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

Gluten analysis
and gluten-free products
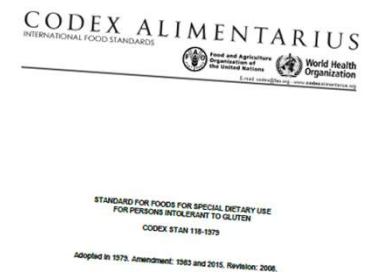
**STANDARD FOR FOODS FOR SPECIAL DIETARY USE
FOR PERSONS INTOLERANT TO GLUTEN**

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.

- 2003 – publication by Enrique Méndez of R5 sandwich ELISA + cocktail extraction
- 2003 – international collaborative study with RIDASCREEN® Gliadin and Cocktail (patented)
- 2006 – Méndez method endorsed as Codex Alimentarius Type I Method
- 2008 – Méndez method became Type I Method and Codex Standard 118-1979 revision

CSX 234-1999

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 |

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.

5.2 Method for determination of gluten

Enzyme-linked Immunoassay (ELISA) R5 Mendez Method.

Gluten Analysis in Codex Alimentarius

Codex Alimentarius Type I method for gluten analysis in food

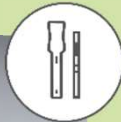Standard CXS 234-1999 (adoption from 2019)

R5 ELISA

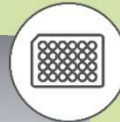RIDASCREEN® Gliadin

Méndez cocktail

for gluten extraction from heat-processed food samples
➜Cocktail (patented)

Most comprehensive gluten testing portfolio …

RIDA®QUICK – Lateral flow tests

- RIDA®QUICK Gluten quant.
  AOAC OMA approval in preparation

- RIDA®QUICK Gliadin
  AOAC-OMA 2015.16
  AOAC-PTM 101702

RIDASCREEN® – ELISA

- RIDASCREEN® Gliadin
  AOAC-OMA 2012.01
  AOAC-PTM 120601

- RIDASCREEN®EASY Gluten
  AOAC PTM approval in preparation

- RIDASCREEN® Total Gluten
  AOAC-OMA 2018.15

- RIDASCREEN® Gliadin competitive
  AOAC-OMA 2015.05

- RIDASCREEN®FAST Gliadin sens.

SureFood® – Real-time PCR

- SureFood ® ALLERGEN Gluten
- SureFood ® ALLERGEN 4plex
  Cereals / Getreide

From farm to fork – gluten testing along the food production chain

Grain harvest and processing

- Procurement and processing of raw materials

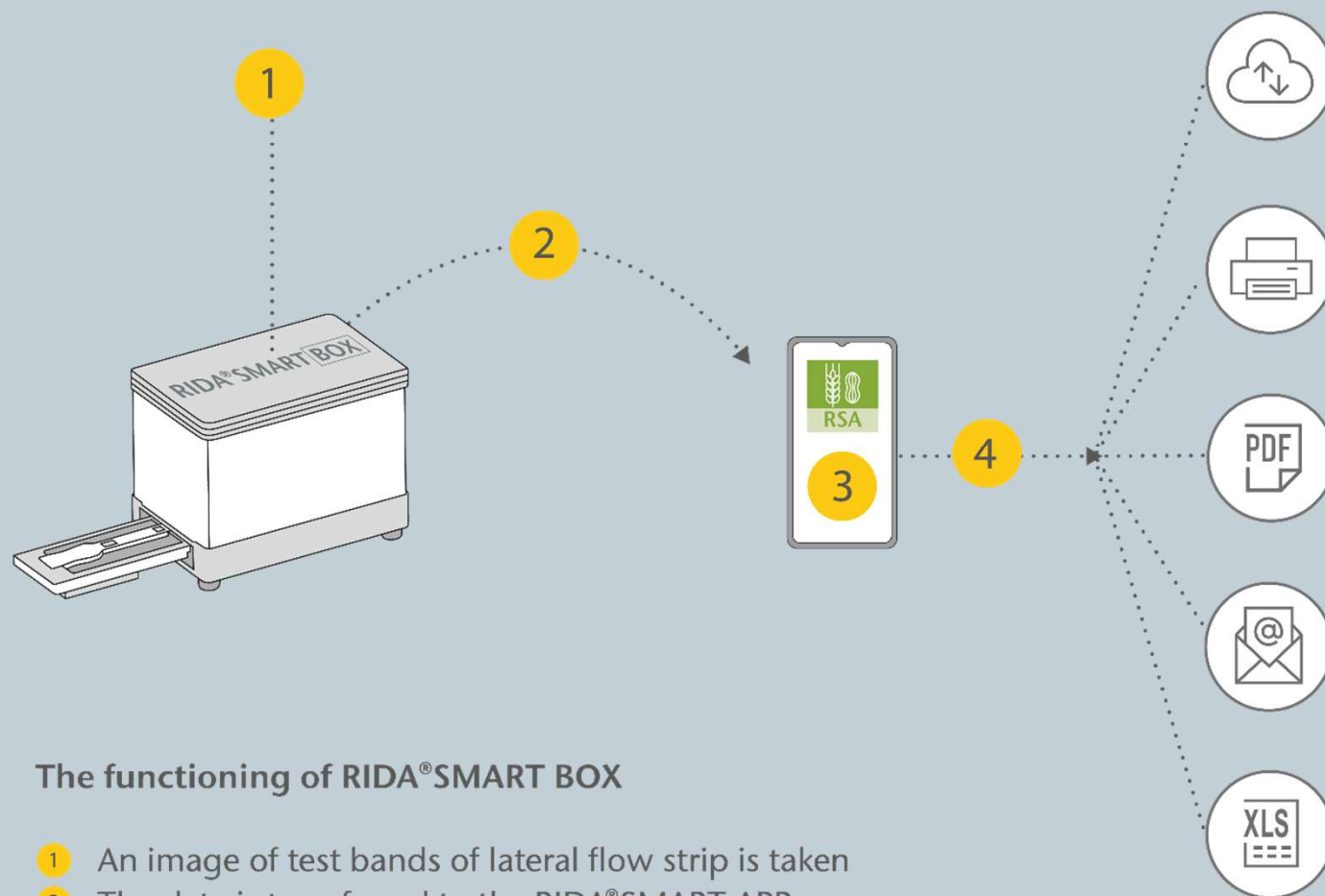- Detection of contamination

RIDA®QUICK **New**
Gluten quant.

RIDA®SMART BOX
Alternative: smart phone
(Android)

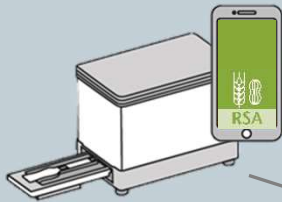Data management

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

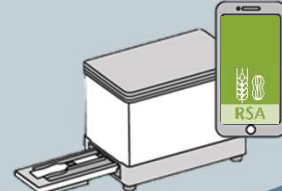# System solution

Customer A

Customer B

Data export

Data export

Private cloud service

Public cloud service

Global QC management

Site C
Data export

Site A
Data export

Site B
Data export

Customer C

Your logo here

Dashboard with real-time data

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

Intermediate trade

Management of raw materials and ingredients for food industry

RIDA®QUICK **New**
Gluten quant.

RIDA®SMART BOX
+ Data management

— or —

Samples to laboratory

Quality management

Ingredients' handling
Cross-contamination

RIDA®QUICK **New**
Gluten quant.

RIDA®SMART BOX
+ Data management

——— or ———

RIDASCREEN® **New**
EASY Gluten
+ Automation/ LIMS

Laboratory

Efficient sample handling 🔍

Fast, improved R5-ELISA method

RIDASCREEN®EASY Gluten

+

New easy extraction method included

ELISA automation

———————— or ————————

Other available ELISA test kits depending on customer needs

Inline control production

- Cross-contamination during production
- Cleaning control

RIDA®QUICK **New**
Gluten quant.

RIDA®SMART BOX
+ Data management

ready-to-swab
RIDA®QUICK Gliadin

— or —

Samples to laboratory

Official control of foodstuffs

Consumer safety

RIDASCREEN® Gliadin

+

Automation/ LIMS

- R5 ELISA with Cocktail (patented) – Méndez ELISA
- Codex Alimentarius type I reference method
- AOAC Official Method of Analysis for foods
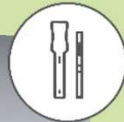- AOAC-PTM 101702

Confirmation/ investigation
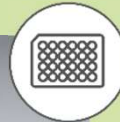
SureFood ® ALLERGEN Gluten

Most comprehensive gluten testing portfolio …

… based on R5 antibody to ensure result comparability between different methods

RIDA®QUICK – Lateral flow tests

- RIDA®QUICK Gluten quant.
  AOAC OMA approval in preparation

- RIDA®QUICK Gliadin
  AOAC-OMA 2015.16
  AOAC-PTM 101702

RIDASCREEN® – ELISA

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  AOAC PTM approval in preparation

- RIDASCREEN® Total Gluten
  AOAC-OMA 2018.15

- RIDASCREEN® Gliadin competitive
  AOAC-OMA 2015.05

- RIDASCREEN® Gliadin sensitive

SureFood® – Real-time PCR

- SureFood ® ALLERGEN Gluten

Codex Type I Method
(Méndez method)

RIDASCREEN® Gliadin
+ Cocktail (patented)

RIDASCREEN® Gliadin
+ Cocktail ECO

➤ more convenient and environment-friendly

RIDASCREEN® EASY Gluten
+ Extraction tablet

➤ fast ELISA method and easy extraction procedure

RIDA® QUICK Gluten quant.
+ Extraction tablet

➤ lateral flow method and easy extraction procedure

RIDASCREEN® Total Gluten
+ Cocktail (patented)

➤ Specialty method for oats according to AOAC SMPR

Quality management
at laboratories

Quality assurance

We recommend strongly

- to run standards and samples in duplicate

- to run control samples with every run
  (e.g. R7012 Set of 3 Processed Gliadin Assay Controls)

Standard curve compliance criteria

- OD of Standard 6 > 1.2

- Lot-specific standard curve from CoA

- It is difficult to indicate exactly further criteria, since

  ➢ each lab uses different equipment

  ➢ each lab has its own environmental conditions (temperature, humidity, …)

  ➢ each lab has other people running the test

How to use the CoA's standard curve information?

CoA	OD	CV	B/Bmax	Diff. between standards
Std. 1	0.131	3.1%	5.3%	
Std. 2	0.591	1.0%	24.1%	18.7%
Std. 3	0.970	3.1%	39.5%	15.4%
Std. 4	1.433	5.0%	58.4%	18.9%
Std. 5	2.027	5.1%	82.6%	24.2%
Std. 6	2.455	4.0%	100.0%	17.4%
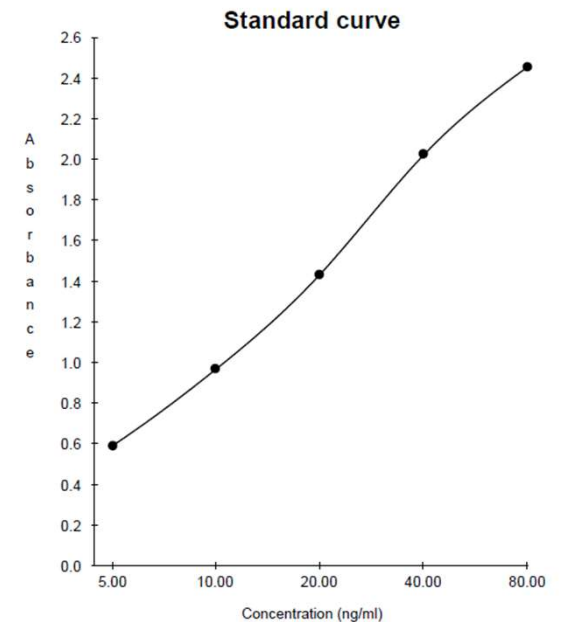
Standard curve

- Your actual standard curve should have a similar shape; only one inflection point
- OD values can be different; B/Bmax and difference between standards should be similar
- CV of duplicates up to 15% are ok, but can be also higher for Standard 1

We recommend

- to calculate mean value, SD, and CV from the first 10 runs of standards in duplicate (20 single OD values)

- to determine B/Bmax for standards and distance between standards from these runs as target and define a target range

- to run control samples (one, better two) with each run

- calculate mean values, SD, and CV as target from the first 10 runs and define a target range; plot values in a diagram

Thank you for your attention!

More about R-Biopharm
Food & Feed Analysis

https://r-b.io/food