Integrated gluten management –
gluten testing along the food production chain

FAMS, Sydney 20th – 22nd of May 2025, Dr. Sigrid Haas-Lauterbach, R-Biopharm AG, Germany

The gluten-free foods market is booming (again)

- Celiac disease is an autoimmune disorder with formation of antibodies (IgM, IgA, IgG), which is induced by gluten from wheat, rye and barley
- The global prevalence of celiac disease is approx. 0.7% - 1.4% (Clin. Gastroenterol Hepatol. 2018 Jun;16(6):823-836)
- For celiac disease patients avoidance of gluten is the only option
- Other patients show a non-celiac gluten/wheat sensitivity (NCGWS) probably mediated by innate immunity

- Some people just feel better when avoiding gluten or even for lifestyle reasons
- Hence, the number of consumers following a gluten-free diet is remarkably high (e.g. 25% in the USA)
- The gluten-free products market is still booming with a CAGR of 8% - 9% (2024 - 2032)

(CAGR=Compound Annual Growth Rate)

Introduction – gluten-free legislation

Irrespective of the customer target group, legislation does apply

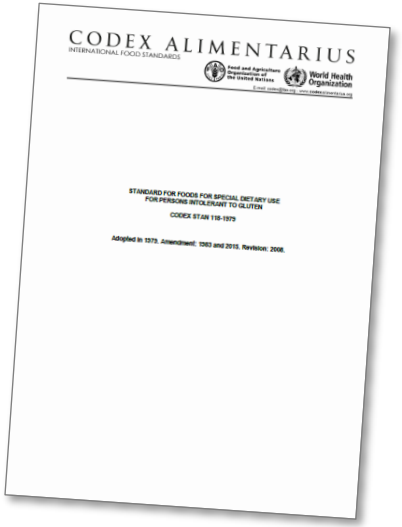
- Codex Stan 118-1979 defines “gluten-free” (i.e. ≤ 20 mg/kg)
- “Commission Implementing Regulation (EU) 828/2014” defines “gluten- free” (i.e. ≤ 20 mg/kg) and “very low gluten” (i.e. ≤ 100 mg/kg)
  → This also applies to non-pre-packed food (e.g. restaurants)
- Gluten-free Certification (like by AOECS – “crossed grain logo”)
- U.S. FDA Final Rule for the (voluntary) use of “gluten-free” if a product contains ≤ 20 mg/kg gluten
- FSANZ (Food Standards Australia New Zealand), the “gluten free claim” means no detectable gluten (LOD 1-3 mg/kg (ppm), LOQ approx. 5 mg/kg (ppm)
- Food products in Australia may be labelled as “low gluten content” if the amount of gluten is below 200 (!) mg/kg

Gluten analysis in Codex Alimentarius

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

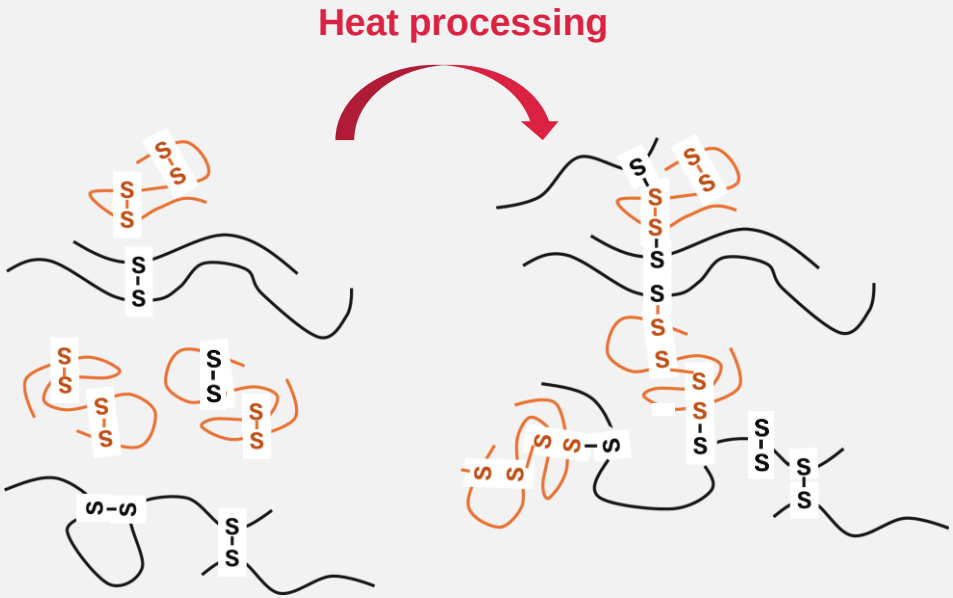
CXS 118-1979

Adopted in 1979. Amended in 1983 and 2015. Revised in 2008.



- Gluten-free foods are dietary foods in which the gluten level does not exceed 20 mg/kg
- Gluten is a protein fraction from wheat, rye, barley, *oats* that is insoluble in water and 0.5M NaCl
- Prolamins are defined as the fraction from gluten that can be extracted by 40 - 70% of ethanol
- The prolamin content of gluten is generally taken as 50%

Food processing influences gluten analysis



Gliadins/Prolamins
Glutenins/Glutelins

Complexed, insoluble gluten proteins

Development of a general procedure for complete extraction of gliadins for heat processed and unheated foods

Enrique García[a], Mercedes Llorente[a], Alberto Hernando[a], Rolf Kieffer[b], Herbert Wieser[b] and Enrique Méndez[a]

European Journal of Gastroenterology & Hepatology 2005, 17:529–539

- Heat processing of gluten leads to formation of intermolecular disulfide bonds
- Simple ethanol extraction is not sufficient
- Disulfide bond breaking reagent is needed for extraction

Gluten analysis in Codex Alimentarius

Codex Alimentarius Type I Method for gluten in foods

Standard CXS 234-1999 (adoption from 2019)

R5 ELISA

e.g. RIDASCREEN® Gliadin

+

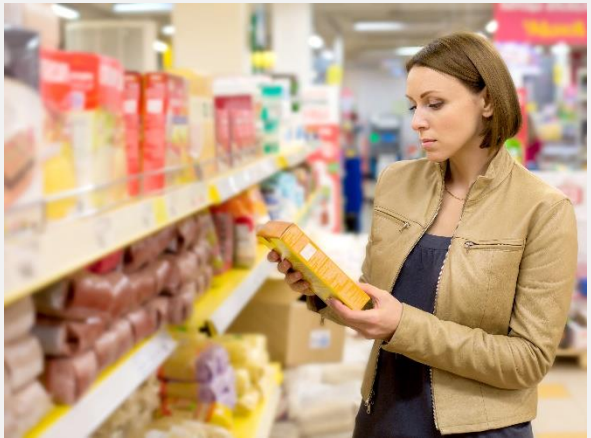
Méndez Cocktail

for gluten extraction from heat-processed foods
➔Cocktail (patented)

Comparing analytical methods for gluten detection

	LFD	ELISA	PCR	LC-MS
Qualitative results	✓	→	✓	✓
Quantitative results	✓	✓	(✓)	(✓)
Sensitivity	High	High; LOQ app. 3 mg/kg	High, based on DNA content	Very High; LOQ app. 0,03 mg/kg
Specificity	Specific for gluten from wheat, rye, and barley together	Specific for gluten from wheat, rye, and barley together	Differentiates individual gluten-containing cereals	Differentiates individual gluten-containing cereals
Target	Prolamin from wheat, rye, and barley	Prolamin from wheat, rye, and barley	Specific DNA sequences	Specific gluten peptides
Fast results	✓✓	✓	↗	→
Multiplexing	→	→	✓	✓
Automation	→	✓	✓	✓
Lab equipment	↓	↗	✓	✓
Requirements for staff training	↓	↗	✓	✓
Device costs	↓	↗	↗	✓

Critical points in gluten management along the food production chain



Grain harvest and processing

- Harvest, transport, milling of gluten-free commodities
- Detection of contamination

Risks:

- Contamination of naturally gluten-free grains and pseudo-grains e.g. polenta, rice, wild rice, millet, golden millet, brown millet, teff, amaranth, buckwheat, cassava, flaxseed, quinoa, or tapioca
- Contamination due to gluten-containing cereals on adjacent fields
- Cross-contamination during harvest, transportation, storage, packaging materials or contaminated equipment
- Crop rotation e.g. maize and winter wheat

Requirements for analysis:

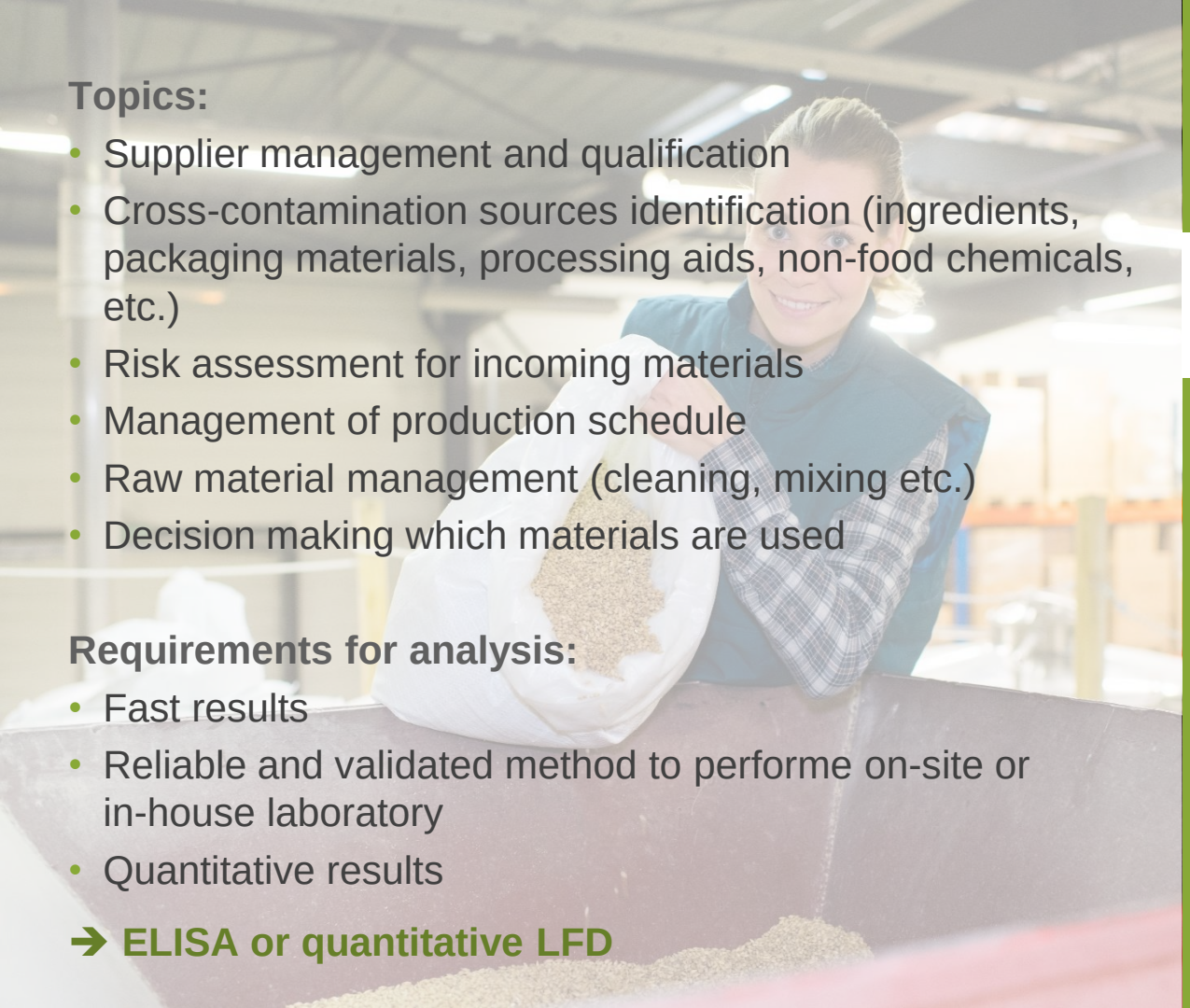
- Fast results to make decisions
- Robust and simple method to performe on-site
- Qualitative or quantitative results

➔ **Qualitative or quantitative LFD**

On site detection

Simple equipment needed

Data management



Topics:

- Supplier management and qualification
- Cross-contamination sources identification (ingredients, packaging materials, processing aids, non-food chemicals, etc.)
- Risk assessment for incoming materials
- Management of production schedule
- Raw material management (cleaning, mixing etc.)
- Decision making which materials are used

Requirements for analysis:

- Fast results
- Reliable and validated method to performe on-site or in-house laboratory
- Quantitative results

➔ **ELISA or quantitative LFD**

Ingredient management

Supplier management
Ingredients‘ handling
Prevention of cross-contamination

- Supplier audits
- Incoming goods control
- Storage management
- Data management

Topics:

- Release of production plant (check productions site and environment before production start for cross contamination)
- Validation of cleaning procedure
- Routine check during cleaning procedure
- Follow up HACCP, identification of critical control points, CIP Water

Requirements for analysis:

- Fast results
- Robust, simple and validated method
- Qualitative or quantitative results

➔ **Qualitative or quantitative LFD or ELISA**

Inline control production

- Cross-contamination during production
- Cleaning control

Cleaning process validation

On-site testing

Hygiene monitoring between production processes

Topics:

- Correct determination of gluten from different sources
- Blending of different oat lots
- High risk of contamination
- when oats are cultivated on fields where previously wheat, rye or barley was grown
- when wheat, rye or barley is grown on neighboring fields
- when oats are stored in silos together with other cereals
- when oats are ground in mills where other cereals are milled
- when equipment used for harvesting, transport, production or packaging is also used for other cereals

Requirements for analysis:

- Reliable and validated methods for detection of gluten in oats and oats products
- Qualitative or quantitative results

➔ **ELISA (quant.) for gluten in oats, PCR (qual.)**

Oats industry

Challenges due to different gluten sources (wheat, rye, barley)

- Different gluten composition in wheat, rye, and barley
- Homogeneity issues
- Identify the potential source of contamination

Topics:

- Product release as gluten free
- Consumer safety according to regulation
- Comply with different regulations and action levels
- Reference and confirmation methods
- Choice of correct method and sample preparation (e.g. highly processed, baked, hydrolysed, fermented…
- Choose the correct method (LC-MS, PCR, ELISA)
- If ELISA is used choose correct assay format (sandwich or competitive)

Requirements for analysis:

- Reliable and validated methods (Codex and/or AOAC)
- Quantitative results

➔ **LC-MS, ELISA, PCR, quant. LFD**

End product testing Labelling and consumer information

Product Quality
Consumer Safety

- Degree of processing (e.g. hydrolysis)
- Interferences with matrix components
- Extraction efficiency and recovery
- Gluten-free labelling
- Different regulations and action levels

Methods in allergen and gluten management

Widely used methods

ELISA

Lateral flow

PCR

LC-MS

… designed for an integrated gluten management along the food production chain

RIDA®QUICK – Lateral flow tests

- RIDA®QUICK Gluten quant. New
  AOAC approval in preparation
- RIDA®QUICK Gliadin
  AOAC-OMA 2015.16
  AOAC-PTM 101702

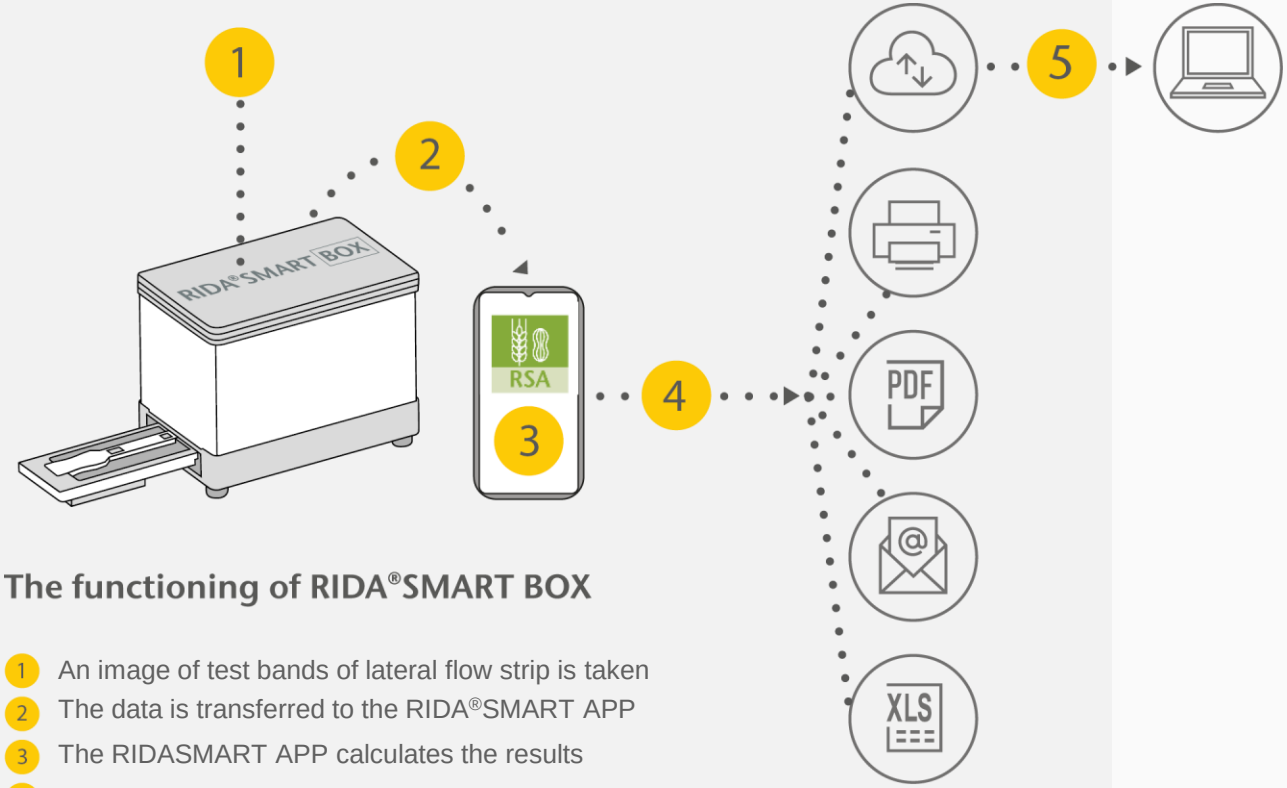
RIDASCREEN® – ELISA

- RIDASCREEN® Gliadin
  AOAC-OMA 2012.01
  AOAC-PTM 120601
- RIDASCREEN®EASY Gluten New
  AOAC 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

Integrated gluten data management – new approach



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 RIDASMART APP calculates the results
4. Full connectivity e.g. cloud, e-mail, pdf, excel, printer
5. Exchange and traceability of information

RIDA®SMART UNIVERSE

User A

User B

Conclusion
How to decide for the right method ?

- Understand the whole process from raw material to finished product
- Define why it is important to test at the single control points, e.g. descion for raw material, control cleaning process…
- Define which type of sample will be tested in this particular step, e.g. hydrolysed, baked, milled samples etc…
- Decide whether a qualitative or quantitative result is needed at a certain control point
- Decide whether a fast result or a result giving more information e.g. the amount of contamination or about the source of the contamination is needed
- Choose the method according to the individual requirements at the specific control point, e.g. method for oats and oats products or hydrolysed samples
- Choose the right format of the method, e.g. for ELISA sandwich (intact or aggregated molecule) or competitive (protein fragments)
- Check the valiadation data of the method to make sure that it works for your type of sample

Thank you for your attention!

More about R-Biopharm
Food & Feed Analysis

https://r-b.io/food