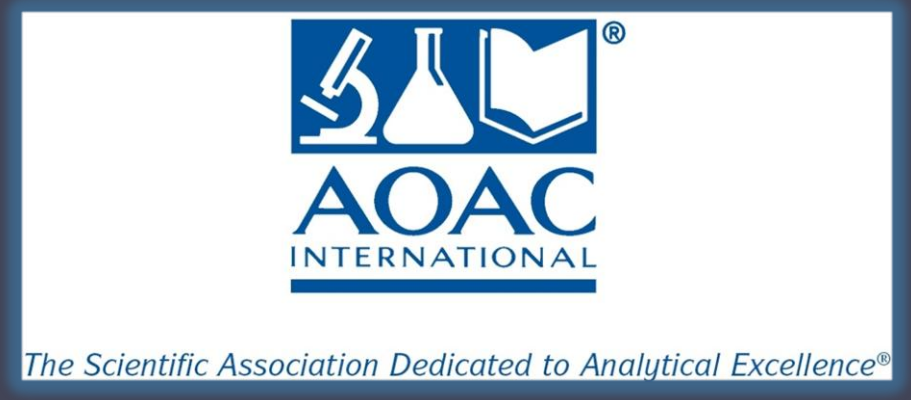

Expert Review Panel for SPSFAM Select Food Allergen Methods

(SPSFAM)

MONDAY, SEPTEMBER 19, 2016, 1:30 p.m.
Room: State 1

DALLAS SHERATON HOTEL
400 NORTH OLIVE STREET
DALLAS, TEXAS, UNITED STATES

contact: spsfam@aoac.org

The Scientific Association Dedicated to Analytical Excellence®

AOAC Stakeholder Panel on Strategic Food Analytical Methods

Food Allergens Expert Review Panel

Monday, September 19, 2016, 1 :30 p.m. – 3 :30 p.m.

Sheraton Dallas Hotel, Room State 1

A G E N D A

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1. Welcome and Introductions
   John Szpylka, Mérieux NutriSciences (ERP Chair)

2. Review of AOAC Volunteer Policies & ERP Proccess Overview and Guidelines
   Deborah McKenzie, AOAC INTERNATIONAL

3. Review of Methods
   For each method, the assigned ERP members will present a review of the revised method manuscripts, after which the ERP will discuss the method and render a decision on the status for each method.

   A. ALL-01
      a. Linda Monaci Review
      b. Sneh Bhandari Review
      c. Other Submitted Reviews
      d. Discussion and Vote

   B. ALL-02
      a. Melanie Downs Review
      b. Tomasz Tuzimski Review
      c. Other Submitted Reviews
      d. Discussion and Vote

4. Final Action Requirements for Approved Method(s)

5. Adjourn

The Scientific Association Dedicated to Analytical Excellence®

AOAC Stakeholder Panel on Strategic Food Analytical Methods:

Expert Review Panel

AOAC Candidate Method #ALL-01

Detection and Quantitation of Selected Food Allergens using LCMS/MS

- Author(s): Lee Sun New, Hua-Fen Liu, Andre Schreiber, Vincent Paez
- Submitted by: Andre Schreiber, SCIEX
- Enclosures: 0
- Submitter notes: None

Primary Reviewer: Linda Monaci

Secondary Reviewer: Sneh Bhandari

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-16 10:12:14
Name	Linda Monaci
E-mail	linda.monaci@ispa.cnr.it
Organization	CNR
Title of Method	Detection and Quantitation of Selected Food Allergens by LCMS/MS
AOAC Candidate Method Number (e.g. ALN-01)	ALL-01
Applicable SMPR	no
Summary:	The method proposed is an LC-MS/MS based method for the detection of egg, milk, peanut and hazelnut allergens in different food commodities. This method is based on a preliminary defatting step followed by protein extraction with an extraction buffer and a denaturant solution, followed by enzymatic digestion of the protein mixture with trypsin. The resulting peptide mixture is partly purified on 10 KDa cut-off filters and successively the filtrate analysed by HPLC MS/MS. The instrument in use is a triple quadrupole MS, operated in MRM mode; a minimum of two peptides for each targeted protein and two transitions for each peptide are monitored.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	The method partly meet the applicability criteria reported in the SMPR paper. Despite the different food matrices successfully analysed it is not specified which form o allergenic material is used in the given tables. According to what reported is never specified if hazelnuts or peanuts employed in the study are raw or roasted. This can significantly change the peptide pattern generated so an in depth investigation should be carried out to seek for common peptides, in that case. According to the requirements for standard method performance the form of the matrix under investigation should be detailed. Another crucial aspect that does not meet the SMPR is the choice of the target allergen that is a basic ste in method development. From what reported it appears that the allergen spiked into the food matrices are the single standard proteins (egg elbumin and the single caseins) as stated in the protocol described. If this is confirmed I do deem that all the parameters calculated are nor consistent with what required from the protocol since the main target differ significantly in protein composition.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes in part. It is not specified which kind of experiment were carried out for recovery calculation; it is not clear at how the spike was realized throughout the procedure and if it was done before the defatting step or not. This might tremendously change the final result of the analysis. How was the LOD calculated? Which was the time window of the noise selected? This should be better detailed. Also the LOD reached and real chromatogram of the LOD reached should also be provided for the different matrices investigated. According to the figures provided it would be hard to really reach in a few cases so challenging LODs by peering at the intensity of the noise along the chromatographic traces.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Again, unless there are missing information in the document, the allergenic material used for the calibration curves appears different form what recommended in the SMPR paper. Calibration curve should be obtained aganist the whole food as reported in the guidelines namely milk, egg powder... This change in allergen material will influence final sensitivity and LODs of the method. In a different case, the correct information about the proper material used for obtaining the calibration curve should be reported.

4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	The information about safety precautions redirect the operator to the consultation of MSDs for each single reagent used. No other information are provided about this aspect along the protocol.
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	No. In case of the starting material used for spikinf the food matrices (not specified the form and not taken into consideration the whole allergenic ingredient), the calculation of the recovery.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	No the RM for allergens were not taken into consideration in this study. The use of CRM or incurred materials are strongly recommended in order to check method performance: e.g. recovery, etc.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	The results provided in the table are not fully supported by the work performed, according to what shown and reported in the protocol. The experimental work carried out has not been properly detailed and some information are missing.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	Yes especially when manipulating strong acids and MMTS (corrosive to tissues)

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	It is not well detailed.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	The method is clearly written but there are several information missing. The proper allergen material (whole allergenic food and roasted peanuts and hazelnuts) to be used Recovery studies Use of QC points Describe how LOD was calculated and provide a criteria for establishment of MDL (use of a specific quantifier peptide? The total analysis time is now specified
5. Based on the supporting information, what are the pros/strengths of the method?	Strengths: The method could be able to monitor traces of all nuts in different food commodities also including soy that might be an added value to method extension.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Weaknesses: The LODs and LOQs reflect a different type of spike reralized in the food therefore the LODs achieved are not in agreement with the perormance requirements detailed in the SMPR document. The calculation of the recovery is also not in line with what expected. The protocol is not properly described and experimental steps not well detailed. It is not clear which was the most sensitive peptide found.
7. Any general comments about the method?	There are several parts that should be improved and some part of the work carried out.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	No; there several experimental details missing and points along the method that must be improved to meet requirements of SMPR.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-13 20:26:54
Name	SNEH BHANDARI
E-mail	sneh.bhandari@mxns.com
Organization	Silliker Laboratories
Title of Method	Detection and Quantitation of Selected Food Allergens using LCMS/MS
AOAC Candidate Method Number (e.g. ALN-01)	ALL-01
Applicable SMPR	AOAC SMPR 2016.002

I. Summary

Summary:

Food samples are defatted and extracted for proteins. Proteins are digested into peptides. The peptides are separated by LC and detected by MS/MS using multiple, characteristic MRM transitions. The method uses selective Multiple Reaction Monitoring (MRM) of characteristic transitions of precursor ions to fragment ions of multiple proteins and peptides to uniquely identify each allergen.

Scope:

LC-MS/MS based method for the detection of egg, milk, peanut, and hazelnut food allergens in finished food products and ingredients

II. Review of Method Only

1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes

III. Review of Information in Support of the Method

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	The supporting information provided does not have any data on evaluation of the method accuracy employing Reference materials. The evaluation of the method performance by analysis of the available CRMs like those mentioned in the SMPR 2016.002 and others like IFAAN reference std. for peanut, and from Food Allergen Lab for egg, hazelnut and milk may help establishing the method accuracy.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Results for the method performance evaluation regarding the analysis of egg, milk, peanut and hazelnut in selected matrices are listed in Tables 14 to 17. Recoveries and RSDr are given for the MQL at 5 and 10 ppm, respectively. RSDr was estimated by analyzing batches in triplicates. Few allergen-matrices combinations were only analyzed in duplicates RSDr not given for these. The analytical range of the method in various matrices listed but no data to support those ranges. The available information about the method performance with respect to analytical range, MQL, MDL, Recovery (%) and RSDr are with in the SMPR specifications. The information regarding the following questions about the method performance is not available in the report provided. 1. Data indicating response of the specific peptides is linear to their concentration particularly in the targeted matrices. 2. Spike recovery at multiple level of spiking in different matrices. The data available are at a single spike level. 3. No data provided to support the mentioned analytical range of the method for different allergens. 4. The author's may clarify whether the method repeatability data are based on single days analysis or multiple days. 5.The authors may clarify whether the the method MDL and MQL calculated based on the quantifier ion of the most sensitive transition of the most sensitive peptide. How are the values at this level confirmed in the absence of consistent response from other transitions.

IV. General Submission Package

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	The SMPR mentions check standards at the lowest point and midrange point of the analytical range for system suitability. No calibration data given in the provided information.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	The separate information about the system suitability evaluation not provided. Blank samples are assumed to have been investigated to check MDL. No data about the method calibration and its reproducibility provided.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	The method as written is not very clear in its procedure of the method calibration at multiple concentration for targeted allergens and also in the procedure of the method suitability evaluation.
5. Based on the supporting information, what are the pros/strengths of the method?	The method has been applied on a variety of matrices and recovery and % RSDr are within SMPR's requirements.
6. Based on the supporting information, what are the cons/weaknesses of the method?	1. The supporting information provided does not have any data on evaluation of the method accuracy employing Reference materials. The evaluation of the method performance by analysis of the available CRMs like those mentioned in the SMPR 2016.002 and others like IFAAN reference std. for peanut, and from Food Allergen Lab for egg, hazelnut and milk may help establishing the method accuracy. 2. The information regarding the following questions about the method performance is not available in the report provided. i). Data indicating response of the specific peptides is linear to their concentration particularly in the targeted matrices. ii). Spike recovery at multiple level of spiking in different matrices. The data available are at a single spike level. iii). No data provided to support the mentioned analytical range of the method for different allergens. iv). The author's may clarify whether the method repeatability data are based on single days analysis or multiple days. v).The authors may clarify whether the the method MDL and MQL calculated based on the quantifier ion of the most sensitive transition of the most sensitive peptide. How are the values at this level confirmed in the absence of consistent response from other transitions. vi). Matrix composition may affect the tryptic digestion of the sample and ionization of the peptide fragments. How is the the matrix effect on digestion on MS quantitation normalized without the internal std. if linearity of the transition response against concentration not established.
7. Any general comments about the method?	It's very promising method but some additional information may be required.

Recommendation for the Method

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.

The method is a very promising candidate for First Action but answers to some of the questions mentioned in earlier section may help in this process.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-16 11:49:10
Name	Melanie Downs
E-mail	mdowns2@unl.edu
Organization	University of Nebraska-Lincoln
Title of Method	Detection and Quantification of Selected Food Allergens using LC-MS/MS
AOAC Candidate Method Number (e.g. ALN-01)	ALL-01
Applicable SMPR	2016.002
Summary:	The authors have submitted a method which they claim detects and quantifies egg, milk, peanut, and hazelnut in various food matrices, in accordance with AOAC SMPR 2016.002. However, the authors have left out significant information about how the method performance was evaluated. It is also difficult to tell from the presentation of the method whether the authors have an understanding of the core concepts of food allergen detection.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	It is unclear throughout the method whether the terms and units used by the authors are the same as those outlined in the SMPR. In particular, the definition of the food allergens in the SMPR does not seem to be consistently applied and/or the terms are not described at all in the method. For example, the SMPR gives a definition for whole egg, but the only material described in the method for egg, is purified egg albumin (ovalbumin), and when the term “egg” is used throughout the method, it is unclear exactly what the authors are referring to.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	No. See previous comments regarding unclear use of definitions and reporting units. Absolutely clear and correct units must be used in order to determine whether the method meets any of the performance requirements.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	The authors present almost no actual data to support the claims they make with respect to the method performance. The authors fail to even present basic information about how the method performance studies were conducted. They give no information about how the food matrices used for performance evaluation were formulated or prepared, no information about what allergenic food materials were used in those matrices, and no information about how the spiked (or incurred) foods were prepared. The authors also present no information regarding how they arrived at their quantitative values from the data that would have been generated from the method. Given this tremendous lack of information about how the studies were conducted, the tables provided by the authors as attempts to verify the performance characteristics of the method are inadequate.In addition, as was noted in previous answers, the lack of clarity on reporting units makes it difficult, if not impossible, for this reviewer to evaluate the supposed method performance.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	No. See previous answer regarding the ability to evaluate the reported method performance values, given the lack of methodological information and actual data.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	The descriptions of system suitability tests are inadequate. From what little can be interpreted, the methods may have major issues with correct definitions and measurement units to align with the SMPR.

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	No. There is almost no data to assess whether system suitability tests and controls worked in accordance with the SMPR. Also, see previous answer regarding lack of information about how the system suitability tests were performed.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	While the initial part of the method is written clearly and concisely, once it comes to the critical point of data analysis, the method provides no information. The authors must provide information about how they conducted the quantification. Did they use external calibration curves of the food allergen? If so, how were these prepared? Did they use all transition peak areas for calculating the quantification data? If so, how was this done? If not, how was the quantification data calculated?
5. Based on the supporting information, what are the pros/strengths of the method?	The sample preparation descriptions for the method are by and large written in a clear, stepwise format that could be followed by an end user.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The supporting information is inadequate in its description of how the method performance was evaluated. What food allergen materials were used for spiking? What was the formulation/composition/preparation of the food matrices? How were the spiking procedures performed? The authors also provide some protein conversion factors but give no indication of when and how those were used by the authors or how and when they would be implemented by an end user.
7. Any general comments about the method?	The presentation of the method and supporting information are insufficient to adequately evaluate the performance of the method. In addition, the decision of the authors to not include the peptide sequences in the method makes it difficult for reviewers, as well as end users, to determine whether there is any potential for lack of specificity when analyzing specific foods for the presence of the target peptides. The target peptide sequences used are critical pieces of information for the user and should not be considered proprietary information. Overall, this method requires significant revision and additional information before its suitability can be determined.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	No. As indicated throughout this review, the presentation of information about the method and the performance evaluation is lacking in key information needed in order to determine whether the method performs adequately.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-13 16:42:01
Name	Stefan Ehling
E-mail	stefan.ehling@abbott.com
Organization	Abbott
Title of Method	Detection and Quantitation of Selected Food Allergens using LCMS/ MS
AOAC Candidate Method Number (e.g. ALN-01)	ALL-01
Applicable SMPR	2016.002

I. Summary of Method

Summary:	The method describes the analysis of four allergens (milk, egg, hazelnut, and peanut) in a variety of matrices. Matrix-matched calibration is performed with isolated proteins, which are then converted to whole allergenic food.

II. Review of Method Only

1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	yes; applicability to chocolate matrix is questionable
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	yes

II. Review of Information in Support of the Method

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	There is no data on the analysis of reference materials. The method performance was evaluated with isolated proteins, which doesn't demonstrate extractability, digestibility, and processing stability of allergens in various matrices. The method doesn't describe what hazelnut and peanut reference materials were used and how they were used, even though method performance data is presented for hazelnut and peanut. There is no data on linearity. Recovery data is presented only at the LOQ.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes. The method doesn't perform adequately in chocolate matrix.

IV. General Submission Package

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Table 9 – content doesn’t appear to make any sense. Spiking volumes are too high and target concentrations are not consistent with spiking volumes. Information is missing on the analysis of hazenut and peanut allergens.

5. Based on the supporting information, what are the pros/strengths of the method?	Calibration and spiking are relatively easy because isolated proteins are used.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Stability/extractability/digestibility of allergenic protein in matrix should be demonstrated through the analysis of CRMs or incurred samples. Only peptides which are heat-stable (stable to processing) should be considered. Information is missing on the analysis of hazenut and peanut allergens. Needs conversion factor of isolated protein (not total protein) to allergenic food.
7. Any general comments about the method?	• Stability/extractability/digestibility of allergenic protein in matrix should be demonstrated through the analysis of CRMs or incurred samples. Spiking isolated protein into matrix is not enough. • Use of TCEP/MMTS reagents is deviation from all previously published works. It should be justified or otherwise commercial interest could be suspected. • 1.5 mL microcentrifuge tubes are not needed. • Would a horizontal shaker be acceptable? • Explain functional roles of urea, OGS (both denaturants) and CaCl2 (activation of trypsin?). • No information is offered on peanut and hazelnut proteins used for calibration. • VIII) System suitability test spike mixture is prepared using a 1:8 dilution of the stock solutions instead of the stated 1:10 dilution. • Table 9 – content doesn't appear to make any sense. Spiking volumes are too high and target concentrations are not consistent with spiking volumes. • Is cryogrinding necessary? • Give temperatures used during centrifugation steps. • Give an estimate of total sample preparation time. • Amount of trypsin is not adjusted to protein content, which might be acceptable. • Gradient program of 20 min is too long for a 100 mm column. • How were peptides selected and how were MRMs determined? How were MS parameters optimized? • Give peptide sequences. • Indicate which is milk protein 1, milk protein 2, etc. • For egg, only one protein is used, which could miss egg yolk or egg white. • Does MDL allow for confirmation? Is it determined based on the least intense MRM or the most intense MRM? • Tables 14 and 15 – recoveries are good in red wine and chocolate, even though stated otherwise. • How was individual protein converted to whole allergenic food? • Data on linearity is lacking. • Recovery information at higher spiking levels would be helpful.

Recommendation for the Method

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	The method shows potential but quite a few issues need to be addressed in order to be recommended as First Action method.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-15 17:31:36
Name	John Lawry
E-mail	john.lawry@covance.com
Organization	Covance
Title of Method	Detection and Quantitation of Selected food Allergens using LC-MS/MS
AOAC Candidate Method Number (e.g. ALN-01)	ALL-01
Applicable SMPR	AOAC SMPR 2016.002
I. Summary:	Multiplexed LC/MS/MS for food allergens
II. Review of Method Only	
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	yes
III. Review of Information in Support of the Method	
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	yes

IV. General Submission Package

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	yes

5. Based on the supporting information, what are the pros/strengths of the method?	tryptic peptides only; simpler sample prep in general meeting SMPR objectives.
6. Based on the supporting information, what are the cons/weaknesses of the method?	no guidance on ensuring a proper protease digestion occurred prior to LC/MS/MS analysis
7. Any general comments about the method?	submitting separate one page document that summarizes two methods together to suggest where each method could help inform the other.

Recommendation for the Method

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	YES, The conditions as stated in SMPR 2016.002 were analytically met.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-12 02:33:45
Name	Susanne Siebeneicher
E-mail	s.siebeneicher@r-biopharm.de
Organization	R-Biopharm AG
Title of Method	Detection and Quantitation of selected food allergens using LCMS/MS
AOAC Candidate Method Number (e.g. ALN-01)	#ALL-01
Applicable SMPR	2016.002

I. Summary of Method

Summary:	This method looks like a pre-trial for the detection of food allergens in food, it is not according to appendix 3. Furthermore, results are not traceable. Method with standard addition is a ver good approach. Also raw data is missing.

II. Review of Method Only

1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes it does, however, only spiked matrices were analysed. Also real processed food should be used. Please delete the further parameters in the method.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Sequences of the allergens are not defined, please specify them. How did they calculate the MDL and MQL, furthermore, why is the MQL the same in every matrix. In case of ELISA, every matrix has its own LOQ due to matrix effects. MS shows also matrix effects, please indicate more in detail. How did they calculate the recovery? With standard addition that seems to be impossible. RSDr was calculated wrong, please calculate according to Appanedix 3F table A3. Please do not use alpha-Casein, since it is not completely soluble in water. Thes use of it may cause wrong results.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes

III. Review of Information in Support of Method

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Calculation of all definitions are not traceable. Need to be recalculated and explained with raw data.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	No reference material was used.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes it does, however, the results are not clear and inconsitent. Table 15 shows recoveries of different matrices. However, it is explained that chocolate has a low recovery, but infant formula is much lower, while it is not mentioned? Furthermore, it is explained that the sensitivity is poor, but why is the MQL the same? Same counts for white wine in table 14.

IV. General Submission Package

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	No blank samples are completely missing.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	As explained before, results are very inconsistent.

4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	No, several points are missing, sequences are not clear.
5. Based on the supporting information, what are the pros/strengths of the method?	Standard addition as a method is a very good approach.
6. Based on the supporting information, what are the cons/weaknesses of the method?	It is not mentioned how often experiments are repeated. How was the analytical range tested or was it assumed? How did they looked for linearity, it is not mentioned in the method? The material needs to be analysed before, because with standard addition, you need to know the amount of allergen in your blank matrix. Spiking solution must be the same protein as in the analyte, because standard addition is used. If there is another protein in an unknown matrix, the the result may be wrong. How would they like to analyse the robustness, it is not mentioned in the method. Only one single buffer was used for the extraction of all analysed proteins, before the use of that buffer, was there an evaluation of different buffers? Many experts could show that there is a different extraction efficiency of different buffers, how do the authors know, if their buffer has the same extraction capacity for all analysed allergens?
7. Any general comments about the method?	There is no indication on the pH of the buffers, please add the information. Lipid removal in the method may lead to the removal of Oleosines, allergens in the lipid phase. For peanut they are known as Ara h 10, 11, 14 and 15. Investigations on hazelnut are ongoing. These Oleosines are potent allergens. The sample is diluted very high, is the sensitivity of the method adequate for taht dilution? Or do the authors need to dilute due to the buffer components? It is mentioned that the parameters GS1 and GS2 are not validated, please specify.

Recommendation for the Method

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	There is no recommendation as a first action method, because many data is missing and the method looks like a pre-version, not a final version. A lot of work needs to be done to adopt this method as a first action.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-16 10:45:50
Name	TOMASZ TUZIMSKI
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Organization	MEDICAL UNIVERSITY IN LUBLIN
Title of Method	Detection and Quantitation of Selected Food Allergens using LCMS/MS
AOAC Candidate Method Number (e.g. ALN-01)	ALL-01
Applicable SMPR	AOAC SMPR 2016.002
Summary:	The proposed method entitled 'Detection and Quantitation of Selected Food Allergens using LCMS/MS' described by Lee Sun New, Hua-Fen Liu, Andre Schreiber, Vincent Paez is applicable for the detection and quantitation of egg, milk, peanut, and hazelnut food allergens in finished food products and ingredients by LC-MS/MS. The method uses triple quadrupole mass spectrometry and selective Multiple Reaction Monitoring (MRM) of characteristic transitions of precursor ions to fragment ions of multiple proteins and peptides to uniquely identify each allergen. Characteristic signature peptides were chosen for each alergen, and MRM transitions for each signature peptide were determined based on their uniqueness compared to background proteins and their sensitivity of detection. For each allergen multiple unique peptides were chosen from unique proteins, and two MRM transitions per peptide were chosen. The method performance requirements were met for the detection of egg, milk, peanut and hazelnut in a number of food matrices. Food samples were defatted and extracted for proteins. Proteins were digested into peptides and peptides were separated by LC and detected by MS/MS using multiple, characteristic MRM transitions on a SCIEX QTRAP® 4500 LC-MS/MS system. The target analytical range of 5-1000 and 10-1000, respectively, recoveries between 60 and 120% and RSDr % of less than 20% were achieved for all allergens in selected matrices. RSDR % of less than 30% were achieved for all allergens in bread dough and bread matrix (three labs with different instruments and operators). The developed method was evaluated following the definitions of AOAC SMPR 2016.002 with respect to Method quantitation limit (MQL), Method detection limit (MDL), Linearity, Repeatability, Reproducibility, Recovery. Analytical data was collected for allergen/matrix combinations listed in AOAC SMPR 2016.002. In Tables 14 to 17 the authors listed the results for egg, milk, peanut and hazelnut. Recoveries, RSDr and RSDR are given for the MQL at 5 and 10 ppm, respectively. RSDr was estimated by analyzing batches in triplicates. Few allergen-matrices combinations were only analyzed in duplicates RSDr not be given for these. The authors are convinced that the method can be easily extended to the detection of other allergens, including soy and other tree nuts (almonds, Brazil nut, cashew, pine nut, pistachio, pecan, and walnut). The authors also declared that an application of this method to matrices not covered by the scope will require additional validation.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	YES: The applicability of the method is adequate to the applicability of the SMPR.

2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	YES: The analytical techniques in the method are adequate and meet the SMPR.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	YES: Definitions, which are specified in the SMPR, were listed in the description, also were applied appropriately in the method.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes: The method contains all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous.
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	YES: The definitions specified in the SMPR were used and applied appropriately in the supporting documentation (manuscripts, method studies, etc.).
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	YES: There are information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Material stated in the SMPR.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	YES: There are information demonstrating that the method performs within the SMPR Method Performance Requirements table specifications for all analytes in the SPMR applicability statement.

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	In my opinion there is no need.
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	YES: There are.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	YES: There are information demonstrating that the method system suitability tests and control as specified in the SMPR worked appropriately and expected.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	The method is well described and substantively prepared. The project of the method is well integrated and includes a clear and concise description.
5. Based on the supporting information, what are the pros/strengths of the method?	The developed method was evaluated following the definitions of AOAC SMPR 2016.002 with respect to Method quantitation limit (MQL), Method detection limit (MDL), Linearity, Repeatability, Reproducibility, Recovery. Analytical data was collected for allergen/matrix combinations listed in AOAC SMPR 2016.002. Specificity is another important analytical parameter. Characteristic signature peptides were chosen for each allergen, and MRM transitions for each signature peptide were determined based on their uniqueness compared to background proteins and their sensitivity of detection. For each allergen, multiple unique peptides were chosen out of unique proteins, and two MRM transitions per peptide were chosen.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The cons/weakness of the method may be costs. But I think it is inevitable.
7. Any general comments about the method?	My remarks to the AOAC Candidate Method #ALL-01 are as follows: Page 13, LC-MS Separation: The authors should answer the following question: How long stationary phase was conditioned by mobile phase? Moreover, they should add details about the condition of the stationary phase: - before the analysis, - between each of the analyses in gradient elution mode (if the additional conditioning was applied for balancing of chromatographic system, despite the fact that concentrations of both components of mobile phase on the start and the end of gradient are the same). I have no additional comments.

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	In my opinion, the AOAC Candidate Method #ALL-01 entitled ‘Detection and Quantitation of Selected Food Allergens using LCMS/MS’ described by Lee Sun New, Hua-Fen Liu, Andre Schreiber, Vincent Paez is applicable for the detection and quantitation of egg, milk, peanut, and hazelnut food allergens in finished food products and ingredients by LC-MS/MS. The AOAC Candidate Method #ALL-01 should be adopted in its present form as a First Action and recommended for publication in the Official Methods of Analysis of AOAC INTERNATIONAL.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-14 05:51:16
Name	sudhakar yadlapalli
E-mail	sudhakar@firstsourcels.com
Organization	First source labotatory solutions LLP
Title of Method	Detetcion and quantitation of Selected Food Allergens using LC-MS/,MS
AOAC Candidate Method Number (e.g. ALN-01)	ALL-01
Applicable SMPR	AOAC SMPR 2016.002

I. Summary of Method

Summary:	Food samples are defatted and extracted for proteins .Proteins are digested into peptides and peptides are separated by LC and quantified by using LC-MS/MS with MRM conditions.

II. Review of Method Only

1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Method met the SMPR except %RSD of Egg allergen in Dough, Milk allergen in Chocolate ,Peanut allergen in Cereal and Chocolate and hazelnut allergen in Cereal and Chocolate.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	No, As per method investigator, poor sensitivity and low recovery in chocolate and cereal noticed to reach the target MQL (as per SMPR).
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes

II. Review of Information in Support of Method

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Yes, however Standard addition method used in this study may impact the performance of the method.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	No, more supportive data require to comment.

IV. General Submission Package

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	yes, As the method involved multiple steps with time bound reactions more precautions are needed.
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	NO, .more information on peptides used in the method quantitation is require to asses the system suitability.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	NO, no information available in the method about Controls.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	No , more information is require about MRM transitions about ionization state and recovery studies.

5. Based on the supporting information, what are the pros/strengths of the method?	Strengths: Most confirmative method as it involves LCMSMS and each peptide with two transitions.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Limitations: 1. Less number of peptides used for confirming targeted allergen by using LCMSMS 2. MRM Transitions are not clear, may need to specify the ionization states of the transition ions. 3. The response of the MQL for some peptides are very low(for ex . walnut _peptide A1 and pecan _ peptide A2) 4. The method involves multi steps and time bounded reaction. 5. More clarity require on recovery and linearity experiments.
7. Any general comments about the method?	Suitability of the method need to be thoroughly checked against SMPR.

Recommendation for Method

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	NO

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-13 15:43:05
Name	Jerry Zweigenbaum
E-mail	j_zweigenbaum@agilent.com
Organization	Agilent
Title of Method	Detection and Quantitation of Selected Food Allergens using LCMS/MS
AOAC Candidate Method Number (e.g. ALN-01)	ALL-01
Applicable SMPR	2016.002

I. Summary of Method

Summary:	This method describes the enzymatic digestion and detection of "signature" peptides by LC/MS/MS indicating the presence of the food allergens milk, egg, hazelnut and peanut in cookies, bread, dough, salad dressing, white wine, infant formula, ice cream, cereal, and chocolate.

II. Review of Method Only

1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	It is not clear in the method how the allergens are being determined. "Signature" peptides are being measured, but there is no indication of quantitative response and calibrations curves. The SMPR specifies measurement of the whole allergenic food. For MDL, MQL and recoveries this method specifies taking the "allergenic mixture and combining it with the test food sample as shown in Table 9." However, there is no description of the allergenic mixture.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	There is a lack a repeatability data as some are based on three replicates and others only two (not statistically valid). It is recommended that MDL and MQL be based on 7 replicates.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	It is vague, refers the MSDS of all reagents. There should be some indication of particular hazards whereas details can be found in the MSDS of each reagent.

III. Review of Information in Support of the Method

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	There are no supporting documents.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	There are summary information but no real data. Because the method measures peptides from digested proteins, the lack of detail makes it difficult to determine whether the method meets the performance criteria. The summary information indications it does. However, it does not appear any reference materials were used.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes.

IV. General Submission Package

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	There is little supporting information
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes, but the system suitability is based on proteins from only two of the four allergenic targets (milk and egg). In addition, no information is given on the peptides measured and therefore it is not possible to evaluate the effectiveness of those peptides for suitability and uniqueness.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	There is no information on how to use the system suitability preparation except to process it along with samples. There is no data showing that the system suitability worked well or criteria to evaluate how well it works.

4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	There needs to be more detail about how to prepare calibration standards. There needs to be more data to show the performance of the method.
5. Based on the supporting information, what are the pros/strengths of the method?	It is difficult to evaluate based on the lack of supporting information.
6. Based on the supporting information, what are the cons/weaknesses of the method?	It is difficult to evaluate based on the lack of supporting information.
7. Any general comments about the method?	The method is lacking detail. Is recovery based on the measurement of one peptide or an average of all that are listed? Why are there multiple proteins selected for milk, peanut and hazelnut, but only one for egg? What is the source of the peanut and hazelnut standards?

Recommendation for the Method

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Not in its present state. There needs to be more detail on how the method is to be executed, what is being measured, and its performance characteristics.

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Expert Review Panel

AOAC Candidate Method #ALL-02

Multiplexed LC-MS Method for the Detection and Quantitation of Selected Food Allergens (milk, hazelnut, peanut and whole egg)

- Author(s): Jennifer Sealey Voyksner, Jerry Zweigenbaum and Robert Voyksner
- Submitted by: Jennifer Sealey Voyksner, ImmunogenX
- Enclosures: 2 (Cover letter and data)
- Submitter notes: None

Primary Reviewer: Melanie Downs

Secondary Reviewer: Tomasz Tuzimski

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-16 11:57:06
Name	Melanie Downs
E-mail	mdowns2@unl.edu
Organization	University of Nebraska-Lincoln
Title of Method	Multiplexed LC-MS Method for the Detection and Quantitation of Selected Food Allergens (milk, hazelnut, peanut and whole egg)
AOAC Candidate Method Number (e.g. ALN-01)	ALL-02
Applicable SMPR	2016.002
Summary:	The authors of this submission present a method for the LC-MS/MS detection and quantification of peptides derived from the pepsin-trypsin/chymotrypsin digestion of food allergens (egg, milk, hazelnut, and peanut) present in the matrices specified in AOAC SMPR 2016.002.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	No. The authors are unacceptably inconsistent and incomplete with their use of units throughout the method submission. The use of units for concentrations of food allergens must be absolutely clear and complete. See comments regarding the supporting information for further description of issues with units.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Based on the presentation of information in the submission, it is tremendously unclear whether the authors are applying the definitions of “food allergens” from the SMPR appropriately throughout the document. All indications of concentrations of food allergens must have absolutely clear units applied. For example, several different milk-based ingredients are used in the method and supporting data. The use of “50 ppm milk” is insufficient and should be revised to indicate precisely what the authors mean. Does it mean 50 ppm fluid whole milk in a specific matrix/solution or 50 ppm lyophilized whole milk in a specific matrix/solution or 50 ppm nonfat dry milk in a specific matrix? The absence of clarity on these definitions of units makes it impossible to determine whether the method meets the requirements from the SMPR based on the data presented. The differences between fluid whole milk (the SMPR definition of “milk”), lyophilized whole milk, and nonfat dry milk make a highly significant impact on determining limits of detection and quantification.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	The authors used alternative sources of reference materials to evaluate the method performance, as there are few certified reference materials for food allergens. Instead of the actual materials being the primary issue, this section is yet again woefully lacking in concise, accurate information about the correct units for these reference materials and what units the authors are implementing for their own results. For example, the authors indicate that they utilized iFAAM peanut reference materials that contained 40 ppm peanut, but this is incorrect. The iFAAM reference material contains 40 ppm peanut protein in reconstituted dessert matrix. The descriptions of the other reference materials are similarly unclear or incorrect. Throughout this section of data, the authors then apply mystery correction factors, which are not sufficiently justified or explained, particularly given the misstatement of concentrations for some of the reference materials. The authors indicate that some of these correction factors are calculated based on Bradford assay protein determinations, but no information is given regarding how or when said protein determinations were performed. Also, the applicability of soluble protein concentrations as opposed to total protein concentrations (i.e. as determined by Kjeldahl or Duma methods) for these materials must be clearly justified.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	As noted above, the unclear presentation of information and particularly measurement units in the submission makes this reviewer unable to determine whether the method meets any of the SMPR specifications.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No.
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	The authors did not specifically indicate system suitability tests were performed.

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	See previous answer indicating no specific system suitability tests apparent.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	The method was submitted as two separate documents, which makes it difficult for a reader to understand the final method workflow proposed by the authors. In addition, the method is not presented in a way that would be easy for an end user to read and follow. The authors should submit a more precise, stepwise explanation of the method from start to finish (e.g. including all final parameters for extraction, digestion, clean-up/SPE, LC-MS/MS, and data analysis. The method does not provide sufficient information as to how the authors conducted the data analysis or how an end user would conduct the data analysis. For example, which peptides and transitions were used for quantification? How should an end use choose those? In the transition list table, the authors indicate at least two peptides that would be used for quantification, but the supporting data only shows results from one peptide from each allergenic food. How was that selection made, and how would end users make that selection? Also, one would assume that the authors are calculating the peak areas for the monitored transitions, but no information is given about the parameters the authors used or what end users should do to derive the quantitative information. Did the authors require a minimum number of points across the peak, for example? The method also needs to have substantially more information about how calibration curves were prepared and how end users would prepare calibration curves for the method. What exact material (e.g. lyophilized whole milk or fluid whole milk) was used for the calibration curves and what were the curves prepared in (in water or in some sort of buffer or in matrix-matched extracts)? Was each concentration extracted and prepared separately or were dilutions performed following digestion and SPE cleanup? How do the authors plan to address the presence of target peptides in species other than the target species (hen's egg peptides in turkey; hazelnut peptide h4 in peach; and Bos Taurus peptides in water buffalo and wild yak?
5. Based on the supporting information, what are the pros/strengths of the method?	It is possible that this submission could be the beginning of a sound method, but the lack of clarity on several key issues noted in this review, makes it difficult to actually assess the method.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The final procedures for the method are unclear, in part due to two separate documents being submitted at different times and, in part, due to a general lack of precision and clarity in the writing and presentation of information. While there are a number of items that need to be clarified, the largest overarching issue with the presentation of the method is the lack of complete, accurate, and clear quantitative units and descriptions of materials used throughout presentation.
7. Any general comments about the method?	Clear and accurate units must be provided not only for reviewers to evaluate the effectiveness of the method, but also so that the method delivers correct and usable data to end users. This method requires significant revision on the issue of units as well as general clarity before it should be considered again.

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	No. Due to the numerous unclear, inconsistent, and incorrect pieces of information in this method submission, the method should not be adopted as First Action. The method requires significant revision before the performance can even be adequately evaluated by reviewers. As the method submission currently stands, it cannot be determined whether the method meets any of the requirements from the corresponding SMPR.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-16 10:54:39
Name	TOMASZ TUZIMSKI
E-mail	tomasz.tuzimski@umlub.pl
Organization	MEDICAL UNIVERSITY IN LUBLIN
Title of Method	Multiplexed LC-MS Method for the Detection and Quantitation of Selected Food Allergens (milk, hazelnut, peanut and whole egg)
AOAC Candidate Method Number (e.g. ALN-01)	ALL-02
Applicable SMPR	AOAC SMPR 2016.002

Summary:

The methodology proposed by the authors provides us with the much-needed means to successfully characterise food products, in order to minimise hidden allergenic reactions in people and to ensure accurate food labelling.
The method described is applicable for the detection and quantitation of milk, hazelnut, peanut and whole egg by performing an extraction and enzymatic digestion of proteins identified as allergenic and then the LC-MS/MS analysis of peptide markers specific and unique to those proteins. The method is used for the detection of eggs, milk, peanut, and hazelnut in other food products.
Sensitivity and selectivity are greatly enhanced by performing an enzymatic digestion and then analysing peptides at the molecular level by LC-MS/MS that are specific to that protein.
This requires a proteomic approach where after digestion, peptides indicative of selected proteins are identified that:
- are consistent with the digestion approach,
- are found in the protein sequence,
- and are not found in other proteins so that false positives are avoided.
This method employs two digestion steps that simulate the in-vivo digestion process.

Other research has focused only on those peptides that are the most sensitive for mass spectrometry (MS) analysis. Specificity is another important analytical parameter. To achieve sequence specificity, the authors chose peptides with a minimum of 6 amino acids. Larger peptides can offer more uniqueness, but result in reduced MS sensitivity. Each peptide sequence was searched against the NCBI nr protein database.
The peptide sequences were selected based on uniqueness to that protein. Peptide markers for detection of the four food allergens were selected from the allergenic proteins (listed in Table 1).
[Synthesized peptide markers and stable labeled isotopes as internal standards will greatly enhance the robustness of any method but the cost is prohibitive. Acceptance of a method would make pursuing this more feasible.]
Peptides that are representative for these allergen proteins are highlighted in the protein sequence and labeled (m=milk, H=hazelnut, p=peanut and ew=egg whites and ey= egg yoke).
The LC/MS/MS analysis was performed using an Agilent 1290 Infinity 2 LC system and an Agilent 6495 triple quadrupole using positive ion detection with electrospray ionization (Agilent Technologies, Santa Clara, CA, USA). The peptides released from the proteolytic digestion were separated on an Agilent Poroshell 120 (2.1 x 50 mm, 2.7 µm) column (Agilent part # 699775-902) using a gradient mode elution (Table 2) at flow rate of 0.350 mL/min. (These low levels of TFA did not result in any observed ion suppression in electrospray on the Agilent equipment and offered better chromatography peak shape compared to formic acid.)
The triple quadrupole was operated in dynamic MRM mode with three transitions per peptide. In Table 2 the authors listed the source conditions and MRM parameters for the target peptides monitored. The collision energy was optimized for each peptide and MRM transition.
As minimum criteria for confirmation of each food allergen, at least 2 peptides - each from 2 allergenic proteins - were selected by the authors. In the LC-MS/MS analysis of each peptide at least 2 transitions were selected for monitoring, one as the quantifier transition and the other as a qualifier for positive confirmation. For most peptides the authors have selected 3 transitions (2 qualifiers for confirmation of identity). Using these criteria, the minimum confirmation limit (MCL) becomes the detection limit of the poorest responding peptide transition (qualifier) of the 4 peptides selected. However, using these criteria a lot of food allergens represented as the whole foods will not meet the minimum method detection or method quantification limits (MDL and MQL). For screening, the MDL and MQL are calculated based on the quantifier ion of the most sensitive transition of the most sensitive peptide.

Quantitative analysis is then obtained by calibration against the whole food by either a cross calibration using pure synthetic

peptides or by measuring the peptides after digestion of a known amount of the allergenic food, whole milk, whole egg (blended white and yolk), peanut, and hazelnut. Taking a known amount of food allergen from the MQL to the limit of dynamic range, e.g. whole white cow milk from 0.1 ug/mL to 1000 ug/mL, prepared in the same way as a sample, will yield a calibration curve for the selected peptides providing the direct concentration of the food in a sample. This will not correct for recovery, the sum of extraction efficiency of the proteins from a specific food matrix, digestion efficiency in that matrix, and LC-MS/MS ion suppression or enhancement in that matrix. Therefore, using this procedure, spike recoveries must be performed to validate each food matrix.
Alternatively, matrix matched calibration using a blank food matrix with the food allergen added will provide a more accurate routine analysis, but would require a calibration curve for each matrix tested. Due to the variety of food matrices and potential to hinder reproducible digestion the authors employed a quality control digest standard to monitor the digestion efficiency, which releases the marker peptides for each food commodity.
Data analysis was performed using Mass Hunter Quantitative analysis software version B.06.00, using a calibration curve with a quadratic fit and 1/x weighing. This calibration curve was used to determine the concentration of the food allergens in the food matrix.

This multiplexed approach provides the means to test food for hidden allergenic compounds with accuracy and sensitivity to satisfy both inspection and labelling purposes. Proteins unique to eggs, milk, peanut, and hazelnut have been extracted, subjected to trypsin digestion and analysis by liquid chromatography/quadrupole time-of-flight mass spectrometry, in order to find highly conserved peptides that can be used as markers to detect components in the food.

1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	YES: The applicability of the method is adequate to the applicability of the SMPR. The LC-MS/MS method for eggs, hazelnut, milk and peanut allergens in various matrices specified in the AOAC SMPR 2016.002 was developed using several representative peptides. I provided my additional remarks in the part '6. Based on the supporting information, what are the cons/weakness of the method?'
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	YES: The analytical techniques in the method are adequate and meet the SMPR. Quantitative analysis is then obtained by calibration against the whole food by either a cross calibration using pure synthetic peptides or by measuring the peptides after digestion of a known amount of the allergenic food, whole milk, whole egg (blended white and yolk), peanut, and hazelnut. Taking a known amount of food allergen from the MQL to the limit of dynamic range, e.g. whole white cow milk from 0.1 ug/mL to 1000 ug/mL, prepared in the same way as a sample, will yield a calibration curve for the selected peptides providing the direct concentration of the food in a sample. In the LC-MS/MS analysis of each peptide at least 2 transitions are selected for monitoring, one as the quantifier transition and the other as a qualifier for positive confirmation. For most peptides we have selected 3 transitions (2 qualifiers for confirmation of identity). Using these criteria, the minimum confirmation limit (MCL) becomes the detection limit of the poorest responding peptide transition (qualifier) of the 4 peptides selected. However, using these criteria a lot of food allergens represented as the whole foods will not meet the minimum method detection or method quantification limits (MDL and MQL). For screening, the MDL and MQL are calculated based on the quantifier ion of the most sensitive transition of the most sensitive peptide.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	YES: Definitions, which are specified in the SMPR, were used and applied appropriately in the method. I provided my additional remarks in the part '6. Based on the supporting information, what are the cons/weakness of the method?
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes: The method contains all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	YES: The definitions specified in the SMPR were used and applied appropriately in the supporting documentation (manuscripts, method studies, etc.).
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	YES: There is a piece of information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Material stated in the SMPR.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	YES: There is a piece of information demonstrating that the method performs within the SMPR Method Performance Requirements table specifications for all analyses in the SPMR applicability statement. In the method proposed by the authors, the specification of the following data according to the SMPR Method Performance Requirements table specifications is as following: Calibrations were performed over a range of 1-1000 ppm for each allergen. The calibration linearity was greater than 0.9978 with calibration residuals within 13% of the actual value over the calibration range. Table 1 presents the MQL and MDL for the selected allergen marker peptides. The MQL met the Method Performance Requirements and equalled 1 on the basis of the calibration residual results. Going to 0.3 ppm resulted in calibration residuals above 20% difference from the actual level. The MDL's ranged from 0.1 to 0.05 based on a signal to noise of 3:1 extrapolated from the 1 ppm calibration level. Spike recoveries for egg, hazelnut, milk and peanut in the allergen matrix combinations listed in the AOAC SMPR 2016.002 method performance document was performed a 5 ppm and 50 ppm. The Tables 2 and 3 list the recovery results for each specified allergen matrix combination. The 5 ppm spike recovery data ranged from 75-101% with a standard deviation of 15% or less. The 50 ppm spike recovery data ranged from 90-104% with a standard deviation of less than 12%. The repeatability of the method was tested on seven separate extractions-digestions and LC/MS/MS analysis of the food allergens in a cookie at 5 ppm. Table 5 presents the results for one peptide for each allergen. The repeatability ranged from 4.5-9.4 percent standard deviation for the four allergens. I provided my additional remarks in the part '7. Any general comments about the method?'
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	In my opinion, there is no need to implement any additional steps in the method evaluated.

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	YES: Yes, there are. (Suitable methods will include blank check samples and check standards at the lowest point and midrange point of the analytical range).
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	YES: There is a piece of information demonstrating that the method system suitability tests and control as specified in the SMPR worked appropriately and expected.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	The method is well described and substantively prepared. The concepts, analyses, and methodology are adequately developed. The method proposed is well integrated and well-reasoned.

5. Based on the supporting information, what are the pros/strengths of the method?

In my opinion the pros/strengths of the method can be found on the each of proposed steps:

Background information and method optimization
The advantage of the PTC (pepsin, trypsin and chymotrypsin) approach is the initial digestion in 0.01M HCl with pepsin breaks the protein down resulting in higher amounts of protein and peptides in solution compared to urea extraction and digestion only the alcohol soluble portion of the protein. Figure 1 shows a factor of ~2 improvement in sensitivity for the pepsin extraction step. Secondly, PTC digestion resulted in smaller peptides, in the 8-18 amino acid range, thus keeping the ion current primarily in the +1 to +2 scharge states, resulting in better sensitivity compared larger peptides which show a larger range of chargé states (e.g. +2, +3, +4). Also the shorter peptides are less expensive to have synthesized.
The PTC digestion conditions were evaluated to determine the effective protein to enzyme ratio and digestion time had on the results. The optimal conditions chosen were the mid-range conditions of protein to enzyme ratio of 100:1 with a reaction time of 1 hour. In addition, linearity of the assay was demonstrated preparing a calibration curve from 0.2-500 ppm for egg, hazelnut, milk and peanut protein followed by digestion a fixed amount enzyme (with a linear correlation coefficient greater than 0.997).

Monitoring the repeatability/effectiveness of the enzymatic digestion process
As the authors proposed in Figure 5, the results using the digestion standard in the analysis for various foods, demonstrating the peaks were within the standard deviation for the mean response, indicating an effective and repeatable digestion process. Additionally, the digestion standard showed good repeatability when analysing different levels of allergens in a cookie matrix.
Incorporation of a solid phase concentration-clean up step
To achieve the required MDL of 5 ppm for the target allergens can prove difficult if detection is based on 4 peptides and 3 transitions per peptide. Each allergen has several representative peptides that show good LC/MS response, so the direct analysis can achieve the MDL of 5 ppm. However, other peptides have lower response (possibly due to the level of the protein that the peptide represents) as well as weakest transition MRM transitions. To overcome this limitation and to reduce chemical noise, solid phase extraction (SPE) sample clean-up was evaluated after the PTC digestion step. Three different solid cartridges were evaluated including a strong anion exchange (SAX), octadecyl (C18) and strong cation exchange (SCX).
The strong cation exchange (SCX) cartridge worked best showing greater than 70% recovery for all the marker peptides. The SCX cartridge also provided greater than 70% peptide recovery for allergens in a most difficult matrix (chocolate), as figure 6 presents.
Figure 7 shows that the SCX cartridge resulted in about 86% recovery of the target peptides for 1000 uL sample loading. When reconstituted in 100 uL, the concentration of the sample increased by ~9 X, resulting in a very strong LC-MS/MS signal. The SCX concentration/clean up step is now incorporated to enable detection of multiple peptide markers and 3 MRM transitions for each marker peptide at 5 ppm levels and lower. This approach for detection of low levels of allergens in certified reference materials (CRM) is described in section 6 of the supplemental data from Aug 18/2016.
Additional peptide stability data (28 days)
The results indicate the peptides are most stable at -20C and -80C and greater than 80% after 28 days.
Selection of quantitation peptides, identification of MRM ion identities
and MS/MS spectra
Marker peptides for the various allergens were selected based on the PTC digestion of egg, hazelnut, peanut and milk and analyzed by LC-MS/MS on a q-TOF-MS. The accurate mass measurement and MS/MS spectra were searched using Spectrum Mill, against the allergen nomenclature data base. This data base lists known protein allergens and is approved by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) http://www.allergen.org/index.php.
Peptides that exhibited the best sensitivity were selected and they did show the same peptide fragment in other species based on a NCBInr data base search of the sequence. Preference was given to peptides that produced [M+2H]+2 ions and MRM transitions that were above the m/z of the parent ion.
Spike recoveries and analysis of certified reference materials
Certified reference materials (CRM) for egg, hazelnut and milk were purchased from FA Food Allergens Laboratory. These CRM's were analysed to demonstrate the method sensitivity and spike recoveries were performed to demonstrate the absence of matrix effects. The CRMs, were 100 ppm for egg and milk (in a cereal flour matrix), 50 ppm for hazelnut (in white chocolate) and 40 and 4 ppm for peanut (in a chocolate dessert mix). These standards were also diluted 10 fold in the corresponding matrix to get information close to the required MDL.
In Table 3 the authors demonstrated the results for the analysis of the CRM's. The mean spike recovery was 104.8% +/- 4.8 %.
There appears to be no suppression for the main quantifying peptides for the respective allergen. Figure 9 has shown the MRM chromatograms for the quantifying and 2 confirming ions for egg (10 ppm), hazelnut (5ppm), milk (10ppm) and peanut (4 ppm) in the CRM matrices.
To improve method detection limits and to increase the number of peptides that will meet the MQL of 10 ppm (5 ppm for egg), the initial evaluation of SCX sample concentration and cleanup was evaluated on several CRM's. The conditions used for the clean-up and concentration were listed in Table 1. The evaluation concentrated 1000uL of PTC digest with a reconstituted volume of 100 resulting in a 10 fold increase. Figure 10 shows the chromatograms for 4 ppm peanut in chocolate and 5 ppm hazelnut in white chocolate. The improvement in signal could be seen when comparing the same samples presented in Figure 9 with Figure 10. The average recovery for the SCX step when compared to the direct analysis of the same samples was 82.3%.
Data from a second LC/MS platform (q-TOF)
To test the method on second instrument calibrations and some spiked recoveries were performed on an Agilent 6530 q-TOF MS. The quantitation was performed on the

To test the method on second instrument calibrations and some spiked recoveries were performed on an Agilent 6530 q-TOF MS. The quantitation was performed on the molecular ion within a 50 ppm mass window and 2 MS/MS full scan spectra for confirmation were acquired when that ion is present.
Figure 11 presents the calibrations for egg, hazelnut, milk and peanuts as well as the calibration residuals over the concentration range of 1-1000 ppm. The residuals for all calibrations were between 85-120% and the linear correlation coefficient was greater than 0.996.
In Table 4 the authors presented the spike recoveries results from the q-TOF for the matrices in the AOAC SMPR 2016.002 at the 50 ppm level. The spike recoveries ranged from 74% to 112% with an overall average of 89.4 % with a standard deviation of 12.6%. Due to the lower sensitivity of the q-TOF compared to the triple quadrupole, the 5 ppm spike recovery was not evaluated.
I agree with the authors who suggested that a third platform (SCIEX API 3000) triple quadrupole would also be used to generate a set of data, which will then produce data from 3 instruments and 2 laboratories.

Specificity is one of most important analytical parameter
The specificity of the method was demonstrated by verifying the absence of the peptide markers for each allergen in the food matrices listed in the AOAC SMPR 2016.002. Furthermore, the authors showed that there were no interferences detected for the MRM transition monitored from the matrices.

Specificity is another important analytical parameter. Peptide selection is based on selectivity and sensitivity. Peptides that are too small will have little selectivity, thus sequences less than six amino acids are avoided. Larger peptides can offer more uniqueness, but result in reduced MS sensitivity. Too large a peptide and sensitivity decreases due to multiple charging and costs of synthesis increase, thus peptides larger than 20 amino acids were usually not selected. Also, peptide sequences were selected based on uniqueness to that protein. Each peptide sequence was searched against the NCBI nr protein database, in order to verify that the sequences were unique, and while, are common ingredients in food products.

Peptide markers for detection of the four food allergens were selected from the allergenic proteins listed in Table 1. Synthesized peptide markers and stable labelled isotopes were used as internal standards. Peptides that are representative for these allergen proteins are highlighted in the protein sequence and labelled (m=milk, H=hazelnut, p=peanut and ew=egg whites and ey= egg yoke).
In Figure 4 the authors provide us with the information that LC/MS/MS MRM chromatograms for all the matrices (bread, cookie, dough, cereal, ice cream, milk chocolate, dark chocolate, salad dressing, wine and infant formula) and a 3 ppm standard of peanut peptide NAQRPDNR (p2) and milk peptide HQGLPQEVL (m3). Figure 5 shows the LC/MS/MS MRM chromatogram for all the marker peptides for egg, hazelnut, milk and peanut showing the 3 transitions monitored.

Conclusion
In conclusion, the proteomic approach described here and proposed by the authors uses LC–MS/MS to specifically detect allergens in one analysis. Proteins unique to eggs, milk, peanut, and hazelnut have been extracted, subjected to trypsin digestion and analysis by liquid chromatography/quadrupole mass spectrometry, in order to find highly conserved peptides that can be used as markers to detect components in the food.
This multiplexed approach provides the means to test food for hidden allergenic compounds with accuracy and sensitivity to satisfy both inspection and labelling purposes. Analytical methodology can be implemented by specialist inspectorates. Innovative procedure of separating and quantitative analysis of hidden allergenic compounds can be used for the correct identification and quantitative analysis of the allergens also by the European Food Safety Agency (EFSA).

6. Based on the supporting information, what are the cons/weaknesses of the method?

Synthesized peptide markers and stable labelled isotopes as internal standards will greatly enhance the robustness of any method but the cost is prohibitive.
Therefore, the cons/weakness of the method may be the costs (e.g., the costs of synthesis peptide markers). Although, I think it is inevitable.
Tryptic digestions may result in missed cleavage sites that can result in interfering peptides. But the authors of the method also confirmed the specificity of each "analyte" peptide. Peptides were identified with their associated proteins searching NCBI nr and then cross searches used to eliminate those peptides that appear in other plants or animals (as with milk and eggs).
According to the authors, the proposed enzymatic digestion process results in samples that contain some interferences decreasing MDL values. In order to overcome this imitation additional SPE pre-concentration/clean-up step is required, which makes whole sample preparation procedure more labour-intense.
A full method performance validation is still required for the all marker peptides. As the authors declared, they also plan to submit data from analysis on a Sciex LC-QQQ system in future experiments. I suppose that having done the experiments planned, the results will be expressed as the reproducibility standard deviation (SDR); or % reproducibility relative standard deviation (%RSDR).

7. Any general comments about the method?

The following aspects of the method (summary, presentation of the topic, definition of research objectives, presentation of research methodology and detailed presentation of method) are described in a satisfactory manner.

My remarks to the AOAC Candidate Method #ALL-02 are as follows:
Page 9, LC-MS/MS Analysis:
The authors should answer the following question:
How long stationary phase was conditioned by mobile phase?
Moreover, they should add details about the condition of the stationary phase:
- before the analysis,
- between each of the analyses (balancing of the chromatographic system).

Page 47, AOAC LC/MS allergen detection supplemental data for ImmunogenX (Aug 18/2016), Table 1 Procedure followed for the SPE of the PTC digested food samples, line specifications/procedure:
'rotoevaporate to dryness' What was the duration of this step of the procedure?
Please add the information, e.g., no more than 2 minutes.
In my opinion: Too long drying is not recommended on the grounds that it may cause the loss of analytes.

Page 58, AOAC LC/MS allergen detection supplemental data for ImmunogenX (Aug 18/2016), Figure 6
Peptide recovery for the listed allergen marker peptides from water and a chocolate matrix using a strong anion exchange (SAX), C18 and strong cation exchange (SCX) SPE cartridge:
In the figure, ranges of RSD values should also be marked (in the same manner as in Figure 8 on p. 60)

Additional remarks:
Page 7, Apparatus, pH Meter/pH paper:
'pH indicator strips'
The authors should elaborate on this phrase. In my opinion, the phrase 'pH indicator strips' is not precise enough.

Page 8, Sample and Test Portion Preparation:
'The ground material is frozen at -20°C and then placed in a cryogenic grinding chamber with a magnetic bar.'
If possible, please add the details about the magnetic bar (e.g., the kind, the manufacturer).

Page 9, Sample and Test Portion Preparation, The third sentence from the end (before the next part 'Determination'):
'Transfer 200 µL to a 96 well taking only the upper layer avoiding transfer of any solids.'
What was the pipette used – what was the maximum value of the volume?
Alternatively, in your answer you can include words such as : 'using a pipette, eg. 200 ul' (add the manufacturer).

Analytical methodology can be implemented by specialist inspectorates and other services dealing with the routine check of allergens at their trace level.
Innovative procedure of separating and quantitative analysis of hidden allergenic compounds can be used for the correct identification and quantitative analysis of the allergens, also by the European Food Safety Agency (EFSA).

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	The proteomic approach described here and proposed by the authors uses LC–MS/MS to specifically detect allergens in one analysis. Proteins unique to eggs, milk, peanut, and hazelnut have been extracted, subjected to trypsin digestion and analysis by liquid chromatography/triple quadrupole mass spectrometry, in order to find highly conserved peptides that can be used as markers to detect components in the food. The multiplexed LC/MS analytical method of detection of these marker peptides in foods was based on retention time of analytes, accurate mass and product ions from MS/MS. LC with electrospray ionization and tandem MS operating in the multiple reaction monitoring mode provide high sensitivity and selectivity for trace analysis. This multiplexed approach provides the means to test food for hidden allergenic compounds with accuracy and sensitivity to satisfy both inspection and labelling purposes. In my opinion base on my knowledge, I believe that the proposed method might be considered as comprehensive LC/MS method for detecting and quantifying peptide markers specific and unique to those proteins. In my opinion, the AOAC Candidate Method #ALL-02 entitled ‘Multiplexed LC-MS Method for the Detection and Quantitation of Selected Food Allergens (milk, hazelnut, peanut and whole egg)’ described by Jennifer Sealey Voyksner, Jerry Zweigenbaum and Robert Voyksner can be adopted in its present form as a First Action and recommended for publication in the Official Methods of Analysis of AOAC INTERNATIONAL.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-14 16:17:02
Name	Stefan Ehling
E-mail	stefan.ehling@abbott.com
Organization	Abbott
Title of Method	Multiplexed LC-MS Method for the Detection and Quantitation of Selected Food Allergens (milk, hazelnut, peanut and whole egg)
AOAC Candidate Method Number (e.g. ALN-01)	ALL-02
Applicable SMPR	2016.002

I. Summary of Method

Summary:	The method describes the analysis of four allergens (milk, egg, hazelnut, and peanut) in a variety of matrices. Matrix-matched calibration is performed whereby whole allergens are incorporated into the matrix. A two-step enzymatic digestion is employed, which is a departure from other methods. Method performace relative to SMPR is excellent and supported by the analysis of a CRMs for peanut in chocolate.

II. Review of Method Only

1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	yes

III. Review of Information in Support of the Method

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	The SMPR doesn't specify the required confirmation criteria (how many proteins per allergen, how many peptides per protein, how many MRMs per peptide). In this light, it's difficult to judge if the method meets the SMPR. If the most intense MRM is considered then the method definitely meets the SMPR; however if 4 different MRMs are required for confirmation then the method will fail for certain allergen/matrix combinations.

IV. General Submission Package

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes

4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Gradient composition table is ambiguous. The presentation of the gradient should be revised.
5. Based on the supporting information, what are the pros/strengths of the method?	Matrix-matched calibration whereby the whole allergen (milk, egg, etc.) is incorporated into the matrix. This demonstrates extractability, digestibility, and stability of peptide markers. Sample processing time is potentially shorter than in other methods because overnight enzymatic hydrolysis is not used. Method performance was demonstrated with a CRM.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Matrix-matched calibration whereby the whole allergen (milk, egg, etc.) is incorporated into the matrix by cryogrinding is very cumbersome. For certain allergens their extracts are used to spike the matrix. In this case the extraction yield of allergenic protein from allergenic food is not known. Sample size is too small (25-35 mg).
7. Any general comments about the method?	• Confirmation criteria are not met (all MRMs are not detectable at MDL). • Need of freeze-drying and cryogenic grinding to prepare calibrants and test samples is very cumbersome. • It is not critical to use 96-well plates. Autosampler vials could be used as well. • Give an estimate of total sample preparation time. • Why are milk chocolate, ice cream, and soy infant formula spiked differently from the other matrices (allergen extracts as opposed to grinding with freeze-dried allergen)? Extraction yield of allergenic protein from allergenic food is not known. • Sample size (25-35 mg) is extremely small. Could they use at least 1 g? • What is the IS solution? • How were MS parameters optimized? • Gradient composition table is ambiguous. Describe the gradient clearly. • Gradient program of 15 min is very long for a 50 mm column. • Table 1 – is MDL based on the quantifier (most intense MRM)? • Digestion with pepsin gives 2-fold improvement in sensitivity. This might not be very significant and could vary between labs and instruments. • Digestion with pepsin results in smaller peptides, in the 8-18 amino acid range. All published methods using trypsin alone for digestion have used peptides in this range, so the claim cannot be substantiated. • Overall it is questionable if the use of pepsin is justified given the added cost and complexity to the sample prep. However if it cuts down on analysis time then it's worthwhile. • Regarding the effect of fat on signal suppression: the amount of ranch dressing was varied, not the amount of fat. In this context one cannot conclude that the fat caused the signal suppression. It could be anything else from the ranch dressing matrix. • The cleanup using SCX and SAX cartridges doesn't make use of a strong wash step with 100% organic solvent, which is the most effective at removing interferences. In this view the most important achievement during the SPE step is sample concentration and not sample cleanup.

Recommendation for the Method

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	The method shows great potential. A few details need to be ironed out, such as confirmation criteria and preparation of calibrants (cryogrinding whole alergenic food or spiking with allergenic food extract). Sample size should also be increased.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-15 17:53:05
Name	John Lawry
E-mail	john.lawry@covance.com
Organization	Covance
Title of Method	Detection and Quantitation of Selected food Allergens using LC-MS/MS
AOAC Candidate Method Number (e.g. ALN-01)	ALL-02
Applicable SMPR	AOAC SMPR 2016.002
I. Summary:	Multiplexed LC-MS Method for the Detection and Quantitation of Selected Food Allergens (milk, hazelnut, peanut, and whole egg)
II. Review of Method Only **1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.**	yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	yes
III. Review of Information in Support of Method:	
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	yes
IV. General Submission Package **1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?**	no
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	note: leaving this question blank due to temporary lack of network access to flipping book.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	note: leaving this question blank due to temporary lack of network access to flipping book.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	yes

5. Based on the supporting information, what are the pros/strengths of the method?	Peptide sequences provided to end user, detailed guidance provided for monitoring efficiency of protease digestion of samples.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Added complexity of three enzyme digestion scheme to quantitate known proteins. It is not obvious why/how this helps with compliance to the SMPR 2016.002 metrics.
7. Any general comments about the method?	submitting separate one page document that summarizes two methods together to suggest where each method could help inform the other.

Recommendation for the Method

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	YES, The conditions as stated in SMPR 2016.002 were analytically met.

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-16 12:40:37
Name	Linda Monaci
E-mail	linda.monaci@ispa.cnr.it
Organization	CNR-ISPA
Title of Method	Multiplexed LC-MS method for the detection and quantitation of selected food allergens
AOAC Candidate Method Number (e.g. ALN-01)	ALL-02
Applicable SMPR	Yes
Summary:	The method submitted is a LC-MS/MS method for the multiplex detection and quantitation of milk, hazelnut, peanut and egg in different food matrices. The method proposed is based on protein extraction and subsequent digestion with three proteolytic enzymes (pepsin, trypsin, chymotrypsin); the final digest is partly purified before HPLC separation and MS/MS detection. The method was run on three different MS instrument: 2 triple quadrupole Mass Spectrometers in MRM mode and on a Q-TOF MS (selecting the precursor ion)
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes, the applicability of the method is in line with applicability of the SMPR.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes general precautions to be taken are specified.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes all information required are reported in the paper.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Yes
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Yes

4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	The method could be written more concisely without compromising the quality of the work performed.
5. Based on the supporting information, what are the pros/strengths of the method?	Strengths: The time required for the whole analysis appears to be shortened compared to the existing literature. The LODs reached are very challenging. The evaluation of the recovery has been done correctly and accruing the goodness of the method. The possibility to extend the method also to other categories of nuts. The method was successfully run on three different MS platforms.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Weaknesses: The use of three different enzymes. It is expensive and add another variable to the whole procedure. Maybe a combination of two enzymes could be investigated to originate peptides with a medium length.
7. Any general comments about the method?	The method is very well described and the experimental work has been properly carried out. The final sensitivity and recovery calculated are very promising.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Yes with small modifications

AOAC SPSFAM ERP REVIEW: MAIN FORM

Submission Date	2016-09-09 05:36:26
Name	Susanne Siebeneicher
E-mail	s.siebeneicher@r-biopharm.de
Organization	R-Biopharm AG
Title of Method	Multiplexed LC-MS method for the detection and quantification of selected food allergens (milk, hazelnut, peanut and whole egg)
AOAC Candidate Method Number (e.g. ALN-01)	#ALL-02
Applicable SMPR	2016.002

I. Summary of Method

Summary:	Gernerally, the method is acceptable, however, in some points it is still a little bit unclear. The method needs to be rewritten, because currently it is a little messy. Two different methods in one single manuscript. SPE should be explained more in detail in the methods.

II. Review of Method Only

1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes it does, however, only spiked matrices were analysed. That does not display the real situation. Also real samples need to be analyzed.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes, however, why were only allergenic peptides used. Sometimes only minor components are then recognized. Due to processes in the fodd preparation these minor componets may get lost, however, others will be present. These components will not be detected approproiately due to the wrong decision in marker peptides. Furthermore, it is essenstial to also look at peptide alterations like oxidation of cysteins, degradation of tryptophan, deamidation of asparagine and glutamine, reaction with sugar (maillard reaction).
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Roasting grade of the nuts and supplier is missing. What is dig STD, is it a chemical or something else. If not please explain in the method.

III. Review of Information in Support of Method

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	The used material is no reference material as stated in the ISO Guide 35.
3. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	There is no specific tabel as described in the SMPR. PLease add the table.

IV. General Submission Package

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	No neagtive controls were used, only spiked matrices, please add a negative control.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	The recovery is good for all spiked samples is good, however, there was a problem with the so called standard material. In table 3 page 9 there are inconsistent results. Why was the table included, what is the occassion?

4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	No, it needs to be rewritten due to inconsistency. Please use AOAC format.
5. Based on the supporting information, what are the pros/strengths of the method?	Nice stability data. Excellent preparation of the data. The most suitable buffer was used. A lot of controls were performed.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Protein concentration was analyzed via Bradfort, this should be done with Kjeldahl or Dumas. Bradfort results in different values for same protein content. Therfore the method is not suitable for these kind of analyses. The calibration curve should be equidistant, because at the high values small changes may change the curve in the lower values. Or prepare two different standard curves, one for low and one for high results.
7. Any general comments about the method?	For sample preparation only small amounts of sample were used. The sample should be adjusted to a certain pH. With an amount of 1 ml, that is impossible. Please check the volumes. What is the internal standard? Please show residues as figure. Then a calculation and visiulazation is better. The used Material of the peanut ring trial contains peanut protein, the ELISA of R-Biopharm measures peanut: please check the calculation, there is something wrong. How is the recovery calculated? In some graphs the is the same peak high for 4 and 10 mg/kg. (sample compared to standard) There are different methods for the calculation of results, do they refer to the standard, to Bradford results or the CRM?

Recommendation for the Method

Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Yes with changes in the format, it could be possible. However, real sample testing is still missing and that is a major point in the requirements of the SMPR.

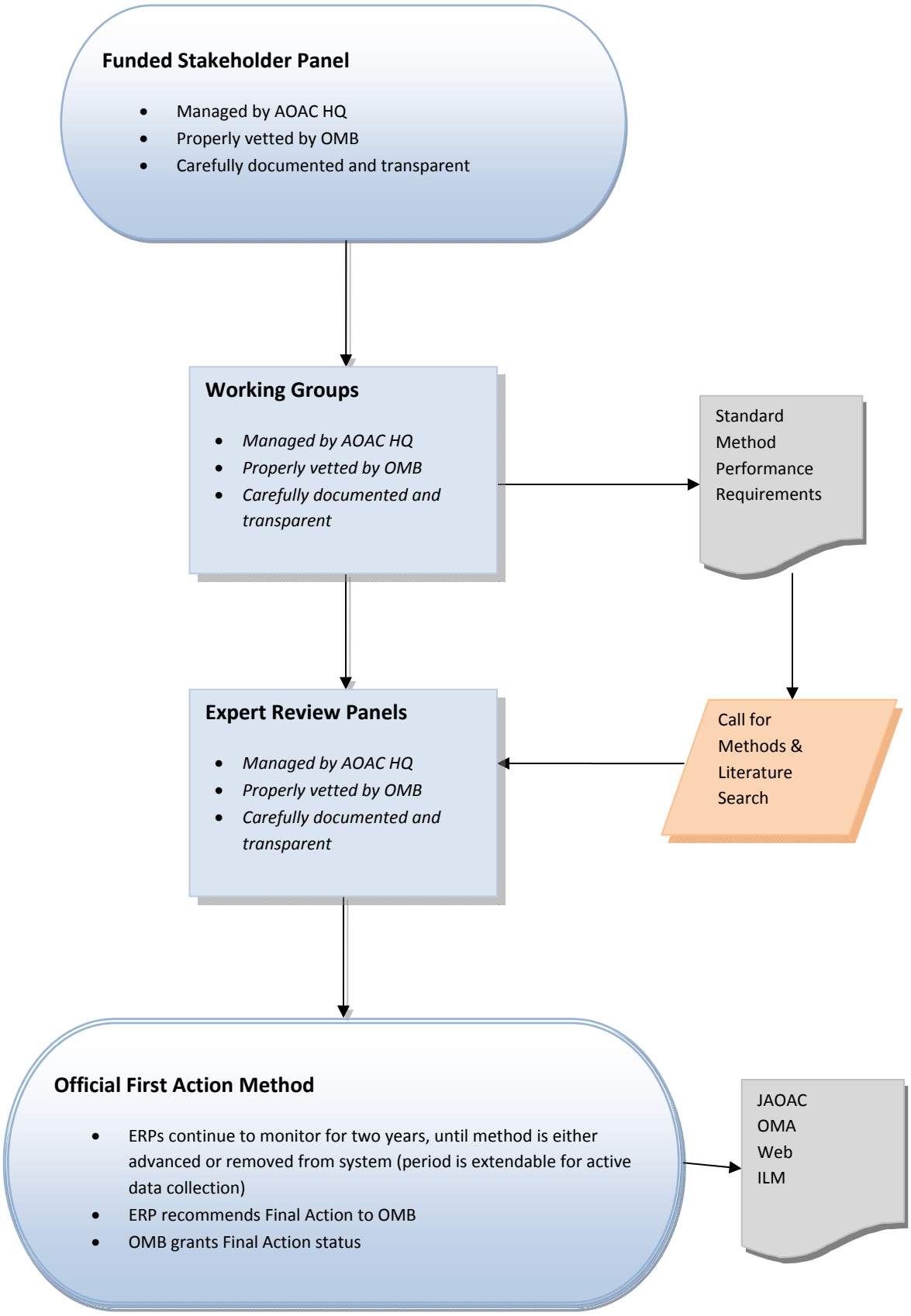
AOAC INTERNATIONAL

First Action Official Methods of Analysis[SM]

Guidance Documents

I. Process Flowchart
II. Process Guidelines
III. Expert Review Panel – Policies & Procedures

Alternate Pathway to Official First Action Method Status



Updated 2011-5-11

AOAC INTERNATIONAL (updated 2011-05-11 by APOFAMS Task Force)

ALTERNATIVE PATHWAY to OFFICIAL FIRST ACTION METHOD STATUS REQUIREMENTS

Expert Review Panels

-Must be supported by relevant stakeholders.

-Constituted solely for the ERP purpose, not for Standard Method Performance Requirements (SMPR) purposes or as an extension of an SMPR.

-Consist of a minimum of seven members representing balance of key stakeholders.

-ERP constituency must be approved by the Official Methods Board (OMB).

-Holds transparent public meetings only.

-Remains in force as long as method in First Action Status.

Official First Action Method Status decision

-Must be made by an ERP constituted or reinstated post 2011-03-28 for Official First Action Status Method Approval (OFASMA).

-Must be made by an ERP vetted for OFASMA purposes by OMB post 2011-03-28.

-Method adopted by ERP must perform adequately against the SMPR set forth by the stakeholders.

-Method must be adopted by unanimous decision of ERP on first ballot, If not unanimous, negative votes must delineate scientific reasons.

-Negative voter(s) can be overridden by 2/3 of non-negative voting ERP members after due consideration

-Method becomes Official First Action on date when ERP decision is made.

-Methods to be drafted into AOAC format by a knowledgeable AOAC staff member or designee in collaboration with the ERP and method author.

-Report of OFAMS decision complete with ERP report regarding decision including scientific background (references etc) to be published concurrently with method in traditional AOAC publication venues.

Method in First Action Status and Transitioning to Final Action Status

-Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.

-Two years maximum transition time (additional year(s) if ERP determines a relevant collaborative study or proficiency or other data collection is in progress).

-Method removed from Official First Action and OMA if no evidence of method use available at the end of the transition time.

-Method removed from Official First Action and OMA if no data indicative of adequate method reproducibility is forthcoming as outlined above at the end of the transition time.

-ERP to recommend Method to Official Final Action Status to the OMB.

-OMB decision on First to Final Action Status

EXPERT REVIEW PANELS

--Policies and Procedures—

Introduction

Expert Review Panels (ERP) are created to provide stakeholders with an expert resource to evaluate analytical solutions to identified needs and concerns.

The ERP will be tasked to search for appropriate methods, issue a "Call for Methods" in the ILM and other avenues, and critically evaluate all collected methods. The ERP will then recommend appropriate methods (as submitted or modified) for adoption as Official First Action methods or for further validation. The ERP, if requested by the Committee/Topic Advisor, would be expected to assist in identifying appropriate materials to be used in the validation studies and in reviewing the protocols for such studies.

Outline of ERP establishment process

An Expert Review Panel is established as follows: A stakeholder or stakeholder body submits a request for the creation of an ERP to the AOAC staff. The request includes a description of the subject area, the desired outcome, and should include a list of recommended subject experts with supporting documentation (see "Qualifications of Expert Reviewers"). Included with this list of recommended subject experts could be a recommendation for an ERP Chair. The request is forwarded to the appropriate AOAC Chief Science Officer (CSO) who identifies potential members for the ERP from a recognized Pool of Experts, a Call for Experts on the AOAC website, and from the stakeholder recommendations. The candidate list and supporting documentation are forwarded to the Chair of the OMB who will assign the review to at least two OMB members. The OMB reviewers will review the candidates for expertise and perceived conflicts of interest and the OMB may then approve the members of the ERP. A Chair for the ERP is also selected. The Chair of the ERP will organize meetings of the ERP to discuss and make recommendations relative to method recommendations, the method(s) to be further validated, and the materials to be used in the validation studies. The conclusions and recommendations of the ERP will be transmitted by the ERP Chair to the OMB and stakeholder body. The stakeholder body will proceed with implementation of the ERP's recommendations by organizing the appropriate SLV study and other items needed for application.

Pool of Potential Expert Reviewers:

Candidates for ERPs are pulled from the following sources. Upon acceptance of the request for the formation of an ERP, a Call for Experts is posted on the AOAC website for a minimum of two weeks. Candidates can then contact AOAC with their interest and credentials. Also, AOAC maintains a Pool of Experts database containing a list of

Approved by Official Methods Board, November 13, 2008
Approved by AOAC Board of Directors, December 9, 2008
Appeals Process Appended – September 2009
Revisedby AOAC Board of Directors, May 25, 2011

AOAC members willing to serve as experts and cataloging their education, experience, and other applicable credentials. Candidates can also be recommended by the stakeholder(s). Note: Candidates (except for the chair) do not need to be members of AOAC. The appointment of experts to an ERP will be for a minimum of 3 years.

Qualification of Expert Reviewers: To qualify as an Expert Reviewer, the candidate must meet one of the following requirements: (1) Demonstrated knowledge in the appropriate scientific disciplines. (2) Demonstrated knowledge regarding data relevant to adequate method performance. (3) Demonstrated knowledge of practical application of analytical methods to bona fide diagnostic requirements. These qualifications must be clearly described in a CV submitted to the CSO and kept on file at AOAC headquarters.

Duties: Members of the Pool of Experts will be called upon to serve on ERPs as needed, and to review documents prepared in the course of the project. These documents may include: (1) procedural documents on how methods will be selected and how single laboratory validation studies will be done; (2) methods submitted for consideration as Official First Action Methods; (3) methods submitted for selection for further validation studies; (4) protocols to be used for single laboratory validation studies; (5) the selection of methods to be considered for full collaborative studies; and (6) validation study reports.

Expert Review Panel:

The CSO selects candidates for an ERP from the Pool of Experts database, the Call for Experts on the AOAC website, and from candidates recommended by the stakeholders. Selection of ERP candidates is based upon their knowledge and experience to adequately evaluate the scope of the study and the anticipated number of submitted methods. The size of the ERP will be sufficient to assure the necessary expertise is present. The CSO may recommend one of the Panel members to serve as Chair.

The CSO submits the following to the OMB Chair: The original submission package, a list of all candidates considered for inclusion on the ERP, the slate of recommended candidates, and a list of possible alternates. Explanations for the ERP choices may be included by either the CSO or a stakeholder if desired. The OMB Chair will delegate two members of the OMB to perform a review. The reviewers submit their recommendations in writing to the OMB. The OMB then votes on the reviewers' recommendations. This vote can be either by email or during an OMB meeting. The OMB may choose not to select one or more individuals on the Panel as submitted and may or may not accept the recommendation of the CSO for the panel Chair. A majority of those voting will be required for approval. The vote of the Chair will break any tie. The CSO, ERP members, and stakeholder body are notified of the vote within one week.

Conflict of Interest: It is incumbent upon each ERP member to avoid any known or potential conflicts of interest and make these known to the CSO and OMB Chair. Each pool member chosen for an ERP will be asked to agree to the AOAC Policies and Procedures on Conflicts of Interest evidenced by completing a Conflict of Interest Form.

If a Pool member being considered to serve on any particular panel is an author, or his/her laboratory is the source of a method under consideration by the Panel, they must so indicate to the CSO or OMB Chair. At the discretion of the CSO or OMB, the names of such Pool members may be removed from consideration, or they may be considered to serve on the ERP with the understanding that a deliberate effort will be required to avoid any known or potential conflicts of interest. In these latter cases, assignments of individual methods for peer review will be made in such a way by the Chair that ERP members will not review any method for which they are an author or co-author, or for which their laboratory is the source; and, most importantly, the Chair will require that they abstain from voting on such a method during the final method selection process. The CSO or OMB may also allow Pool members that qualify under the requirements of expert reviewers, but for whom there is a known or potential conflict of interest to be present as an observer on any particular Panel. In these cases, and only at the discretion of the Chair, observers may provide comments, but only if and when called upon by the Chair to do so.

Non-disclosure Statement: All members of an ERP must have signed the AOAC Volunteer Acceptance Form. For certain contracts, each Pool member or observer chosen may be asked to sign a non-disclosure statement agreeing not to discuss or disclose confidential information presented and discussed during meetings of the ERP.

Meetings of the ERP: The ERP Chair will organize meetings of the ERP, to review the methods and accompanying validation data, score them numerically, and prepare a summary report. Meetings of the ERP can include voting members of the Panel, and non-voting members (AOAC staff, stakeholder members, and observers).

The CSO may assist the Panel Chair in facilitating meetings. The members of the Panel are to review distributed documents before the meeting. To facilitate the process, the Chair may assign primary and secondary reviewers for each method. The primary and secondary reviewers prepare a short critique of the method that is distributed or presented to the ERP. If both the primary and secondary reviewers conclude that the method should not be considered further, the ERP Chair may call for a vote by the Panel; if a unanimous vote to drop a method without further discussion results, the Chair removes the method from further consideration. The Panel then discusses each of the remaining methods in turn.

Method Selection Process: The ERP will evaluate all of the methods in a scientifically unbiased manner.

Occasionally, a large number of analytical methods of variable quality are encountered. When this occurs, the following “pre-screening” procedure is suggested to eliminate methods that are not satisfactory. The Chair of the ERP with the assistance of at least one other member of the ERP may review all of the methods and remove unsatisfactory methods from consideration. The remainder of the methods would be sent to the ERP members for review.

The basic requirements for selection of methods for further validation studies will be: fitness for purpose, applicability to the scope needed, clarity of method description, satisfactory performance characteristics, and single laboratory validation data. To assist the Panel, the AOAC will provide a "Methods Selection Worksheet," which may be modified at the discretion of the ERP. ERP members will identify the best method(s) for further validation, and identify any modifications to be made to the method. An example of the Method Selection Worksheet is attached.

Samples: The ERP will be asked to recommend the specific materials (matrices) to be included in the subsequent validation studies, along with detailed justifications.

Summary Report: The Chair of the ERP prepares a Summary Report clearly enunciating the recommendations of the Panel, the manner in which these conclusions were reached, any modifications of the method(s) chosen, and the materials (matrices) to be included in the validation studies. The report is to be submitted to the ERP in a timely fashion after the concluding ERP meeting. Comments are also due back to the ERP Chair in a timely fashion. The report is then sent to the stakeholders and a copy is forwarded to the Chair of the OMB.

Post-ERP Activities: AOAC retains the right to call on the panelists, as well as members of the Industry Groups, for continued assistance in the subsequent validation studies. This may include (1) help in obtaining the required samples for use in the subsequent validation studies, as well as participating laboratories; (2) help in developing and reviewing the validation study protocols; and (3) help in reviewing the data resulting from the validation studies and reviewing the manuscript describing the results. These activities will be coordinated by the CSO.

Method Selection Worksheet

Method Title:
Method Number:

Overall evaluation score (1being lowest, 10 being highest):

Additional Factors to Consider:

Recommendation:

Signature (date):

Expert Review Panel Selection Criteria:

1. AOAC paid consultants and AOAC staff should not act as Chairs of ERPs.

2. Members of the BoD may act as voting members but it is recommended that they sit as non-voting members of the panel, unless the CSO can demonstrate that there are so few experts in the field available to the community that they are needed to move the project forward.

3. Paid consultants of AOAC and AOAC staff may not serve as voting members on ERPs.

4. If a single business location is represented by more than one person on an ERP, that location shall have only one vote.

5. The Chair of the ERP must be a member of AOAC INTERNATIONAL.

Appeals Process:

ERP - Openness of Process and Appeals:

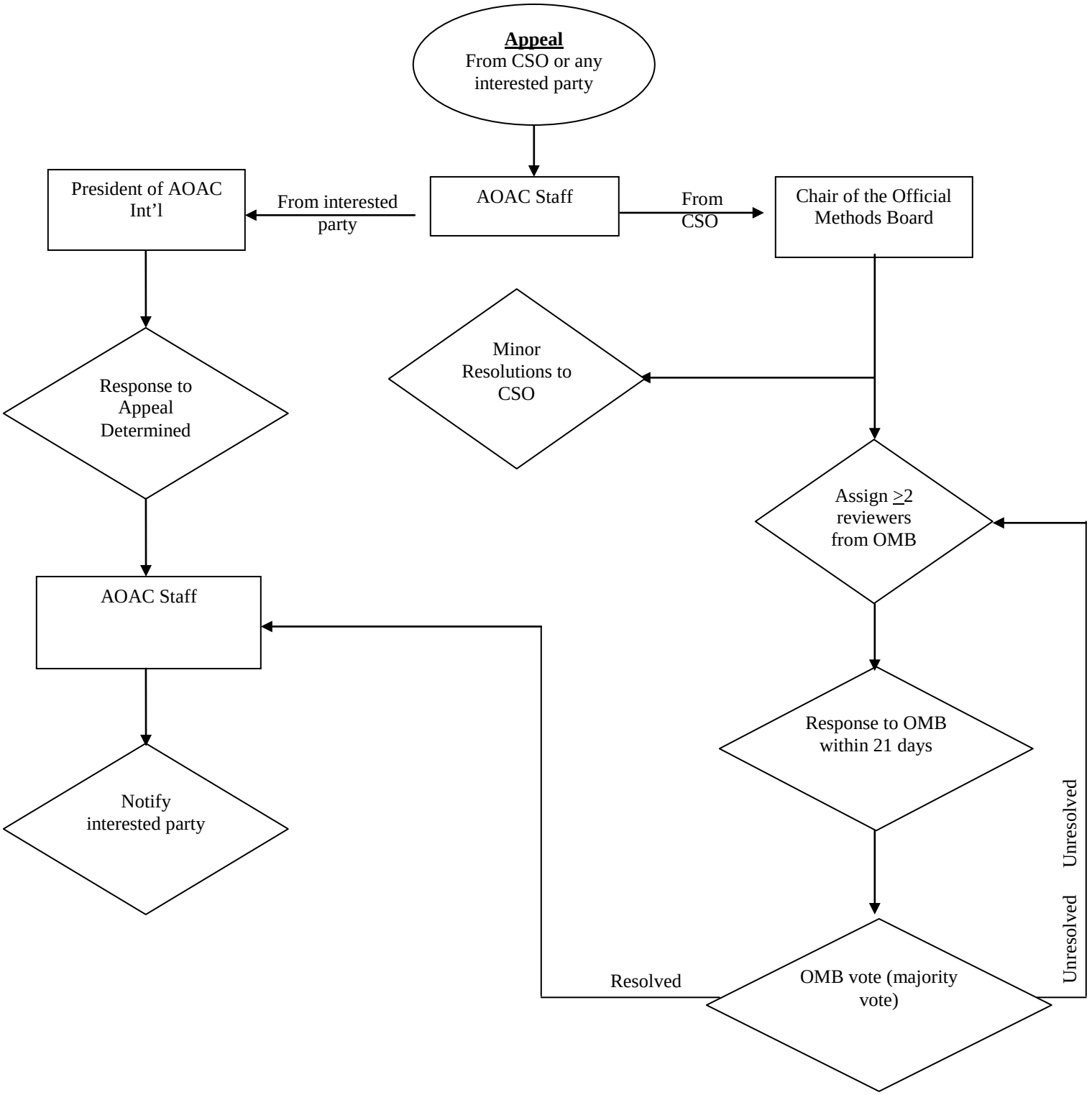
The entire ERP review process is fully open. Any interested party (person, agency, organization, association, company, Chief Scientific Officer (CSO), or group) shall have the right to comment.

Appeals or comments are sent to the AOAC Staff.

Technical decisions by the ERP are final and are not subject to review or appeal. Other questions or issues regarding procedures, conflict of interest, or impropriety may be appealed to the President of the AOAC INTERNATIONAL.

All written concerns will be considered and given a response.

If there is disagreement between the CSO and the Official Methods Board reviewers, the CSO may appeal to the Chair of the Official Methods Board for consideration. The Official Methods Board can select an impartial panel to review the issue, which must report to the Official Methods Board with a resolution within 21 days of its assignment.



Approved by Official Methods Board, November 13, 2008
Approved by AOAC Board of Directors, December 9, 2008
Appeals Process Appended – September 2009
Revisedby AOAC Board of Directors, May 25, 2011

Appendix G: Procedures and Guidelines for the Use of AOAC Voluntary Consensus Standards to Evaluate Characteristics of a Method of Analysis

Expert Review Panels, Official Methods Board, First and Final Action *Official Methods*℠

In early 2011, an AOAC Presidential Task Force recommended that AOAC use Expert review panels (ERPs) to assess candidate methods against standard method performance requirements (SMPRs) to ensure that adopted First Action Official Methods℠ are fit for purpose.

Formation of an ERP

AOAC ERPs are authorized to adopt candidate methods as First Action *Official Methods* and to recommend adoption of these methods to Final Action *Official Methods* status. Scientists are recruited to serve on ERPs by a variety of ways. Normally, a call for experts is published at the same time as a call for methods is posted. Interested scientists are invited to submit their *curriculum vitae* (CV) for consideration. Advisory panel, stakeholder panel, and working group members may make recommendations to AOAC for ERP members. All CVs are reviewed and evaluated for expertise by the AOAC Chief Scientific Officer (CSO). The CVs and CSO evaluations are forwarded to the OMB for formal review. Both the CSO and OMB strive to ensure that the composition of a proposed ERP is both qualified and represent the various stakeholder groups. The recommended ERP members are submitted to the AOAC president who then appoints the ERP members.

Review of Methods

Methods submitted to AOAC in response to a call for methods are collected and compiled by AOAC staff. The AOAC CSO and working group chair perform a preliminary review of the methods and classify them into three categories: (*1*) fully developed and written methods that appear to meet SMPRs; (*2*) fully developed and written methods that may or may not meet SMPRs; and (*3*) incomplete methods with no performance data. Method submitters are apprised of the evaluation of their methods. Method developers with submissions that are classified as Category 2 or 3 are encouraged to provide additional information if available. A list of all the submitted methods and their classifications are posted for public review.

Usually, two ERP members (sometimes more) are assigned to lead the review of each Category 1 method. An ERP meeting is convened to review the methods. ERP meetings are open to all interested parties, and are usually well-attended events with about 50–60 attendees common. Each Category 1 method is reviewed and discussed by the ERP. If stakeholders have designated the method to be a dispute resolution method (as stated in the SMPR), then the ERP is asked to identify the single best candidate method to be adopted as a First Action *Official Method*. If the SMPR does not specify the need for a dispute resolution method, then the ERP may choose to adopt all methods that meet the SMPRs, or may choose to adopt the single best method in their collective, expert opinion.

In addition, an ERP may choose to require changes to a candidate method as part of its First Action adoption and/or identify issues that are required to be resolved prior to adoption as a Final Action *Official Method.*

Methods adopted by an ERP as First Action *Official Methods* may not be in AOAC *Official Methods* format. Method developers/authors are asked to assist AOAC to rewrite the method and accompanying manuscript into an AOAC-acceptable format.

Two-Year First Action Evaluation Period

Under the new pathway, a method may be designated as a First Action *Official Method* based on the collective judgment of an ERP. *Official Methods* remain as First Action for a period of about 2 years. During the First Action period, the method will be used in laboratories, and method users will be asked to provide feedback on the performance of the method.

As previously described, two (or more) ERP members are assigned to lead the review of candidate methods for adoption as First Action *Official Methods*. After a method has been adopted as First Action, these lead reviewers are expected to keep track of the use of and experience with the First Action *Official Method*. At the conclusion of the 2-year evaluation period, one or both of the lead reviewers will report back to the ERP on the experience of the First Action *Official Method.*

The presiding ERP will monitor the performance of the method, and, at the completion of the 2-year First Action evaluation period, determine whether the method should be recommended to the OMB for adoption as an AOAC Final Action *Official Method*.

It is also possible that First Action *Official Methods* are not recommended for Final Action. There are two possibilities for an ERP to decide not to proceed with a First Action method: (*1*) feedback from method users indicates that a First Action method is not performing as well in the field as was expected; or (*2*) another method with better performance characteristics has been developed and reviewed. In either case, the ERP may choose to repeal the First Action status of a method.

OMB Review

The OMB will review all methods recommended for Final Action or repeal by the ERP, and will consider a number of factors in their decision. A guidance document for factors to consider is provided on the AOAC website at http://www.aoac.org/vmeth/OMB_ERP_Guidance.pdf. Some of the factors identified by the guidance document for OMB consideration are (*1*) feedback from method users, (*2*) comparison to the appropriate SMPR, (*3*) results from single-laboratory validation, (*4*) reproducibility/uncertainty and probability of detection, (*5*) availability of reference materials, and (*6*) safety concerns.

Conclusion

The new pathway to *Official Methods*℠ is deliberately designed to avoid creation of elaborate review systems. The intent of the model is for method experts to use their scientific knowledge, experience, and good judgment to identify and adopt the best methods possible for the analytical need.

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These methods are then published as First Action *Official Methods,* and used by analysts while additional information about the method is collected.

Method reviewers may consider other forms of information in lieu of the traditional collaborative study to demonstrate method reproducibility.

Additional Information

Coates, S. (2012) "Alternative Pathway," *Inside Laboratory Management* **16**(3), pp 10–12

Expert Review Panels, Policies and Procedures, AOAC INTERNATIONAL, http://www.aoac.org/News/EXPERT%20REVIEW%20PANELS%20final%20revision.pdf

Standard Format and Guidance for AOAC Standard Method Performance Requirement (SMPR) Documents, AOAC INTERNATIONAL, http://www.aoac.org/ISPAM/pdf/3.5%20SMPR%20Guideline%20v12.1.pdf

Guidance Documents

Requirements for First Action Official Methods℠ Status

See Figure 1 for process flowchart.

Expert Review Panels

(*1*) Supported by relevant stakeholders.

(*2*) Constituted solely for the ERP purpose, not for SMPR purposes or as an extension of an SMPR.

(*3*) Consist of a minimum of seven members representing a balance of key stakeholders. A quorum is the presence of seven members or 2/3 of total vetted ERP membership, whichever is greater.

(*4*) ERP constituency must be approved by the OMB.

(*5*) Hold transparent public meetings only.

(*6*) Remain in force as long as method in First Action status.

First Action Official Method℠ Status Decision

(*1*) Must be made by an ERP constituted or reinstated post March 28, 2011 for First Action *Official Method℠* status approval.

(*2*) Must be made by an ERP vetted for First Action *Official Method℠* status purposes by OMB post March 28, 2011.

(*3*) Method adopted by ERP must perform adequately against the SMPR set forth by the stakeholders.

(*4*) Method must be adopted by unanimous decision of ERP on first ballot. If not unanimous, negative votes must delineate scientific reasons.

(*5*) Negative voter(s) can be overridden by 2/3 of voting ERP members after due consideration.

(*6*) Method becomes Official First Action on date when ERP decision is made.

(*7*) Methods to be drafted into AOAC format by a knowledgeable AOAC staff member or designee in collaboration with the ERP and method author.

(*8*) Report of First Action *Official Method℠* status decision complete with ERP report regarding decision, including scientific background (references, etc.), to be published concurrently with method in traditional AOAC publication venues.

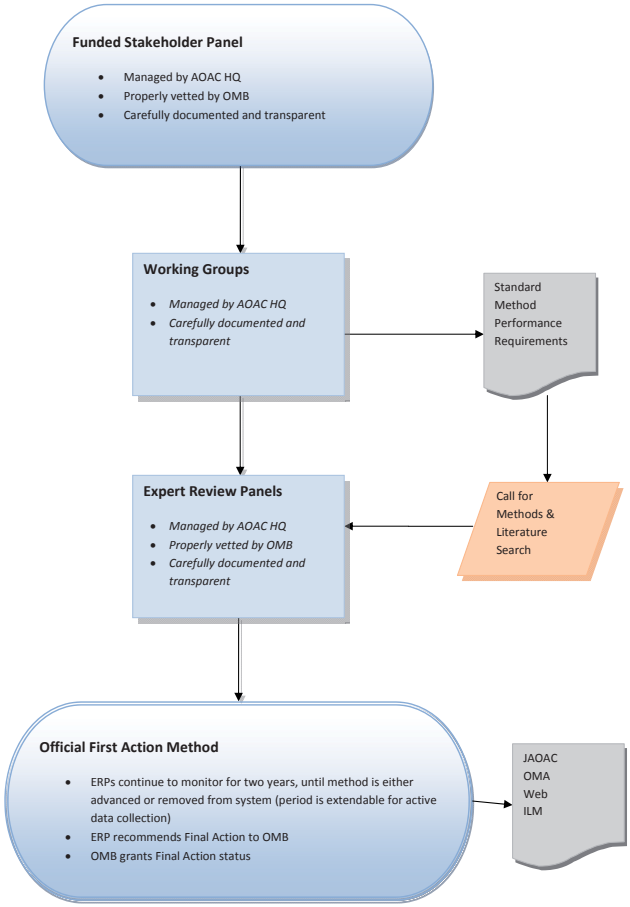

Figure 1. Summary of standards development through *Official Methods of Analysis*.

Method in First Action Status and Transitioning to Final Action Status

(*1*) Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.

(*2*) Two years maximum transition time [additional year(s) if ERP determines a relevant collaborative study or proficiency or other data collection is in progress].

(*3*) Method removed from Official First Action and OMA if no evidence of method use available at the end of the transition time.

(*4*) Method removed from Official First Action and OMA if no data indicative of adequate method reproducibility is forthcoming as outlined above at the end of the transition time.

(*5*) ERP to recommend method to Final Action Official status to the OMB.

(*6*) OMB decision on First to Final Action status.

These guidance documents were approved by the AOAC Board of Directors on May 25, 2011. Revised in February 2014 to include the definition of a quorum under the section *Expert Review Panels*, item (*3*).

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First Action to Final Action Methods: Guidance for AOAC Expert Review Panels

In December 2011, the Official Methods Board (OMB) approved a guidance document for ERPs to support their work as they deliberate on methods, adopt methods as Official First Action, and, subsequently, track method usage and performance between First Action status and Final Action consideration. The guideline is based on parameters of a method that the OMB will consider when deliberating on methods recommended for Final Action status. ERPs are to use this guideline in their deliberations.

ERPs working within the AOAC process may recommend a First Action status method be elevated to Final Action status. Such a recommendation leverages the ERP's high level of expertise supported by data from the initial evaluation, and results from the subsequent 2-year method performance evaluation period.

The OMB receives the recommendation with supporting documentation, and determines if Final Action status is warranted. OMB's review verifies the method process was conducted in compliance with the guidelines and protocols of the Association.

For transparency and to expedite the review process, the main areas OMB will review when evaluating ERP recommendations to promote methods to Final Action are listed below. Documentation of the areas listed below will also increase confidence in method performance and assist users to properly and safely perform the methods at their locations.

A. Method Applicability

(**a**) A method's applicability to the identified stakeholder needs is best assessed by the stakeholder panel and should be a part of the process from the onset. OMB liaisons will remind stakeholder panels to maintain this focus point.

(**b**) OMB may ask ERPs and stakeholder panels for feedback to improve the applicability of the method, such as potential method scope expansions and potential points of concern.

B. Safety Concerns

(**a**) A safety review must be performed for a method to be recognized as First Action.

(**b**) All safety concerns identified during the 2-year evaluation period must be addressed.

(**c**) Guidance and support can be obtained from the AOAC Safety Committee.

C. Reference Materials

(**a**) Document efforts undertaken to locate reference materials. Methods may still progress to Final Action even if reference materials are not available.

(**b**) Guidance and support can be obtained from the AOAC Technical Division on Reference Materials.

D. Single-Laboratory Validation

(**a**) Data demonstrating response linearity, accuracy, repeatability, LOD/LOQ, and matrix scope must be present. Experimental designs to collect this data may vary with the method protocol and the intended use of the method.

(**b**) Resources can be identified by the AOAC Statistics Committee.

E. Reproducibility/Uncertainty and Probability of Detection

(**a**) For quantitative methods, data demonstrating reproducibility and uncertainty must be present. Experimental designs to collect this data may vary with the method protocol, available laboratories, and the intended use of the method (i.e., collaborative studies, proficiency testing, etc.).

(**b**) For qualitative methods, data must be present demonstrating the probability of detection at specified concentration levels as defined by the SMPR. Experimental designs to collect this data may vary with the method protocol, available laboratories, and the intended use of the method.

(**c**) Guidance and support can be obtained from the AOAC Statistics Committee.

F. Comparison to SMPR

(**a**) Document method performance versus SMPR criteria. Note which SMPR criteria are met. For SMPR criteria not met, the ERP documents the reasoning why the method is still acceptable.

(**b**) Data is present to assure the matrix and analyte scopes are covered. This is critical for methods used for dispute resolutions.

G. Feedback from Users of Method

(**a**) Document positive and negative feedback from users of the method during the trial period.

(**b**) Feedback from users demonstrating method ruggedness should be documented.

(**c**) Assess the future availability of vital equipment, reference materials, and supplies.

H. ERP Recommendations to Repeal First Action Methods

Recommendations to repeal First Action methods shall be accompanied with detailed reasons for the decision.

The First to Final Action guidance for ERPs was approved by the OMB in December 2011 and effective as of February 1, 2012.

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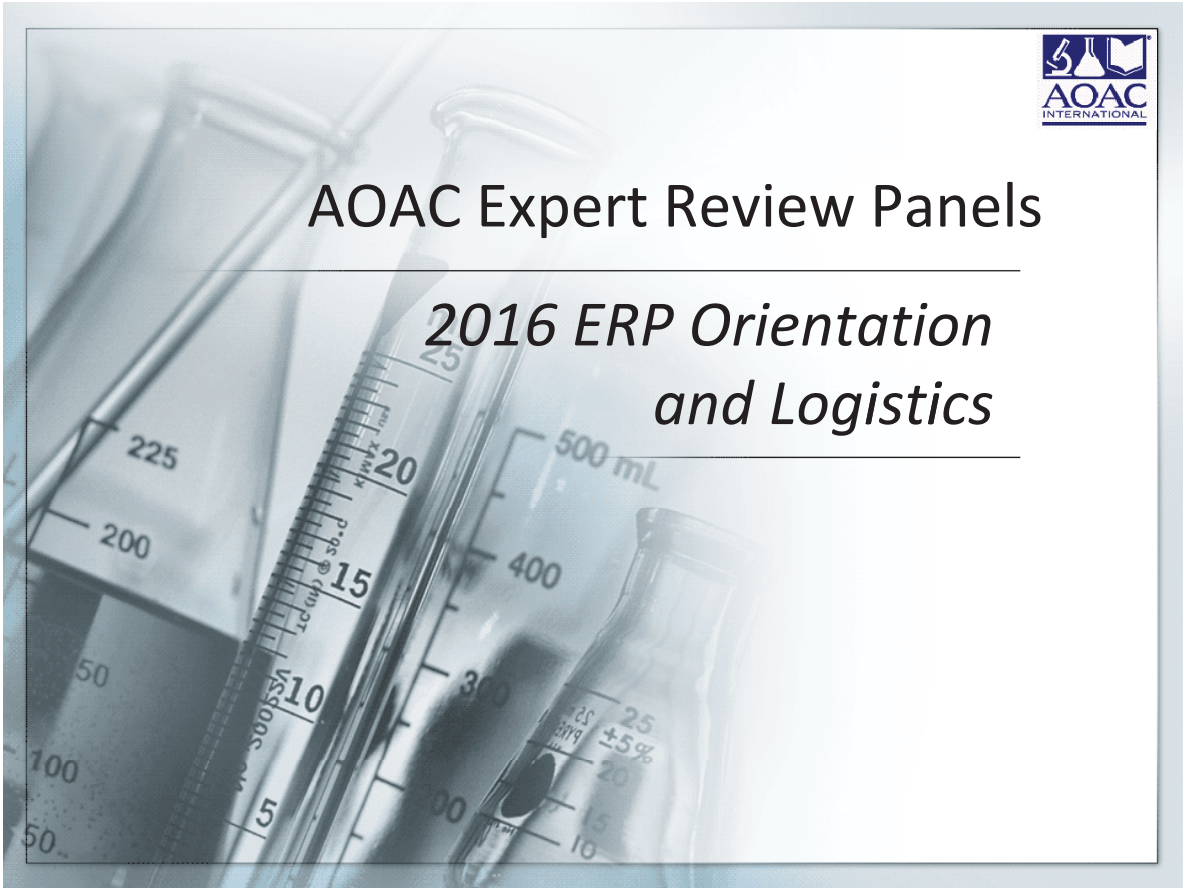


AOAC Expert Review Panels

2016 ERP Orientation and Logistics

Session Syllabus

1. AOAC Method Submission
2. Recruitment of ERP Members
3. ERP Composition & Vetting Expertise
4. ERP Method Assignments
5. ERP Meeting
6. ERP Consensus
7. Post ERP Meeting
8. First Action to Final Action status
9. Method Modifications
10. Publications
11. Documentation
12. Summary of Responsibilities

TRACT 1

METHOD SUBMISSION

Paths to AOAC *Official Methods*

- AOAC Official Methods through AOAC Standards Development

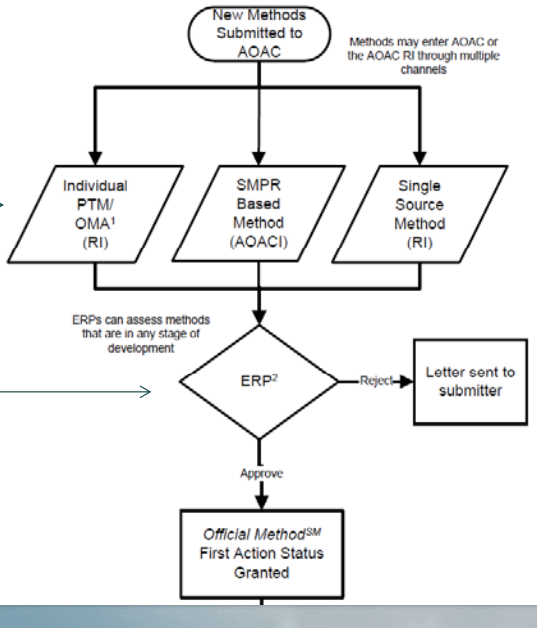
Road to First Action OMA Status

Terms:

- PTM – *Performance Tested Methods℠*
- RI – Research Institute
- ERP – Expert Review Panel
- OMB – Official Methods Board
- SP – Stakeholder Panel
- SMPR – Standard Method Performance Requirement

Three modes of entry and (program administration)

Expert Review Panels will review all methods for all three modes of entry.

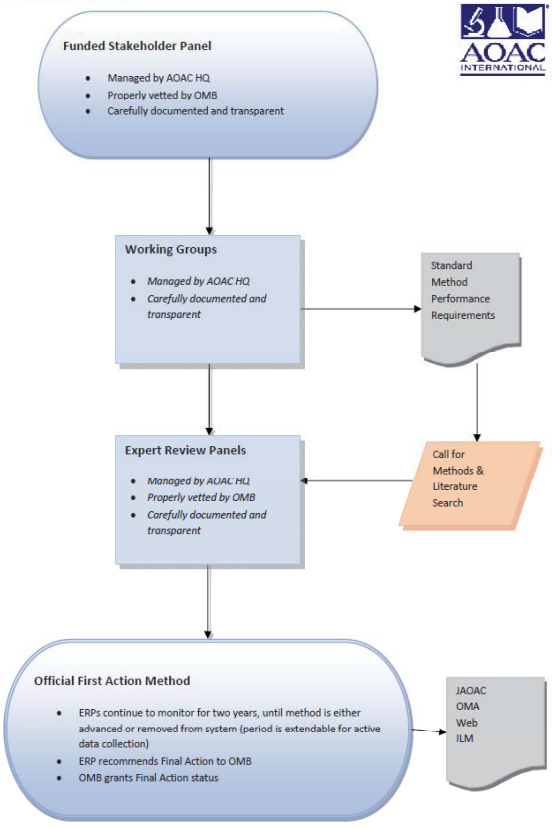


Note: Appeals process always available; see Alternative Pathway Guidelines for appeals process.

1 PTM certification previously issued, PTM reviewers will be ERP members
2 Unless otherwise provided for under a contractual agreement, AOAC will regularly convene ERPs twice a year: once during the Mid-Year Meeting and again during the Annual Meeting

Recap of the Overall Process for Methods Submitted in response to SMPRs or Call for Methods – aka "alternative pathway"

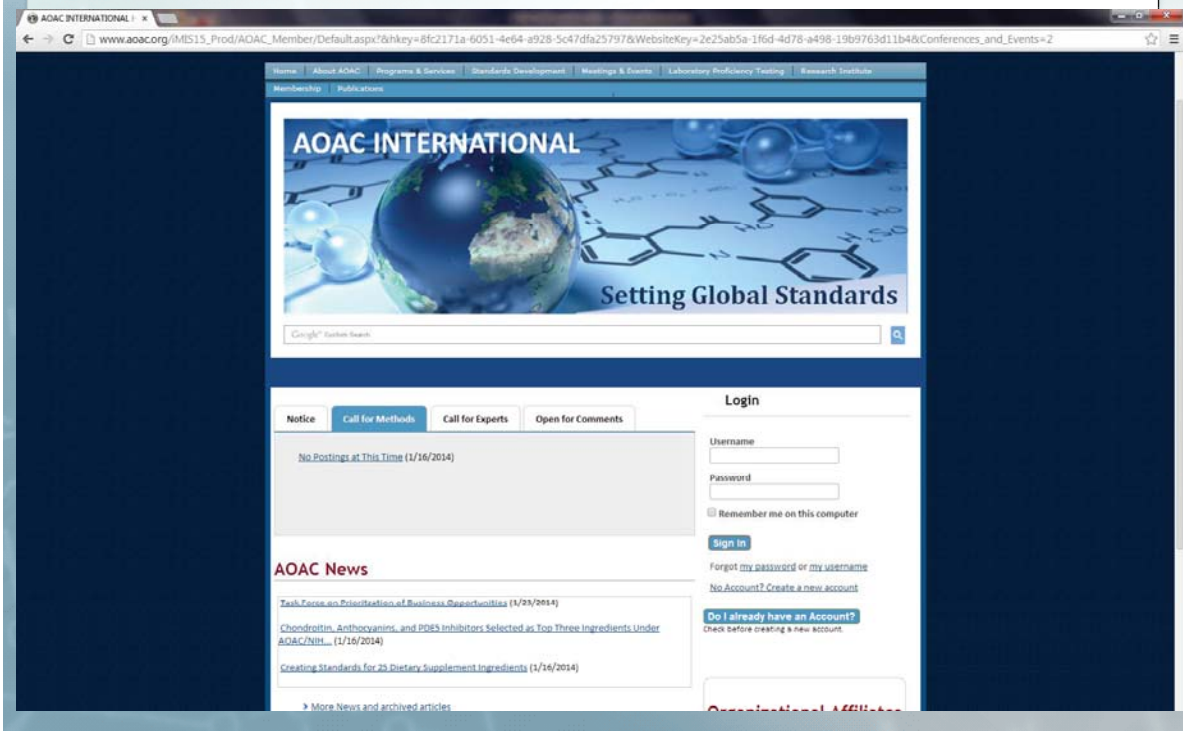
1. Allows AOAC to focus on projects addressing an urgent need of a critical mass of stakeholders.
2. Drives AOAC processes forward faster.
3. Assembles stakeholders (industry, government and academia) to neutral place to articulate and reach consensus on requirements and resolve conflicts.
4. Those requirements are codified and are published as "Standard Method Performance Requirements" (SMPRs).
5. Methods are solicited that purport to meet those requirements.
6. Expert review panels (ERPs) judge the methods against the SMPRs. Method(s) that best meet the SMPRs are adopted and designated "First Action" *Official Method of Analysis*.
7. Process for First Action status to Final Action status follows as the same process for all AOAC First Action *Official Methods*.



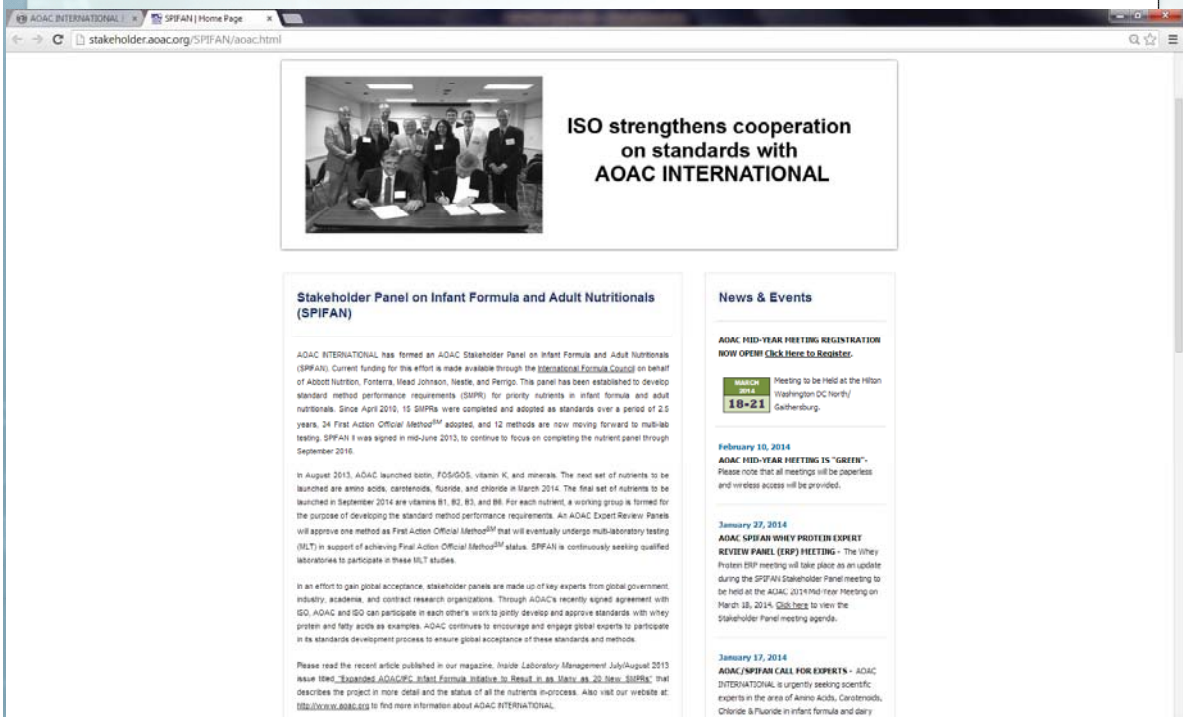
Method Submissions

- Method developers responding to an AOAC issued Call for Methods or to adopted standard method performance requirements (SMPRs) should submit their methods to AOAC INTERNATIONAL
- All other methods should be submitted to the AOAC Research Institute.
- Contact AOAC staff for details.

Calls for Methods



Call for Methods



December 19, 2013 AOAC/SPIFAN Community Update

STAKEHOLDER PANEL ON INFANT FORMULA & ADULT NUTRITIONALS (SPIFAN) NEWS

AOAC/SPIFAN CALL FOR CARNITINE METHODS EXTENDED

AOAC INTERNATIONAL invites method developers to submit Carnitine methods for consideration through the AOAC *Official Methods*℠ Program. Methods should meet or exceed the Standard Method Performance Requirement (SMPR). Click here to view Carnitine Call for Methods.

Interested method developers should provide a description and data demonstrating that the method will meet the SMPR. Click here to submit method(s). Deadline for submissions to be considered is **Friday, January 17, 2014**.

AOAC/SPIFAN CALL FOR EXPERTS

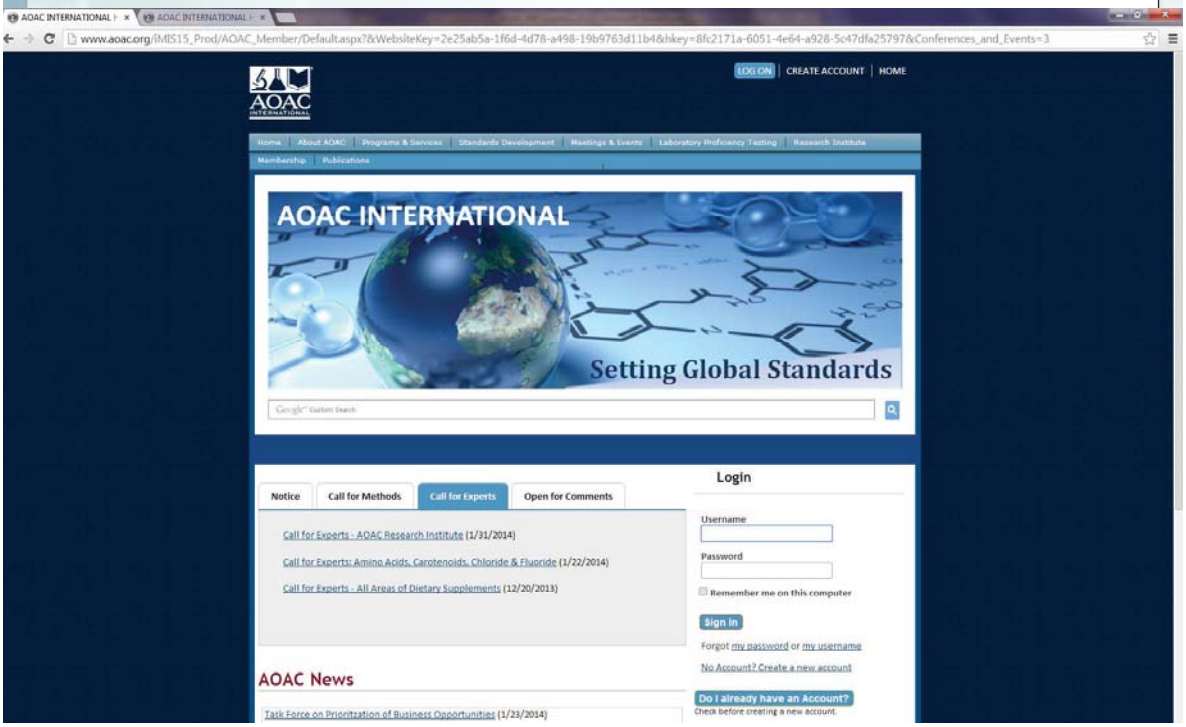
AOAC INTERNATIONAL is urgently seeking scientific experts in the area of Amino Acids, Carotenoids, Chloride & Fluoride in infant formula and dairy products to establish standard methods performance requirements (SMPRs). Click here to view Call for Experts.

SPIFAN ACTIVITIES AT AOAC INTERNATIONAL MID-YEAR MEETING (March 18-19, 2014)

TRACT 2

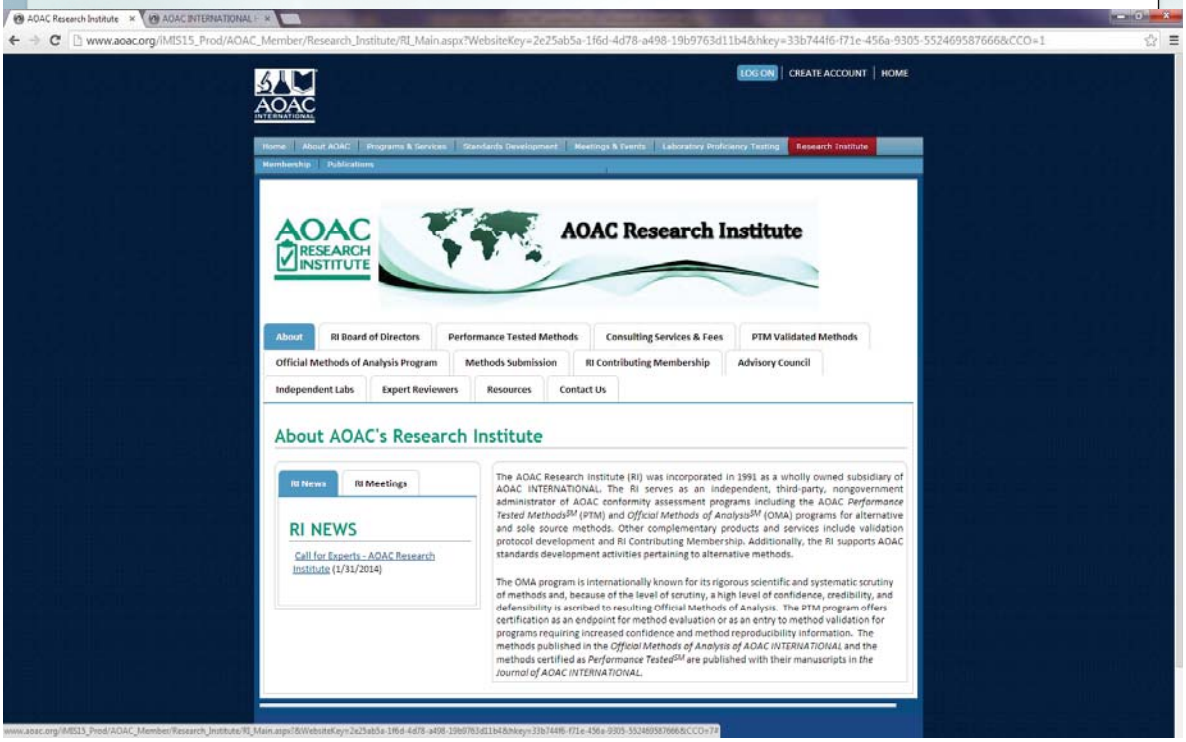
RECRUITMENT OF ERP MEMBERS

CALL FOR EXPERTS



CALL FOR EXPERTS

CALL FOR EXPERTS



Other Forms of Recruitment

- Official Methods Board
- Email Blasts to AOAC network
- Leveraging networks of Advisory Panel members, Working Group Members, AOAC Communities and Sections

REQUIREMENTS FOR ERP SERVICE

- Must have demonstrated expertise in the method, technology, analyte/matrix, etc… Be a subject matter expert.
- Must be able to attend ERP meetings
- Must be able to complete assigned reviews on time
- Must be prepared to speak on the method and share reviews during the meeting
- Must be proactive in tracking assigned First Action *Official Methods*
- Must be able to assist in peer reviewing paper for publication
- Must sign and submit AOAC Volunteer Acceptance Form

AOAC Policies

- AOAC INTERNATIONAL Antitrust Policy
- AOAC INTERNATIONAL Policy On The Use Of The Association Name, Initials, Identifying Insignia, Letterhead, And Business Cards
- AOAC INTERNATIONAL Policy And Procedures On Volunteer Conflict Of Interest
- Volunteer Acceptance Form

Antitrust Responsibilities

- AOAC activities frequently involve cooperative undertakings and meetings where competitors may be present, it is important to emphasize the ongoing commitment of our members and the Association to full compliance with national and other antitrust laws
- Association's structure is fashioned and its programs are carried out in conformance with antitrust standards.
- An equal responsibility for antitrust compliance - which includes avoidance of even an appearance of improper activity - belongs to the individual.
  - The appearance of improper activity must be avoided because actual proof of misconduct is not required only whether misconduct can be inferred from the individual's activities.
- Compliance with AOAC policy and guidelines involves not only avoidance of antitrust violations, but avoidance of any behavior which might be perceived as such.

Antitrust Policy Document

- The document states antitrust laws in general terms, and 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 and activities.
- Signing the AOAC INTERNATIONAL Volunteer Acceptance Form means that the signer has read, understand and agrees to comply with the policy.

USE OF THE ASSOCIATION NAME, INITIALS, IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS

- to protect the reputation, image, legal integrity and property of the Association.
- "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.
- 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;
- Please review instructions on use and sanctions for violations.
- Signing the AOAC INTERNATIONAL Volunteer Acceptance Form means that the signer has read, understand and agrees to comply with the policy.

Volunteer Conflict Of Interest

- 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.

Volunteer Conflict Of Interest Policy Document

- Contains illustrations of apparent or direct conflicts of interest, but not all inclusive
- Contains guidance on Dos and Don’ts for volunteers
- Signing the AOAC INTERNATIONAL Volunteer Acceptance Form means that the signer has read, understand and agrees to comply with the policy.

TRACT 3

ERP COMPOSITION & VETTING EXPERTISE

ERP Composition

- Call for Experts or Volunteers is issued.
- Members must be vetted by AOAC Official Methods Board (OMB).
  - Demonstrated expertise
  - Diversity and balance of the overall expert review panel
- AOAC volunteer appointment
  - Serve at the pleasure of the President of AOAC INTERNATIONAL
- Additional members may be added.
- Can have non-voting members
- OMB assigns an OMB member to serve as a representative on each ERP

ERP SELECTION PROCESS

- AOAC paid consultants and AOAC staff should not act as Chairs of ERPs.
- Members of the BoD may act as voting members but it is recommended that they sit as non-voting members of the panel, unless the CSO can demonstrate that there are so few experts in the field available to the community that they are needed to move the project forward.
- Paid consultants of AOAC and AOAC staff may not serve as voting members on ERPs.
- If a single business location is represented by more than one person on an ERP, that location shall have only one vote.
- The Chair of the ERP must be a member of AOAC INTERNATIONAL.

Vetting Process

AOAC Chief Science Officer

- Reviews all candidates and supporting documentation for expertise
- Makes a recommendation for an ERP slate

Official Methods Board

- Reviews proposed recommended ERP slate
  - Expertise
  - Balance of panel
  - Conflicts of interest
- Renders decision on proposed ERP members and a Roster is formed.

TRACT 4

ERP METHOD ASSIGNMENTS

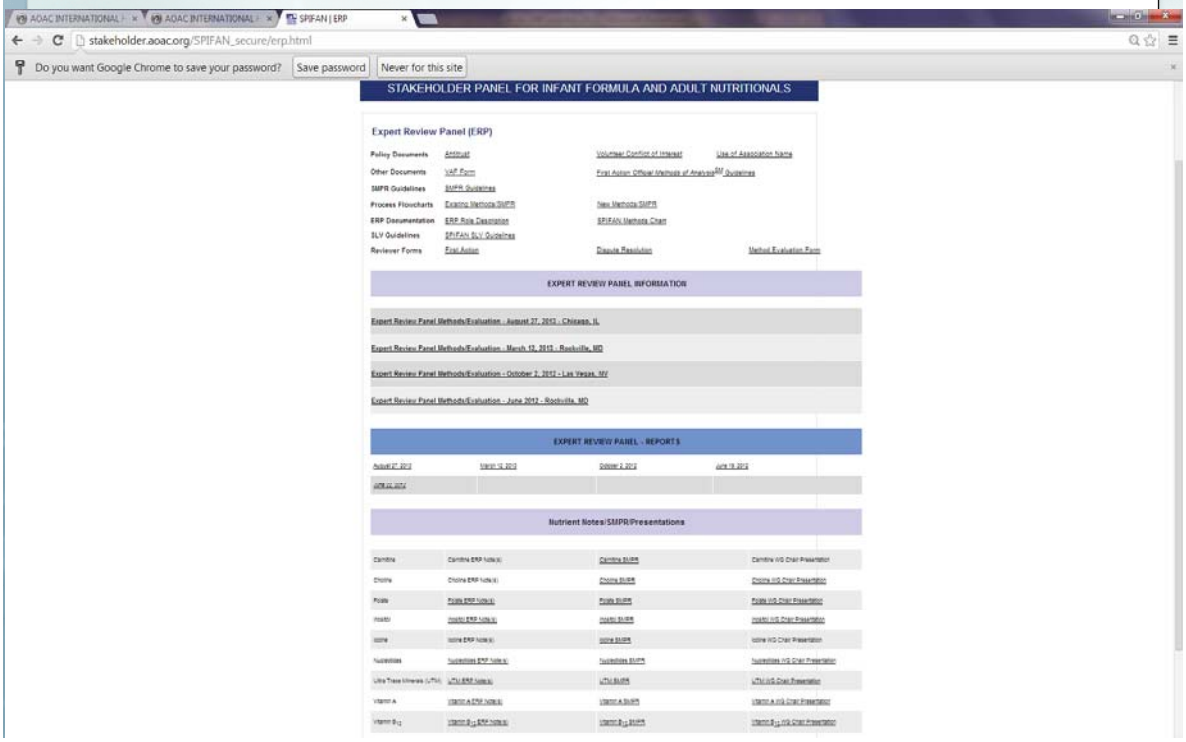
ERP Method Assignments

- A primary and secondary reviewer is assigned to every method.
  - In depth review via review form
  - Prepare to attend and speak on the method and make a recommendation for ERP discussion and consideration.
  - Review forms are completed and returned to AOAC staff in advance of the meeting.
- Members of both Committee on Safety and Committee on Statistics serve as advisory resources for all ERPs

ERP REVIEWS

- Primary and Secondary Reviewers and/or entire ERP conduct in-depth review of method and any supporting information.
  - In-depth review is done electronically through password protected website access and is completed prior to the in-person meeting.
  - Deadlines for submission of reviews
  - Depending on the number of methods 15 to 30 days for review
  - Track and present feedback on assigned First Action *Official Methods*.
  - Present on the method during the meeting and can make the motion to adopt the method.
  - Can recommend additional feedback or information for Final Action consideration

ERP REVIEWS



AOAC INTERNATIONAL

ERP REVIEWS

- **In your judgment, does the method sufficiently meet the Standard Method Performance Requirements (SMPR)?**

- In your judgment, is the method scientifically sound and can be followed?
- In your judgment, what are the strengths and weaknesses of the method?
- In your judgment, how do the weaknesses weigh in your recommendation for the method?
- In your judgment, will the method serve well the stakeholder community that will use the method?
- In your judgment, what additional information may be needed to further support the method meeting the SMPR?

TRACT 5

ERP MEETINGS

ERP Meetings

- ERPs will meet in person at a minimum of twice a year and up to four times per year:
  - AOAC Mid-Year meeting (DC metro area)
  - AOAC Annual Meeting.
- At the ERP meeting:
  - **Primary and secondary reviewers or entire ERP** will present their reviews and makes a motion/recommendation to the ERP whether or not to adopt the method as First Action OMA.
  - ERP discusses the method.
  - ERP renders a decision on First Action status.
  - ERP renders decisions on modifications to First Action methods only.
- If the method is adopted
  - ERP decides on what additional information is needed to recommend the method for Final Action status

ERP MEETINGS

- MEETINGS ARE HELD IN-PERSON, HOSTED BY AOAC
- A QUORUM IS THE PRESENCE OF SEVEN (7) MEMBERS OR 2/3 OF THE TOTAL VETTED ERP, WHICHEVER IS GREATER.

IF NO QUORUM, THEN NO MEETING!

ERP MEETINGS

- REVIEWERS PRESENT THEIR REVIEWS AND MAY INITIATE A MOTION TO ADOPT THE METHOD IF THEY CHOOSE
  - Chair recognizes the reviewers
  - Primary and secondary / ERP reviews are presented.
    - If in favor, they may make and second a motion to adopt or not adopt the method
    - Chair can then entertain discussion on the method
    - Chair can call for a vote once deliberation is complete

ERP MEETING - Discussions

- In your collective judgment, is the method scientifically sound and can be followed as written?
- **In your collective judgment, does the method sufficiently meet the Standard Method Performance Requirements (SMPR)?**
- In your collective judgment, what are the strengths and weaknesses of the method?
- In your collective judgment, do the weaknesses outweigh the strengths in your recommendation for the method?
- In your collective judgment, is the method safe and can it serve well the stakeholder community that will use the it?
- In your collective judgment, is additional information needed to before considering this method for First Action OMA status?

TRACT 6

ERP CONSENSUS

ERP CONSENSUS

- First Action Official Methods status is granted:
- Method must be adopted by unanimous decision of ERP on first ballot, if not unanimous, negative votes must delineate scientific reasons.
- Negative voter(s) can be overridden by 2/3 of voting ERP members after due consideration.
- Method becomes First Action on the date when ERP decision is made.

ERP CONSENSUS

- The ERP may then reach consensus on any additional information that it needs to review to be able to make a recommendation for Final Action *Official Methods* status.
- This is a separate motion.

TRACT 7

POST ERP MEETING

Post ERP Meeting

- An ERP report with the decisions of the ERP will be drafted
  - Review and approval by ERP chair
  - Posted on website within 15 business days after the ERP meeting
- AOAC staff will send notification to method authors/submitters regarding outcomes on specific methods

TRACT 8

FIRST ACTION TO FINAL ACTION STATUS

ERP Tracking

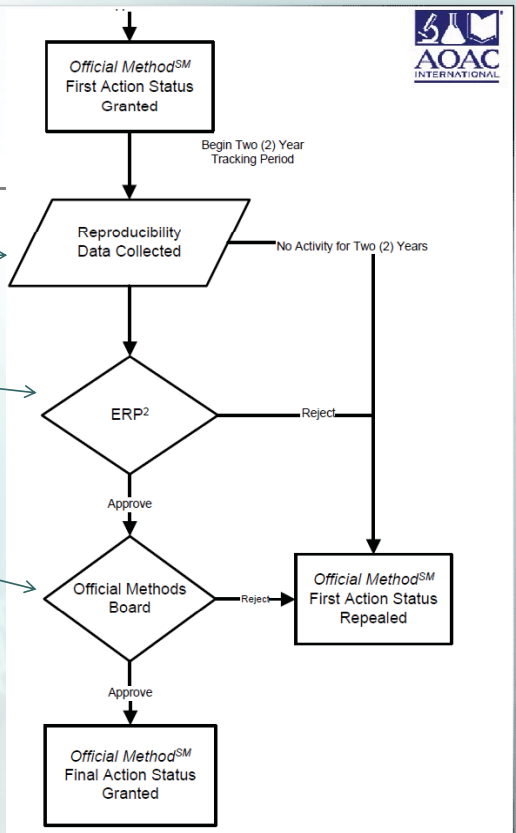
- Between First Action and Final Action:
  - The primary and secondary reviewers track the methods on behalf of the ERP over this time period.
    - Based on information from method authors, laboratories using the method, general community feedback, additional laboratory work
    - Are ERP recommendations being fulfilled?
    - Is the method meeting the standard criteria more closely?
    - How well is community guidance and OMB guidance being reflected?
  - Updates on the method are given by the primary and secondary reviewers during the ERP meetings.
  - At the end of two years, ERP makes a recommendation to OMB for Final Action status, repeal, or continuance.

Road to Final Action OMA Status

Method reproducibility must be demonstrated before Final Action consideration.

ERP determines if sufficient evidence merits a recommendation for Final Action status or repeal.

- Only the OMB promotes a method to "Final Action" status or repeal the method.
- Methods that did not meet the bar would be repealed.
- Same for all method submissions



Terms:

- PTM – *Performance Tested Methods℠*
- RI – Research Institute
- ERP – Expert Review Panel
- OMB – Official Methods Board
- SP – Stakeholder Panel
- SMPR – Standard Method Performance Requirement

Note: Appeals process always available; see Alternative Pathway Guidelines for appeals process.

1 PTM certification previously issued, PTM reviewers will be ERP members
2 Unless otherwise provided for under a contractual agreement, AOAC will regularly convene ERPs twice a year: once during the Mid-Year Meeting and again during the Annual Meeting

Path to Final Action

Review of ERP Method Recommendations

What to Expect from AOAC Official Method Board (OMB)

Standard Method Performance Pathway

1. Standard Method Performance Requirements authored by Working Groups and established by Stakeholders
2. Expert Review Panel (ERP) vetted by OMB
3. ERP approves methods for First Action
4. Method reproducibility data collected
5. ERP monitors method performance
6. ERP recommendations sent to OMB within 2 years
   - Final Action, First Action continuation, or Repeal

OMB Liaison

- OMB member or designee is assigned to your ERP
- Liaison monitors First Action to Final Action process
- Monitors ERP's documentation of all items in OMB Guidance document (OMA Appendix G)

Method Applicability

- Determine how method meets stakeholder's needs
  - scope, accuracy, precision, etc.
- Are ERP recommendations & improvements implemented?
- Assess method limitations & concerns

Safety Concerns

- Safety review completed for First Action
  - Participation by Safety Committee
- All safety issues identified during 2 year review addressed
  - Participation by Safety Committee

Reference Materials

- Identification of potential reference materials (RM)
  - If none found, define alternative options
- RM performance expectations

Available resource is the AOAC Technical Division on Reference Materials (TDRM)

Single Laboratory Validation

Chemistry

- Linearity
- Accuracy
- Repeatability
- LOD / LOQ
- Matrix scope
- Selectivity

Microbiology

- Inclusivity/Exclusivity
- Robustness
- Repeatability
- POD or equivalent
- Matrix scope

AOAC Committee on Statistics is your resource

Quantitative Reproducibility/Uncertainty

- Experimental designs may vary
  - Collaborative study
  - Proficiency Testing data
  - Multi-lab study variations
- Committee on Statistics
  - is available to discuss new study design protocols
  - Formalized tools were presented at the 2013 Annual Meeting

Qualitative Reproducibility/Uncertainty

- Experimental designs may vary
- Committee on Statistics is available to discuss new study protocols designs

Compare to SMPR

- Method meets Performance Criteria
- Method does not meet Performance Criteria
  - Acceptable or not? List reasoning
- Document acceptability to Stakeholders

Feedback from Users

- Solicit and document user feedback
  - ERP Chair determines mechanism
  - May take form of
    - Proactive calls to users
    - Tally of incoming calls
    - Emails
    - Web surveys

Feedback from Users

- Method performance
- Safety Concerns
  - Warnings
  - Alternatives
- Equipment and supply availability
  - Readily available
  - Practicality
  - Suggested improvements
  - Failures
- Reference material availability

September 20, 2004

ERP SUMMARY FOR FIRST TO FINAL ACTION METHOD RECOMMENDATION

AOAC No.	NAME OF METHOD

GUIDANCE FOR AOAC ERPS - APPENDIX G[1]	**Considered?**	**Comments/Reference if applicable**
Method Applicability		
ERP First Action to Final Action recommendations & improvements		
Draft Final Action method reviewed by ERP		
Safety Concerns		
Reference Materials		
Single Laboratory Validation		
Reproducibility/Uncertainty and Probability of Detection		
Comparison to SMPR (SMPR criteria met?)		
Feedback from Users of Method		

DOCUMENTATION	**Available?**	**Comments**
Safety Evaluation		
Reference Materials		
SLV or PTM		
Approved Validation Protocols		
Statistics Review		
Method Published in OMA		
Method Performance vs SMPR criteria		
Feedback Information		
Additional Recognition(s)		
ERP Reports		
Manuscript(s) Published in JAOAC		
ERP Method Recommendation (Final Action/Repeal/Continuation)		

[1] *Official Methods of Analysis of AOAC INTERNATIONAL*, Appendix G: Procedures and Guidelines for the Use of AOAC Voluntary Consensus Standards to Evaluate Characteristics of a Method of Analysis, p.3 *"First Action to Final Action Methods: Guidance for AOAC Expert Review Panels."*

ERP SUMMARY FOR FIRST TO FINAL ACTION METHOD RECOMMENDATION

AOAC 2012.25	**Residues of Three Triphenylmethane Dyes and Their Metabolites (Malachite Green, Leuco Malachite Green, Crystal Violet, and Brilliant Green) in Aquaculture Products** **Liquid Chromatography/Tandem Mass Spectrometry**

GUIDANCE FOR AOAC ERPS - APPENDIX G[1]	**Considered?**	**Comments/Reference if applicable**
Method Applicability	Yes	Triphenylmethane dyes as specified in applicability statement.
ERP First to Final Action recommendations & improvements implemented/addressed	Yes	
Draft Final Action method reviewed by ERP	Yes	
Safety Concerns	Yes	Completed and discussed during ERP meeting
Reference Materials	Yes	Currently no reference materials available for these types of drugs
Single Laboratory Validation	Yes	Hurtaud-Pessel et al., *J. AOAC Int.* **96**, 1152(2013) Andersen et al., *J. AOAC Int.* **98**, 636(2015) – modification – matrix extension
Reproducibility/Uncertainty and Probability of Detection	Yes	Schneider & Andersen *J. AOAC Int.* **98**, 658(2015)
Comparison to SMPR (SMPR criteria met?)	Yes	SMPR 2009.001 – SMPR for Quantitative Methods for Drug Residues in Shrimp, Tilapia, Catfish, and Salmon; SMPR criteria met according to ERP
Feedback from Users of Method	Yes	Discussed in ERP Meeting

DOCUMENTATION	**Available?**	**Comments**
Safety Evaluation	Yes	Completed; Discussed in ERP meeting
Reference Materials	No	None specified in SMPR; none available
SLV or PTMs	Yes	Hurtaud-Pessel et al., *J. AOAC Int.* **96**, 1152(2013) Andersen et al., *J. AOAC Int.* **98**, 636(2015)
Approved Validation Protocols	No	Used SMPR; OMA appendix D, and help from Chemical Contaminants Community subgroup
Statistics Review	Yes	Completed
Method Published in OMA	Yes	2012.25
Method Performance vs SMPR criteria	Yes	SMPR 2009.001 – SMPR for Quantitative Methods for Drug Residues in Shrimp, Tilapia, Catfish, and Salmon
Feedback Information	Yes	Discussed in ERP meeting
Additional Recognition(s)	No	
ERP Reports	Yes	10/2012; 12/2015
Manuscript(s) Published in JAOAC	Yes	Hurtaud-Pessel et al., *J. AOAC Int.* **96**, 1152(2013) Andersen et al., *J. AOAC Int.* **98**, 636(2015) Schneider & Andersen *J. AOAC Int.* **98**, 658(2015)

ERP Method Recommendation (Final Action/Repeal/Continuation)	**Final Action**	**Method scope expanded and the latest version of the method approved by ERP is in Collaborative Study Manuscript published in 2015 by Schneider and Andersen.**

EXAMPLE

ERP Recommendations

- Supply all documentation to AOAC by established deadline
  - Documentation includes ERP review details
- Representative from ERP present at OMB review meeting
- If method to be repealed, document reasoning

TRACT 9

PUBLICATIONS

Publication of First Action Methods

- Any approved method(s) along with supporting manuscript(s) and documentation sent to AOAC Publications after the meeting.
  - AOAC Official Methods number assigned.
  - Method and method manuscript prepared for publication in the *Official Methods of Analysis of AOAC INTERNATIONAL* and in Journal of AOAC INTERNATIONAL
  - Updates on methods approved or status changes are published in the Inside Laboratory Management magazine and on the AOAC website

Format for AOAC *Official Methods of Analysis*

The language of the method should be concise and completely free from ambiguity. Conciseness is desirable, both to ensure clarity and to save space. Whenever there is a conflict between clarity and style, clarity is more important.

Present Tense and Imperative Mode
- Check sentences that do not begin with a verb and change them, if feasible, to the imperative mode (e.g. Pipet 10 mL..., Stir..., etc.). Exceptions are: use of adverb modifier ("Accurately weigh..."), prepositional clause ("For refined sugars, use..."), permissive statements ("Ferric hydroxide may be used..."), and statements in the "Principle" section.

Abbreviations
- Most abbreviations are the same as those used by Chemical Abstracts. Do not use abbreviations in titles and headings. See the *Definitions of Terms and Explanatory Notes*.

Repetition and Redundancy
- Eliminate repetition and redundancy as far as possible; use only for emphasis. Do not use "distilled" with water, "concentrated" with common acids, "95%" with alcohol, or "ACS" with reagents covered by ACS specifications. These are understood by definition.

Terminology, Formulae and Chemical Names
- For names of chemical compounds, use the spelling, hyphenation, and word division given in Chemical Abstracts. Use a national pharmacopeia for names for drugs. Use ISO nomenclature for pesticides and Codex nomenclature for names of food additives and color additives.

Consistency
- Watch for internal contradictions in the text: volumes that do not add up or that exceed the capacity of the container; too abrupt a transition from one operation to another (a line may be omitted); and impractical or impossible numbers (e.g., 100 g NaCl will not dissolve in 100 mL water).

Cross-references
- All new AOAC methods should be written as complete and self-contained as practical. Do not refer to other AOAC methods. If part of a procedure in an *Official Method*℠ is taken from material previously published elsewhere, incorporate those steps in the method rather than referring the analyst to another publication.

Definitions
- The section "Definition of Terms and Explanatory Notes," *Official Methods of Analysis of AOAC INTERNATIONAL*, is the basic guide to conventions and consistency.

Illustrations and Tables
- If symbols are used on the figure, include an explanation in the caption or text. Provide descriptive titles for tables. Explain any obscure headings in a footnote.

Bibliographic References
- Check all references for accuracy. Use standard Chemical Abstracts abbreviations for *Journal* titles. In general avoid references in method. Cite background references in the "Introduction" or "Discussion" section of the collaborative study manuscript -- not in the method. If part of a procedure in an *Official Method*℠ is taken from material previously published elsewhere, incorporate those steps in the method rather than referring the analyst to another publication.

Safety
- All methods must be reviewed for safety and potential hazards. Methods should automatically incorporate cross-references to the safety statement(s), or present questioned conditions to the attention of the Committee on Safety for resolution.
- Decisions regarding inclusion of safety statements should be practical, recognizing that overuse will be self-defeating.
- Methods that create toxic, obnoxious or environmentally hazardous fumes and wastes should contain practical directions for disposal.

Checking Edited Copy and Proofreading
- The author must review a copy of the original version and edited copy to ensure that there has been no change in meaning, to correct typographical errors, and to answer any questions posed by the editor. The author must review the typeset method for accuracy.

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Online Technical Resources

Method Development, Optimization & Validation
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- Homogeneity
- Guide for Writing Methods in AOAC Format
- Statistics Protocol Review Form
- OMA - Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis
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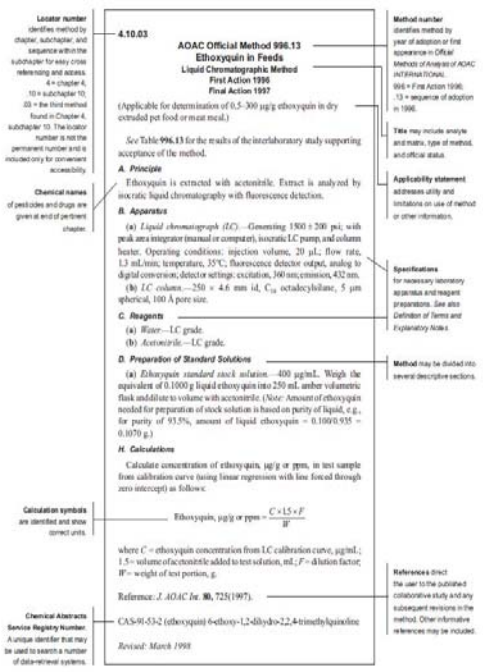
All resources are accessible at
http://www.aoac.org/imethguidelines.htm

For questions, please contact:
P 301-924-7077 x157 E dmckenzie@aoac.org

Guide to Method Format

(Method shown is incomplete to allow space for description.)



The AOAC style used for preparing methods for publication in the *Official Methods of Analysis of AOAC INTERNATIONAL* includes the following essentials:

- ✓ Standardized format that follows the order of laboratory operations.
- ✓ Use of the imperative mode.
- ✓ Cross-references to identical reagents, apparatus, and operations.
- ✓ Use of standardized definitions, terminology, and style.
- ✓ Use of accepted abbreviations and simplifications.
- ✓ Use of SI units
- ✓ Methods should be written as complete and self-contained as practical.
- ✓ Normality should be referred in terms of Molarity.
- ✓ ppm should be changed to mg/kg or mg/L
- ✓ ppb should be changed to ng/g or ng/mL
- ✓ ppt should be changed to pg/g or pg/mL

FORMAT OF AOAC® *OFFICIAL METHODS of ANALYSIS OF AOAC INTERNATIONAL*

Title:
- Includes analyte being determined, type of matrix (matrices), and analytical technique used for analysis.

Applicability:
- Includes list of matrix(es) along with specific matrix types and range or limits of determination or detection.

Precautions:
- Makes an analyst aware of hazardous materials used in analysis.

Data Collection:
- Table(s) that presents performance parameters including matrices tested in a collaborative study, levels of analyte(s), % recovery, RSD_r, RSD_R, s_r, s_R, HORRAT, number of observations, etc

Principle:
- Explains scientific premise on which the method is operates specifically the mechanism of the analysis.

Apparatus:
- Lists the equipment that requires assembly or that has specifications critical to the method performance. Describe equipment in terms of performance characteristics.

Reagents:
- List the reagents with amounts and appropriate units needed to conduct the analysis and describe the reagents in terms of performance characteristics.

Sample and Test Portion Preparation:
- Describe the preparation of samples and the test portion.

Determination:
- Describes the actual analysis.

Calculations:
- Section that explains how to calculate final results; presented in a form of equation or description.

Other sections as needed

REFERENCING *AOAC® OFFICIAL METHODS*℠

When referencing *AOAC® Official Methods*℠, only the method number should be used as seen in the following example:

(1) *Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method **2008.01**

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Publication of First Action Methods

NO OMA NUMBER ASSIGNED

UNTIL ALL DOCUMENTATION SUBMITTED

1. Method incorporating ERP revisions (preferably in AOAC Format)
2. Method Manuscript incorporating ERP revisions (in AOAC Format)
3. Signed AOAC Copyright Authorization form

Format for AOAC First Action *Official Methods* Manuscripts and Protocols

FORMAT FOR FIRST ACTION OMA MANUSCRIPTS

TITLE: Title of manuscript includes method title which includes the analyte(s), matrix(es), and analytical technique, if applicable. It may also include a *common* method name and ends with "Collaborative Study."

AUTHOR(S): Provides authors' full (e.g. no initials) names and contact information.

ABSTRACT:
- ✓ Specific information on the method and study.

INTRODUCTION:
- ✓ Information on why collaborative study was conducted, how many collaborators participated in the study, previous work done, and information on compound or process that was studied.

COLLABORATIVE STUDY:
- ✓ Information on matrices and number of test samples tested, test sample preparations, instructions for collaborators, etc.

METHOD:
- ✓ Written in AOAC style.

COLLABORATORS' COMMENTS:
- ✓ Any comments and suggestions received from collaborators and information on how they were addressed, e.g., incorporating instructions into the method, etc.

RESULTS AND DISCUSSION:
- ✓ Information on type of statistical analyses performed on raw data, reasons for rejecting some of the data, discussion of results with references to tables and figures, discussion of the method performance, etc.

RECOMMENDATION:
- ✓ Recommendation to adopt method First Action.

ACKNOWLEDGMENTS:
- ✓ Full names and addresses of all collaborators that participated in the study.

REFERENCES:
- ✓ Included all references cited in the text.

APPENDICES or **FIGURES AND TABLES:**
- ✓ Include any figures and tables that may make the manuscript and the performance of the method easier to understand and interpret.

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All resources are accessible at
http://www.aoac.org/vmethodguidelines.htm

For questions, please contact:
P 301-924-7077 x157 E dmckenzie@aoac.org

TRACT 10

MODIFICATIONS

Modifications of Methods

- During First Action and Final Action, methods can be modified or extended to additional matrixes and/or analytes.

Submitting a Modification

Standards Development

- Contact staff and they will let you know the best way to submit the modification information and any additional requirements.
  - Staff will inform of the appropriate mechanism to submit a modification.
- Fully revised method manuscript and a revised version of the AOAC OMA method, both in OMA format, must be submitted.

Research Institute

- Submit request for modifying a method through the AOAC website.
  - AOAC > Research Institute > Method Submission
  - AOAC RI Application for Method Change or Modification
- Fully revised method manuscript and revised method, both in OMA format, must be submitted.

Processing Modifications

ERPs from Standard Development and Research Institute

- Review of the modification will undergo a preliminary review by at least the AOAC CSO.
  - Comments to be shared with method author.
- Original ERP reviewers will be assigned to review the method
- Method will be added to ERP agenda for their next meeting

Approval of Modifications

- If ERP approves a method modification including extensions, then the method begins a new two (2) year period.

- If the method modification is to correct an editorial error, then the method, then there is no change.

Method modifications require substantiation of the modification or extension with proof of method performance as deemed suitable by the EPR.

TRACT 11

DOCUMENTATION

Reports and Documentation

- AOAC staff or designee will capture the decisions and action items into an ERP report.
- The draft report will be sent back to the ERP Chair whose responsibility it is to sign off on the report once approved.
- The report is then distributed to the ERP.
- ERP is responsible for a drafting a written recommendation to the OMB for each method at a maximum of two years following adoption as First Action OMA
- Approved methods from the ERP meetings are published in the OMA and in the *Journal of AOAC INTERNATIONAL*.
- Meeting overviews are published in the AOAC *Inside Laboratory Management* magazine.

TRACT 12

SUMMARY OF RESPONSIBILITIES

Roles and Responsibilities

- Expert Review Panel:
  - Review methods and meet in person to discuss and render decisions on methods for First Action *Official Methods* status.
  - Track First Action *Official Methods*
  - Modify First Action methods if necessary
  - Make recommendations on First Action methods no more than 2 years after adoption to OMB.
- Official Methods Board:
  - Vet and approve ERP membership
  - Assign OMB liaison to be a resource to the ERP
  - Review ERP recommendations and render decisions (*Final Action, Repeal or remain First Action*) on First Action OMAs
- AOAC Staff
  - Coordinate the ERP and meetings, facilitate reviews, document ERP actions/decisions.
  - Issue necessary calls for experts and methods

Task Force on Communication/ ERP Best Practices

Recommendations for Staff

- Regularly debrief with ERP Chairs for input after meetings
- ERP background and training materials on website
- Offer orientation on a regular basis, to all ERP chairs and potential members, wider distribution of training materials
- Execute post training surveys
- Clearly outline expectations of reviewers prior to meeting: attendance is mandatory, cursory review of all methods to be discussed
- Encourage all method authors to attend ERP: helps process move smoothly and authors will only be privy to full discussion if they attend
- Establish a codification system in OMA for “dispute resolution methods” *
- Investigate ways to elevate the level of prestige for participation in an ERP.

** Project specific*

Task Force on Communication/ ERP Best Practices

Best Practices for ERP Chairs

1. Work closely with staff during the orientation period for ERP
2. Clearly understand consensus and quorum rules
3. Discourage abstentions unless a true conflict of interest is present; *use discretion as necessary when determining if a vote allows a method move forward.*
4. Encourage ERP reviewers to be fully prepared
5. Add brief orientation to ERP meeting agenda
6. Where in a stakeholder panel community requires only one method is desired, a 2 step process that considers multiple methods may be adopted as First Action and assessment of the best method is determined during follow up ERP meetings.
7. When considering methods for repeal, advise ERP members that repeal does not discredit method, it is simply a procedural determination that a method will not be moved forward.

Expert Review Panels

The ERPs review and approve appropriate methods (as submitted or modified) for adoption as First Action Official Methods or for further validation. ERPs also make recommendations regarding Final Action Official Methods status.

Expert Review Panels

- Must be supported by relevant stakeholders.
- Constituted for the review of methods, not for Standard Method Performance Requirements (SMPR) purposes or as an extension of a Working Group.
- Consist of a minimum of seven (7) members representing a balance of expert stakeholders. **Quorum is a minimum of 7 members present or 2/3 of the total vetted members, whichever is greater.**
- ERP constituency must be approved by the Official Methods Board (OMB).
- Holds transparent public meetings only.
- Remains in force as long as method in First Action Status.

First Action Official Method Status decision

- Must be made by an ERP constituted or reinstated post 2011-03-28 for First Action Official Method Approval (FAOMA).
- Must be made by an ERP vetted for FAOMA purposes by OMB post 2011-03-28.
- Method adopted by ERP must perform adequately against the SMPR set forth by the stakeholders. Or demonstrate performance or characteristics that meet the scope, applicability and/or claims of the method.
- Method must be adopted by unanimous decision of ERP on first ballot, If not unanimous, negative votes must delineate scientific reasons.
- Negative voter(s) can be overridden by 2/3 of non-negative voting ERP members after due consideration
- Method becomes First Action Official Methods on date when ERP decision is made.
- Methods to be drafted into AOAC format by a knowledgeable AOAC staff member or designee in collaboration with the ERP and method author.
- Report of FAOMS decision complete with ERP report regarding decision including scientific background (references etc) to be published concurrently with method in traditional AOAC publication venues.

Method in First Action Status and Transitioning to Final Action Status

- Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.
- Two years maximum transition time (additional year(s) if ERP determines a relevant collaborative study or proficiency or other data collection is in progress).
- Method removed from First Action Official Methods and OMA if no evidence of method use available at the end of the transition time.
- Method removed from First Action Official Methods and OMA if no data indicative of adequate method reproducibility is forthcoming as outlined above at the end of the transition time.
- ERP to recommend Method to Official Final Action Status to the OMB.
- OMB decision on First to Final Action Status

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Online Technical Resources

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All resources are accessible at
http://www.aoac.org/vmethguidelines.htm

For questions, please contact:
P 301-924-7077 x157 E dmckenzie@aoac.org

About Expert Review Panels (ERPs)

ERP OVERVIEW:

An Expert Review Panel (ERP) is assembled to review and adopt methods as Official First Action. ERPs will track Official Methods for two years or until such time as reproducibility has been demonstrated and cumulative feedback on method use and performance are obtained. ERPs will make a recommendation regarding Final Action method status for all OMAs to the Official Methods Board (OMB).

All ERP members are expected to serve with the highest integrity and without direct or indirect conflicts of interest. A method assignment can last two years. All members of the ERP are expected to actively participate in ERP meetings and to perform duties and reviews in timely fashion. All members should maintain strict adherence to review timelines and deadlines. AOAC staff documents ERP deliberations.

ESTABLISHING AN EXPERT REVIEW PANEL:

- AOAC staff issues a Call for Experts:
  - Based on voluntary consensus standards and methods submitted to AOAC INTERNATIONAL that may meet the standards.
  - Proprietary and sole source method developers submit individual methods to the AOAC Research Institute.
  - Candidates are asked to submit a CV or information that demonstrates expertise to AOAC staff if not already part of a recognized pool of experts.
- AOAC Chief Scientific Officer (CSO) reviews the documentation for the candidates and make recommends a slate for an expert review panel including the chair to the Official Methods Board.
- The candidate list and supporting documentation are forwarded to the Chair of the OMB who will assign the review to at least two OMB members.
- The OMB reviewers will review the candidates for expertise and perceived conflicts of interest and the OMB may then approve the members of the ERP. A Chair for the ERP is also approved.

EXPERT REVIEW PANEL (ERP):

- Review, discuss and demonstrate consensus on methods for Official First Action method status.
- Participate in the publications process of First Action methods.
- Track and discuss feedback all First Action methods for two years.
- Reach and demonstrate consensus on recommendations for Final Action method status.
- Actively participate in the broader stakeholder effort.

ERP CHAIR:

- Lead ERP discussions in the review and adoption of methods for First Action Official Methods.
- Participate in stakeholder panel activities.
- Review and approve ERP report.
- Work with AOAC staff, working groups and other stakeholder panels to ensure a thorough understanding of the standard method performance requirements and the methods to be assessed.
- Implement the OMB First Action to Final Action Guidelines with the ERP members.
- Advise and review First Action methods and post First Action publications.
- Represent the ERP in presenting the ERPs recommendation to the Official Methods Board regarding Final Action method status.

MECHANICS OF AN AOAC EXPERT REVIEW PANEL

- AOAC CSO assigns methods for review to the expert review panel members.
- For each method, 2 ERP members are assigned as primary and secondary reviewers and present at the ERP meeting.
- All members are expected to actively participate and review methods for First Action Official Method status - conducting thorough and prompt review of methods and being prepared to speak on assigned methods at ERP meetings
- The ERP chair and the 2 reviewers for each method are expected to participate in the publications peer review process for First Action methods.
- ERP reviewers track assigned methods that were adopted as First Action Official Methods and update ERP on method use during two year period between First Action and Final Action
  ERP members are expected to participant in the stakeholder panel activities and/or community at large .

- *ERPs can work with topic advisors (aka, subject matter experts)*
- *OMB can recognize a pool of experts from which ERP members can be selected*

Eligibility Criteria for Expert Reviewers

Be a key expert and/or thought leader of the method or priority under consideration.

- Demonstrated knowledge in the appropriate scientific disciplines.
- Demonstrated knowledge regarding data relevant to adequate method performance.
- Demonstrated knowledge of practical application of analytical methods to bona fide diagnostic requirements.

Be approved by the Official Methods Board

- Qualifications must be clearly described and submitted to AOAC headquarters.

Duties of Expert Reviewers

Members of the Pool of Experts will be called upon to serve on ERPs as needed and to review documents .These documents may include:

- Procedural documents on how methods will be selected and how single laboratory validation studies will be done;
- Methods submitted for consideration as First Action Official Methods;
- Methods submitted for selection for further validation studies;
- Protocols to be used for single laboratory validation studies;
- Selection of methods to be considered for full collaborative studies; and
- Validation study reports

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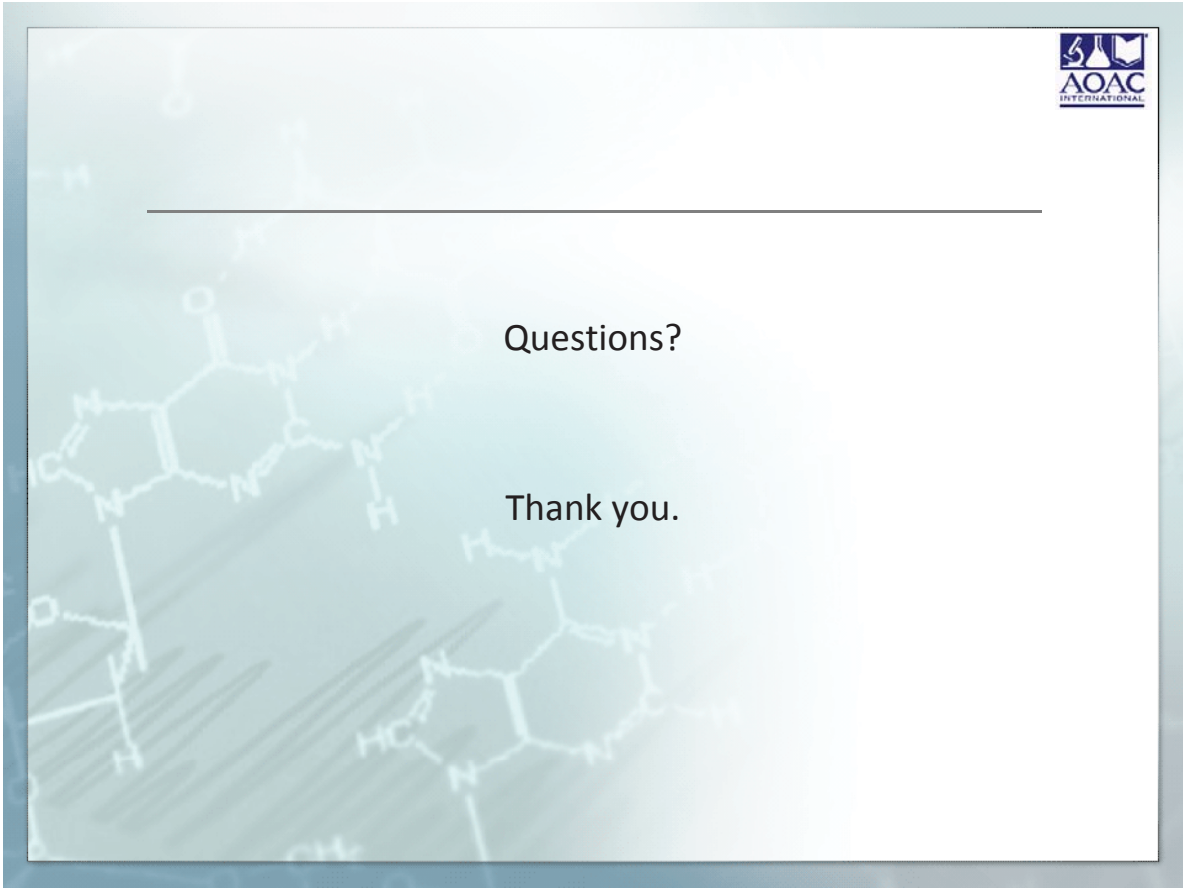


First Action Method Updates

Expert Review Panel Tracking and Recommendations of First Action Methods

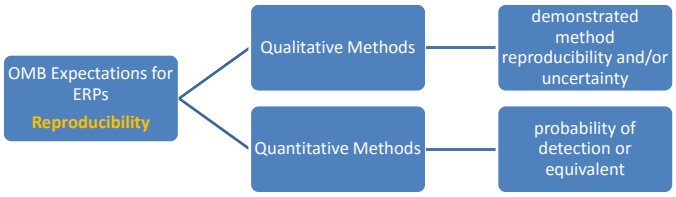
AOAC Policies & Procedures

- Policy on Antitrust
- Policy on Use of Association Name, Identifying Insignia, Letterhead, Business Cards
- Policy on Volunteer Conflict of Interest
- Expert Review Panel Policies and Procedures
- OMA Appendix G

OMA, Appendix G

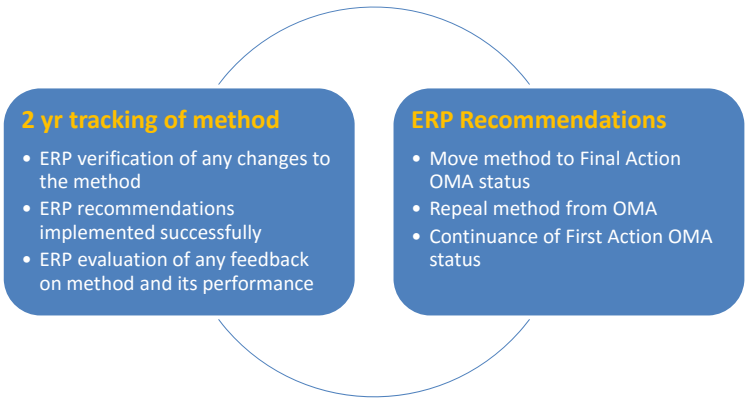
Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.

- ERP is looking to verify if method reproducibility has been appropriately assessed and satisfactorily demonstrated



OMA, Appendix G

Two years maximum transition time (additional year(s) if ERP determines a relevant collaborative study or proficiency or other data collection is in progress).



OMA, Appendix G

Method removed from Official First Action and OMA if no evidence of method use available at the end of the transition time.

First Action OMA Tracking

- Tracking period is ≤ 2 years and begins on the date of the ERP's decision to adopt a method for OMA First Action status.

No Use in 2 Years

- Repeal from OMA

OMA, Appendix G

Method removed from Official First Action and OMA if no data indicative of adequate method reproducibility is forthcoming as outlined above at the end of the transition time.

First Action OMA Tracking

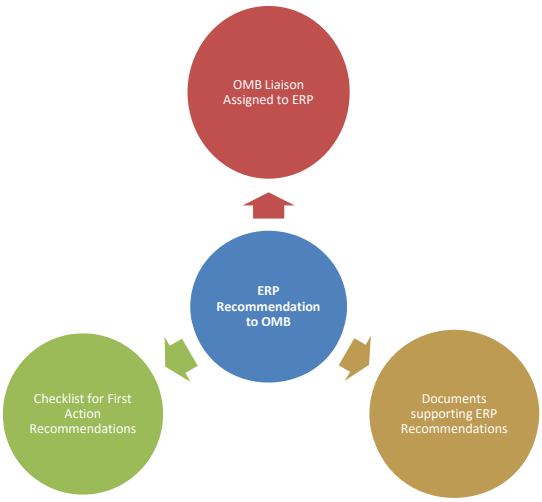
- Tracking period is ≤ 2 years and begins on the date of the ERP's decision to adopt a method for OMA First Action status.

No Demonstration of Method Reproducibility in ≤ 2 Years

- Repeal from OMA

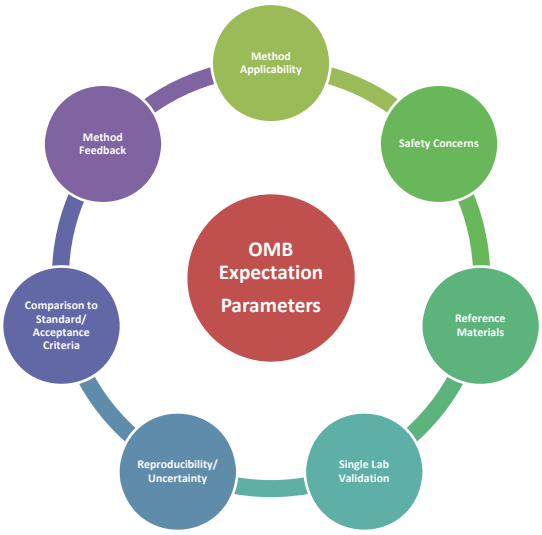
OMA, Appendix G

ERP to recommend Method to Official Final Action Status to the OMB.

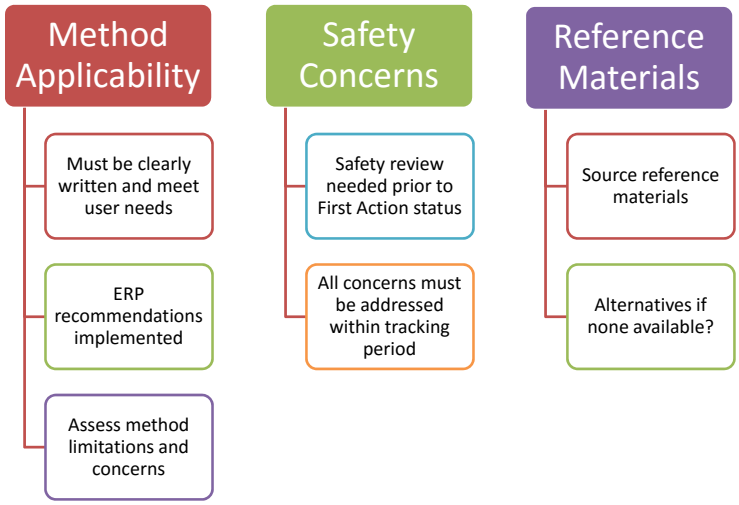


OMA, Appendix G

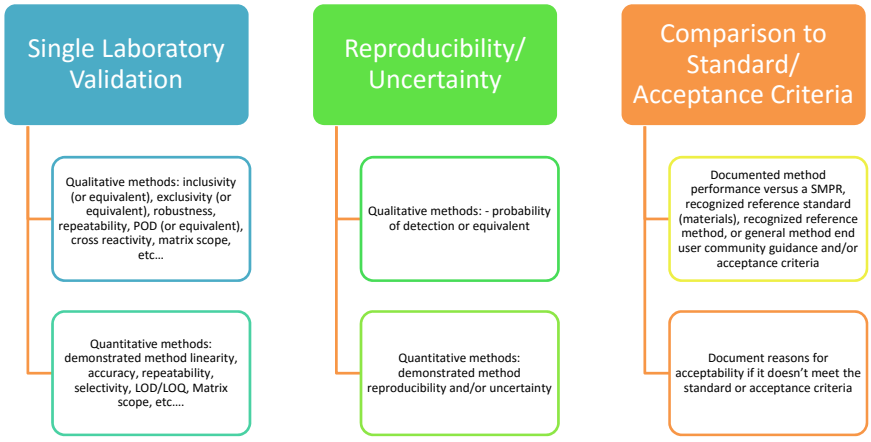
First Action to Final Action Methods: Guidance for AOAC Expert Review Panels



OMB Expectation Parameters



OMB Expectation Parameters



OMB Expectation Parameters

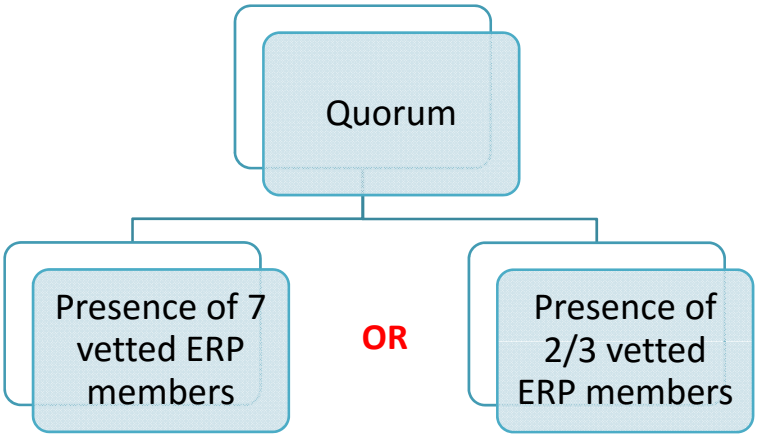
Method Feedback from End Users

Consider any positive or negative feedback on overall method performance, applicability, availability of reference materials, matrix scope, method component sourcing, robustness or ruggedness parameters.

Documentation Needed

- Method Safety Evaluation
- Reference Materials
- Evidence of Single Laboratory Validation or equivalent
- Evidence of Reproducibility Assessment
- Published First Action OMA
- Method Performance versus SMPR *or* acceptance criteria
- Final draft of First Action OMA to be considered for status update
- Rationale or Justification for Repeal or Continuance of First Action OMA

ERP Meetings



WHICHEVER IS GREATER

ERP Meetings

METHOD AUTHOR: present any method feedback obtained and any resulting changes to the method, any reproducibility information, any implemented ERP recommendations, final draft of method proposed for decision

ERP MEMBERS: present any method feedback obtained and discuss any resulting changes to the method, any reproducibility information, any implemented ERP recommendations, review and agree upon final draft of method proposed for decision, and make a recommendation to OMB.

CONSENSUS: 2/3 vote in favor of a motion. Abstentions do not count towards vote; in case of multiple abstentions. Staff will monitor and record consensus voting.

STAFF: Will organize and coordinate meeting, record ERP actions and decisions, draft ERP report and distribute after chair approval, work with chair and OMB liaison to complete checklist and assemble recommendation package for OMB.

Questions?

Thank you.

2016 SPSFAM Food Allergens ERP - Roster

John Szpylka - Chair	Merieux NutriSciences
David Almy	Neogen Corporation
Sneh Bhandari	Merieux Nutrisciences
John Lawry	Covance
Linda Monaci	National Research Council of Italy
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