AOAC INTERNATIONAL

The Scientific Association Dedicated to Analytical Excellence®

AOAC INTERNATIONAL Food Allergen Working Group Comments

December 15, 2016

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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.

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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.

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

AOAC INTERNATIONAL

International Stakeholder Panel on Alternative Methods (ISPAM)

DRAFT MEETING AGENDA

Thursday, December 15, 2016

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

ISPAM Chair: Erin Crowley

(Q Laboratories, Inc.)

I. WELCOME & INTRODUCTIONS (Crowley/McIver)

II. REVIEW OF THE STANDARD METHOD PERFORMANCE REQUIREMENT (SMPR®) COMMENTS (Coates)

III. STANDARD METHOD PERFORMANCE REQUIREMENT (SMPR®) FOR FOOD ALLERGEN ASSAYS - QUALITATIVE/EGG REVISION - VERSION 3 (Coates/Godefroy/Yeung)

IV. NEXT STEPS (Crowley/McIver)

V. ADJOURN (Crowley)

***Requires a vote**
Draft meeting agenda is subject to change w/out notice

DRAFT AOAC Allergen SMPR Version 2; November 30, 2016

Quantitation of Whole Chicken Egg Allergens by ELISA-based Methods

Intended Use: ~~Reference m~~Method for cGMP compliance.

1. **Purpose:** AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC Stakeholder Panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their evaluation of validation study data for method being considered for *Performance Tested Methods* or AOAC *Official Methods of Analysis*, and can be used as acceptance criteria for verification at user laboratories.

2. **Applicability**:
   Quantitation of whole chicken egg allergens in selected finished food products and ingredients as listed in table 2 (may need to revise table to be consistent with OMA Appendix M).

3. **Analytical Technique**:
   Enzyme-linked immunosorbent assay (ELISA) ~~based assays~~ or other binding based technologies.

4. **Definitions**:

   Enzyme-linked immunosorbent assay (ELISA) ~~based assays~~.
   An assay that uses antibodies and color change to identify a substance. ELISA can perform other forms of ligand binding assays instead of strictly "immuno" assays, though the name carried the original "immuno" because of the common use and history of development of this method. The technique essentially requires any ligating reagent that can be immobilized on the solid phase along with a detection reagent that will bind specifically and use an enzyme to generate a signal that can be properly quantified.

   Binding Based Technology

   Allergens

   Commodities

   Limit of Quantitation (LOQ)
   The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

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Comment [D1]: May want to consider adding environmental samples

Limit of Detection (LOD)
Limit of detection (LOD).—The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

~~**Method detection limit (MDL)**~~
~~The minimum concentration of a substance that can be measured (detected) and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte using at least two ion MS/MS transitions.~~[1]

Repeatability
Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility
The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

Recovery
The fraction or percentage of ~~spiked~~ incurred analyte that is recovered when the test sample is analyzed using the entire method.

Whole Egg
A combination of pasteurized [chicken] egg whites and egg yolks from the same production batch blended together in their entirety, in natural proportions.[2]

Comment [D2]: In performance table, be specific or clarify so that it is clear what is meant as to type of egg.

Comment [D3]: Do we need to add a footnote for a definition/reference for "pasteurized"?

5. **Method Performance Requirements**:
See table 1.

6. **System suitability tests and/or analytical quality control:**
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

7. **Reference Material(s):**

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 20th Edition of the AOAC

~~[1] 40 CFR Part 136, Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11~~

[2] Introduction to Egg Products, USDA Food Safety and Inspection Service, website: http://www.fsis.usda.gov/wps/wcm/connect/c5c85914-5055-4f09-8098-1a179a1c6e14/EPT_Introduction.pdf?MOD=AJPERES, accessed 12/15/2015.

INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

Whole Egg

- NIST 8445
- LGC SAL-RSM-5 (Check for characterization level)

8. Validation Guidance:

Method developers should provide data for method performance in all claimed matrixes (listed in table 2).

Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

Appendix M: Valaidation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_m.pdf

Field Code Changed

9. Maximum Time-To-Result: None

Table 1: Method performance requirements

Comment [D4]: May need to consider adding specificity

Parameter	Minimum Acceptance Criteria		
	Cookies, Bread, Dough, Salad Dressing	Wine	Matrix X
Analytical Range (ppm)	~~10 1000~~< 5 - > 10		
LOQ (ppm)	< 5		
MDL (ppm)	< 10		

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Recovery (%)	60-120%		
% RSD_r	≤20 %		
% RSD_R	≤ 30%		
Note: Allergen to be reported by dry weight.			

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Table 2: **Selected Finished Food Products and Ingredients ~~Matrixes~~ (revised per OMA Appendix M)**

cookies
bread
dough
salad dressing
wine
chicken
ice cream
pasta

Comment [D5]: Are these matrices okay for this analyte

Comment [D6]: Matrices listed in OMA Appendix M

Appendix M: Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices

Although there are a number of documents published on method validation (1, 2) which target analytical methods in general, and there are numerous publications on validation of ELISA methods for pesticides, these documents do not address specific areas of concern for food allergen analysis, such as reference materials, spiking methods, or choice of matrixes. In the absence of a universally recognized reference standard for food allergen ELISAs, many organizations and end-users use different validation protocols and different analytical standards. Such inconsistency and duplication inevitably has a negative economic impact on the food allergen community. This document is designed to accompany the *AOAC Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (1), and to provide guidance specific to the validation of quantitative ELISA-based methods for food allergens. This protocol was designed to meet or exceed the minimum requirements set forth in the AOAC guidelines; it was developed with input from a wide range of experts in the area of food allergens, working under the auspices of the AOAC Presidential Task Force on Food Allergens and with the active contribution of the Allergen Working Group, part of the MoniQA network of excellence. This document will focus on developing guidance on a method validation study protocol to validate the performance characteristics of quantitative food allergen ELISA methods. The practical protocol is intended to help method developers in designing a study to generate appropriate validation data that would be suitable for submission to AOAC INTERNATIONAL or regulatory bodies for recognition. Both the study design and data would be subject to scrutiny before acceptance by the AOAC or other authority.

Methods for detecting various food allergens have been available for a number of years. Many of these methods use ELISA-based techniques to detect specific protein markers in food matrixes. The detection of food allergens by ELISA is a unique analytical procedure characterized by the recognition and binding of specific antigens by antibodies. Food allergens are proteins, which are large and complex molecules with defined structures in their native forms, that can induce allergic reactions in sensitized consumers. From the analytical point of view, the integrity of the protein structure is critical to favor protein solubility and promote antibody-allergen binding. Although specificity of antibodies in commercial ELISAs for food allergens varies, in most cases, these methods target a complex mixture of soluble allergenic and nonallergenic proteins, rather than a specific protein. This mixture of target proteins will have diverse structural and chemical properties in the complex mixture of a food matrix. Some food commodities contain several allergenic proteins, e.g., at least eight peanut proteins, such as Ara h 1 and Ara h 2, can potentially cause an immunological response. But other commodities, such as fish, shellfish, and mollusks, contain only one major allergen; still others may consist mainly of allergenic proteins, e.g., all major milk proteins (caseins, β-lactoglobulin, α-lactalbumin, etc.) possess an allergenic capacity.

The ability of an ELISA method to detect food allergen proteins in a test sample is affected by the efficiency with which these proteins are extracted from the sample, as well as the efficiency with which the antibody or antibodies used in the ELISA detect these proteins in the sample extract. The overall performance of an ELISA-based method for the detection of food allergens is a function of these two parameters.

The fact that allergic individuals often react to different protein constituents of the allergenic food further complicates the choice of targets. Because most food products are heat-treated, food production processes like roasting and extrusion can have significant influence on the solubility and extractability of the target proteins, as well as on the ability of the antibody or antibodies used in the ELISA to recognize them. Factors that may influence the test results include: (*1*) interactions with compounds in a food matrix (e.g., polyphenols and tannins); (*2*) reduced solubility and reactivity of heat-denatured proteins; and (*3*) differences in the protein profile of a particular food allergen from different species, varieties, and geographic origins. These factors all contribute to the difficulty in finding appropriate reference materials for food allergens and explaining why the proteins in a sample extract might not be fully comparable to that of the calibrators included with a particular detection method. These topics have been extensively reviewed recently (3).

Availability of validated methods is critical for both method developers and end-users. For method developers, validation of an analytical procedure is used to demonstrate that it is suitable for its

This document provides supplemental guidance on specifications for the development and implementation of studies to validate the performance characteristics of quantitative ELISA methods for the determination of food allergens. It is intended as a companion document to other existing publications on method validation. The guidance is divided into two sections: information to be provided by the method developer on various characteristics of the method, and implementation of a multilaboratory validation study. Certain criteria included in the guidance are allergen-specific. Two food allergens, egg and milk, are used to demonstrate the criteria guidance. These recommendations will be the basis of the harmonized validation protocol for any food allergen ELISA method, whether proprietary or nonproprietary, that will be submitted to AOAC and/or regulatory authorities or other bodies for status recognition. Regulatory authorities may have their own particular requirements for data packages in addition to the guidance in this document. Future work planned for the implementation and validation of this guidance will include guidance specific to other priority allergens.

These guidance and best practices were completed by the AOAC Food Allergens Analytical Community and submitted to AOAC INTERNATIONAL for publication in 2009.

Reference: Abbott, M., Hayward, S., Ross, W., Godefroy, S.B., Ulberth, F., Van Hengel, A.J., Roberts, J., Akiyama, H., Popping, B., Yeung, J.M., Wehling, P., Taylor, S., Poms, R.E., & Delahaut, P. (2010) *J. AOAC Int*. **93**, 442–450

Table 1. Food commodities that should be included in cross-reactivity testing for ELISA methods targeting egg

Adzuki beans	Almond	Barley	Beef	Brazil nut
Buckwheat	Cashew	Chestnut	Chick peas	Chicken
Cocoa	Coconut	Corn	Crustacean/prawn/shrimp	Duck
Fish	Gelatin (bovine)	Hazelnut	Kidney beans	Kiwi
Lecithin	Lentils	Lima beans	Linseed	Macadamia nut
Milk	Oats	Octopus	Peanut	Peas
Pecans	Pine nut	Pistachio	Poppy seeds	Pork
Pumpkin seed	Rice—white and brown	Rye	Sesame	Soybean
Split peas	Sunflower seed	Turkey	Walnut	Wheat

intended purpose. For end-users, validated methods help to ensure reliability, repeatability, accuracy, and precision of the results generated using a particular method.

Method performance is documented using information and data provided by the method developer through interlaboratory validation studies. Minimum requirements for both information and data are included in this guidance, and may be applicable to any priority food allergen, as defined by the Codex Alimentarius Committee on Food Labeling in 1998 (4). However, due to the nature of food allergens, certain aspects, such as reference materials and spiking methods, would need to be addressed on a case-by-case basis. This document addresses these allergen-specific criteria for two food allergens, egg and milk. Further guidance for other priority allergens will be developed and communicated by the AOAC Presidential Task Force on Food Allergens and/or the Food Allergens Analytical Community under the auspices of the MoniQA network.

Required Allergen-Specific Information to be Provided on the ELISA Method

Information relating to the design of a method and its target analytes, as well as method performance characteristics, shall be provided by the method developer when submitting validation data for assessment. This information can be an important part of an overall package of information for evaluating a method. Proprietary information on antibody design or certain aspects of the method do not have to be disclosed. The AOAC guidelines (1) outline requirements for a final collaborative study manuscript. These allergen-specific requirements are additional recommendations that apply only to food allergen ELISA methods during method development and the final collaborative study.

The following information should be submitted along with the interlaboratory validation study data:

Antibody information.—Information on the antibody must include whether the antibody is monoclonal or polyclonal, whether it targets a single protein or multiple proteins, and whether the target protein used to generate the antibody was fractionated, modified, or synthesized in some way. Method developers are encouraged to include as much additional information about the antibody as possible. It is not necessary to reveal proprietary information. An example of antibody characterization for ELISA methods was discussed in a previous communication, specifically targeting mycotoxin/phycotoxin analysis (5). This approach could be adapted for allergen-specific antibodies.

Cross-reactivity.—Cross-reactivity is defined as a positive response to a sample that does not contain any of the target analyte. Method developers must test their allergen detection method for cross-reactivity for the target allergen in a variety of food commodities, which will vary for different target analytes and will depend on a number of factors. Food commodities tested for cross-reactivity should include a wide selection of foods and ingredients, particularly those that are genetically similar to the target allergenic commodity and that are likely to be analyzed for the presence of the target food allergen. The greater the number of items tested for cross-reactivity the better. In general, food items tested for cross-reactivity should be prepared as they would normally be consumed (raw or cooked).

Cross-reactivity testing should be based on the full-strength extracts, i.e., a sample of the item being tested for cross-reactivity should be extracted using the extraction buffer and procedure outlined in the method instructions, then analyzed at full strength to determine if it leads to a positive result. If a positive result is obtained, the extract must be diluted and rerun to characterize the extent of the cross-reactivity.

A minimum list of food commodities that should be included in cross-reactivity testing for egg and milk is provided in Tables 1 and 2, respectively. Many of these commodities will be the same for

Table 2. Food commodities that should be included in cross-reactivity testing for ELISA methods targeting milk

Almond	Barley	Brazil nut	Beef	Buckwheat
Cashew	Chick peas	Cocoa	Corn meal	Crustacean/prawn
Egg	Fish	Hazelnut	Lecithin	Lima bean
Oats	Peas	Peanut	Pecan	Pine nut
Pistachio	Poppy seed	Pumpkin seed	Rice–white and brown	Rye
Sesame seed	Soy bean	Split peas	Sunflower seed	Walnut
Wheat				

Table 3. Matrixes of interest for ELISA methods targeting egg and milk

Egg	Milk
Chicken	Cookies, baked goods
Ice cream	Dark chocolate
Pasta	Drink mixes (ex. alcoholic beverage premix)
Salad dressing	Orange juice
Soy milk	Infant formula
Wine	Wine

all priority allergens, but specific items may be included on some lists, depending on particular concerns, e.g., genetic homology (crustaceans and dust mites) or matrixes of likely exposure. Table 3 lists matrixes of interest for ELISA methods that target egg and milk.

Information on calibrators.—The calibrators provided in the kit must be clearly defined. Information should address the following questions:

What is the calibrator that is supplied with the kit and used to generate the calibration curve? How was the calibrator prepared and assayed? Is the calibrator made from raw or processed material? Was the calibrator extracted or purified and if so how? Is the calibrator in extraction or dilution buffer?

It is very important to identify how the concentration of the calibrator is being expressed, what the units are, and whether it refers to the whole commodity or to a level of protein. If the calibrator is expressed as a level of protein, it should be clarified whether it refers to total protein or soluble protein and how the level of protein was determined, e.g., bicinchoninic acid assay with bovine serum albumin as the standard. Information on whether the calibrator is commercially available should also be provided.

Information on matrixes.—ELISA methods can be susceptible to matrix effects or perform differently in different matrixes. The method developer should clearly identify which matrixes the method is applicable for, on the basis of their in-house data, recognizing the variability of specific formulations. The developer should also identify any matrixes that the method is known to have difficulty with, and identify clearly which states of the food allergen (raw, cooked, or both) the method is capable of detecting.

LOQ, LOD, and lower limit of application (LLA).—LOD is defined as the lowest concentration or mass of analyte in a test sample that can be distinguished from a true blank sample at a specified probability level. LOQ is the lowest level of analyte in a test sample that can be reasonably quantified at a specified level of precision.

Manufacturers or method developers are free to define an LLA at whatever level of confidence they choose. This value may be higher than the LOQ and represents a level below which the method developer does not support or recommend use of the method.

Before conducting an interlaboratory study (precollaborative), a single-laboratory validation study of the ELISA-based allergen detection method should be carried out in-house by the method developer. Guidelines for single-laboratory validation of methods of analysis are readily available (2). The LOD should be estimated by a statistical analysis of the calibration data according to the ISO standard ISO 11843-2 (6) for linear data, or ISO 11843-5 (7) for linear and nonlinear data, using as default probabilities $\alpha = \beta = 0.05$, where α and β represent the probability of a false positive and false negative, respectively. When doing this estimation, care should be taken to include as many sources of variation as possible within a single laboratory. Calibration data from at least three analysts over a minimum of three different runs should be included, preferably using different instruments, if possible.

Ruggedness and lot-to-lot variability of method performance.—Ruggedness refers to the ability of a method to resist changes in the final results when minor deviations are made in the experimental conditions described in the procedure. The ruggedness of the method should be investigated by performing experiments in which specific parameters are changed to determine the impact on the experimental result. In particular, the effect of deviations in incubation times, reagent volumes, extraction conditions (time and temperature) should be investigated. It is recommended that deviations for time and volume be investigated at ±5% or more, and incubation temperatures tried at ±3°C or more. If any of these experimental conditions are particularly important in achieving consistent results, this should be mentioned in the kit insert information.

The shelf life should include the stability of all the reagents provided with the test kit, ideally through real-time testing of reagents under normal storage conditions. Accelerated stability testing at higher than normal storage temperatures can also be used to estimate stability. An expiration date for each test kit should be clearly indicated, along with appropriate conditions for storage before use.

A small number of test kits from each lot should be set aside for comparison with previous or future lots. When a new lot of test kits is produced, it should be tested against the previous lot. New lots should have characteristics similar to those of the previous lots. For example, a positive control sample, such as an incurred test sample or spiked sample, should be analyzed with each new lot to be sure that consistent results are achieved. Information on lot-to-lot variability should be provided by the kit manufacturer as part of the data submission package.

Key Elements of Interlaboratory Validation

Number of Laboratories Required

The required number of participating laboratories will be based on AOAC *Appendix D* guidelines (1), currently set at a minimum of eight laboratories contributing usable data at the end of the study.

In order to encourage participation from as diverse a group of laboratories as possible, the AOAC Presidential Task Force on Food Allergens and the Allergen Working Group of the MoniQA network require that, to minimize bias, no more than one-fourth of the total number of laboratories contributing data which is used in the final analysis of the study may be from the same organization. For the purposes of this requirement, the term organization refers to a particular company, such as the method developer or kit manufacturer, or to any other body, such as a regulatory body or other government agency.

Recruiting enough qualified laboratories to conduct a proper validation study for food allergens is difficult. However, the purpose of an interlaboratory validation study is to document the performance of the method in the hands of other laboratories, and this could not be accomplished if many of the laboratories participating in the study were from the same organization. If method developers use laboratories from their own organization as part of the validation study, the results generated by these laboratories shall have the same dispersion of results as those generated by other participating laboratories.

Table 4. Example of raw data

	0 ppm		0.5 ppm		1.0 ppm		2.5 ppm		5 ppm	
Lab	A	B	A	B	A	B	A	B	A	B
1	0.61	0.46	1.10	1.13	1.24	1.97	3.08	2.80	3.65	3.61
2	–0.27	–0.41	0.41	0.29	0.57	0.71	2.80	2.07	4.51	4.84
3	0.37	0.21	0.62	0.11	0.45	0.70	2.82	2.93	4.24	3.93
4	0.13	0.13	1.06	0.62	0.79	0.41	1.95	2.37	5.22	4.96
5	0.24	–0.10	0.29	0.29	1.60	1.56	3.24	3.54	5.59	5.82
6	–0.23	–0.30	0.89	0.72	1.11	1.07	2.32	2.36	4.67	5.22
7	0.15	0.07	0.04	0.25	0.35	0.01	2.09	2.01	5.37	5.55
8	0.02	0.10	0.67	0.47	0.46	0.19	1.52	1.58	6.35	5.53
9	–0.02	–0.18	1.19	0.64	1.40	1.42	2.37	1.56	4.28	3.75
10	–0.10	–0.09	0.68	0.79	0.87	0.77	1.98	2.52	3.04	3.74

The AOAC Presidential Task Force on Food Allergens and the MoniQA food allergen community will attempt to develop a list of external laboratories from around the world that method developers could enlist to participate in validation studies. This will mitigate issues associated with the quality of results generated by the laboratories, or shipping of study samples across borders.

Number of Matrixes, Concentration Levels, and Replicates Required

The food allergen working group recommends that minimum requirements for any validation study include two matrixes, four concentration levels per matrix, and two replicate samples of each concentration per matrix in each laboratory. This is in compliance with AOAC *Appendix D* requirements for a minimum of five materials. For the concentration levels, one of the levels must be the zero level or blank. As an example, for a study using the minimum four concentration levels, two replicates and two matrixes, each participating laboratory would receive 16 samples for analysis.

In addition to a blank or zero level, one of the remaining concentration levels must be less than or equal to two times the LLA stated for the kit so that at least one of the concentration levels is at the lower end of the calibration curve. The remaining non-zero levels should be evenly distributed throughout the range of the calibration curve.

In general, more replicates per laboratory will result in greater certainty in the estimates of both repeatability and reproducibility. As with most estimates of variation, there is a law of diminishing returns with respect to increasing the sample size: the greatest advantage is made in the first few increases in sample size (replicates), but not much afterwards. These decisions are eventually made based on the tradeoffs between improved statistical estimates and resources needed to manage and perform the study. For allergen ELISA methods, the food allergen working group has concluded that a minimum of two replicates per laboratory will optimize the statistical confidence while not imposing undue burden on study participants.

Acceptance Criteria

Acceptance criteria are defined as numerical limits, ranges, or other suitable measures for acceptance of the analytical results to which a food allergen method should conform to be considered acceptable for its intended use. Acceptability of method performance is generally based on a number of factors, including percent recovery for spiked or incurred samples.

Ideal percent recovery levels would range from 80 to 120%. Recovery levels are affected by both the efficiency of the extraction step and the ELISA procedure. With ELISA methods for food allergens, this level of recovery is not always possible, particularly when certain difficult matrixes are analyzed. In addition, the recovery from incurred samples can be substantially different from those obtained using spiked samples. For this reason, recoveries between 50 and 150% will be considered acceptable so long as they can be shown to be consistent.

Data Analysis for Interlaboratory Studies

The ISO standard for method validation, ISO 5725-2 (8), and the AOAC *Official Methods of Analysis* (9) are the standards that outline how to analyze data stemming from interlaboratory trials in the context of analytical method validation. Each matrix/level combination should be treated as a separate experiment. For each matrix/level combination, the following analyses should be performed: Outliers should be tested sequentially by Cochran's and Grubbs' tests, as indicated in AOAC *Official Methods of Analysis*, *Appendix D* (1). Mean, accuracy (if applicable), repeatability (S_r), reproducibility (S_R), RSD of repeatability (RSD_r), and RSD of reproducibility (RSD_R) should be calculated and reported.

For each matrix, the LOD and LOQ of the method should be estimated using the sample S_R by the methods described in the IUPAC Nomenclature guidelines for LOD and LOQ (10). These guidelines call for a probabilistic estimation of LOD based on the variance observed at zero or near-zero concentration levels. If all assumptions are met (variance is constant and normally distributed, and the blank distribution is centered on zero), the LOD can be estimated as 3.3 times the SD of the distribution of blank results. This corresponds to false-positive and false-negative risks of 5% each ($\alpha = \beta = 0.05$), which is the recommended level for LOD estimation. LOQ can be set at 10 times the S_R.

Example of LOD Estimation for ELISA Collaborative Study Data

The following example uses data from a hypothetical collaborative study performed with an ELISA allergen test kit and shows the various steps required to calculate the LOD and LOQ for the method in a particular matrix as well as how to construct an operating characteristic (OC) curve for the method at a given concentration, such as the LOQ. Because different matrixes could give different results, data from each matrix in the study should be analyzed separately. The example is for samples spiked at nominal

Table 5. Example of data analysis following AOAC/ISO 5725 Standard

		0 ppm	0.5 ppm	1.0 ppm	2.5 ppm	5 ppm
Total number of laboratories	p	10	10	10	10	10
Total number of replicates	Sum(n(L))	20	20	20	20	20
Overall mean of all data (grand mean)	$\bar{\bar{x}}$	0.040	0.612	0.882	2.395	4.694
Repeatability SD	s_r	0.108	0.211	0.220	0.305	0.325
Reproducibility SD	s_R	0.269	0.350	0.536	0.580	0.913
Repeatability RSD	RSD_r	273.438	34.456	24.888	12.721	6.925
Reproducibility RSD	RSD_R	680.549	57.203	60.711	24.228	19.455
HorRat value	HorRat	26.164	3.322	3.724	1.727	1.535

levels of 0, 0.5, 1.0, 2.5, and 5 ppm. The samples were analyzed in duplicate by 10 laboratories. It should be noted that these values may not reflect the full range of the calibration curve for this ELISA method, which could go much higher than 5 ppm. The results of the collaborative study and an example of how to use the data to calculate LOD are as follows:

Step 1: Collect data (*see* Table 4).

Step 2: Data analysis following AOAC/ISO 5725 standard (*see* Table 5).

Step 3: Model (S_R) by mean as per ISO 5725 (*see* Table 6).

Figure 1 gives an example plot of S_R versus mean. This model uses an ordinary least square estimate. Weighted least square analysis would also be acceptable.

Step 4: Estimate LOD and LOQ. Basic formula:

$$LOD = 3.3 \times s(0) = 1.0 \text{ ppm}$$

$$LOQ = 10 \times s(0) = 3.0 \text{ ppm}$$

Advanced formula to adjust for increase in s_R as mean increases: slope = 0.1285; intercept = 0.3081; xbar(0) = 0.039553; LOD = (xbar(0) + 3.3 × intercept)/(1–1.65 × slope); LOD = 1.3405; LOQ = 3 × LOD = 4.0215. These estimates are likely to be more accurate than those obtained following the simple formula.

Step 5: Construct OC curve based on results of Steps 3 and 4. Calculate the SD over a range of concentrations bracketing the LOQ using the formula:

$$SD = 0.1285 \times \text{concentration} + 0.3081$$

where 0.1285 and 0.3081 are the slope and intercept of the curve from Step 3.

Use a normal distribution calculation function to calculate the probability of obtaining a result higher than the LOQ (4.0) for the given concentration using the calculated SD and assuming a normal distribution. The probability thus calculated is plotted against the concentration to obtain the OC curve.

The curve below was calculated in Excel using the following equation to calculate the probability of a result higher than LOQ:

$$= 1 - \text{NORMDIST}(LOQ, \text{mean concentration}, S_R, 1)$$

where the LOQ is set at 4.0 ppm, the mean concentration is on the *x* axis, and the S_R is calculated from the mean concentration using the equation from Step 3.

Figure 2 presents an example of the OC curve. This OC curve shows the probability of obtaining a result above 4 ppm based on the concentration present in a sample. When the concentration in the sample is 4 ppm, there is a 50% chance the result will be above 4 ppm.

It is very important for collaborators to report all results obtained by the method without censoring to a predetermined LOD or LOQ. For nonspiked samples, this may mean half of the responses are negative numbers. It is critical to keep this information in the data set, as censoring will result in biased LOD/LOQ estimates.

For the results of the interlaboratory study, model S_R by concentration mean as detailed in ISO 5725-2. If the slope is significantly greater than zero, it should be taken that variance of the method increases with increased concentration. In this event, LOD estimates will need to be corrected with a general formula, which is shown above. If the general formula for LOD is used, LOQ can be estimated as three times LOD.

Additional guidance on the handling and analysis of data generated during interlaboratory studies will be provided through implementation studies conducted following this validation protocol.

Allergen-Specific Criteria

Certain criteria are dependent upon the specific target food allergen. For example, reference materials, spiking methods and food matrixes will vary from one food allergen to the next. General guidance on allergen-specific criteria and specific guidance for milk and egg allergens are as follows:

Reference materials.—Choosing a reference material for use in an allergen method validation can be extremely challenging. A perfect representative material rarely exists. Different species of the same food commodity may have different protein profiles. Processing methods can also drastically affect protein content, conformation, solubility, and reactivity. In general, a reference material is representative of the allergenic food commodity, is well-characterized, can be produced or supplied with robust reproducible

Table 6. Example of (S_R) modeling

Level	Mean	s_R
0	0.039553	0.26918
0.5	0.612395	0.350308
1.0	0.882414	0.535725
2.5	2.395355	0.580356
5.0	4.693936	0.913203

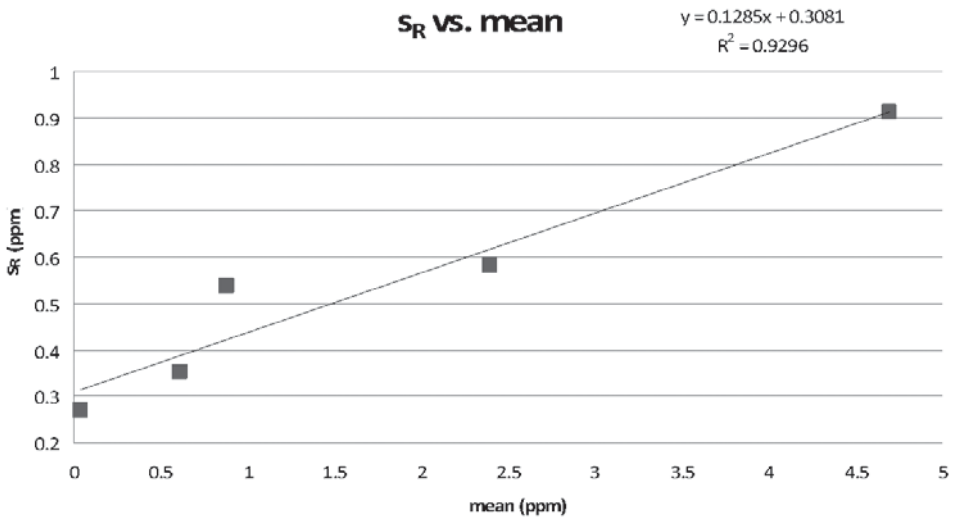

Figure 1. Example curve of S_R versus mean.

characteristics, and can be used as a calibration standard, control, or spiking material. Food allergens can be present in many different forms, processed or unprocessed, depending on the food matrix in which they are found, and with very divergent characteristics and functions in a food. It is unlikely a single material can represent many different possibilities at once. However, a widely available reference material will provide a common reference point for data comparison purposes between kits designed for the same food allergen.

For egg detection methods, based on a preliminary multilaboratory study, a suggested material is the National Institute of Standards and Technology (NIST) egg powder (NIST RM-8445). This is the first NIST reference material specifically intended for use in food allergen testing. The kit manufacturer is expected to provide a conversion factor relative to the NIST egg powder if a different material is used.

For milk detection methods, a suggested material is the NIST nonfat milk powder (NIST RM-1549). Although this reference material was not specifically intended for use in food allergen testing, it has been used in the past for method validations and has performed well as a reference material for milk ELISAs. The kit manufacturer is expected to provide a conversion factor relative to the NIST milk powder if a different material is used.

Spiking methods.—The best source of information on method performance for allergen detection methods is an incurred sample, which is defined as one in which a known amount of the food allergen has been incorporated during processing, mimicking as closely as possible the actual conditions under which the sample matrix would normally be manufactured. This kind of real-life sample would give the most accurate representation of the recovery and response of a particular method for that particular matrix. Whenever possible, validation studies for allergen detection tests should be run using incurred samples. Unfortunately, incurred samples can be difficult and costly to obtain, particularly in larger quantities required for a validation study.

Because of these limitations, validation studies using samples with food allergens added to them after manufacturing (spiked

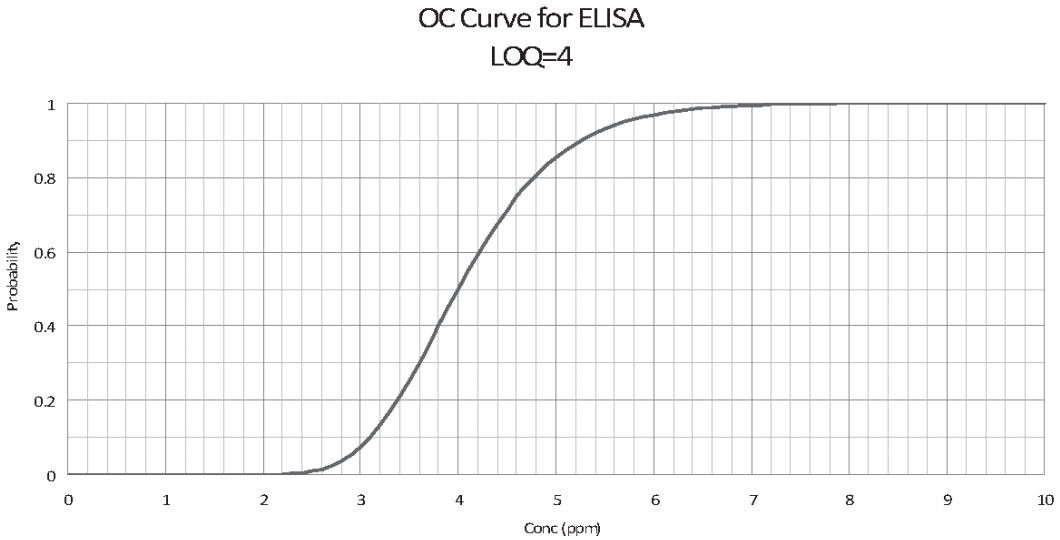

Figure 2. Example of OC curve.

samples) are still considered an acceptable way to generate information about the performance of a method in specific matrixes. However, spiked samples may result in an artificially higher recovery than would be obtained from incurred samples; hence, some regulatory bodies may be unwilling to consider approval of validation data without the inclusion of data generated with incurred samples prepared with known and controlled amounts of the reference material for the allergen being targeted.

There are several ways to prepare spiked samples. One way involves the preparation of a large batch of a food sample that contains a specific food allergen, then gradual dilution of the allergen by mixing with more of the food sample that does not contain the allergen. This kind of sample preparation works best for samples that can be mixed well in an attempt to reach homogeneity, such as liquids or fine powders. An example would be the use of pasta containing a known amount of egg that had been ground to a fine powder and was then mixed with non-egg-containing pasta (also ground to a fine powder) stepwise until the desired concentration of egg was reached. Considerable effort is required to ensure sufficient mixing and to verify the homogeneity of the final batch of material, but this method of sample preparation has the advantage of being relatively similar to an incurred sample.

Because it can be difficult to mix a large batch of samples at a low spiking level to make a homogeneous mixture, the most precise way to spike samples is to add a known amount of a food allergen to each individual sample or test portion. This method results in each sample receiving an accurate amount of analyte, and addresses the issue of homogeneity of the spiked samples. Such a spiking method has been successfully used in the AOAC peanut *Performance Tested Method*℠ study (11). In that study, individual test portions were weighed out and spiked before being sent out for analysis. This method of spiking results in a small part of the actual procedure (weighing of samples) being completed before the samples are distributed to study participants, and eliminates any weighing errors that may be introduced if study participants have to weigh the samples. Although this procedure is not ideal, the AOAC and MoniQA food allergens communities believe it is acceptable in order to overcome problems with production of large batches of food samples homogeneously spiked at a low level with a particular allergen. This type of sample preparation is the most artificial method and least representative of real-life samples.

When spiking samples, unaltered reference material should be used instead of a protein extract of the reference material. If the reference material is completely soluble in the buffer used for spiking, a solution of the reference material can be prepared and diluted to the appropriate level. The spike should be delivered in the same volume for each of the spiking levels.

The stability of the spiking material in the matrix of interest should be investigated by spiking several samples, and then extracting and analyzing them over the same period of time that will be required to complete the entire study. If the response changes significantly over time, this must be accounted for in the study design. Samples will have to be prepared, shipped, and analyzed within a defined time frame to avoid any decrease in response.

The suggested reference materials (NIST RM-1549 for milk and NIST RM-8445 for egg) are both powders that could be used with either of the spiking methods mentioned earlier (spiking a large batch of the matrix followed by serial dilution in a blank matrix, or spiking individual test portions using a spiking solution). Although the NIST nonfat milk powder (NIST RM-1549) is soluble in water or phosphate-buffered saline, the NIST egg powder (NIST RM-8445) is not. However, use of a tissue grinder, such as the Potter-Elvehjem type, will facilitate dispersion of the egg powder to form a homogeneous suspension. Thus, for both egg and milk, a stock solution of the reference material can be made, followed by dilution to the appropriate spiking levels. A recommended starting concentration for the stock solution is 1 mg/mL. In all cases, the method chosen for preparation of the spike and the spiking method should be documented in the validation report.

Food matrixes.—The matrix being analyzed can have a large impact on the performance of an ELISA method. Ideally, methods would be able to analyze all matrixes with equally reliable results. In reality, methods may work better for some matrixes than for others. The choice of matrixes included in a validation study is left to the method developer to meet customer demands. Although no matrixes are mandatory, some are of particular interest for each food allergen and are based on which food products are most likely to be contaminated with a particular allergen. Table 3 lists matrixes of interest for egg and milk. Method developers are encouraged to include as many of these matrixes as possible in their validation studies. However, good performance in one or even several matrixes does not guarantee good performance in others.

Conclusions

The food allergen analytical community is challenged to develop detection methods for multiple allergens in various food products to protect allergic consumers and promote consumer confidence. This protocol reflects the consensus reached through input from various food allergen analytical experts and contains recommendations based on the current knowledge of ELISA methods. Specific recommendations have only been included for two priority allergens, egg and milk. The general considerations of the protocol will be applied to other priority allergens in the future. Meeting the challenges of developing reliable food allergen detection methods requires conscientious and continuous support from the allergen community. Future work is planned for the implementation of this guidance document for egg and milk ELISA methods and for the development of similar guidance pertaining to other priority food allergens.

Acknowledgments

Under the auspices of the AOAC Presidential Task Force on Food Allergens and with the support of the MoniQA network of excellence (www.moniqa.org), the working group represents major food allergen test kit manufacturers and experts from regulatory agencies in Europe, Australia, Japan, Canada, and the United States, and the AOAC general referee for food allergens.

The participation of the following collaborators is greatly appreciated.

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ISPAM WORKING GROUP CHAT COMMENTS/QUESTIONS:
November 30, 2016
11:00-1:00pm ET

Yumin Chen: since this is an ELISA method. do we need the term of "two ion MS/MS transition" in Method detection Limit? Does it mean a MS method is needed to confirm the method detection limit?

Laura Allred: I don't have the Abbott paper in front of me. Does it cover a definition of "Whole Egg Allergen", or do we need to discuss that here?

Yumin Chen: In light of FSMA, do we envision it is to be used for FSMA compliance?

Laura Allred: I am in agreement with Paul.

Laura Allred: Do we want to use "allergen" instead of "protein"?

France Cho: MDL is a statistically calculated value. We will need to spike at that level to verify if the amount can be seen or not. If recovery is low, result will be biased low, therefore false negative

Markus L: I agreee with Laura: proteins

Bert Popping: It should include both, as Paul said, provided validation data are available for both

Laura Allred: environmental swabbing should be covered in the qualitative SMPR

Bert Popping: Yes

Diana Kavolis: Can we consider applicability as quant of whole chicken egg. (not specify allergens perse as we test a whole entity and would need to validate on spikes with whole items not purified proteins.

France Cho: I propose whole and separated egg components to cover those who use whole egg or egg white

Lisa Monteroso: Delia, I will send my comments to you

Diana Kavolis: Should have validation data for classes of matrices that may be a challenge - ovaltine is used in cocoa products and high tannin products are not represented in the matrices section

France Cho: Validating using specific matrices will make sure that method is fit for lspecific or group listed.

Bert Popping: In my view manufacturer can choose. But it has to be indicated that only those matrices have been validated.

Laura Allred: would a list of items to be checked for cross-reactivity be more beneficial than listing required validation matrices? Maybe this list is "priority" matrices, not required ones.

Lisa Monteroso: I agree that manufacturer should choose.

Lisa Monteroso: YES. Please add cross reactivity. It is missing in this document

France Cho: agree with Lisa

Bert Popping: Good point! Milk in orange juice is not uncommon either

Yumin Chen: how about eggnog?

Paul Wehling: yes agree with Bert

Bert Popping: (whey rather than milk)

Paul Wehling: I think Cross-reactivity is covered in the guidance paper

Paul Wehling: We might want to bring some of that into the SMPR

Lisa Monteroso: Is the guidance paper you are referring to appendix M?

Lisa Monteroso: Sorry I want to make sure I am following

Paul Wehling: I cant find Appx M anywhere.

Delia Boyd: We will send it out to the working group after the meeting

Lisa Monteroso: I can send to you.

Lisa Monteroso: Ha. Or Delia will

Lisa Monteroso: ;)

France Cho: Recovery range should be tighter in my opinion, 40% indicates that 60% may not be detected it present

Lisa Monteroso: Sent to you now Delia! Please forward

France Cho: Oops, should be 60% and 40% (not 40 and 60%)

masahiro shoji 2: Recovery range should be changed in spiking case and naturally incured case. Japan uses natually incurred samplen abd range is 50-150%.

Laura Allred: yes, that's why it should be protein, not the allergen. People with allergens can react to different epitopes, but a monoclonal antibody will only ever see one.

France Cho: Agree. Incurred products would give a better picture of what is really recovered in actual products.

Laura Allred: this is where we need a section on method limitations for SMPRs and validations - any method based on one or two protein markers may notwork in fractionated materials.

Laura Allred: this section could also cover the applicability of the method to raw vs cooked material,which is important in egg.

Bert Popping: Would that not exclude methods recently developed by Ghidhari?

Markus L: Whats about reader-based quantitative LFDs?

Bert Popping: I think we need to widen this

Laura Allred: ligand-based assays

Laura Allred: with an appendix specific to ELISA

Hirotoshi Doi: The recovery range should be changed. Spiking case is 80-120%, and incured case is 50-150% according to Appendex M.

Laura Allred: good point

Bert Popping: Carmen and I will drop out now as our flight is boarding. We look forward to receiving the meeting minutes.

Delia Boyd: Thanks Bert & Carmen for your input. Have a great flight

Laura Allred: is there a suitable conversion from dried to liquid egg?

Markus L: there are values from the literature

Laura Allred: so we leave it to the developer to state which they are reporting? And if they use liquid egg as their reporting units, do we allow them to use a conversion when using dried reference materials?

Markus L: yes we should do that

Laura Allred: what form has been used for most challenge studies? if challenges (and future thresholds) are based on whole liquid egg, expressing everything in terms of dried eggs will create confusion.

Laura Allred: that's why protein would be better than commodity

Markus L: all results for challenge studies are given in mg/serving size protein

Yasutaka Nishiyama: I agree with Laura. Protein is better

Laura Allred: that's why the units should be egg protein

Laura Allred: several egg kits now can quantitate to 1 ppm (for raw egg)

Laura Allred: r-biopharm low standard is 0.5 ppm

Markus L: Laura, I need to look for that but I think this is dried NIST material

Laura Allred: ah, not protein?

France Cho: The average moisture of whole egg can be used. E-mail me of you need help.

Laura Allred: yes, Markus, that's 0.5 ppm whole dried egg, which converts to 0.13 ppmegg white protein

Laura Allred: you can make incurred baked goods

Laura Allred: sounds good

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-02 14:37:25
First & Last Name	Laura Allred
Organization	GFCO/GIG
E-mail Address	laura.allred@gmail.com
Date Submitted	12-02-2016
Question/Comment-1	Section 2 lines 18-19. Change this section to read "Quantitation of whole chicken egg protein in selected food products and ingredients." Protein would be a more achievable and more easily standardized target than allergens, of which there may be many, and some of which may be unknown. This would remove the difficulty of defining "Allergen" as listed on line 38, and would lead to the removal of the statement that allergen should be reported by dry weight at the bottom of Table 1. While we may want to recommend priority matrices, we may not want to tell assay developers that they must validate their kit for a fixed set of matrices, so perhaps we could omit the reference to Table 2, or rename Table 2 as a list of priority matrices. Some current ELISA methods have been shown to have difficulty detecting or accurately quantitating cooked egg material. Do we want to allow manufacturers the option to validate their kit for one or the other? Or do we want to say it must be validated for both? That might mean changing the wording here to "Quantitation of cooked and raw whole chicken egg protein in selected food products and ingredients."
Question/Comment-2	Section 3 lines 23-24. The group has agreed to open up this SMPR to include other binding-based assays. In Section 4 lines 29-30, we have defined ELISA as encompassing other ligand binding assays, but in line 29 we have kept the requirement for color change as being part of an ELISA method. There are other reporter systems, such as fluorescent markers, that are non-enzymatic and non-color based, but assays that use these reporters are still commonly called ELISAs. Do we want to either widen the definition of an ELISA, or alternatively remove it altogether and state that the applicability is for "Protein binding assays, such as ELISA"?
Question/Comment-3	Section 6 (line 85). Would this section be a good place to list required cross-reactivity checks, perhaps by referencing Table 1 of Appendix M?
Question/Comment-4	Table 1. Analytical range should be more in the range of <0.5 to >5 ppm whole egg protein for most products (with special requirements as needed for other matrices such as wine). Most kits on the market now have an LOQ below 1 ppm egg protein, and this range would be more in scale with the VITAL reference dose/action level system. Do we want to provide conversions here to whole liquid egg, liquid egg whites or egg white protein (e.g. <0.5 ppm whole egg protein = <1 ppm dried whole egg = <3.8 ppm liquid whole egg = <0.55 ppm dried egg white = <5 ppm liquid egg white)?
Question/Comment-5	Table 1. Acceptable recovery % is skewed towards false negatives, which would not be preferable for public safety. I didn't see it in Appendix M, but i believe the Abbott paper recommended a recovery range of 50-150%, and many manufacturers have operated based on this. It would be nice to tighten this range, recognizing that it can be difficult to get excellent recovery across multiple matrices with a kit that has one extraction buffer and extraction protocol. Can we review recent PTMs and OMAs for ELISA methods and see if recoveries closer to 75-120% are realistic?

Question/Comment-6	A section should be added to all validations that describes the method limitations. For instance, if a kit manufacturer realizes their egg assay works well for raw egg but not cooked, in addition to only validating the kit for unheated foods, there should also be a statement that the kit is not suitable for testing cooked products. Similarly if the kit works well in some matrices but not others, this should be stated in the validation, since labs tend to take AOAC methods and use them for every situation.

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-05 11:35:16
First & Last Name	Virginie Barrere
Organization	Université Laval
E-mail Address	virginie.barrere@fsaa.ulaval.ca
Date Submitted	12-05-2016
Question/Comment-1	If environmental samples are not mentioned, the intended use has to be changed to Method for food testing for example. If the intended use is for cGMPs compliance, environmental samples have to be included in this SMPR. cGMPs involve sanitation, cleaning and control of cross contamination.
Question/Comment-3	Yes. FAO CXP_015e Pasteurization – a microbiocidal control measure where eggs or egg products are subjected to a process, using heat to reduce the load of pathogenic microorganisms to an acceptable level to ensure safety.
Question/Comment-4	If specifity is added, should cross reactivity be tested as well and a list of food matrices be added for this purpose?

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-07 11:50:53
First & Last Name	Yumin Chen
Organization	PepsiCo
E-mail Address	Yumin.Chen@pepsico.com
Date Submitted	12-07-2016
Question/Comment-1	Cover environmental and sanitation samples (Proposed for group discussion) i. Because quantatitive ELISA will be used to calibrate the analyte used to validate qualitative method (Agreed by several group members). ii. If a food contains food allergen, the label should describe that. The most valuable use of an allergen method is acutally to assess allergen footprint in order to clear a production line to run a second non-allergen containing product.
Question/Comment-2	Create a category of alcohol containing beverage so that wine and eggnog can both go under
Question/Comment-3	Definition – Specificity: need to connected to the binding technology that implied by the ELISA definition.
Question/Comment-4	Spiking – According to the AOAC appendix M, the best spiking sample is an incurred sample.

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-09 14:10:08
First & Last Name	Melanie Downs
Organization	University of Nebraska-Lincoln
E-mail Address	mdowns2@unl.edu
Date Submitted	12-09-2016
Question/Comment-1	Title, Line 3; Section 2, Line 18: With respect to the use of both the words "chicken" and "whole", a bit more clarity in the intent would be beneficial. By inserting "chicken" to describe the source of egg, it may unintentionally disqualify ELISA methods that detect chicken egg but also react with other bird eggs (e.g. duck, turkey, etc.). While the primary purpose of the methods would be to detect and quantify chicken egg, the SMPR should perhaps address what types and/or levels of cross-reactivity with other species will be acceptable. The use of "whole" may imply that egg white and egg yolk should be detected equivalently. Most methods, however, would primarily detect egg white proteins, even when whole dried egg is use as the method calibrant. It may be beneficial to discuss and describe the extent to which methods are required to detect egg yolk and egg white fractions independently.
Question/Comment-2	Section 4, Lines 38-40: In the Definitions section, it would be useful to give some thought as to whether definitions for both "allergens" and "commodities" are necessary. If "allergens" are to be defined as allergenic source foods (similar to how most regulations define food allergens), then a definition for commodities may create additional confusion.
Question/Comment-3	Section 4, Lines 76-78: The definition given in this section seems too specific to a particular product for the purposes of this SMPR. The definition given is that of refrigerated liquid whole eggs, as defined by the USDA FSIS. Given the complicated regulatory authority for eggs in the United States (i.e. the FDA regulates in shell eggs, while the USDA FSIS regulates egg products), it may be difficult to apply a regulatory definition of whole egg for the purposes of this SMPR. (The FDA also does not have a regulatory definition for "eggs", per 21 CFR 160.100.) It would be beneficial for this working group to agree upon a simple definition for egg that suits the purposes of the SMPR.

Question/Comment-4	Table 1: The concentration units in this table should be much more specific. The note at the bottom of the table is more confusing than helpful in this regard. The units of "ppm" really must be clearly described somewhere, for example: "ppm indicates mg whole dried egg per kg product". The note at the bottom of the table could be interpreted to mean that the units should be expressed on a dry weight basis (i.e. mg whole dried egg per kg dry weight product), which we would not want. For the top end of the analytical range, it might be more clear to state something like, "at least ten times the LOQ", rather than just ">10", which could lead to somewhat narrow analytical ranges (e.g. 3-13 ppm whole dried egg). MDL should be changed to LOD, and the minimum value should be changed. (The LOD should be less than the LOQ.) It may also be worthwhile to discuss the actual utility and applicability of requiring an LOD for a quantitative method. Results below the LOQ and above the LOD often cause additional confusion for end users, as it then becomes difficult to interpret the information and evaluate the risk associated with such a result when quantitative information is lacking. There should be some requirements added regarding specificity and cross-reactivity. (Referring to OMA Appendix M may be sufficient.)

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-08 16:42:42
First & Last Name	Michael Farrow
Organization	Abbott Nutrition
E-mail Address	Michael.Farrow@Abbott.com
Date Submitted	12-08-2016
Question/Comment-1	Section 3 Line 23: Strike "based assays" and include "with consideration of other ligand binding technologies."
Question/Comment-2	Section 4: Include definitions for (1)Allergen: A food or substance which may initiate an antibody-mediated immune response in certain individuals despite the substance not being otherwise harmful; (2) Antigen: Any substance that is recognized and bound by antibodies; (3) Rewrite ELISA as follows: An assay that uses an immobilized solid phase component, antigen-antibody interactions, and color change to identify a substance. (Strike the rest); (4) Ligand-Binding Assay (definition to be determined); (5) Include proposed LOD text and strike MDL; (6) Part per million (ppm): microgram of detected food antigen per gram of protein.
Question/Comment-3	Table 1: Analytical Range: 0.5-500 ppm; LOQ: 0.5 ppm; LOD: 0.1 ppm; Recovery: 60-140%; Small r RSD: 15% ALSO these values should be adjusted to the food matrices that are being considered and should be adjusted for typical serving sizes. (Multiple tables may be necessary especially with the inclusion of environmental samples)
Question/Comment-4	Table 2: Adjust matrix types to general food categories: e.g. Baked Goods, Beverages (Non-alcoholic and alcoholic), Environmental Samples; Meats and Processed Foods, etc. Include examples within each category.
Question/Comment-5	Section 2 Line 18: Add environmental samples; This is vital as ELISA-based quantitative technologies are often part of the method validations for qualitative technologies such as lateral flow devices. It may be pertinent to validate cleaning through demonstrating an X-fold reduction in the specific antigens used at a facility. Surfaces with and without dilute cleaning solutions can be problematic matrices for antibody-based assays.
Question/Comment-6	Line 5: Would it be necessary to strike Reference from intended use section? Wouldn't reference status be at the discretion of AOAC committees once a novel method is up for review? Question 7: Where would information on Cross-Reactivity be captured?

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-05 15:18:53
First & Last Name	Diana Kavolais
Organization	The Hershey Company
E-mail Address	dkavolis@hersheys.com
Date Submitted	12-05-2016
Question/Comment-1	Delia - Please see email that contains the document you sent as the comments I have are more readily understood in the context of the body of the document.

DRAFT AOAC Allergen SMPR Version 2; November 30, 2016

Quantitation of Whole Chicken Egg Allergens by ELISA-based Methods

Intended Use: ~~Reference m~~Method for cGMP compliance.

1. **Purpose:** AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC Stakeholder Panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their evaluation of validation study data for method being considered for *Performance Tested Methods* or AOAC *Official Methods of Analysis*, and can be used as acceptance criteria for verification at user laboratories.

2. **Applicability**:

   Quantitation of whole chicken egg allergens in selected finished food products and ingredients as listed in table 2 (may need to revise table to be consistent with OMA Appendix M).

3. **Analytical Technique**:

   Enzyme-linked immunosorbent assay (ELISA) ~~based assays~~ or other binding based technologies.

4. **Definitions**:

   Enzyme-linked immunosorbent assay (ELISA) ~~based assays~~.

   An assay that uses antibodies and color change to identify a substance. ELISA can perform other forms of ligand binding assays instead of strictly "immuno" assays, though the name carried the original "immuno" because of the common use and history of development of this method. The technique essentially requires any ligating reagent that can be immobilized on the solid phase along with a detection reagent that will bind specifically and use an enzyme to generate a signal that can be properly quantified.

   Binding Based Technology

   Allergens

   Commodities

   Limit of Quantitation (LOQ)

   The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

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Comment [D1]: May want to consider adding environmental samples

Limit of Detection (LOD)
Limit of detection (LOD).—The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

~~**Method detection limit (MDL)**~~
~~The minimum concentration of a substance that can be measured (detected) and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte using at least two ion MS/MS transitions.~~[1]

Repeatability
Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility
The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

Recovery
The fraction or percentage of ~~spiked~~ incurr[illegible]alyte that is recovered when the test sample is analyzed using the entire method.

Whole Egg
A combination of pasteurized [chicken] egg whites and egg yolks from the same production batch blended together in their entirety, in natural proportions.[2]

Comment [D2]: In performance table, be specific or clarify so that it is clear what is meant as to type of egg.

Comment [D3]: Do we need to add a footnote for a definition/reference for "pasteurized"?

5. **Method Performance Requirements**:
See table 1.

6. **System suitability tests and/or analytical quality control:**
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

7. **Reference Material(s):**

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 20th Edition of the AOAC

[1] ~~40 CFR Part 136, Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11~~

[2] Introduction to Egg Products, USDA Food Safety and Inspection Service, website: http://www.fsis.usda.gov/wps/wcm/connect/c5c85914-5055-4f09-8098-1a179a1c6e14/EPT_Introduction.pdf?MOD=AJPERES, accessed 12/15/2015.

INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

Whole Egg

- NIST 8445
- LGC SAL-RSM-5 (Check for characterization level)

8. Validation Guidance:

Method developers should provide data for method performance in all claimed matrixes (listed in table 2).

Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

Appendix M: Valaidation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_m.pdf

Field Code Changed

9. Maximum Time-To-Result: None

Table 1: Method performance requirements

Comment [D4]: May need to consider adding specificity

Parameter	Minimum Acceptance Criteria		
	Cookies, Bread, Dough, Salad Dressing	Wine	Matrix X
Analytical Range (ppm)	~~10-1000~~< 5 - >		
LOQ (ppm)	< 5		
MDL (ppm)	< 10		

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Recovery (%)	60-120%		
% RSD_r	≤20 %		
% RSD_R	≤ 30%		
Note: Allergen to be reported by dry weight.			

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Table 2: Selected Finished Food Products and Ingredients ~~Matrixes~~ (revised per OMA Appendix M)

cookies
bread
dough
salad dressing
wine
chicken
ice cream
pasta

Comment [D5]: Are these matrices okay for this analyte

Comment [D6]: Matrices listed in OMA Appendix M

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-07 09:54:02
First & Last Name	Sefat E Khuda
Organization	US FDA
E-mail Address	sefat.khuda@fda.hhs.gov
Date Submitted	12-07-2016
Question/Comment-1	Title: Quantitation of Whole Egg Allergens by ELISA-based Methods My suggestion: Quantitation of chicken whole Egg proteins by Antibody-based or Immunochemical Methods Because under analytical techniques, there are different techniques like ELISA or other binding based technologies. Guessing that, other binding based technologies are also utilizing antibodies like ELISA. Regulation requires labeling of egg based on the presence of egg proteins in food. ELISA against whole chicken egg detects both allergenic and non-allergenic proteins from egg.

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-08 14:05:05
First & Last Name	Terry Koerner
Organization	Health Canada
E-mail Address	Terry.Koerner@hc-sc.gc.ca
Date Submitted	12-08-2016
Question/Comment-1	Line 103 Appendix M requires two or three matrices in the initial study.
Question/Comment-2	Line 122 In my opinion we need to assess the level requirements differently based on consumption information and known clinical information. A 5 ppm level may be fine for some matrices, but it may be inadequate for others (bread, drinks, etc...)

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-08 13:59:40
First & Last Name	Terry Koerner
Organization	Health Canada
E-mail Address	Terry.Koerner@hc-sc.gc.ca
Date Submitted	12-08-2016
Question/Comment-1	Line 17 Applicability Considering that clinical results will be expressed in mg of total egg protein, the results of the assays should be in total chicken egg protein.
Question/Comment-2	Line 18 "food products" If this will be linked with Table 2 then it might be better to put food matrices.
Question/Comment-3	Line 42 Limit of Quantitation Considering this is for egg should we be more specific in our definition? ...concentration or mass of total egg protein in a given matrix...
Question/Comment-4	Line 50 Limit of Detection Same as LOQ. Be more specific to total egg protein
Question/Comment-5	Line 76 Whole Egg We should be clear on how this whole egg definition, which comes from a food inspection service will carry over to the reporting units of total egg protein.
Question/Comment-6	Line 84 System suitability Appendix M is clear about what the testing levels should be.

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-08 06:32:59
First & Last Name	Markus Lacorn
Organization	R-Biopharm
E-mail Address	m.lacorn@r-biopharm.de
Date Submitted	12-08-2016
Question/Comment-1	Table 1: Why do we need an analytical range? A possible user may decide if an analytical range is broad enough. At the moment the LoQ (or sometimes also LoD) is of most interest since we are only interested in presence or absence. This may change when threshold values will be installed (comparable to gluten). Change: Delete the analytical range from the table
Question/Comment-2	Table 1. Change recovery to mean recovery otherwise precision would not be necessary any longer
Question/Comment-3	Table 1. Instead of defining commodities we could separate the table into low-processed samples (e.g. salad dressing, dough, ice cream) and high-processed samples (e.g. bread, cookies, pasta): not fixed number decision by method developer New Table 1 will be sent separately to Delia
Question/Comment-4	Delete Table 2 and include examples in Table 1 as described in another comment
Question/Comment-5	Table 2: Chicken is a possible cross-reacting commodity that needs to be characterized (see also AOAC Guidelines by Abbott et al.). To be discussed in the group if the list stated in the Abbott paper is sufficient.

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-08 06:29:31
First & Last Name	Markus Lacorn
Organization	R-Biopharm
E-mail Address	m.lacorn@r-biopharm.de
Date Submitted	12-16-2016
Question/Comment-1	Definiton: LoQ is not defined in an acceptable way since sufficient precision and acceptable recovery should be mentioned. The terms “sufficient” and “acceptable” depends on the method developer and shall be stated with numbers. Change: to be discussed by the group
Question/Comment-2	Definitions: mention that reproducibility is only characterized when a collaborative test was performed Change: include collaborative tests in the definition
Question/Comment-3	Definition “recovery”: Recovery may be characterized by spiking experiments because incurred materials are not available; this SMPR should allow spiked samples if there is no other possibilities; incurred should be preferred in any case
Question/Comment-4	System suitability: Quantitative ELISA systems always contain calibrators therefore it is not necessary to deliver an additional check sample; instead: It is recommended that every user of these kits establish his own control samples that fits his needs best.
Question/Comment-5	Reference Materials: Delete LGC materials since they are produced by a lab which is NOT ACCREDITED according to ISO Guides! Please refer to the “certificates” this lab delivers (only ISO 9001 is mentioned). Certifcates are available on request. The NIST materials are valuable.
Question/Comment-6	Maximum Time-to-Result: Customers will not accept an assay e.g. with incubation times over night.

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-08 06:27:30
First & Last Name	Makrus Lacorn
Organization	R-Biopharm
E-mail Address	m.lacorn@r-biopharm.de
Date Submitted	12-08-2016
Question/Comment-1	Title: The title mentions ELISA but chapter 3. Analytical Techniques also mention “other binding based technologies”. Furthermore, we always detect proteins and but not allergens in all cases; these proteins may be allergens to sensitized customers; National legislations demand to declare “egg” and not egg allergens. Change title: Quantitation of whole chicken egg proteins by immunochemical methods
Question/Comment-2	Title: Why “whole” egg proteins? One method provider could also measure ovalbumin and recalculate this to whole egg Change: to be discussed by the group
Question/Comment-3	Applicability: If surfaces and cleaning in place solutions should be included this need to taken into account in the whole document Change: to be discussed by the group
Question/Comment-4	Definition “allergens”: Do not define allergens because this is quite broad (they may derive from food but also from dust) but explain for “whole egg” that egg constituents may be allergenic to consumers for an individual extent
Question/Comment-5	Definitions “commodities”: If surfaces and CIP water are included, commodities would not be sufficient as a definition; you may call them matrices but at the end we need to define matrices (food, CIP water) and surfaces
Question/Comment-6	Definition “ELISA”: Several formats are possible for ELISA: 1. Sandwich: analyte ligating agent is bound on surface and second analyte-ligating reagent is coupled to an enzyme: sandwich format 2. Analyte from calibrator or sample is coupled to surfaces (by the user) and ligating agent is coupled to enzyme: calibration curve like sandwich curve 3. Fixed amount of analyte is bound to surface and competes with free analyte in solution for ligating binding sites; this ligand is labelled to a marker (not only an enzyme); competitive format 4. Ligating reagent is coupled to surface: free analyte (from calibrator or sample) is competing with a fixed amount of labelled analyte for binging sites: competitive format These are only examples for microtiterplate based methods; LFD devices are even more complex and other systems may also be Change: Delete ELISA and insert new definition: “Binding-based assays: Antigen or ligand based methods where one of the components is attached to a surface. The measurement signal is directly or inversely proportional to the amount of measurand. ” This will also include quantitative LFDs or dip-sticks.

DRAFT AOAC Allergen SMPR Version 2; November 30, 2016

Quantitation of Whole Chicken Egg Allergens by ELISA-based Methods

Intended Use: ~~Reference m~~Method for cGMP compliance.

1. **Purpose:** AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC Stakeholder Panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their evaluation of validation study data for method being considered for *Performance Tested Methods* or AOAC *Official Methods of Analysis*, and can be used as acceptance criteria for verification at user laboratories.

2. **Applicability**:

   Quantitation of whole chicken egg allergens in selected finished food products and ingredients as listed in table 2 (may need to revise table to be consistent with OMA Appendix M).

3. **Analytical Technique**:

   Enzyme-linked immunosorbent assay (ELISA) ~~based assays~~ or other binding based technologies.

4. **Definitions**:

   Enzyme-linked immunosorbent assay (ELISA) ~~based assays~~.

   An assay that uses antibodies and color change to identify a substance. ELISA can perform other forms of ligand binding assays instead of strictly "immuno" assays, though the name carried the original "immuno" because of the common use and history of development of this method. The technique essentially requires any ligating reagent that can be immobilized on the solid phase along with a detection reagent that will bind specifically and use an enzyme to generate a signal that can be properly quantified.

   Binding Based Technology

   Allergens

   Commodities

   Limit of Quantitation (LOQ)

   The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

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Comment [D1]: May want to consider adding environmental samples

Limit of Detection (LOD)

Limit of detection (LOD). —The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

~~**Method detection limit (MDL)**~~

~~The minimum concentration of a substance that can be measured (detected) and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte using at least two ion MS/MS transitions.~~[1]

Repeatability

Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

Recovery

The fraction or percentage of ~~spiked~~ incurred analyte that is recovered when the test sample is analyzed using the entire method.

Whole Egg

A combination of pasteurized [chicken] egg whites and egg yolks from the same production batch blended together in their entirety, in natural proportions.[2]

Comment [D2]: In performance table, be specific or clarify so that it is clear what is meant as to type of egg.

Comment [D3]: Do we need to add a footnote for a definition/reference for "pasteurized"?

5. **Method Performance Requirements**:
   See table 1.

6. **System suitability tests and/or analytical quality control:**
   Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

7. **Reference Material(s):**

   Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 20th Edition of the AOAC

~~[1] 40 CFR Part 136, Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11~~

[2] Introduction to Egg Products, USDA Food Safety and Inspection Service, website: http://www.fsis.usda.gov/wps/wcm/connect/c5c85914-5055-4f09-8098-1a179a1c6e14/EPT_Introduction.pdf?MOD=AJPERES, accessed 12/15/2015.

INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

Whole Egg

- NIST 8445
- LGC SAL-RSM-5 (Check for characterization level)

8. Validation Guidance:

Method developers should provide data for method performance in all claimed matrixes (listed in table 2).

Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

Appendix M: Valaidation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_m.pdf

Field Code Changed

9. Maximum Time-To-Result: None

Table 1: Method performance requirements

Comment [D4]: May need to consider adding specificity

Parameter	Minimum Acceptance Criteria		
	Cookies, Bread, Dough, Salad Dressing	Wine	Matrix X
Analytical Range (ppm)	~~10-1000~~< 5 - > 10		
LOQ (ppm)	< 5		
MDL (ppm)	< 10		

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Recovery (%)	60-120%		
% RSD_r	≤20 %		
% RSD_R	≤ 30%		
Note: Allergen to be reported by dry weight.			

Table 2: Selected Finished Food Products and Ingredients ~~Matrixes~~ (revised per OMA Appendix M)

cookies
bread
dough
salad dressing
wine
chicken
ice cream
pasta

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Comment [D5]: Are these matrices okay for this analyte

Comment [D6]: Matrices listed in OMA Appendix M

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-08 03:23:07
First & Last Name	Yasutaka Nishiyama
Organization	NH Foods Ltd.
E-mail Address	ya.nishiyama@nipponham.co.jp
Date Submitted	12-08-2016
Question/Comment-1	Table 1 Recovery should be 50-150%. Recovery is defined as "The fraction or percentage of incurred analyte" in line 73. According to Appendeix M, "recoveries between 50 and 150% will be considered acceptable" for incurred samples.
Question/Comment-2	Table 1 Because LOD is usually lower than LOQ, minimum acceptance criteria should be "<5", rather than "<10". In parameter column, "MDL" should be "LOD".
Question/Comment-3	Table 2 (lines 131-133) These matirices are acceptable to be included in the table.

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-07 15:47:29
First & Last Name	Girdhari Sharma
Organization	US FDA
E-mail Address	Girdhari.Sharma@fda.hhs.gov
Date Submitted	12-07-2016
Question/Comment-1	Title: As mentioned in the Chat, protein may be more appropriate than allergens, OR quantifying the egg as a commodity. It may be easier to convert between total protein and total egg based on known protein content in egg. One of the problem with quantifying allergens is to separate non-allergens from total proteins since the reference material will most likely have total proteins. This makes it difficult to calculate recovery of allergens. If changes made, it would also be need to be in Applicability section and Table 1.
Question/Comment-2	Title: The title should be modified to reflect the analytical technique section. Also since ELISA is a binding-based assay, it may not be specified separately if using a broader definition such as binding-based methods. Is the SMPR meant for protein binding-based or covers other techniques such as PCR as well?
Question/Comment-3	Line 50, LOD: would this be 90 or 95% certainty? LOD calculation is presented in Appendix M. Can the false-positive at minimum concentration of analyte be distinguishable from true-positive expected at the LOD concentration? The false-positive would be due to matrix interference.
Question/Comment-4	line 101, Validation Guidance: As discussed in the meeting, there should be provision for additional matrices if desired.
Question/Comment-5	line 122 Table 1: What is Matrix X and why separated from other matrices? As pointed in the meeting, LOD is typically lower than LOQ. Appendix M suggests recoveries of 50-150% as acceptable for incurred samples. Is whole egg referred to as allergen here (Note)? If not, reporting allergen from the material used in incurred samples would be difficult and vary depending on the antibody specificity.

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission Date	2016-12-07 22:34:42
First & Last Name	Masahiro Shoji
Organization	Morinaga Institute of Biological Science
E-mail Address	masahiro.shoji@gmail.com
Date Submitted	12-08-2016
Question/Comment-1	AOAC food allergen activities have already lots of archives (wisdoms of ancestors) as Appendix D, F & M. We shall utilize them as the basis because these were the results of intensive discussion of the experts. What we concentrate to do is to unify them, harmonize with them and input the most recent information.
Question/Comment-2	In order to interpret the analysis result, it will be useful to know the target protein and antibody information. For instance, casein test and Beta-lacto albumin test is not necessarily identical in milk analysis.

AOAC ISPAM "Food Allergen" Working Group Questions/Comments Form

Submission ID	357553503668955068
Submission Date	2016-12-14 14:38:23
Submission IP	184.105.48.66
First & Last Name	Paul Wehling
Organization	General Mills, Inc.
E-mail Address	paul.wehling@genmills.com
Question/Comment-1	Per LOD/LOQ definitions in the SMPR - I note that Appendix M includes these definitions. We should harmonize the SMPR to Appendix M. LOD is defined as the lowest concentration or mass of analyte in a test sample that can be distinguished from a true blank sample at a specified probability level. LOQ is the lowest level of analyte in a test sample that can be reasonably quantified at a specified level of precision.
Question/Comment-2	Per discussion of including cross-reactivity in the SMPR. Appendix M does discuss this. Perhaps good language in the SMPR would be such like - "Cross-reactivity has been investigated as per Appendix M. Method developers should submit cross-reactivity data and include any notable observations." or something like that.
Question/Comment-3	The concept of trueness for an ELISA method is difficult to define, let alone experimentally estimate. We did discuss this on the last call. I think it is important for the developer to evaluate and describe the protein sequences that their antibodies (or other agents) bind to.
Question/Comment-4	In terms of measuring trueness, there should be some attempt made to evaluate this.
Question/Comment-5	For LOD/LOQ, LOD criteria should be lower than the LOQ criterion. I'd expect the procedures described in Appx M to be used.
Question/Comment-6	Criterion for recovery should be symmetrical about 100%, e.g., 60-140%