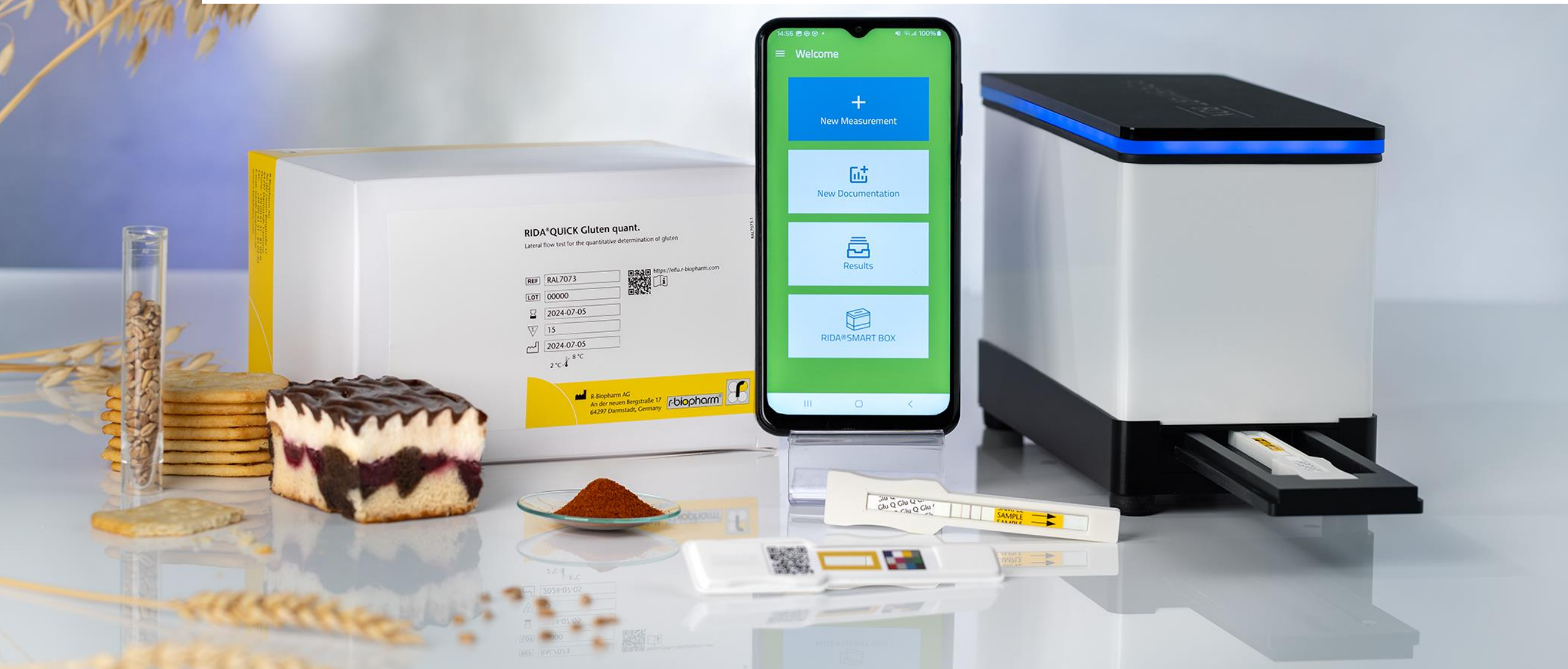
Integrated Gluten Management

Johanna Meder

February 2025

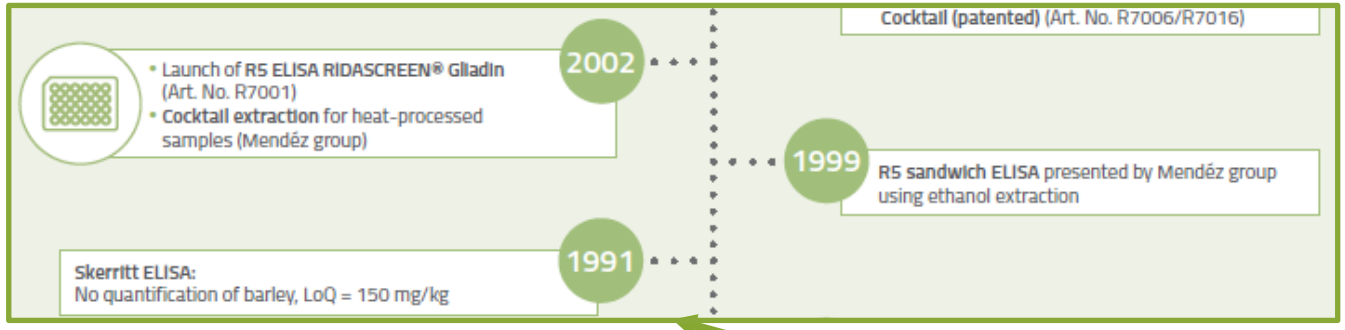


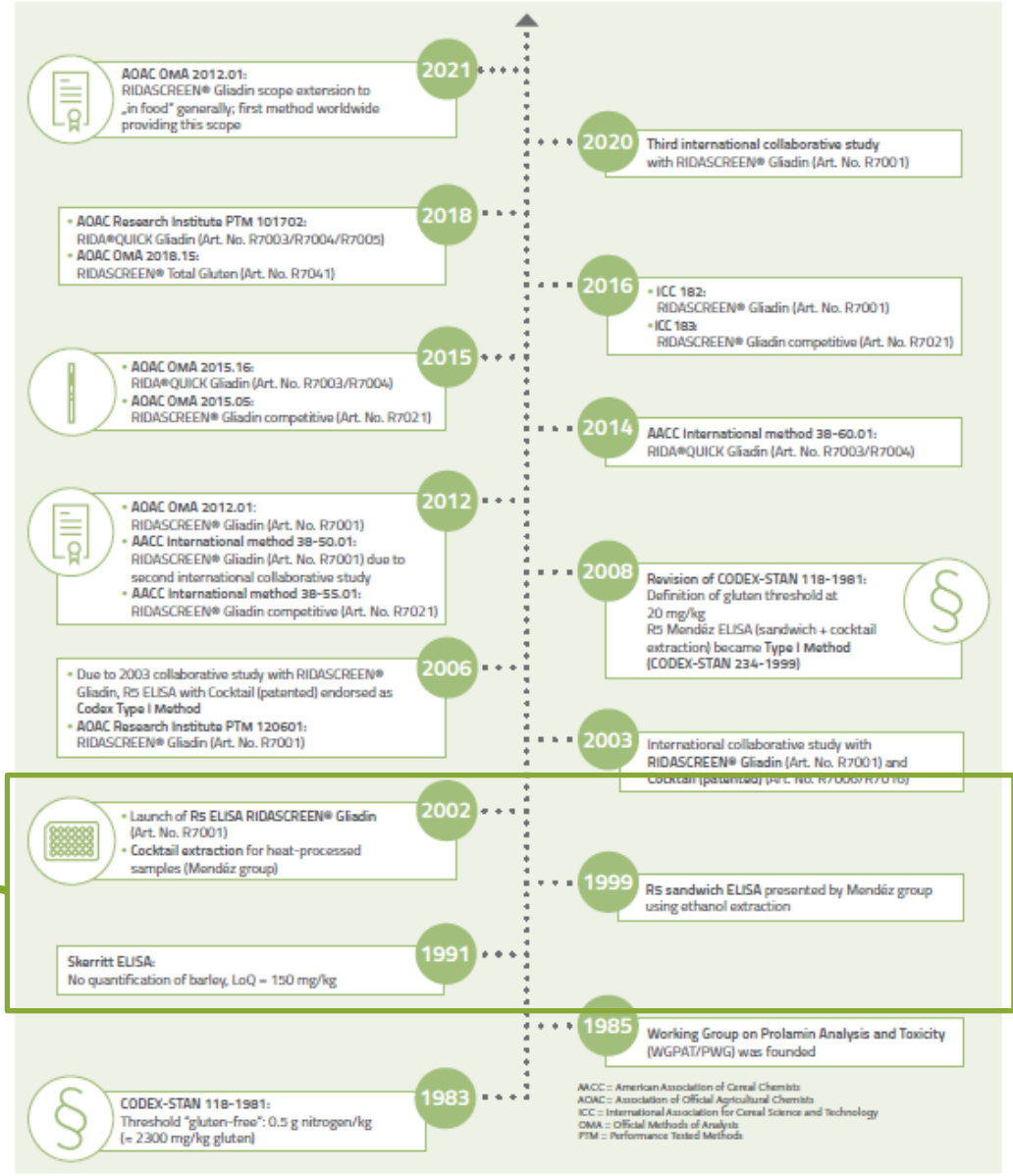
Overview

- History of gluten and Codex Alimentarius
- Methods for gluten detection
- Integrated Gluten Management
  - RIDASCREEN®Gliadin
  - RIDASCREEN®EASY Gluten
  - RIDA®QUICK Gluten quant.

History of gluten

R5 methods: a history of internationally accepted gluten analysis





History of gluten

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

Unidad de Gluten, Centro Nacional de Biotecnología, Madrid, Spain.

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Accepted 27 November 2002

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

History of gluten

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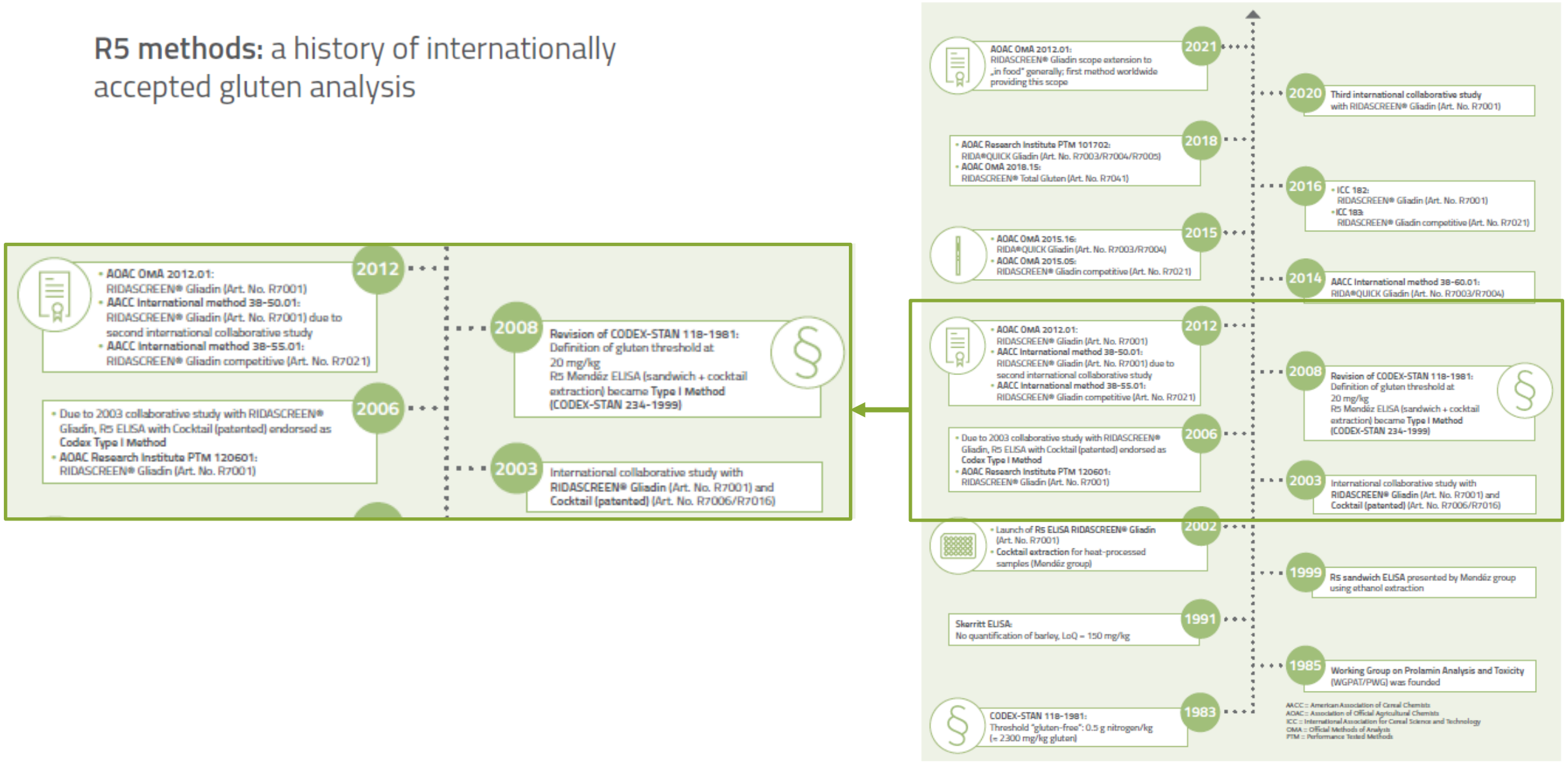


Codex Standard 234-1999

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

Gluten Analysis according to Codex Alimentarius

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

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 42nd Session of the Codex Alimentarius Commission in 2019.

Codex Stan 118-1979

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%.

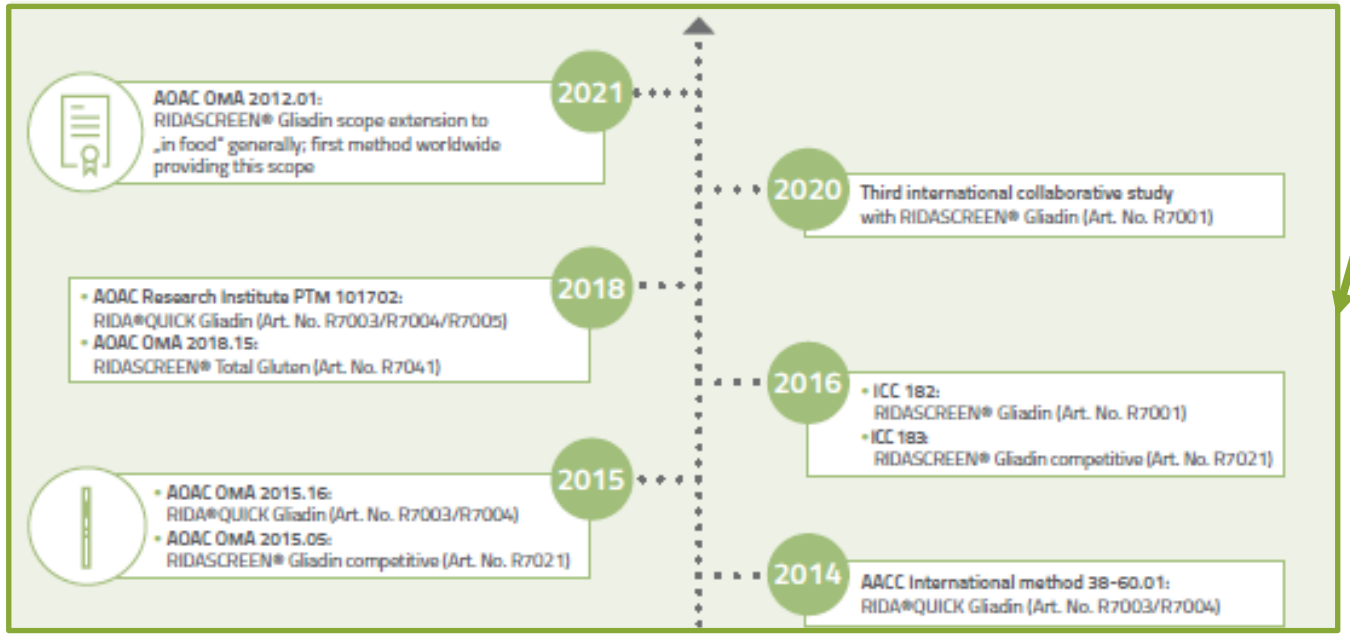
5.2 Method for determination of gluten

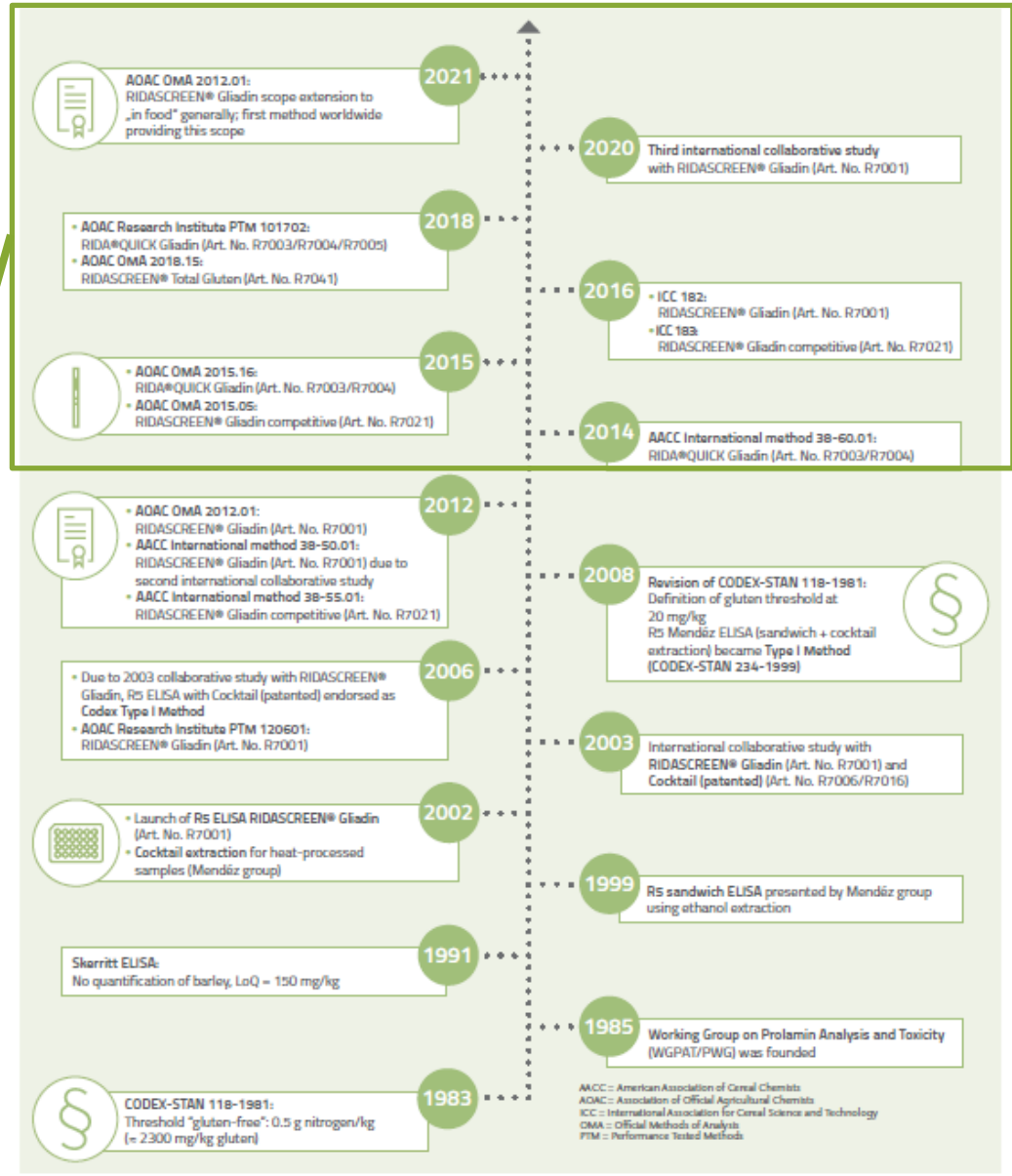
Enzyme-linked Immunoassay (ELISA) R5 Mendez Method.

[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.

History of gluten

R5 methods: a history of internationally accepted gluten analysis





Methods for gluten detection

ELISA

- Detection of gliadin/gluten
- Sandwich or competitive
- Quantitative control of foods
- Confirmation PCR/LFD

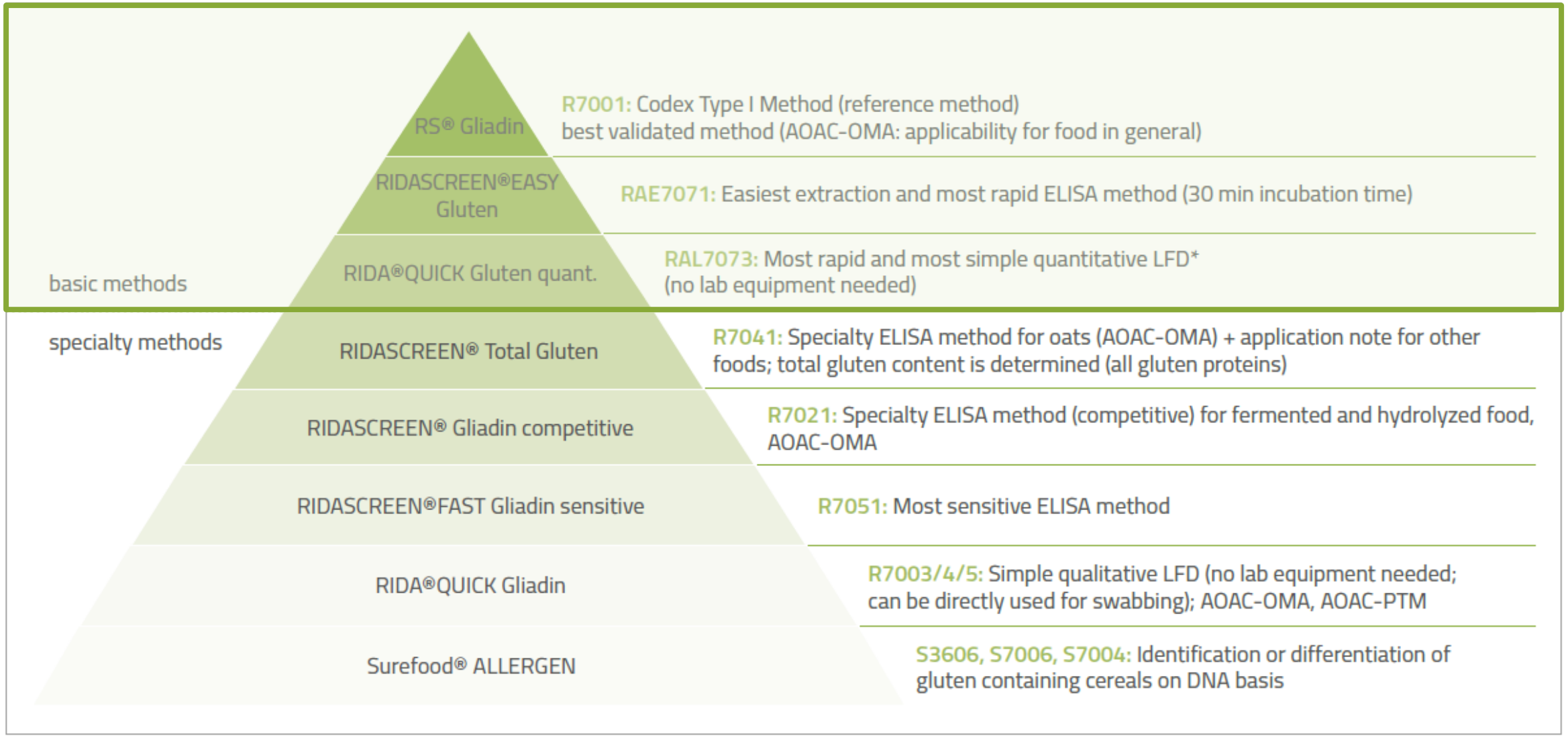
LFD

- Detection of gliadin/gluten
- Quantitative and qualitative control of foods, surfaces and cleansing water
- Screening

PCR

- Detection of DNA
- Quantitative and/or qualitative control of foods
- Confirmation ELISA

Integrated Gluten Management



OMA: Official Method of Analysis; PTM: Proficiency Tested Method

RIDASCREEN® Gliadin

- R5 sandwich ELISA
- Codex Alimentarius Type I method
  - Use of Cocktail (patented)
- AOAC-OMA

→ Applicability for foods in general
→ Focus on incurred matrices

- Automation applications and equipment available

- Based on R5 antibody → result comparability due to common immunological target

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

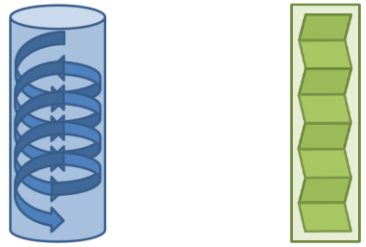
Protein structure and antibody reactivity

Primary structure:

Amino acid sequence

Secondary structure:

α-helix und β-pleated sheet

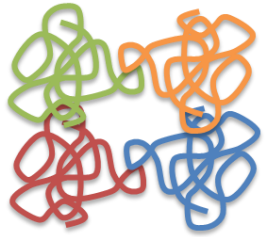


Tertiary structure:

3D-structure of a single protein

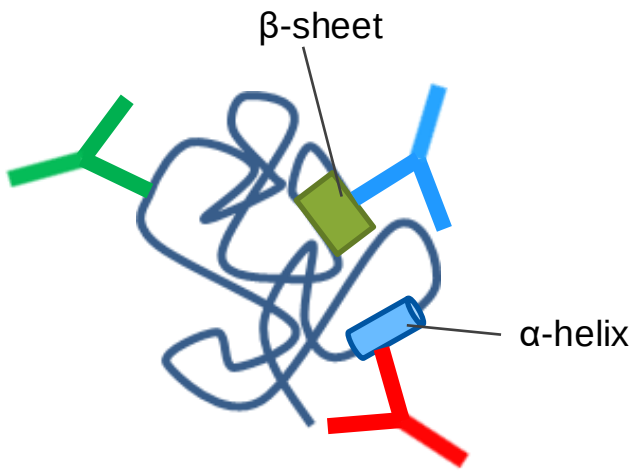
Quarternary structure:

3D-structure of combined protein

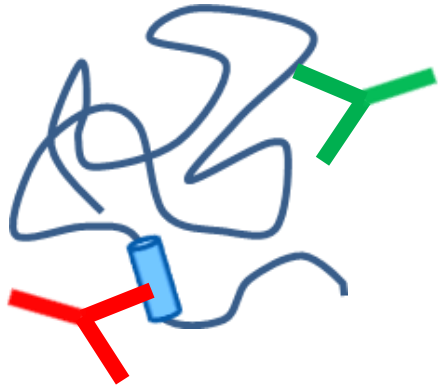


Antibodies react always with one specific epitope only!

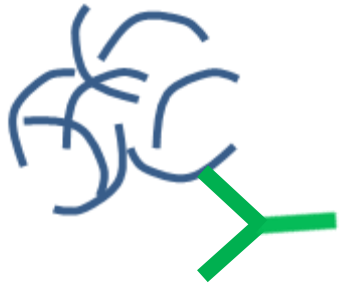
Food processing and influence on protein structure



native



processed



highly processed (hydrolyzed)

loss of structural epitopes

Gradually, depending on the food processing conditions

At what point does a contamination occur?

Impact on:

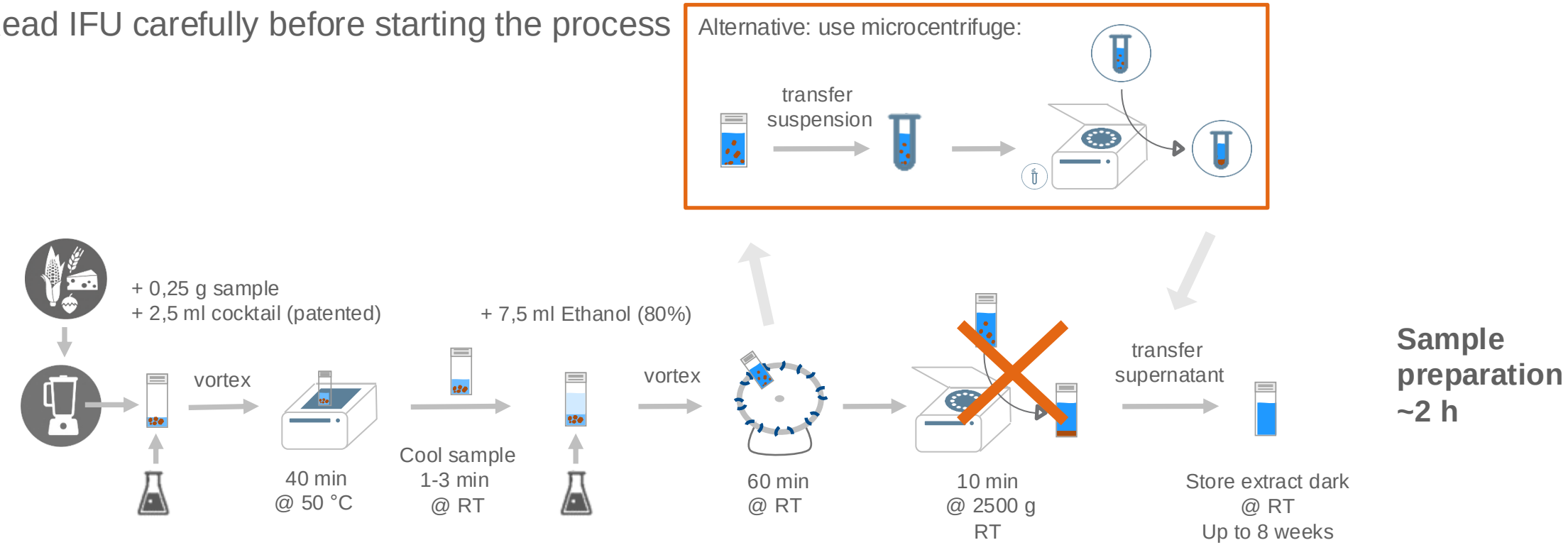
- Protein structure and matrix aggregation
- Extraction procedure
- Recovery

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

RIDASCREEN®Gliadin

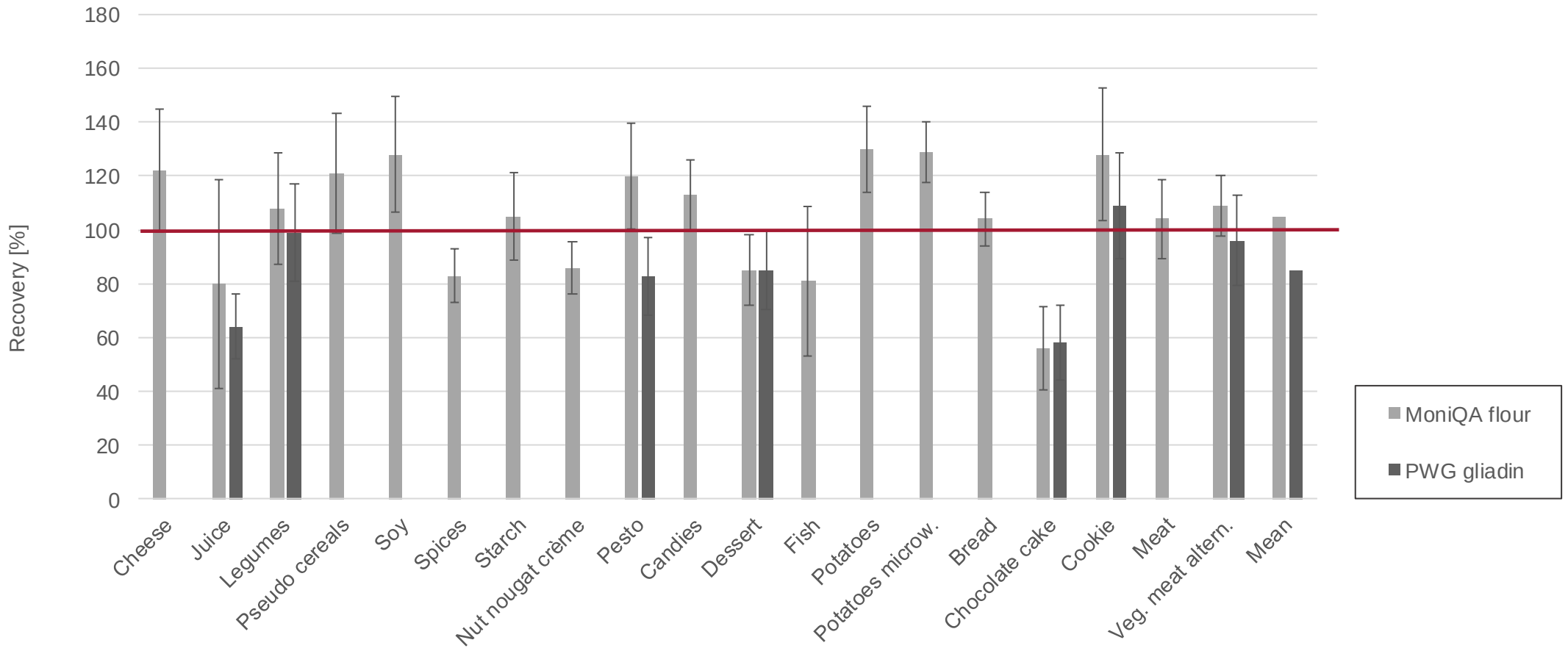
Sample preparation according to the Codex Alimentarius Type I method (use of cocktail)

Read IFU carefully before starting the process



Sample preparation ~2 h

Overall recovery and precision were very good



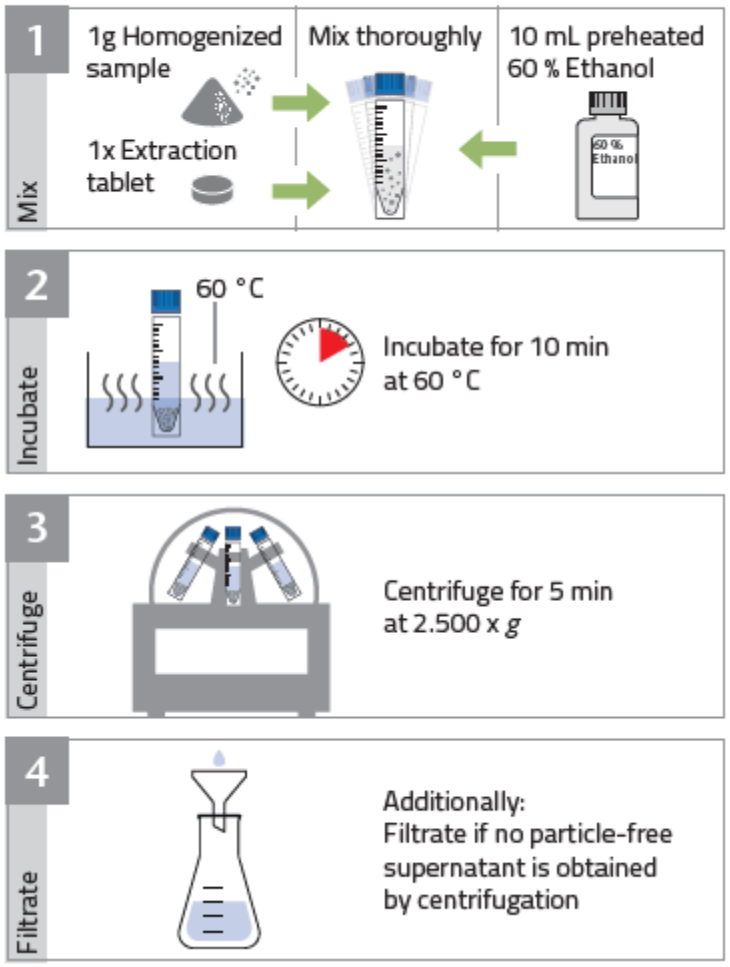
New RIDASCREEN® EASY Gluten

- New R5 sandwich ELISA
  - Calibration to gluten
- Gluten from wheat, rye, barley
- Easy extraction
- Automation applications and equipment available
- Focus on incurred matrices

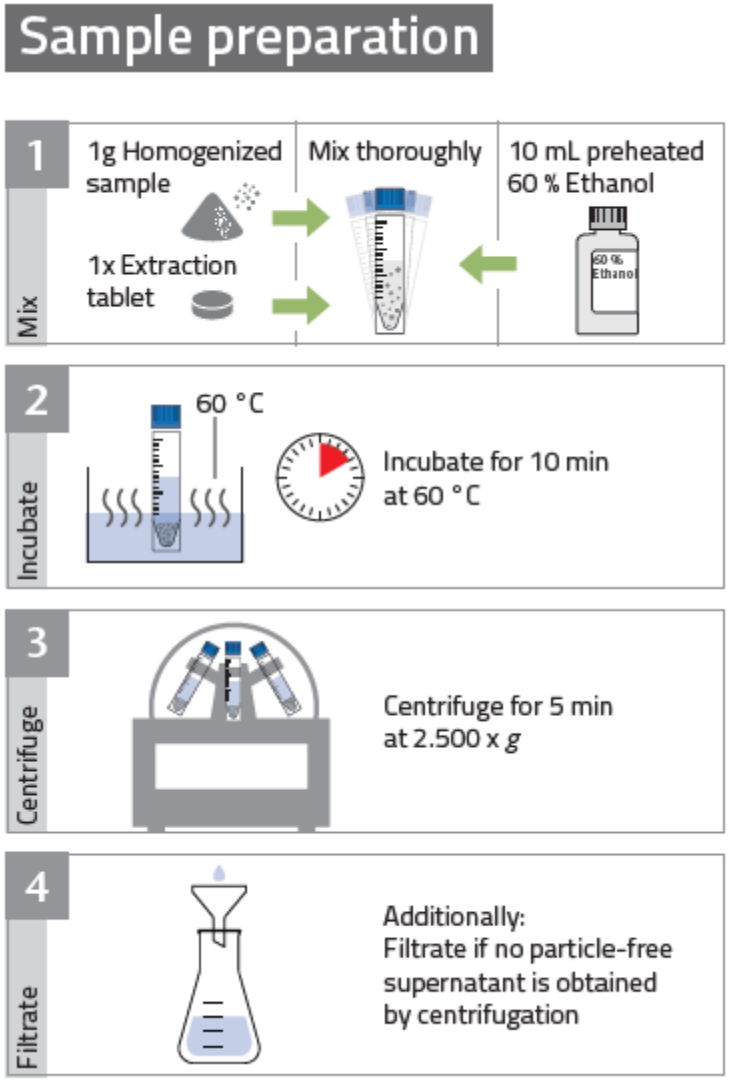
- Based on R5 antibody → result comparability due to common immunological target

RIDASCREEN®EASY Gluten - Sample preparation

Sample preparation



RIDASCREEN®EASY Gluten - Sample preparation



RIDASCREEN®EASY Gluten – ELISA procedure

ELISA procedure

1 Dilute

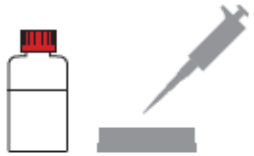
Dilute the particle-free extract 1:50

2 Sample/ standard

Pipet 100 µL diluted extract or 100 µL standard (1-6) into the plate

10 min incubation

3 Conjugate



Wash 4 x 250 µL
Add 100 µL conjugate

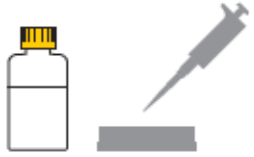
10 min incubation

4 Substrate

Wash 4 x 250 µL
Add 100 µL substrate/ chromogen

10 min incubation in the dark

5 Stop



Add 100 µL stop solution

Read at 450 nm

New RIDA®QUICK Gluten quant.

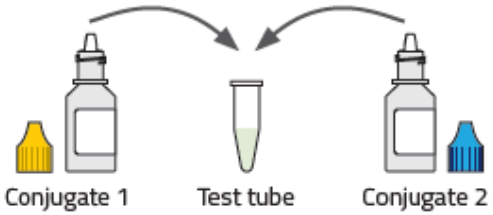
- New quantitative R5 LFD
  - Calibration to gluten
- Gluten from wheat, rye, barley
- Easy extraction, fast analysis
- Focus on incurred samples
- Evaluation with RIDA®SMART APP Allergen
  - Data management, Cloud
- Only quantitative LFD based on R5 antibody → result comparability due to common immunological target

RIDA®QUICK Gluten quant. - Sample preparation

Sample preparation

Swab samples

1 Add 4 drops of each and mix



2 Moisten swab with 8 drops of swabbing buffer

3 Swab area of interest

4 Intensively flush out in the test tube from 1

5 Incubate 5 min

or

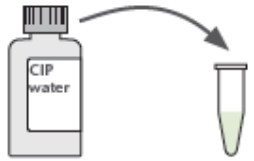
CIP water

1

CIP water can be used directly

2 Add 4 drops of each and mix

3



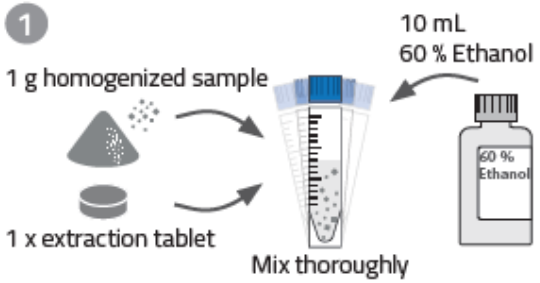
Add 20 µL or 1 drop (with provided pipette) CIP water and mix

4 Incubate 5 min

or

Food samples

1

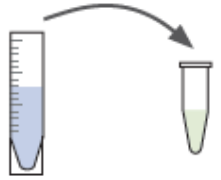


2

Filter

3 Add 4 drops of each and mix

4



Add 20 µL or 1 drop (with provided pipette) filtered extract and mix

5 Incubate 5 min

RIDA®QUICK Gluten quant. - Test procedure

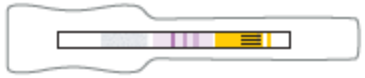
Test procedure

1. Place test strip in test tube

2. Incubate 5 min

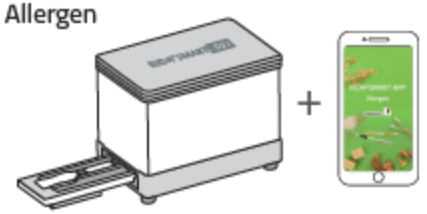
Evaluation

1. Place test strip into the test strip holder

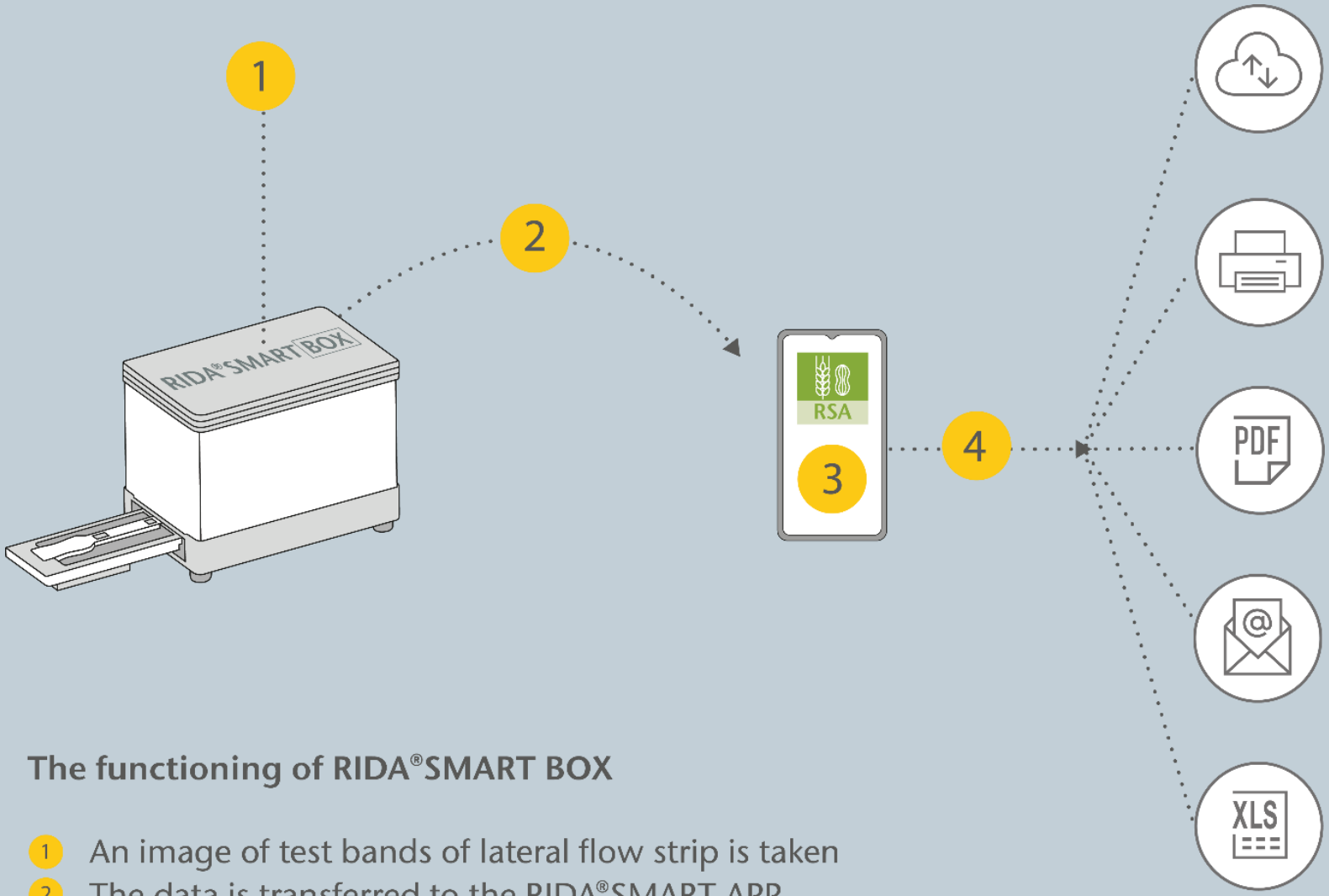


2a. Evaluation with a verified Android smartphone: Cover with the RIDA®SMART APP Cover and evaluate with the RIDA®SMART APP Allergen

2b. Evaluation with the RIDA®SMART BOX and any Android device: Place into RIDA®SMART BOX and evaluate with the RIDA®SMART APP Allergen



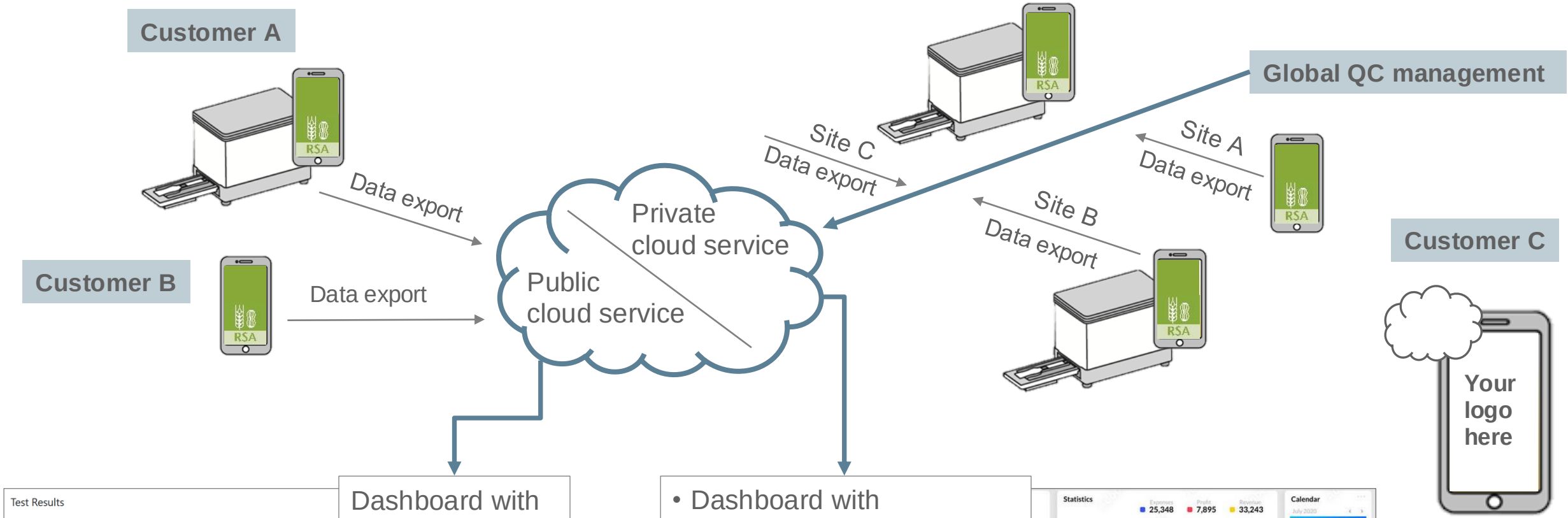
Integrated gluten management

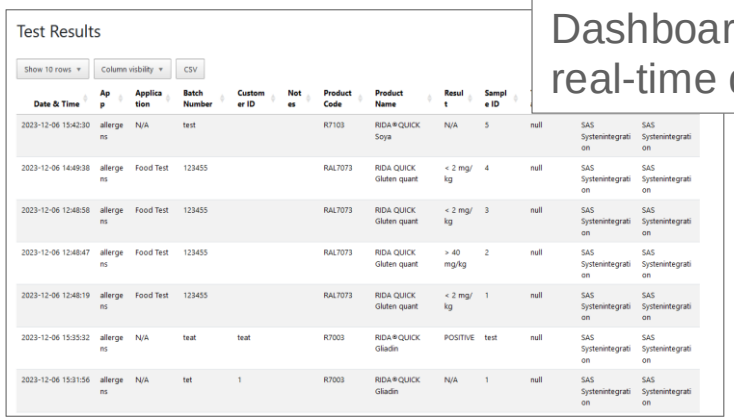


The functioning of RIDA®SMART BOX

1. An image of test bands of lateral flow strip is taken
2. The data is transferred to the RIDA®SMART APP
3. The RIDA®SMART APP calculates the results
4. Full connectivity e.g. cloud, e-mail, pdf, excel, printer

System solution





Dashboard with real-time data

- Dashboard with real-time data and analytics
- Customized APP design
- Data export to your own system via API

Integrated Gluten Management

- All tests are based on R5 antibody (ELISA/LFD)
- Quantitative evaluation (ELISA/LFD quant./PCR)
- Qualititative evaluation (LFD/PCR)
- Optional: Automation available
- Result comparability

Result comparability

Incurred samples:

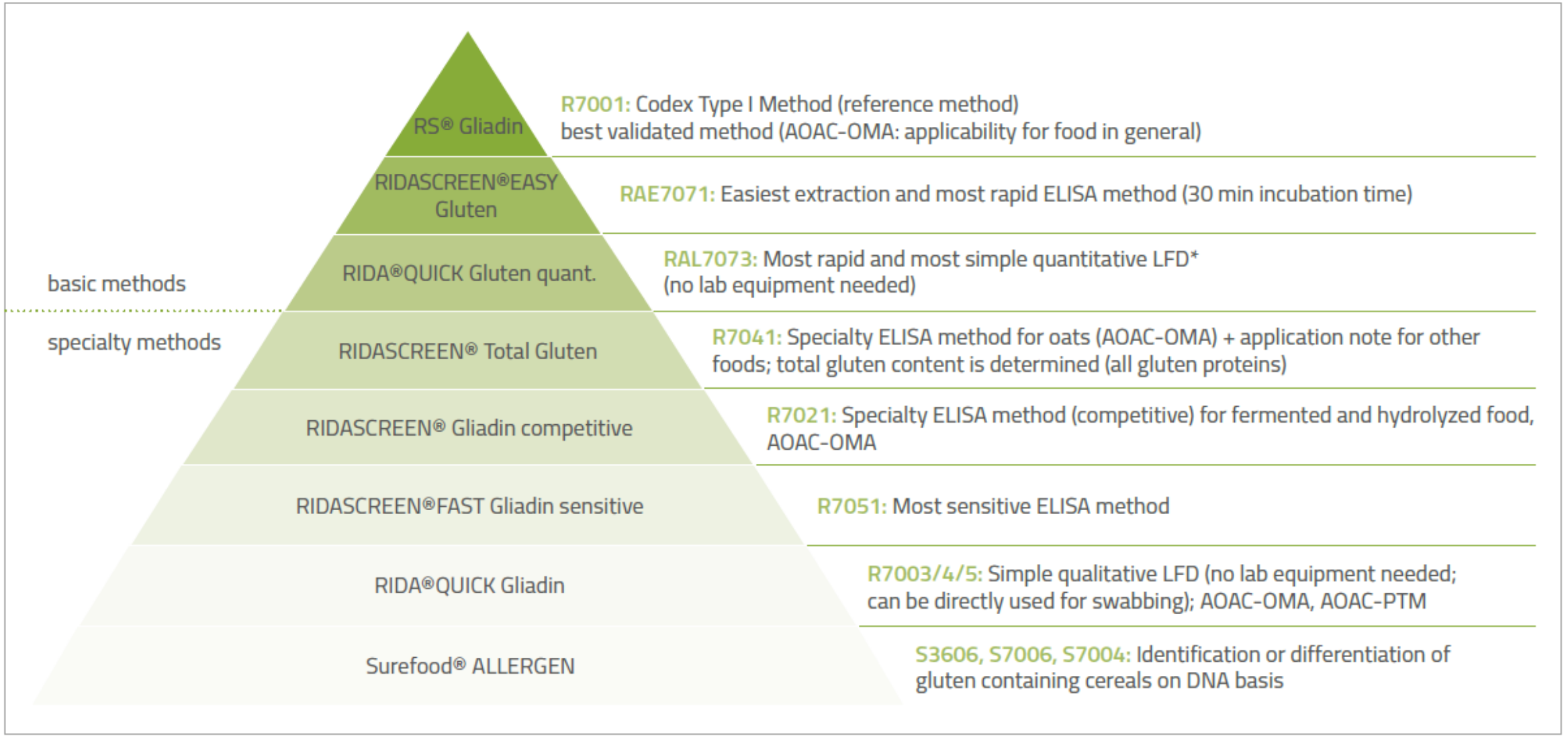
Matrix	Sample	R7001 RS Gliadin Recovery (%)	RAE7071 EASY Gluten Recovery (%)	RAL7073 QUICK Gluten quant. Recovery (%)
Oat cookie	B9	198	148	121
Oat cookie	R9	237	189	146
Oat cookie	W9	140	107	124
Chocolate creme	B9	186	152	193
Chocolate creme	W9	93	93	70
Spice	W9	106	112	92
Cake	R9	209	118	81
Cake	W9	123	91	75
Pesto	W9	125	76	89
mean incurred samples		157	121	110

Result comparability

Incurred samples:

Matrix	Sample	R7001 RS Gliadin Recovery (%)	RAE7071 EASY Gluten Recovery (%)	RAL7073 QUICK Gluten quant. Recovery (%)	R7001 with factor 1.5
Oat cookie	B9	198	148	121	149
Oat cookie	R9	237	189	146	178
Oat cookie	W9	140	107	124	105
Chocolate creme	B9	186	152	193	139
Chocolate creme	W9	93	93	70	70
Spice	W9	106	112	92	79
Cake	R9	209	118	81	156
Cake	W9	123	91	75	92
Pesto	W9	125	76	89	94
mean incurred samples		157	121	110	118

Solutions for gluten analysis



OMA: Official Method of Analysis; PTM: Proficiency Tested Method

Integrated Gluten Management
Gluten testing along the food production chain

Thank you for your attention!

More about R-Biopharm
Food & Feed Analysis

https://r-b.io/food

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