{\left[\frac{s(L)^2}{L} + \frac{s(r)^2}{N}\right]^2}{\frac{\left[\frac{s(L)^2}{L}\right]^2}{L-1} + \frac{\left[\frac{s(r)^2}{N}\right]^2}{N-L}}$$

Step 5.—Calculate 95% confidence limits on LPOD:

If $0.15 \le \text{LPOD} \le 0.85$:

$$LCL = \max\left\{0, LPOD - \frac{t_{0.975,df}\ s(POD)}{\sqrt{L}}\right\}$$

$$UCL = \min\left\{1, LPOD + \frac{t_{0.975,df}\ s(POD)}{\sqrt{L}}\right\}$$

If LPOD <0.15 or LPOD > 0.85:

$$LCL = \frac{x + 1.9207 - 1.9600\sqrt{x - \frac{x^2}{N} + 0.9604}}{N + 3.8415}$$

$$UCL = \frac{x + 1.9207 + 1.9600\sqrt{x - \frac{x^2}{N} + 0.9604}}{N + 3.8415}$$

where x is the number of observed positive outcomes and N is the total number of trials.

If LPOD = 0:

$$LCL = 0$$

$$UCL = 3.8415/(N + 3.8415)$$

If LPOD = 1:

$$LCL = N/(N + 3.8415)$$

$$UCL = 1$$

Step 6.—Calculate 95% confidence intervals for dLPOD:

dLPOD is the difference between any two LPOD estimates, for example to compare a candidate method to a reference method:

$$dLPOD_C = LPOD_C - LPOD_R$$

The associated 95% confidence interval (LCL, UCL) for the expected value of dLPOD = $LPOD_1 - LPOD_2$ is estimated by:

$$LCL = dLPOD - \sqrt{(LPOD_1 - LCL_1)^2 + (LPOD_2 - UCL_2)^2}$$

$$UCL = dL\,POD + \sqrt{(LPOD_1 - UCL_1)^2 + (LPOD_2 - LCL_2)^2}$$

Example

Suppose the reference method in an interlaboratory study gave the following results when 12 replicate test portions were tested in each of 10 laboratories: *see* Table F1.

Here, x = 76, N =120, and LPOD = 0.6333 (= 76/120).

The repeatability standard deviation

$$s_r^2 = \frac{\sum_{i=1}^{L}(n_i - 1)s_i^2}{\sum_{i=1}^{L}(n_i - 1)} = \frac{\sum\left[x_i - \left(x_i^2/n_i\right)\right]}{N - L} = \frac{\left[\left(7 - 49/120\right) + \left(9 - 81/120\right) + \ldots + \left(9 - 81/120\right)\right]}{120 - 10}$$

$$= 0.2242$$

$$s_r = \sqrt{s_r^2} = \sqrt{0.2242} = 0.4735$$

where s_i^2 is the variance of the results from laboratory i, x_i is the number of positive detections from laboratory i, n_i is the number of observations from laboratory i, N is the total number of data, and L is the number of laboratories.

Table F1

	Method R			R
Lab	Positive	Negative	Total	POD
1	7	5	12	0.5833
2	9	3	12	0.7500
3	6	6	12	0.5000
4	10	2	12	0.8333
5	5	7	12	0.4167
6	7	5	12	0.5833
7	5	7	12	0.4167
8	7	5	12	0.5833
9	11	1	12	0.9167
10	9	3	12	0.7500
All	76	44	120	

And $\sqrt{LPOD(1 - LPOD)} = 0.4819$, suggesting s_L will be small compared to s_r.

The among-laboratory standard deviation is

$$s_L^2 = \max\left\{0, \frac{\sum(POD_i - LPOD)^2}{L - 1} - \frac{s_r^2}{n}\right\}$$

$$= \max\left\{0, \frac{\left[(0.5833 - 0.6333)^2 + \ldots + (0.75 - 0.6333)^2\right]}{10 - 1} - \frac{0.2242}{12}\right\}$$

$$= \max\{0, 0.02963 - 0.0187\}$$

$$= 0.01093$$

and $s_L = \sqrt{0.01093} = 0.1045$, which is noticeably less than s_r, as expected.

The reproducibility standard deviation is

$$s_R^2 = s_r^2 + s_L^2$$
$$= 0.01093 + 0.2242$$
$$= 0.2351$$

so $s_R = \sqrt{0.2351} = 0.4849 \approx s_r$

The results are summarized in Table F2.

The "homogeneity test" reported above is the T statistic based on the χ^2 distribution, so the *p*-value of 0.1703 should be compared to 0.10. The test indicates the observed value of s_L = 0.1046 is not statistically significant, so the study was not large enough to reliably detect an interlaboratory effect of this size.

Table F2

Parameter	Value
LPOD	0.6333
s_r	0.4735
s_L	0.1046
s_R	0.4850
p-Value for *T*-test	0.1703

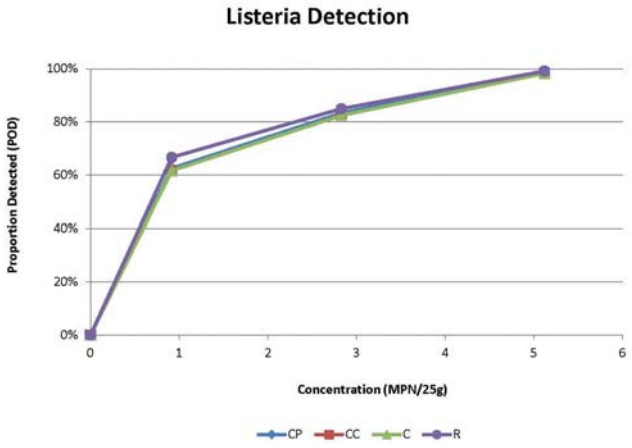
ANNEX G
Data Summary Table Template and Example for Qualitative Method Collaborative Studies

Table G1. Comparative results for the detection of *Listeria innocua* in raw shrimp by the candidate and reference methods in an interlaboratory study

			Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs R	CP vs CC
Statistic	Concn, MPN/25 g	Lab	N	x	POD(CP)	N	x	POD(CC)	N	x	POD(C)	N	x	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
		01	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		02	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		03	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		04	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		05	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		06	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		07	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		08	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		09	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		10	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
Estimate	0.00	All	120	0	0.00	120	0	0.00	120	0	0.00	120	0	0.00	0.00	0.00
LCL	0.00				0.00			0.00			0.00			0.00	–1.00	–1.00
UCL	0.02				0.17			0.03			0.03			0.03	0.03	0.03
s_r					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.17			17			0.17			0.17		
s_L					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.03			0.03			3.00			0.03		
s_R					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.24			0.24			0.24			0.24		
P_T					1.0000			1.0000			1.0000			1.0000		
		01	12	8	0.67	12	8	0.67	12	8	0.67	12	7	0.58	0.08	0.00
		02	12	9	0.75	12	8	0.67	12	8	0.67	12	7	0.58	0.08	0.08
		03	12	8	0.67	12	8	0.67	12	8	0.67	12	6	0.50	0.17	0.00
		04	12	6	0.50	12	6	0.50	12	6	0.50	12	10	0.83	–0.33	0.00
		05	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		06	12	6	0.50	12	6	0.50	12	6	0.50	12	8	0.67	–0.17	0.00
		07	12	8	0.67	12	8	0.67	12	8	0.67	12	6	0.50	0.17	0.00

Table G1. (*continued*)

			Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs R	CP vs CC
Statistic	Concn, MPN/25 g	Lab	N	x	POD(CP)	N	x	POD(CC)	N	x	POD(C)	N	x	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
		08	12	7	0.58	12	7	0.58	12	7	0.58	12	11	0.92	–0.33	0.00
		09	12	8	0.67	12	8	0.67	12	8	0.67	12	9	0.75	–0.08	0.00
		10	12	8	0.67	12	8	0.67	12	8	0.67	12	9	0.75	–0.08	0.00
Estimate	0.92	All	120	75	0.63	120	74	0.62	120	74	0.62	120	80	0.67	–0.05	0.01
LCL	0.73				0.53			0.53			0.53			0.58	–0.36	–0.37
UCL					0.72			0.71			0.71			0.76	–0.04	0.12
s_r					0.50			0.50			0.50			0.47		
LCL					0.44			0.44			0.44			0.42		
UCL					0.52			0.52			0.52			0.52		
s_L					0.00			0.00			0.00			0.04		
LCL					0.00			0.00			0.00			000		
UCL					0.13			0.11			0.11			0.22		
s_R					0.50			0.50			0.50			0.47		
LCL					0.45			0.45			0.45			0.42		
UCL					0.52			0.52			0.52			0.52		
P_T					0.9634			0.9867			0.9867			0.3711		
etc.																



ANNEX H
Logarithmic Transformation of Data from Quantitative Method Single Laboratory and Collaborative Data

Quantitative microbiological count data from experiments spanning multiple dilutions often do not show a Poisson nor a Gaussian statistical distribution. When the underlying physical mechanism allows for "clustering," typically a logarithmic transformation will normalize the data.

Perform a logarithmic transformation on the reported CFU/unit (including any zero results) as follows:

$$Y = \log_{10} [CFU/unit + (0.1)f]$$

where f is the reported CFU/unit corresponding to the smallest reportable result, and "unit" is the reported unit of measure (e.g., g, mL, 25 g).

Examples

(*1*) For the control concentration, the CFU/g is reported as "<0.003." So CFU/unit = 0.0, and $Y = \log_{10} [0.0 + (0.1)(0.003)] = -3.52$.

(*2*) For the low concentration, the CFU/g is 0.042. So $Y = \log_{10} [0.042 + (0.1)(0.003)] = -1.37$.

(*3*) For the high concentration, the CFU/g is 0.231. So $Y = \log_{10} [0.231 + (0.1)(0.003)] = -0.64$.