AOAC Research Institute

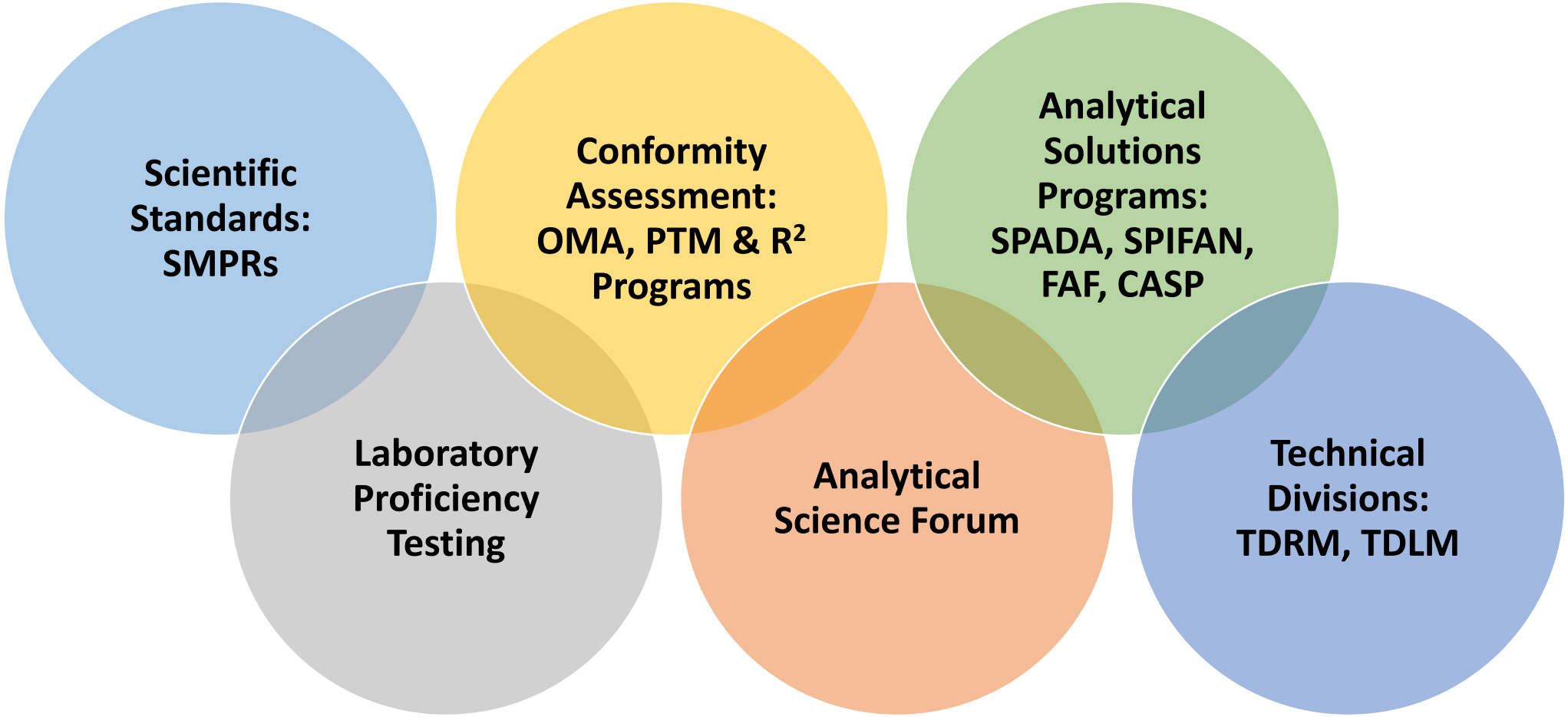
Module P13: AOAC INTERNATIONAL Conformity Assessment Programs – *Reviewed and Recognized*℠ Program

About AOAC INTERNATIONAL

AOAC INTERNATIONAL brings together government, industry, and academia to establish standard methods of analysis that ensure the safety and integrity of foods and other products that impact public health around the world

- Leader of analytical excellence dedicated to developing and validating standards, methods and technologies, of global relevance to advance food safety, food integrity, and public health.
- AOAC INTERNATIONAL is a 501(c)(3), independent, third party, not-for-profit association and voluntary consensus standards developing organization.

Scientific Solutions



Reviewed and Recognized℠ (R^2) Program

Overview

The AOAC Research Institute (AOAC RI) is a division of AOAC INTERNATIONAL that promotes and conducts activities to help develop, improve, and validate proprietary testing methods.

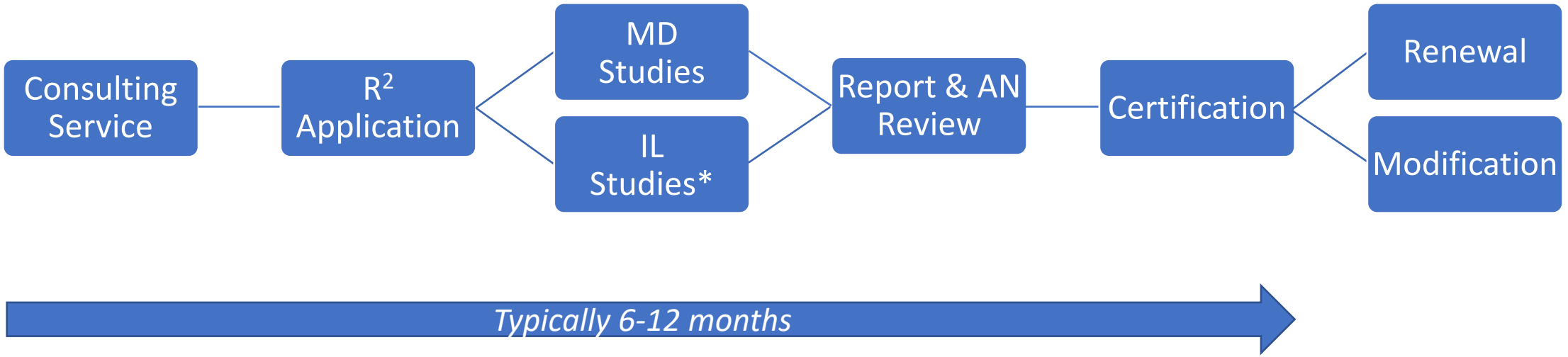
- Incorporated in 1991 as a wholly-owned subsidiary of AOAC INTERNATIONAL
- Merged into AOAC INTERNATIONAL in 2019 as a division
- Administers the *Performance Tested Methods*SM (PTM) Program
- Administers the *Reviewed and Recognized*SM (R^2) Program
- https://www.aoac.org/scientific-solutions/research-institute-ptm/

Overview

Reviewed and Recognized (R^2) Program

- Helps companies and their customers safeguard their businesses, mitigate risk, and protect consumers through trusted, reliable testing methods and products.
- Provides independent third-party review and certification for equipment/component method performance.
  - Analogous to the PTM program, but for non-test kit methods.
- The certification mark assures users that an independent assessment found that method performance meets an appropriate standard for its intended use.
- Can be the first step toward *Official Method*℠ status

R^2 Process and Timeline



MD = Method Developer; IL = Independent Laboratory

*Traditional or On-Site Validation

Consulting Service – Optional Fee for Service

Step 1: Application

- Includes method applicability statement (target analyte(s) and matrixes), method instructions, relevant method safety information, and whether harmonized with another program
- Applicant will be invoiced within 2-3 business days of submission

Step 2: Develop Validation Testing Protocol and Facilitate Review Process

- Validation outline designed to comply with current AOAC Guidelines and/or SMPRs
- Includes appropriate reference materials and methods

Consulting Service

Step 3: Expert Peer Review and Approval

- Technical consultants work with AOAC volunteer experts to ensure review and approval of testing protocols
- Statistical review (if applicable)

Step 4: Approved Testing Protocols and Plans

- AOAC approved testing protocols are valid for one year from the date of approval.
- Testing protocol for Method Developer and Independent Laboratory Studies

Data Requirements - Chemistry

Validation Studies

1. Calibration Model – typically carried out by MD, in some cases also the IL
2. Selectivity - can be carried out by MD or IL
3. Matrix Testing – MD and IL
   a. All matrixes tested by MD + 1/5 tested by IL
   b. All matrixes tested by IL
4. Robustness – can be carried out by MD or IL
5. Equipment/Component Consistency and Stability –MD; may be able to use QA data

See specific training modules for more details on the validation studies

Data Requirements - Microbiology

Validation Studies

1. Inclusivity/Exclusivity – can be carried out by MD or IL
2. Matrix Testing
   a. All matrixes tested by MD + 1/5 tested by IL
   b. All matrixes tested by IL
3. Robustness – can be carried out by MD or IL
4. Equipment/Component Consistency and Stability – typically carried out by MD; may be able to use QA data

See specific training modules for more details on the validation studies

Independent Laboratory Options

- Traditional Process
  - Equipment and components are shipped to the Independent Laboratory (IL)
  - MD provides training to IL on how to perform the method
  - IL performs study

- On-Site Process
  - MD is unable to ship large equipment or method is intended to be performed only by the MD (i.e., fee for service method)
  - IL prepares blinded validation samples and/or test portions
  - Analyst from IL performs study at MD site side by side with MD on blinded validation test portions with AOAC Technical Consultant present as observer

Validation Guidelines

Various guidelines for microbiology and chemistry SLV and MLV studies are available and can also be found as appendices in the AOAC *Official Methods of Analysis*SM *of AOAC INTERNATIONAL*:

http://www.eoma.aoac.org/appendices.asp

Standard Method Performance Requirements

- **Standard Method Performance Requirements (SMPRs®)** are voluntary consensus standards, developed by stakeholders, that prescribe the minimum analytical performance requirements for classes of analytical methods.
- SMPRs are unique to AOAC's processes and were introduced in recognition of the fact that acceptance criteria are critical to evaluating the suitability of any testing protocol for its intended use.
- Methods that meet the performance requirements can claim conformity to the SMPR

AOAC SMPR 2018.009

***Standard Method Performance Requirements* (SMPRs®) for Lactose in Low-Lactose or Lactose-Free Milk, Milk Products, and Products Containing Dairy Ingredients**

Intended Use: Method for Confirming Compliance with Regulatory Standards and Dispute Resolution

1 Purpose

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

2 Applicability

Measure lactose found in milk, milk products, and products containing dairy ingredients that are low-lactose or lactose-free. The analytical method must account for potential interferences (*see* Table 1) in these matrices. This scope includes "lactose-free" infant formulas and adult nutritionals.

3 Analytical Technique

Any analytical technique(s) that measures the analyte(s) of interest and meets the following method performance requirements is/are acceptable.

4 Definitions

Infant formula.—Breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infant during the first months of life up to the introduction of appropriate complementary feeding (Codex Standard 72-1981) Infant Formula and Formulas for Special Medical Purposes – 0–12 month of age; Follow-Up Formula – from 6–12 months and for young children; Young Children – 12–36 months of age; Foods for Special Medical Purposes Nutritionally complete specially formulated food for adults, consumed in liquid form, which may constitute the sole source of nourishment (AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals; 2010). Made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch and amino acids, with and without intact protein.

Lactose.—β-D-galactopyranosyl-(1→4)-D-glucose. CAS No. 63-42-3 (*see* Figure 1).

Limit of detection (LOD).—The lowest concentration or mass of analyte in a test sample that can be distinguished from a true blank sample at a specified probability level.

Limit of quantitation (LOQ).—The lowest level of analyte in a test sample that can be quantified at a specified level of precision.

Milk and milk products.—Milk is defined as the normal mammary secretion of a milk animal, intended for consumption as liquid milk or for further processing

Milk product is defined as a product obtained by any processing of milk. Although a milk product shall be made from milk, the definition does not hinder the milk from being subjected to various processing steps before it becomes an end product.

Composite milk product is a product of a milk product and other food(s) where the milk constituents are an essential part in terms of quantity of the final product. [Bulletin of IDF 397 (2005) *The Codex General Standard for the Use of Dairy Terms, Its Nature, Intent and Implications*]

Recovery.—Fraction or percentage of analyte that is measured when the test sample is analyzed using the entire method.

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

Reproducibility.—Variation arising when identical test materials are analyzed in different laboratory by different operators on different instruments. The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (%RSD_R).

5 Method Performance Requirements

See Table 2.

6 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks, and appropriate check standards.

7 Validation Guidance

Recommended level of validation: *AOAC Official Methods of Analysis*℠.

Method data packages must include relevant data regarding interferences and instabilities, such as listed in Table 2. Not all interferences are likely to occur in all matrices. Method developers are responsible for assessing interferences with the method.

8 Maximum Time-to-Results

None

9 Reference and Harmonization Materials

See Tables 3 and 4 and Figure 2.

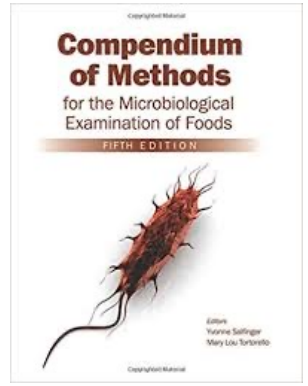
Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 21st Ed. of the *Official Methods of Analysis of AOAC INTERNATIONAL* (2019). Available at http://www.eoma.aoac.org/app_f.pdf

Approved by the AOAC Stakeholder Panel on Strategic Food Analytical Methods (SPSFAM). Final Version Date: August 2018.

Reference Methods

Must be applicable to the analyte/matrix and may include (but are not limited to):

FDA, FSIS, AOAC *Official Methods of Analysis* (OMA), ISO, EPA, Health Canada, American Public Health Compendium of Methods, validated methods from peer reviewed literature (chemistry)



Reference Materials and Reference Standards

Available at (but not limited to):

National Institute of Standards and Technology (NIST), EPA, National Research Council Canada (NRC), US Pharmacopeia (USP), Institute of Reference Materials and Measurements (IRMM), Chemical Suppliers

Statistical Tools

Excel Workbooks are available for analysis of qualitative and quantitative data for SLVs and MLVs.

An Excel Calculator is available for MPN determination.

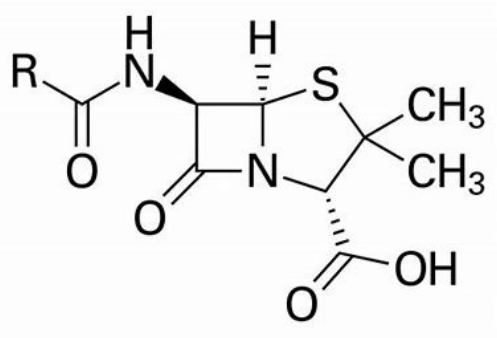
(Note that the MPN calculator is not compatible with iMac.)

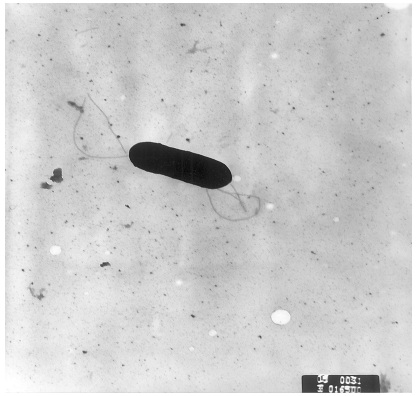
Ask a Technical Consultant for the current versions.

Method Claims

Analyte

- The analyte(s) detected or determined by a method must be clearly stated in the intended use (applicability) statement of the method.
  - For example, metabolites or marker residues of pesticides and animal drugs.
  - *Listeria* spp. claims must specify which species are included in the claim. Recognized *Listeria* species include *L. grayi*, *L. innocua*, *L. ivanovii*, *L. marthii*, *L. monocytogenes*, *L. rocourtiae*, *L. seeligeri*, and *L. welshimeri.*
  - *Salmonella* claims must distinguish between *Salmonella enterica* and *Salmonella* spp. (*S. enterica* + *S. bongori*).
- Any limitations to the analyte claim must be clearly stated in the application note.





Method Claims

Matrix

- The matrix claim is the scope of matrixes included in the validation study and must be expressly stated in the intended use (applicability) statement of the method.
- The R^2 program allows certain general claim statements, but the list of specific matrixes from the validation study must also be included.
- The specific matrixes examined in the validation study are important for ISO 17025 certified laboratories to determine whether verification or validation studies are required when implementing the method for their matrixes.





Matrix Claim – General Claim Statements

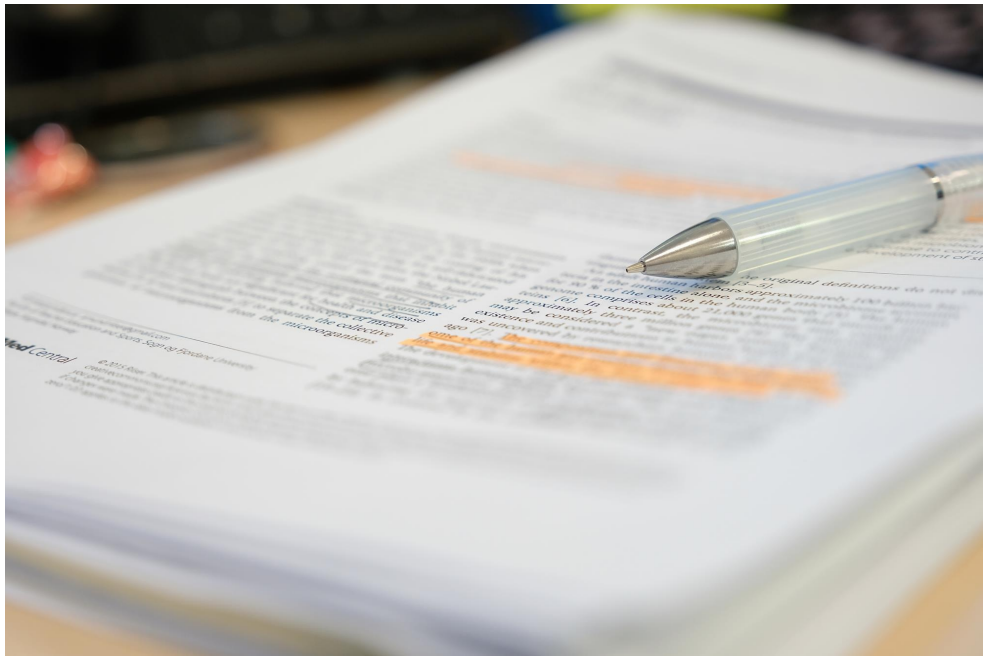
Claim	Number of Matrixes	Number of Categories
Broad Range of Foods	15	5
Variety of Foods	10	5
Selected Foods	5	2
Food Category	5	1
Environmental Surfaces	7	NA
Selected Environmental Surfaces	1-6	NA

Acceptable food category sources include:

1. ISO 16140-2:2016, Annex A: *Classification of sample types and suggested target combinations for validation studies*.
2. FDA 9-sector Food Triangle

R^2 Report

- Upon completion of a validation, method developers are required to prepare and submit a study report supporting the intended use claims of the method
- The report is prepared following a standard template provided by the AOAC Research Institute. The template is intended to facilitate submission to the *JAOAC*
- This report will include the results of both the Method Developer Validation Study and the Independent Validation Study, including all data generated during the studies



R^2 Report

- Title
- Authors
- Abstract
- General information
- Principle of the method
- Definitions
- Safety precautions
- Method
  - Applicability Statement
  - Apparatus and Supplies
  - Reagents
  - Instructions
  - Results and Interpretation
- Validation Studies
  - MD and IL Studies
  - Methodology and results, including tables and figures
- Discussion
- Conclusion
- Acknowledgements
  - Submitting Company
  - Independent Laboratory
  - Reviewers
- References

Application Note

The application note should include the following items, when applicable:

- **Intended User** - Specify the intended user
- **Environmental Factors** - Identify the environment that the test should be conducted
- **Applicability Statement** - Identify the analyte, mode of measurement and matrixes for which the kit has been validated
- **Limitations** – Disclose any known limitations or interferences of the method
- **System Suitability** – Requirements for determining valid equipment and component setup
- **Instructions** - Include complete instructions from sample preparation to data interpretation
- **Detection Limit and Limit of Quantitation** - Express in concentration (parts per million, percent, mg/kg, CFU/g, etc.) the limits of detection and quantitation
- **Precautions** - Provide warnings of safety concerns, disposal instructions, and potentially hazardous steps or components.
- **Technical Assistance** - Provide information (email, internet, telephone and FAX numbers) where the user can obtain technical assistance.

Application Note

Example Applicability Statement:

Method X was certified by the AOAC Research Institute *Reviewed and Recognized*℠ Program for determination of glyphosate, N-acetylglyphosate, AMPA, and N-acetyl AMPA in a variety of matrixes including whole durum wheat, whole oats, groats, chickpeas, dried split green peas, infant cereal, infant formula, blueberries, apple juice, and soymilk.

Example of Method Claim Limitation:

Method not applicable to products with low pH (≤4).

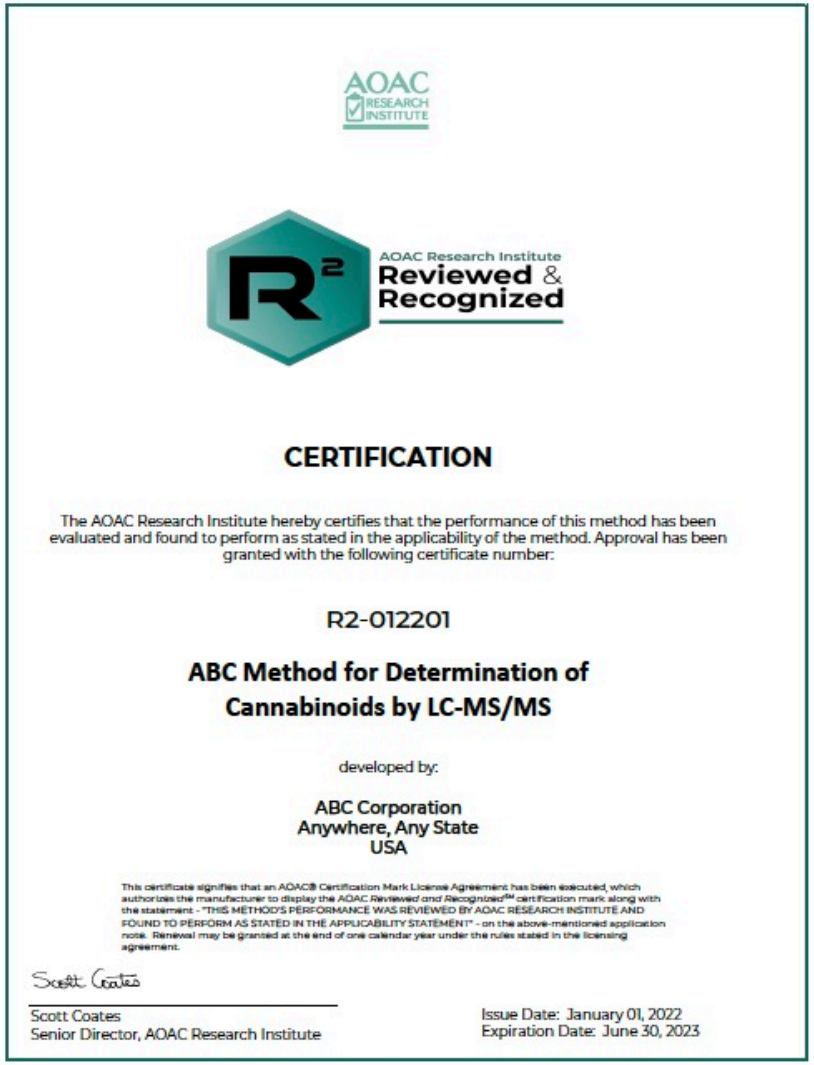
Review

- One Volunteer Expert and 2 Expert Reviewers will review the R^2 Validation Study Report and Application Note to determine acceptability as a *Reviewed and Recognized* method.
- Reviewers will provide recommendations to the AOAC-RI Project Manager for awarding or denying R^2 status.
- Upon receipt, the Project Manager will compile each reviewer's recommendations and comments into a single form for the Method Developer. The Method Developer is responsible for responding to all comments and questions in writing.
- All responses and revised documents shall be submitted to the Project Manager, who will forward them to the AOAC Volunteer Expert and Expert Reviewers for additional comment or approval.
- The process continues until consensus is reached among the three reviewers for either approval or rejection.

Certification

A ***Reviewed and Recognized***[SM] certificate is issued by the AOAC-RI to the Method Developer for each method granted R^2 status. The certificate carries a unique certification number and name of the approved test method. Certificates provide details about the method validation including:

- Overview of the method and validation
- Claimed matrixes
- Selectivity
- Results of method developer and independent laboratory matrix studies



AOAC RESEARCH INSTITUTE

AOAC Research Institute Reviewed & Recognized

CERTIFICATION

The AOAC Research Institute hereby certifies that the performance of this method has been evaluated and found to perform as stated in the applicability of the method. Approval has been granted with the following certificate number:

R2-012201

ABC Method for Determination of Cannabinoids by LC-MS/MS

developed by:

ABC Corporation
Anywhere, Any State
USA

This certificate signifies that an AOAC® Certification Mark License Agreement has been executed, which authorizes the manufacturer to display the AOAC *Reviewed and Recognized*℠ certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND FOUND TO PERFORM AS STATED IN THE APPLICABILITY STATEMENT" - on the above-mentioned application note. Renewal may be granted at the end of one calendar year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates
Senior Director, AOAC Research Institute

Issue Date: January 01, 2022
Expiration Date: June 30, 2023

Certification

R^2 certificates are made available to the public though the AOAC website. Certificates are searchable by:

- Analyte
- Matrix
- Manufacturer
- Method name
- License number

Certification Mark and License Number

- Method Developers of approved methods are licensed to use the R^2 certification mark and R^2 certification number on their packaging, application notes, and promotional materials.
- Use of the certification mark and/or number is entirely optional but highly encouraged.
- Method Developers will receive a copy of the certification mark with a unique certification number as soon as the method is R^2 approved.

R^2 Modifications

- During the life cycle of a method, changes often occur
- As a validated method is modified, its certificate is no longer applicable, regardless of the size of the change
- The AOAC Research Institute (RI) has a process for method developers to modify their methods and maintain their certifications
- These modifications can be submitted to the AOAC RI at anytime during the year and must be reviewed before being implemented

R^2 Modifications

- Modifications or changes to methods typically fall into one of three categories:
  - Changes to labeling
  - Changes to the components or reagents of the method
  - Changes to the manufacturing and/or quality process
  - Changes to the scope of the method
- When a modification to your method occurs, it is the method developer's responsibility to notify AOAC RI

Failure to notify AOAC RI of the changes may result in cancellation of your R^2 Certificate

Renewals

- An annual review is conducted on all approved *Reviewed and Recognized*SM methods.
- Method developers apply for recertification and indicate whether modifications have been made to the validated method or components.
  - Modifications will require additional review time and may require an additional fee.
- Certifications are reviewed and renewed on a yearly basis from June 01 to May 31.

Contributing Membership

AOAC created a non-voting membership category, Contributing Member, for the AOAC Research Institute. The AOAC Research Institute Advisory Council comprises representatives of the Contributing Member organizations in good standing.

Annual dues are $5,000.00 USD and benefits include:

- One seat on the RI Advisory Council
- $1,000.00 USD discount toward RI Consulting fees
- $6,000.00 USD discount toward R^2 Application fees
- Discounted Harmonized Consulting
- One complimentary individual AOAC INTERNATIONAL membership
- **Join as a Contributing Member now**
- For all questions, contact Nora Marshall at **nmarshall@aoac.org**

R^2 Program Fees

AOAC RI Fees	Price[c]	RICM Price[c]
Consulting – R^2	$3000	$2000
Consulting - Harmonized	$4000	$3000
Application – R^2	$21,000[a]	$15,000[a]
Application – R^2/OMA Harmonized	$56,000	$30,000[b]
RI Contributing Membership (RICM)	$5000	
Annual Renewal	$3000	

[a]A $500 discount applies if the Consulting Service was used
[b]If also an AOAC Organizational Member
[c]**Discounted introductory pricing of $10,000 for the R^2 Application in effect through March 31, 2022**.

The cost of the Independent Lab Study depends on the scope and nature of the matrix study and whether other studies (e.g., selectivity) are also included in the Independent Lab work. Your Technical Consultant can provide a nonbinding ballpark estimate for your situation.

METHOD CONFORMITY ASSESSMENT PROGRAMS

	Performance Tested Methods℠	*Reviewed and Recognized℠*	*Official Methods of Analysis℠*
Method Type(s)	Proprietary methods	Non-proprietary methods	Proprietary or non-proprietary methods
Technical Requirements			
Calibration Model (for Quantitative Methods)	✓	✓	✓
Selectivity	✓	✓	✓
Matrix Studies	✓	✓	✓
Robustness	✓	✓	✓
Product Stability	✓		
Product Consistency	✓	✓	
Repeatability	✓	✓	✓
Reproducibility			✓ *(required for Final Action)*
Peer Review	Three reviewers	Three reviewers	Expert Review Panel
Method Format			
Package Insert Requirements	✓		
Application Note Requirements		✓	
AOAC OMA Method Format			✓
Publication (AOAC Journal Format)			✓
Certification Mark	✓	✓	
Compendium Publication			✓
Annual Review	✓	✓	
Final Action Status Review			✓

Contact Us

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We're here to help!