Performance of ELISA-based Analytical Methods Supporting Compliance with Gluten Free Regulations

Stefan Schmidt • 2024-05-30

1. Codex Alimentarius reference method for gluten analysis
2. AOAC Official Method of Analysis for gluten
3. Meaning of incurred samples for assays' validation
4. Results of 3rd international, collaborative study with RIDASCREEN® Gliadin ELISA

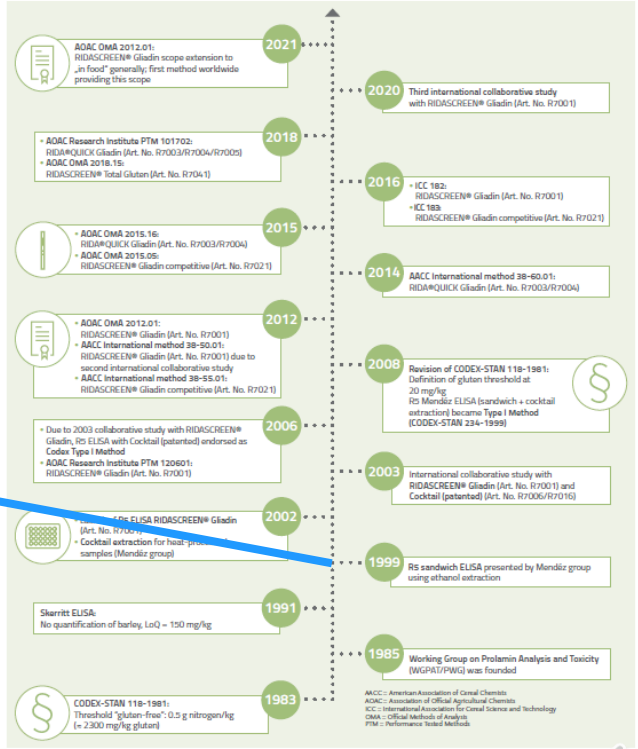
R5 methods: a history of internationally accepted gluten analysis

Cocktail (patented) (Art. No. R7006/R7016)

2002
- Launch of **R5 ELISA RIDASCREEN® Gliadin** (Art. No. R7001)
- **Cocktail extraction** for heat-processed samples (Mendéz group)

1999 **R5 sandwich ELISA** presented by Mendéz group using ethanol extraction

1991 **Skerritt ELISA:** No quantification of barley, LoQ = 150 mg/kg



Innovative approach to low-level gluten determination in foods using a novel sandwich enzyme-linked immunosorbent assay protocol

Israel Valdés, Enrique García, Mercedes Llorente and Enrique Méndez

European Journal of Gastroenterology & Hepatology 2003, 15:465–474

Keywords: gliadin, gluten, coeliac disease, toxic epitope, ELISA

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The method proposed here is also a simple sandwich ELISA, which uses the monoclonal antibody R5 employed as the coating antibody in our original ELISA [4]. The most remarkable feature of this new system is that a single antibody serves as both coating agent and conjugate to HRP for detection. The system's detec-

Independently of the ELISA method used, the main difficulty when analysing cooked foods is that the system needs to be able to extract quantitatively the insoluble aggregated α- and γ-subfractions and also denatured fractions. The use of the new quantitative cocktail extraction procedure [9] and R5-ELISA fulfils both these requirements. As demonstrated here, the cocktail extraction procedure for heat-processed food samples has the advantage that aggregated α- and γ-fractions are solubilized and extracted and can still react specifically with R5. Heat treatment leaves

RECOMMENDED METHODS OF ANALYSIS AND SAMPLING

CXS 234-1999[1]

Adopted in 1999

[1] The most updated version of the method should be used, in application of ISO/IEC 17025. The present list of methods reflects the amendments adopted by the 44th Session of the Codex Alimentarius Commission in 2021.

PART A – METHODS OF ANALYSIS BY COMMODITY CATEGORIES AND NAMES

Gluten-free foods	Gluten	Enzyme-Linked Immunoassay R5 Mendez (ELISA) Method *Eur J Gastroenterol Hepatol* 2003; 15: 465-474	Immunoassay	I

in 2008

Codex Alimentarius Type 1 method for gluten analysis in food

Standard CXS 234-1999 (adoption from 2019)

R5 ELISA

e.g. RIDASCREEN® Gliadin

+

Méndez Cocktail

marketed as
Cocktail (patented)
by R-Biopharm

Gluten in Codex Alimentarius

STANDARD FOR FOODS FOR SPECIAL DIETARY USE FOR PERSONS INTOLERANT TO GLUTEN

CODEX STAN 118-1979

Adopted in 1979. Amendment: 1983 and 2015. Revision: 2008.

RECOMMENDED METHODS OF ANALYSIS AND SAMPLING

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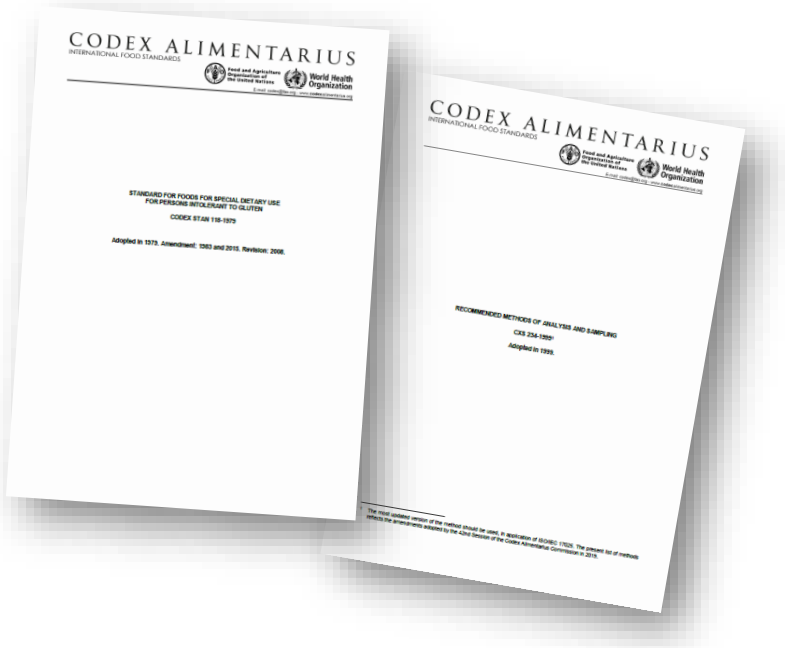


[1] The most updated version of the method should be used, in application of ISO/IEC 17025. The present list of methods reflects the amendments adopted by the 42nd Session of the Codex Alimentarius Commission in 2019.

2.1.1 *Gluten-free foods*

Gluten-free foods are dietary foods

a) consisting of or made only from one or more ingredients which do not contain wheat (i.e. all *Triticum* species, such as durum wheat, spelt, and khorasan wheat, which is also marketed under different trademarks such as KAMUT), rye, barley, oats[1] or their crossbred varieties, and the gluten level does not exceed 20 mg/kg in total, based on the food as sold or distributed to the consumer, and/or

b) consisting of one or more ingredients from wheat (i.e. all *Triticum* species, such as durum wheat, spelt, and khorasan wheat, which is also marketed under different trademarks such as KAMUT), rye, barley, oats[1] or their crossbred varieties, which have been specially processed to remove gluten, and the gluten level does not exceed 20 mg/kg in total, based on the food as sold or distributed to the consumer.

2.2.2 *Prolamins*

Prolamins are defined as the fraction from gluten that can be extracted by 40 - 70% of ethanol. The prolamin from wheat is gliadin, from rye is secalin, from barley hordein and from oats[1] avenin.

It is however an established custom to speak of gluten sensitivity. The prolamin content of gluten is generally taken as 50%.

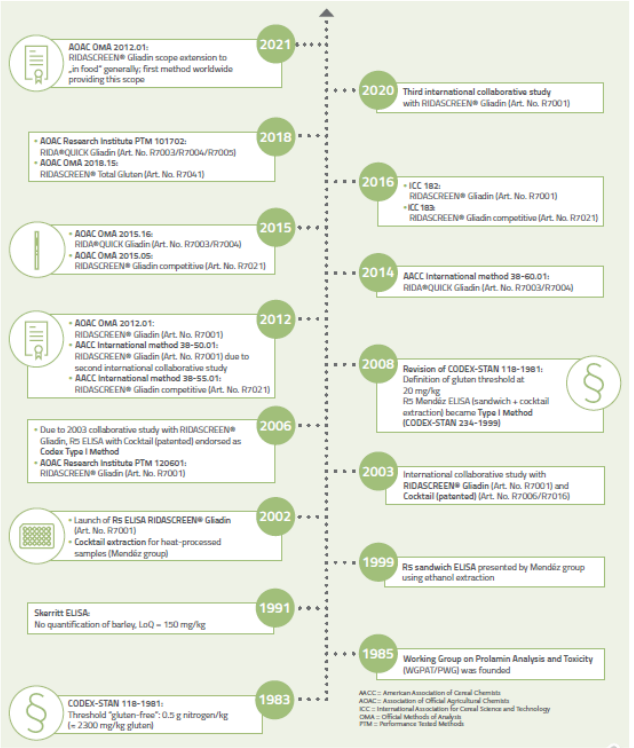
5.2 Method for determination of gluten

Enzyme-linked Immunoassay (ELISA) R5 Mendez Method.

[1] Oats can be tolerated by most but not all people who are intolerant to gluten. Therefore, the allowance of oats that are not contaminated with wheat, rye or barley in foods covered by this standard may be determined at the national level.

R5 methods: a history of internationally accepted gluten analysis

- In 2012, combination of RIDASCREEN Gliadin and Cocktail (patented) became AOAC Official Method of Analysis 2012.01 for foods
- In 2016, new AOAC guidelines limited the method to rice- and corn-based matrices
- In 2020, 3rd collaborative study with a wide range of different matrices to demonstrate its wide applicability with special focus on **incurred samples**



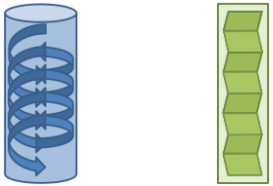
Protein structure and antibody reactivity

Primary structure:

Amino acid sequence

Secondary structure:

α-helix und β-pleated sheet

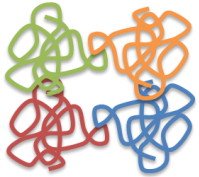


Tertiary structure:

3D-structure of a single protein

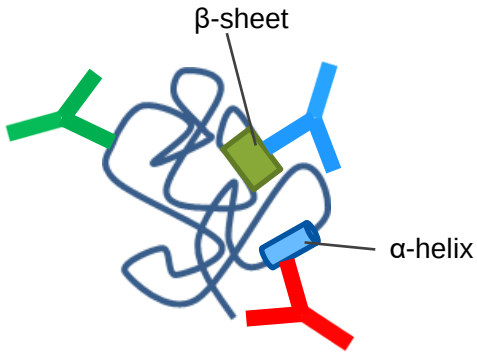
Quarternary structure:

3D-structure of combined protein

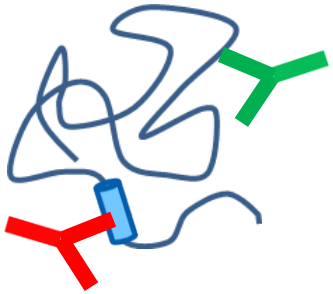


Antibodies react always with one specific epitope only!

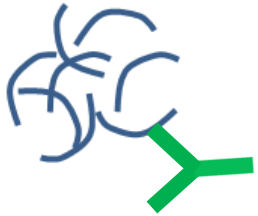
Food processing and influence on protein structure



native



processed

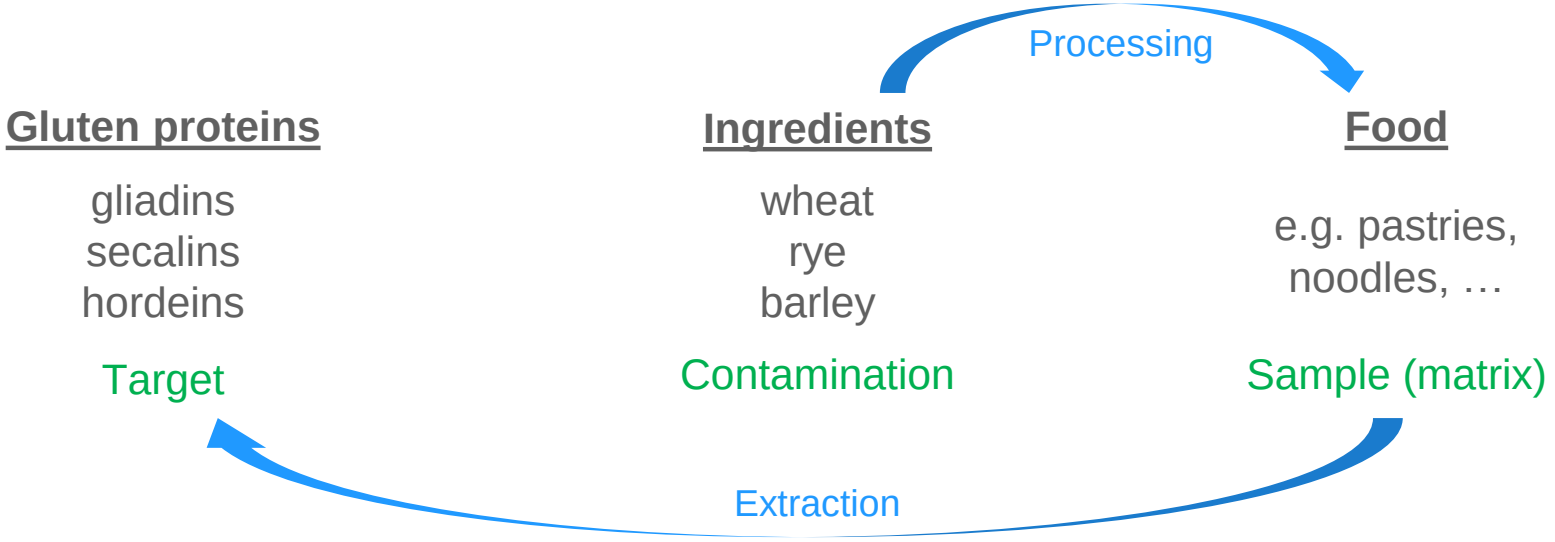


highly processed (hydrolyzed)

loss of structural epitopes

Gradually, depending on the food processing conditions

Impact of food processing and sample extraction on recovery



Both, grade of processing and extraction procedures do influence the protein structure and hence the reaction of the allergen with the detection antibodies in an assay (recovery).

Importance of method harmonization for internationally comparable results

Defined by Codex Alimentarius

- ELISA
- R5 antibody
- Calibration to gliadin
- Extraction procedure (Méndez cocktail)

Foods are not defined by the Codex Standard. It is the kit manufacturer‘s responsibility to show the methods applicability for different types of food (nativ, heat-processed, …)

Different methods show different results for the same sample!

RIDASCREEN® Gliadin 3rd international, collaborative study

to demonstrate its wide applicability with special focus on incurred samples

3rd international, collaborative study with 19 different food matrices

Selection of samples

- Consultation with CD patient societies
- Foods with high risk of gluten contamination
- High relevance in human nutrition
- 19 different real food samples
  (not only dry foods but also foods with a high level of moisture)
- All processed samples were incurred **prior to main processing step**
- All samples were **prepared independently** (Hochschule Geisenheim University)

3rd international, collaborative study with 19 different food matrices

Sample overview

Sample	Processing
Starches	Mixing / blending
Pseudo cereals	Mixing / blending
Legumes	Mixing / blending
Soy	Mixing / blending
Spices	Mixing / blending
Juice	Mixing / blending
Cream cheese	Mixing / blending

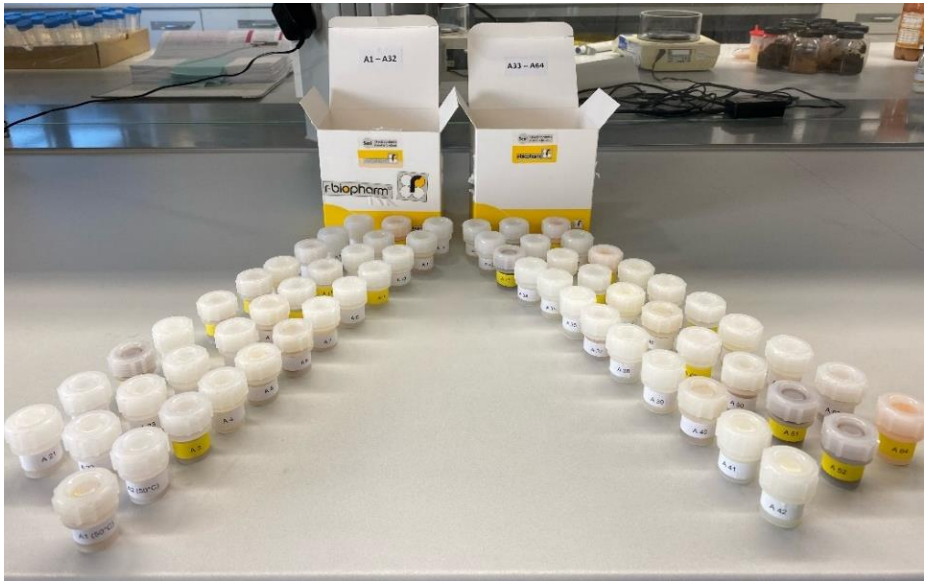
Sample	Processing	Time	Temperature
Nut nougat crème	Heating	60 min	80°C
Pesto	Heating	5 min + 10 min	100°C + 80°C
Candies	Cooking	15 min	100°C
Dessert	Cooking	10 min	100°C
Fish	Cooking	20 min	100°C
Potatoes (gnocchi)	Cooking	15 min	100°C
Potatoes (gnocchi)	Microwaving	2.5 min	1500 W
Meat	Frying	16 min	190°C
Vegetarian meat alternative	Frying	16 min	190°C
Cake	Baking	55 min	170°C
Cookies	Baking	25 min	150°C
Bread	Baking	60 min	180°C

3rd international, collaborative study with 19 different food matrices

Study consisted of 64 blind coded samples analyzed by 14 laboratories, in total 896 samples

14 Laboratories

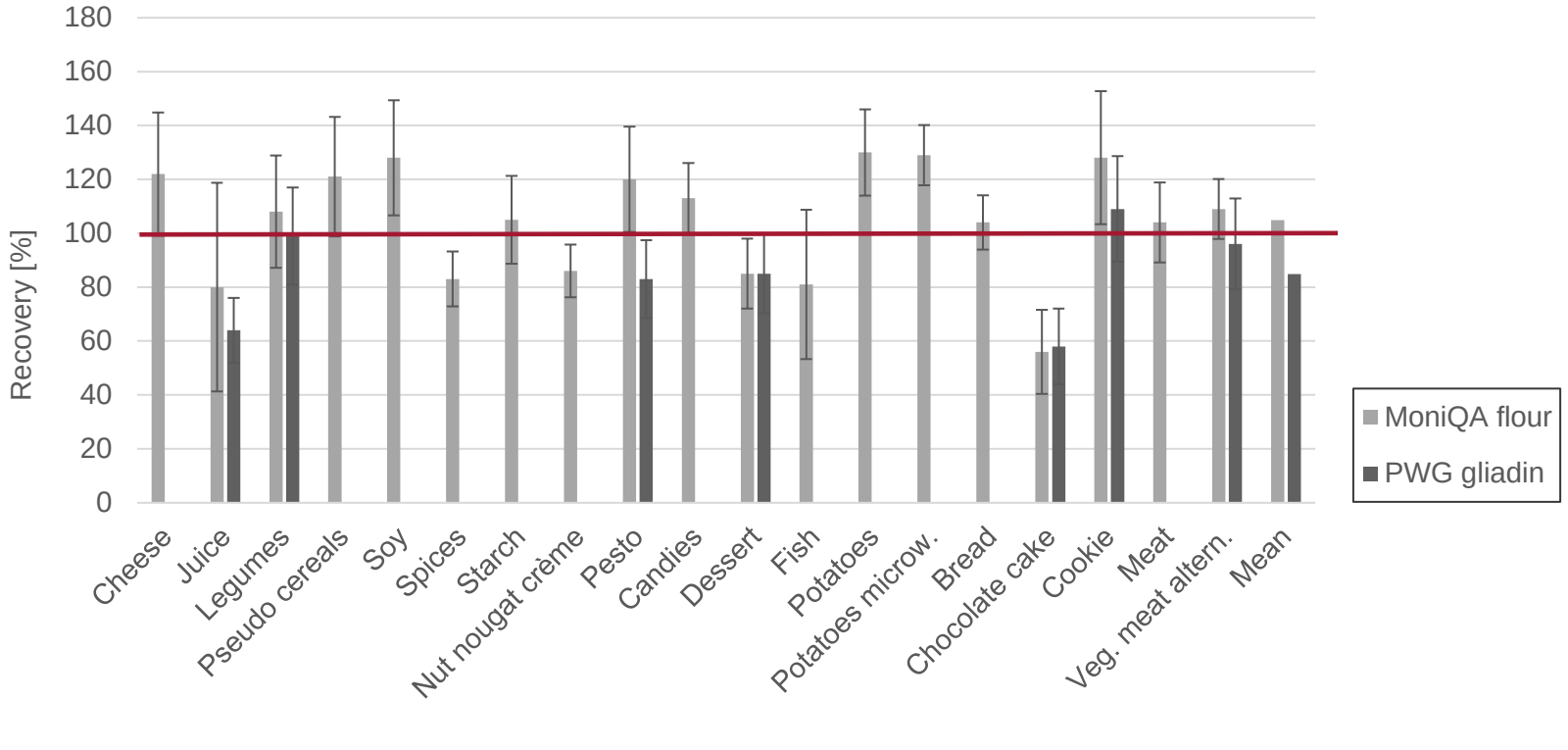
- Austria
- Canada (2)
- Finland
- Germany (4)
- Ireland
- Italy
- USA (4)



32 different samples with blind coded duplicates (1-64)

Samples with skim milk powder extraction with yellow label

Overall recovery and precision were very good



AOAC Official Method 2012.01
Gliadin as a Measure of Gluten in Food
by R5 sandwich ELISA RIDASCREEN® Gliadin

Based on a Specific Monoclonal Antibody to
Celiac Toxic Amino Acid Prolamin Sequences
First Action 2012
Final Action 2016

Applicable for the quantitative measurement of intact gliadin as a measure of gluten in unprocessed and processed matrices from important gluten-free food categories including rice- and corn-based products, soy, starches, pseudo cereals, legumes, spices, juice, nut nougat crème, cream cheese, pesto, meat, vegetarian meat alternative, cookies, dessert, cake, fish, bread, candies, and potatoes. The sandwich ELISA quantifies intact gliadin from wheat and also intact related proteins from rye and barley. This method is not accurate for quantification of fermented or hydrolyzed gluten.

open access: https://doi.org/10.1093/jaoacint/qsab148

Codex Alimentarius Type 1 method for gluten analysis in food

Standard CXS 234-1999 (adoption from 2019)

R5 ELISA

e.g. RIDASCREEN® Gliadin

+

Méndez Cocktail

marketed as
Cocktail (patented)
by R-Biopharm

Further AOAC approved methods for gluten analysis specific for certain conditions

RIDASCREEN® Gliadin competitive (Art. No. R7021)

AOAC Official Method 2015.05
Partially Hydrolyzed Gluten
in Fermented Cereal-Based Products
R5 Competitive ELISA
First Action 2015
Final Action 2018

Further AOAC approved methods for gluten analysis specific for certain conditions

RIDA®QUICK Gliadin (Art. No. R7003, R7004 and R7005)

AOAC RESEARCH INSTITUTE

CERTIFICATION

AOAC® *Performance Tested*℠

Certificate No.

101702

The AOAC Research Institute hereby certifies the test kit known as:

RIDA®QUICK Gliadin

manufactured by

R-Biopharm AG
An der neuen Bergstraße 17
64297 Darmstadt
Germany

This method has been evaluated in the AOAC® *Performance Tested Methods*℠ Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*℠ certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (January 07, 2021 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

January 07, 2021
Date

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At what point does a contamination occur?

Impact on:

- Protein structure and matrix aggregation
- Extraction procedure
- Recovery

→ Hence, it is important to include incurred samples in validation studies and AOAC guidelines were revised accordingly.

Thank you for your attention