مؤتمر عُمان الدولي السادس
لسلامـــة وجـودة الغـــذاء
Oman 6th International Conference
on Food Safety and Quality

Analytical Approaches Applied to Food Allergen Management

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#معاً_لغذاء_آمن
FSQC_OM

Food allergies

- The consumption of food is safe for the majority of people

- But 2-4% of adults and 6-8% of children suffer from food allergies
- A food allergy is a malfunction of the immune system with formation of IgE against certain components (proteins).
- Food intolerances such as lactose intolerance are not accompanied by an immune response (enzyme deficiency).
- Celiac disease has elements of both an allergy and an autoimmune disease
- Number of people affected continues to rise
- Allergic reactions can range from mild to life-threatening, depending on the allergen and the patient's situation

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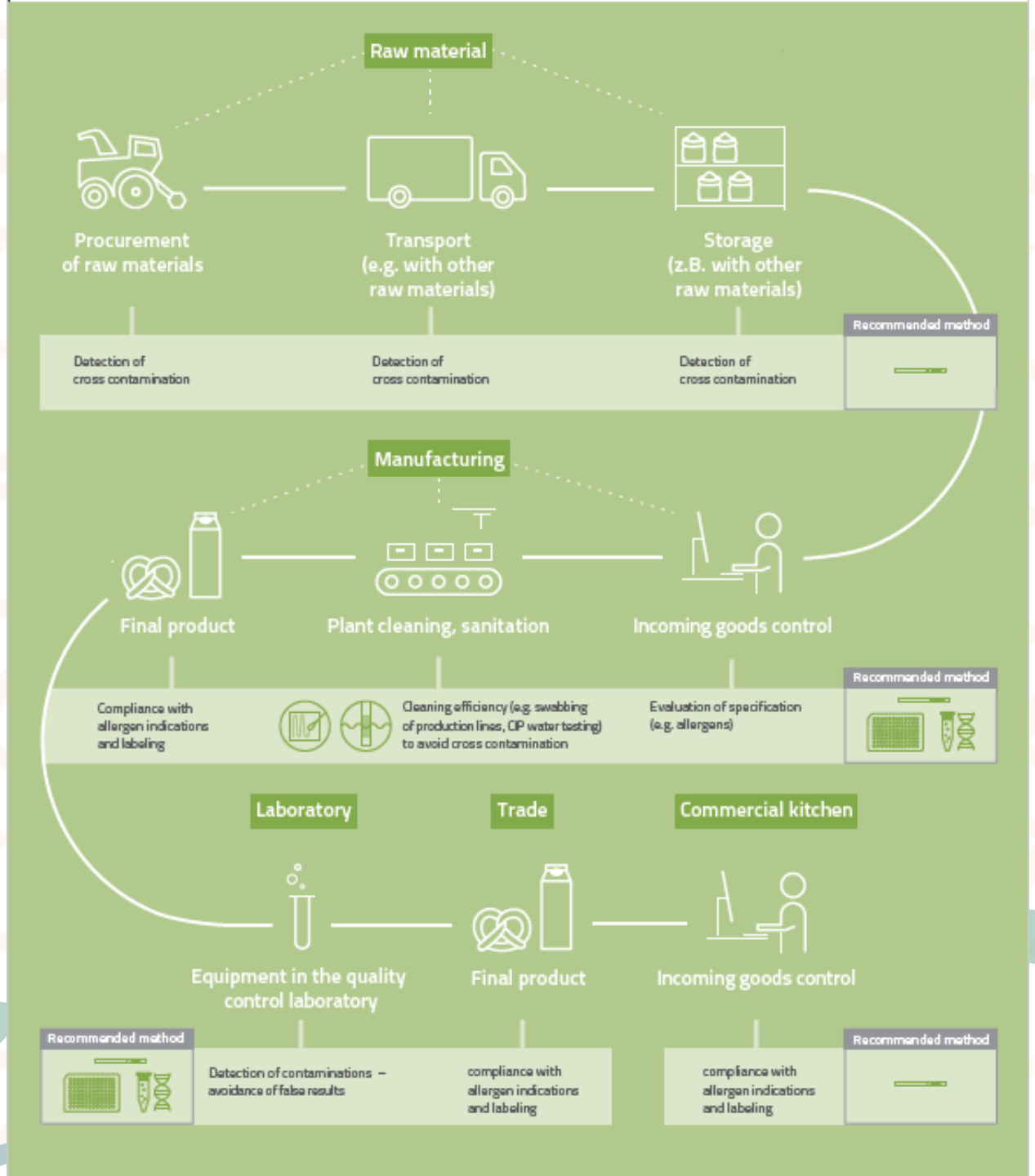
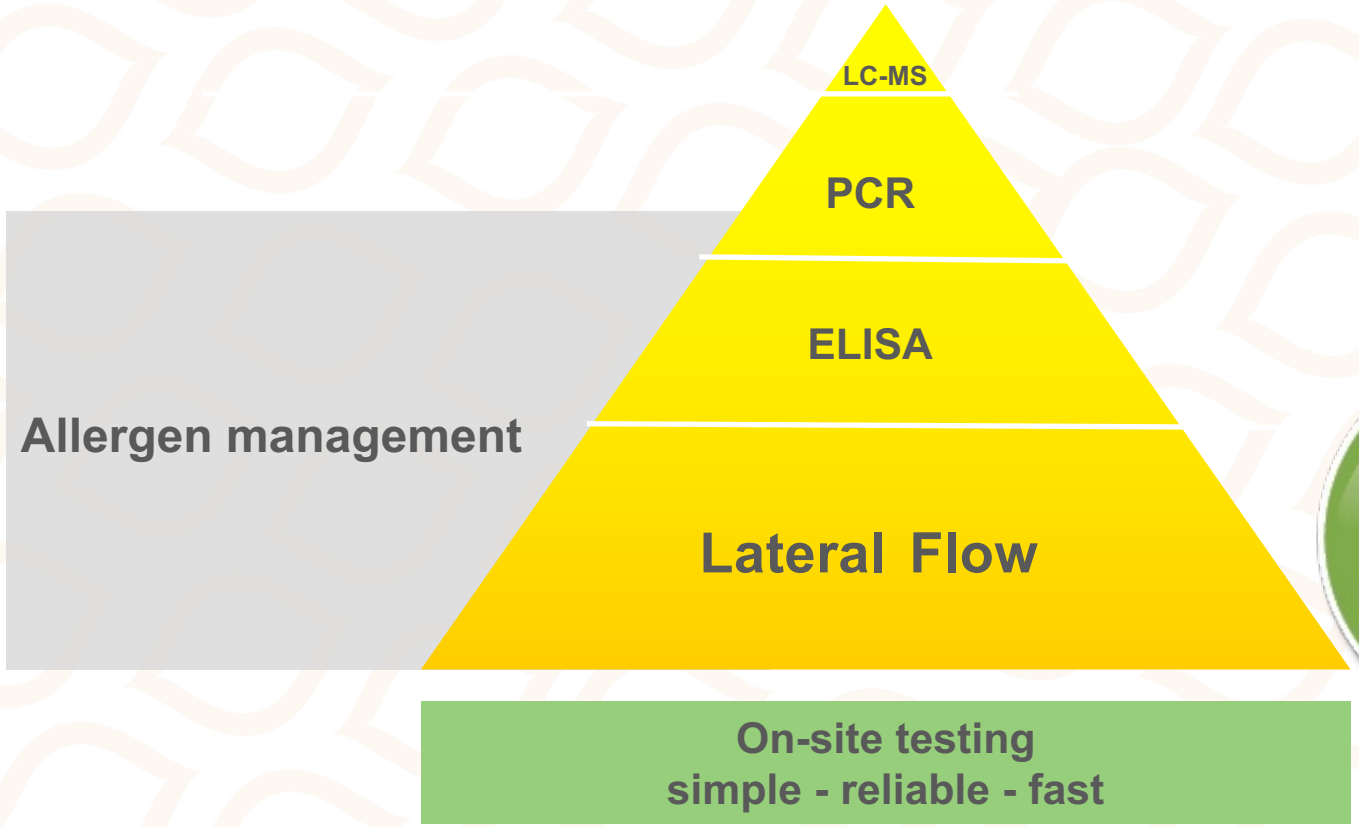
12 - 14 يونيو 2023م - مسقط

Allergen Management: test methods and objectives within the framework of food production

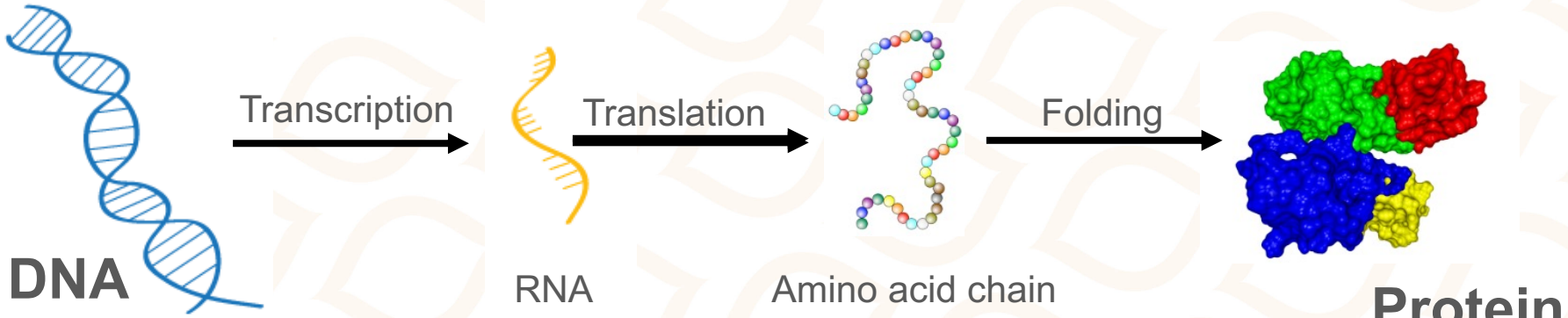
„From Farm to Fork!“



Methods for allergen management



Methods for allergen detection



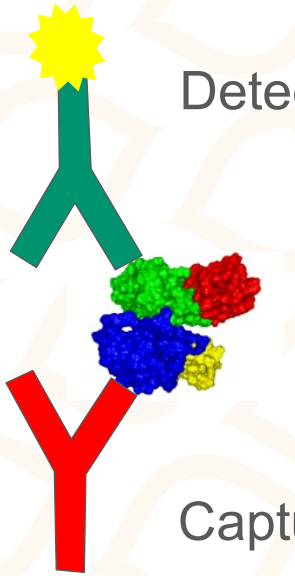
PCR

Immunobased Assays

ELISA

LFD

Detect allergens (proteins) with direct sandwich immuno assays



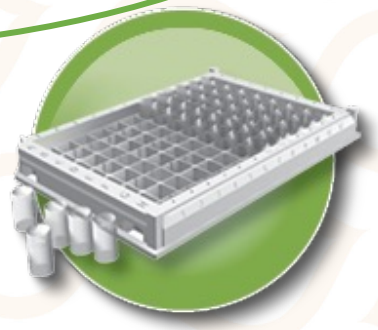
Detection antibody

Antigen (allergen)

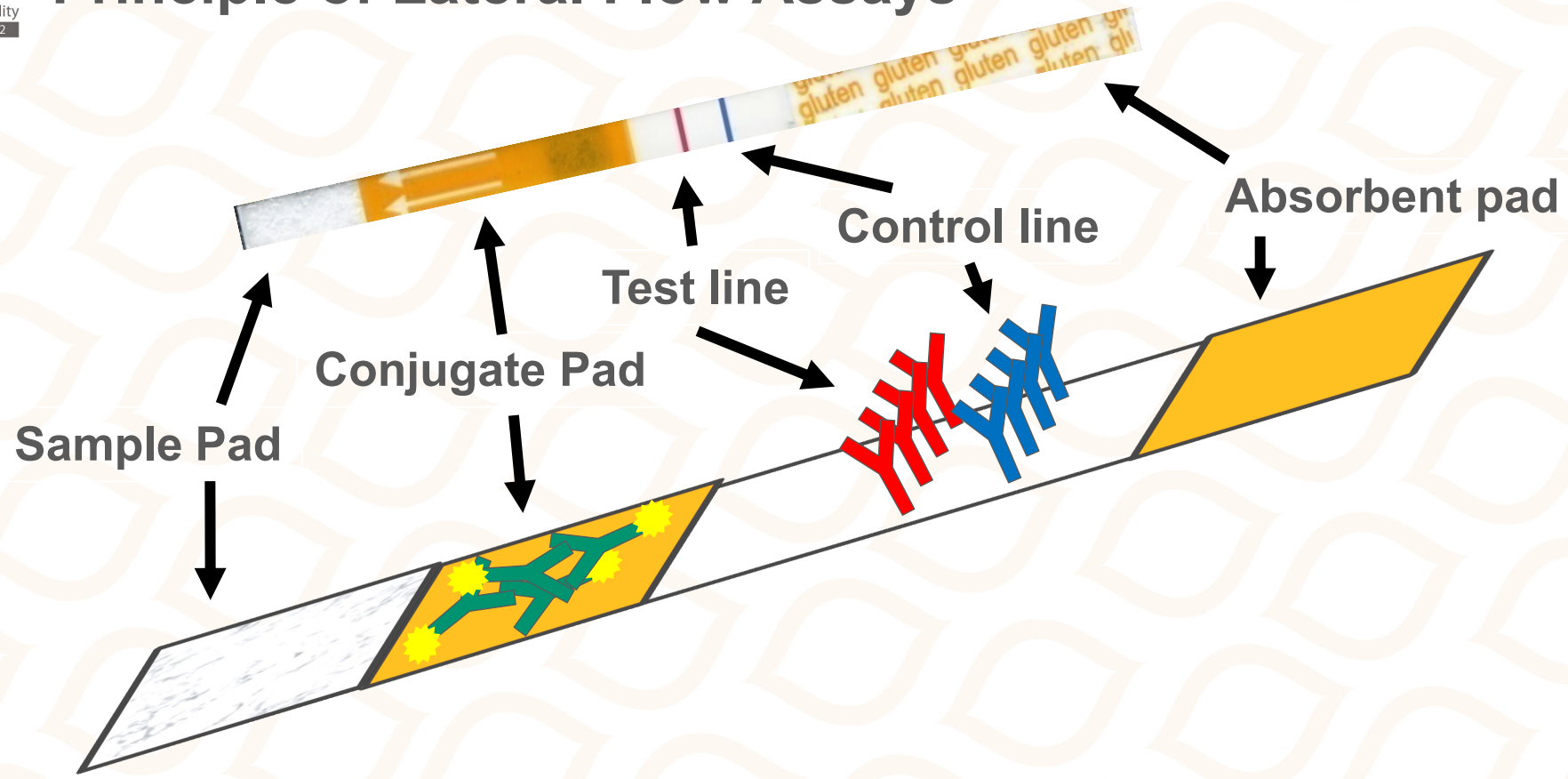
Capture antibody

Gold-labelled
Latex-labelled → Lateral flow

Enzyme-linked → ELISA



Principle of Lateral Flow Assays



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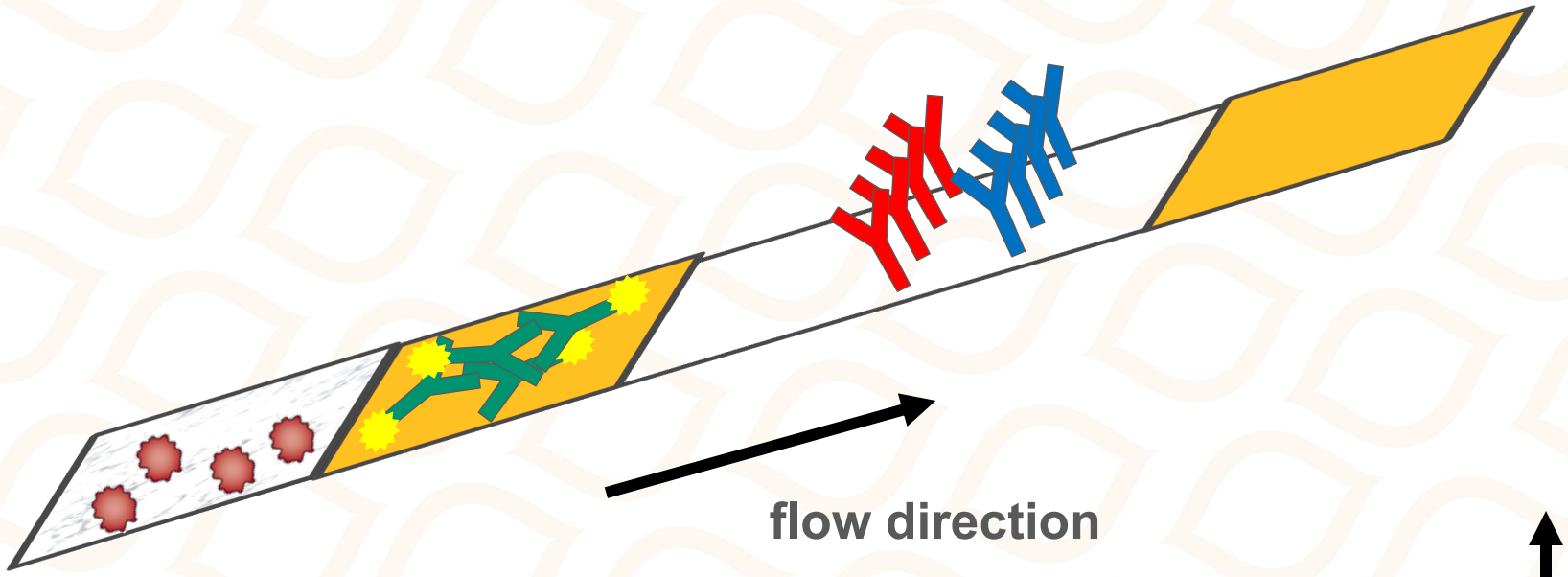

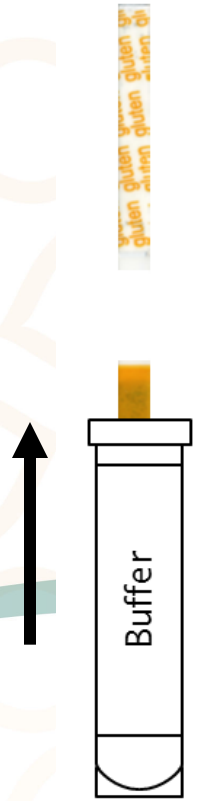

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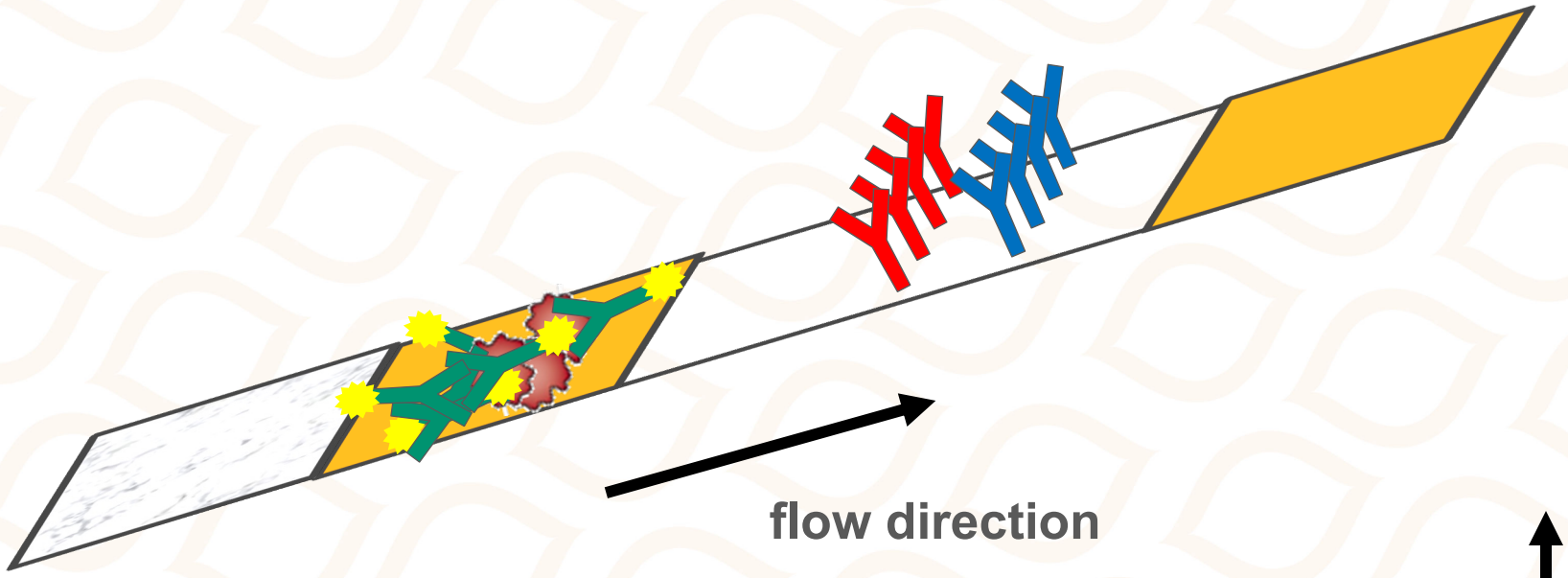

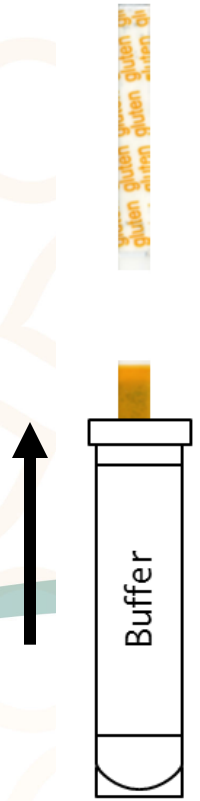

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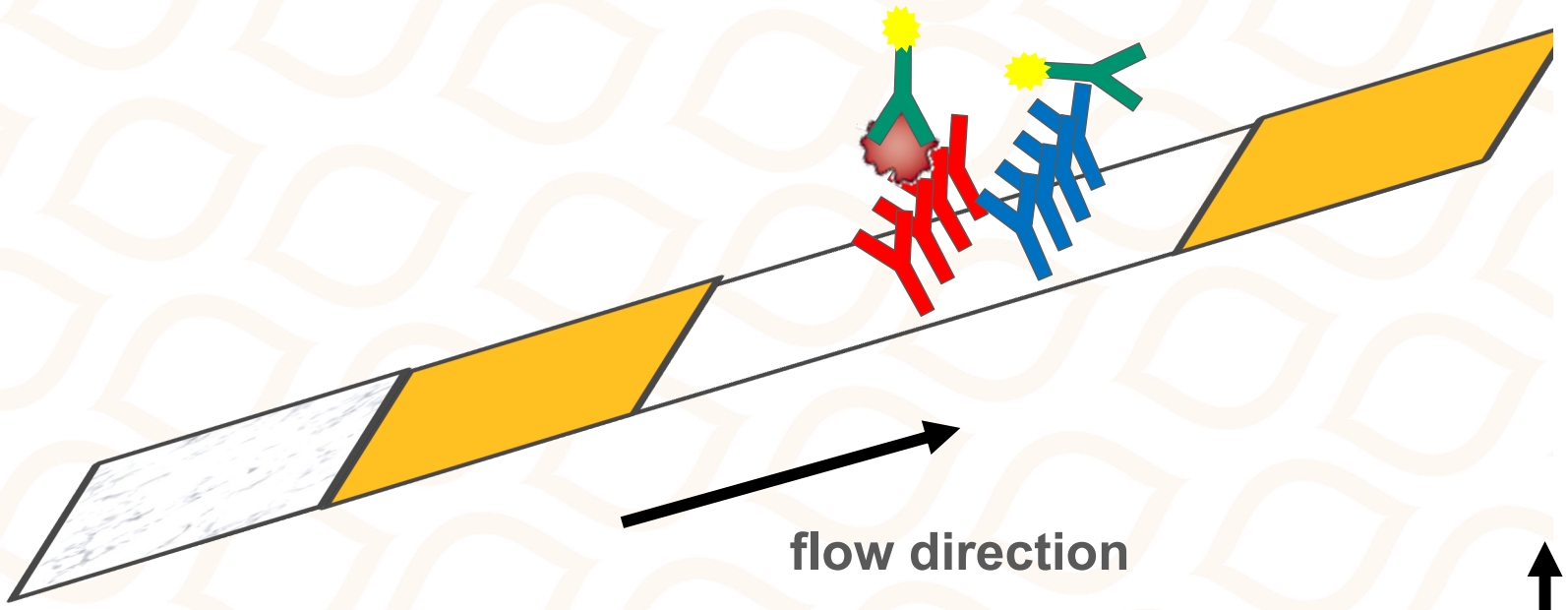

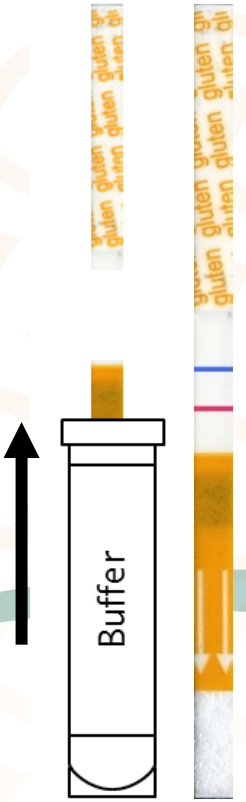

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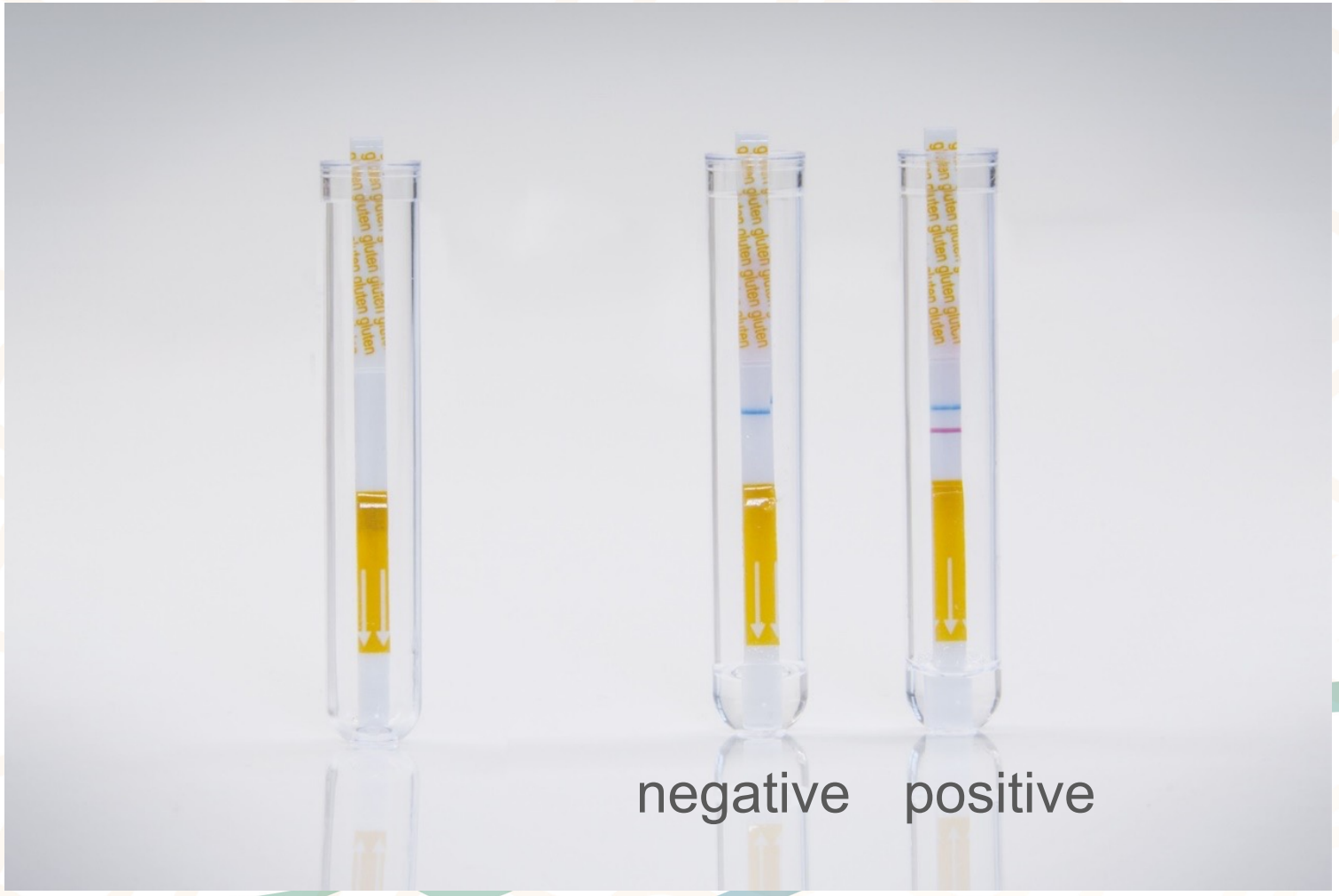


Hook Effect or „Overload Effect“

- May occur when a very high amount of an analyte is present in the sample
- The amount of analyte exceeds the amount of color-labeled antibody → ratio needed to form the test band is unbalanced
- although the analyte is present, only a weak or no test band is visible

- **Problem**: risk of falsely low or negative interpretation.
- **Solution**: included Hook line: a missing Hook line indicates a high allergen content in a sample
- Video about principle of hook effect available on request

Evaluation of LFDs with Hook Line

Negative

→ Only C-Line + H-Line present

Positive

→ C-Line + H-Line + T-Line present

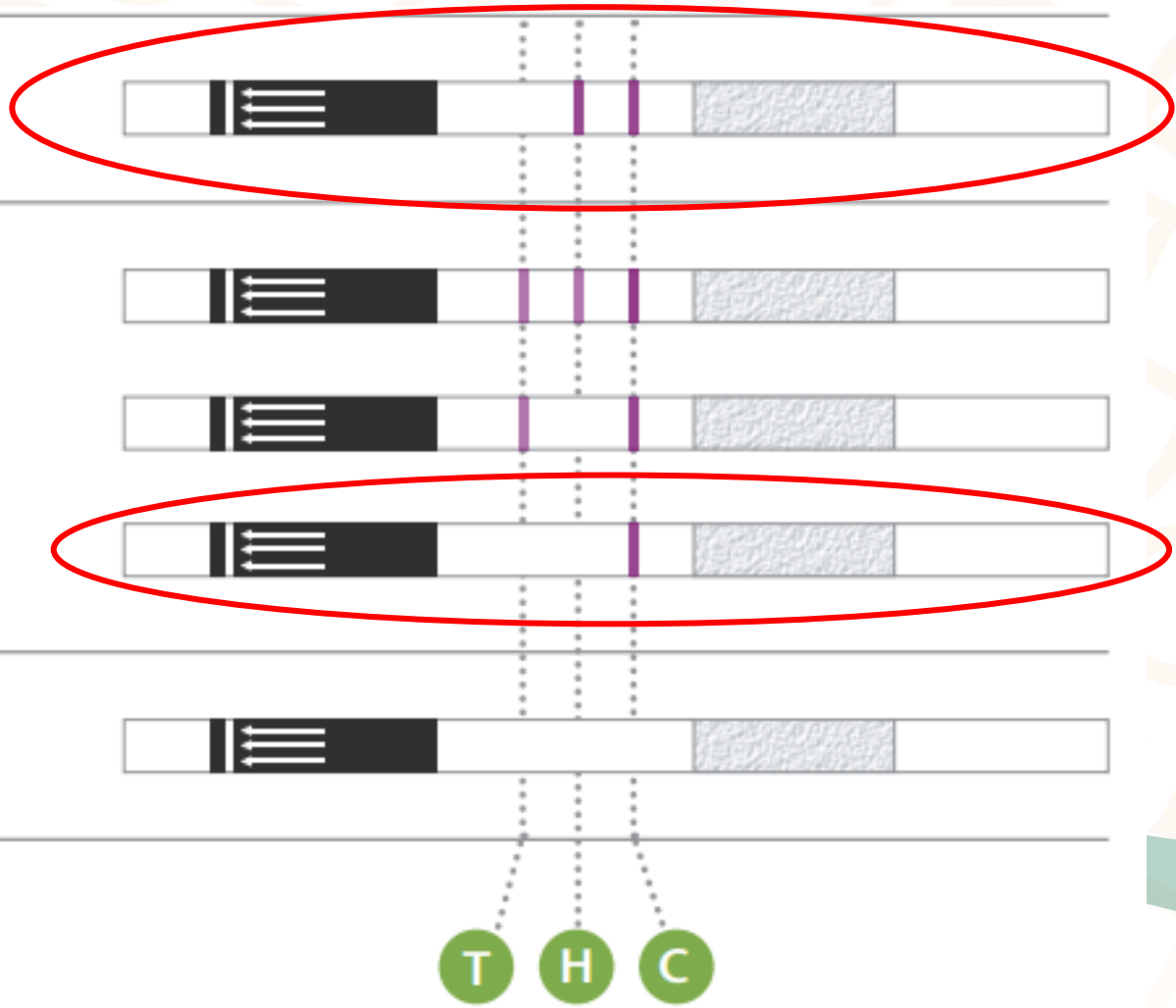
→ C-Line + T-Line present + H-Line faint or absent: high positive result (> 1.000 ppm/10.000 ppm)

→ Only C-Line present: suspected high positive result; repeat test with higher sample dilution

Invalid

→ No C-Line present

C-Line = control line
H-Line = hook line
T-Line = test line



LFD portfolio by R-Biopharm

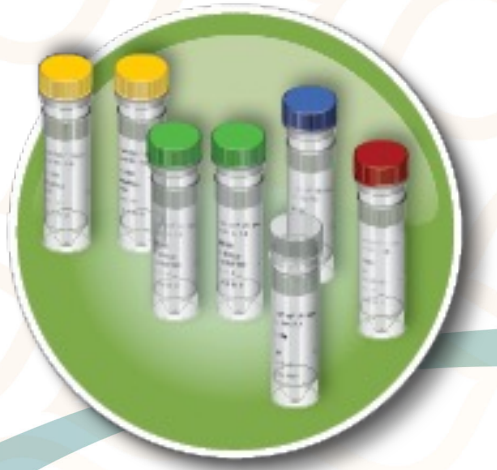
RIDA® QUICK

Bioavid

Methods for allergen detection

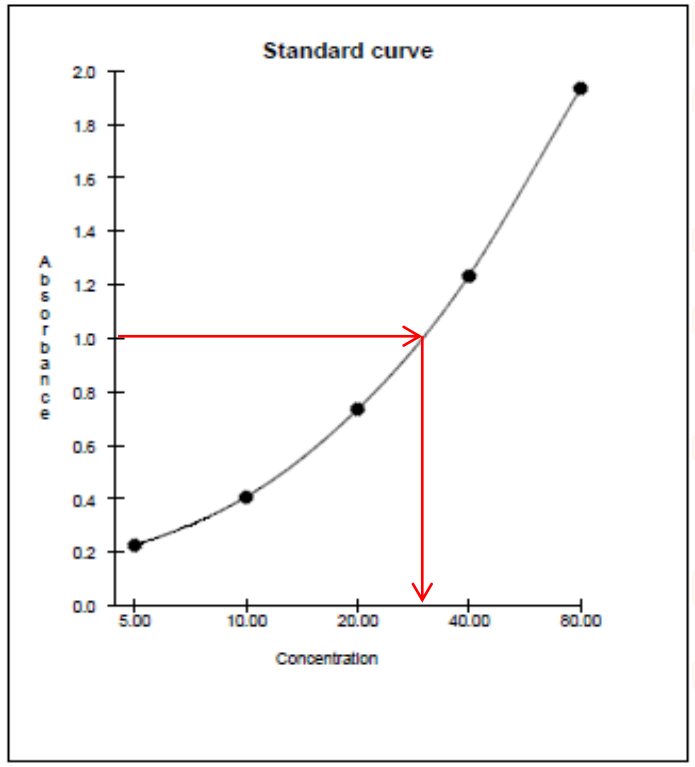
Lateral Flow

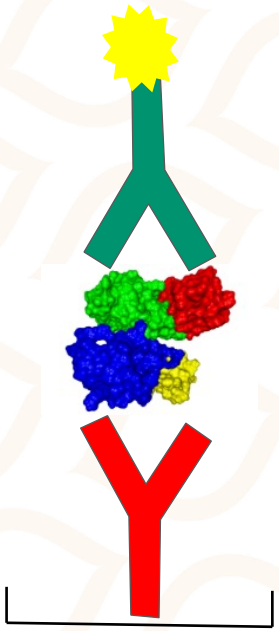
ELISA



PCR

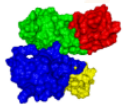
Principle of (sandwich) ELISA





The darker the color, the higher the concentration

- Measurement of 5-6 standard preparations allows plotting of a standard curve
- With the absorbance of the tested sample, the concentration can be read from the curve
- Analysis gives a quantitative result in e.g. mg/kg (ppm)



Intact proteins

Principle of ELISA

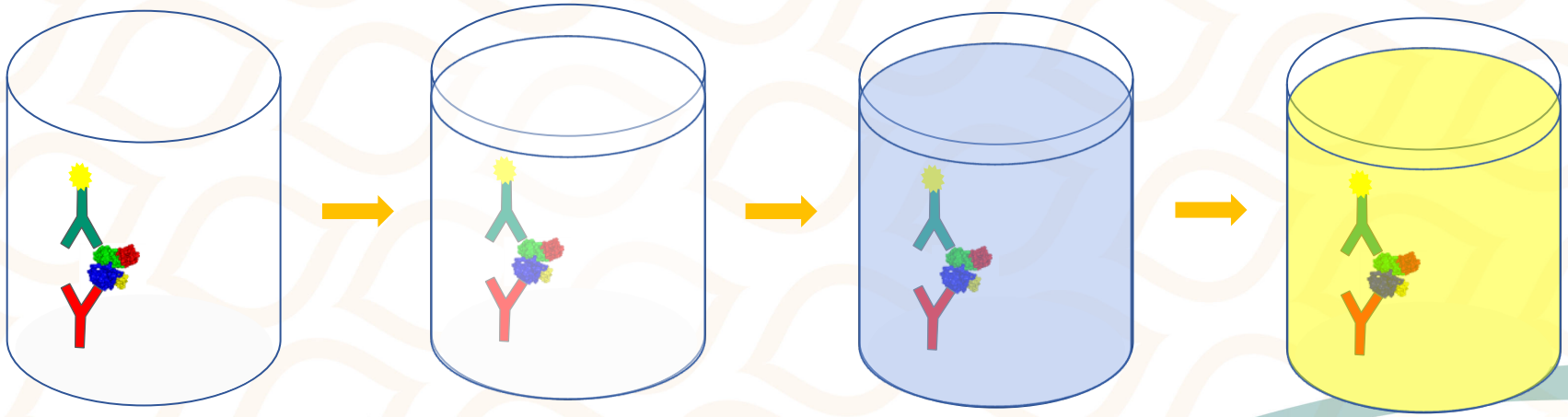
1. Addition and incubation of the sample
2. Washing
3. Addition and incubation of the conjugate
4. Washing

After the last washing step:

5. Add substrate

6. Incubation

7. Stop reaction



Allergen ELISA by R-Biopharm

RIDASCREEN® ELISA – quantitative analysis











→ Automation

Gluten-free products and gluten analysis

Gluten Analysis in Codex Alimentarius

Codex Alimentarius Type 1 method for gluten analysis in food

Standard CXS 234-1999 (adoption from 2019)

R5 ELISA

RIDASCREEN® Gliadin

+

Cocktail (patented)*

Special buffer for gluten extraction from processed food samples

Modified AOAC method adopted in an AOAC ERP meeting on 26th of April 2021:

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.

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

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

AOAC Official Method 2015.16
Gluten in Processed
and Nonprocessed Corn Products
Qualitative R5 Immunochromatographic Dipstick
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*SM

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 Bergstraβe 17
64297 Darmstadt
Germany

This method has been evaluated in the AOAC® *Performance Tested Methods*SM 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*SM 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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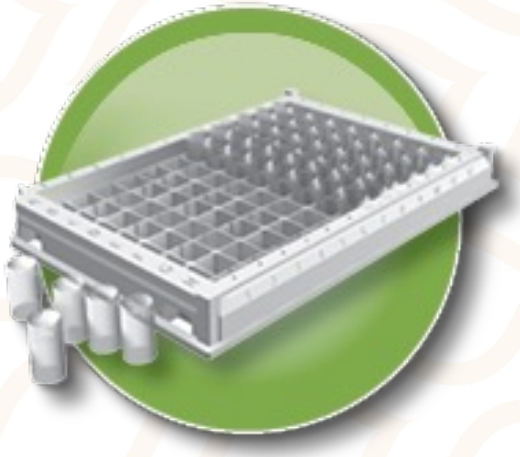
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

Methods for allergen detection

Lateral Flow



ELISA

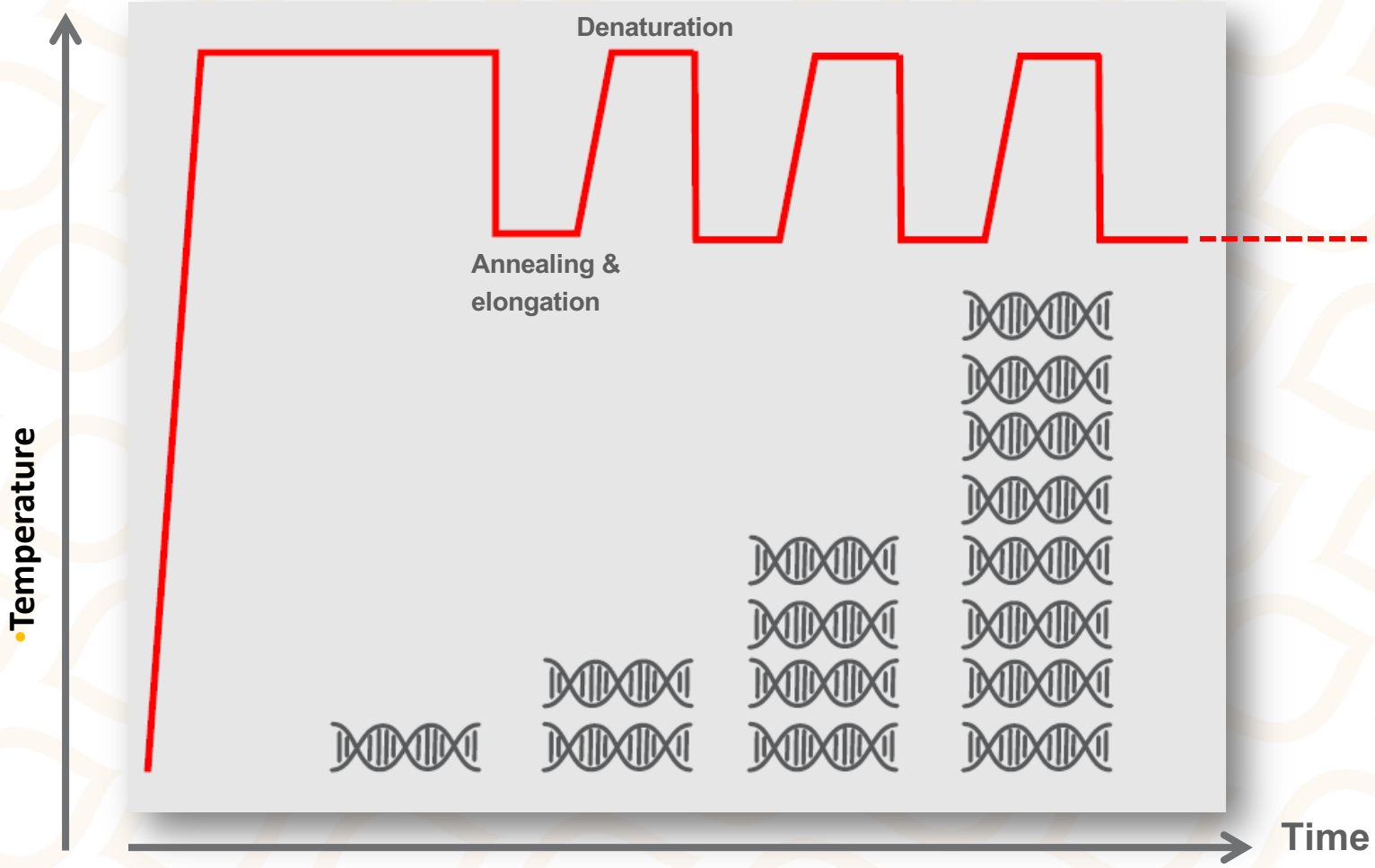
PCR

What is real-time PCR (Polymerase Chain Reaction) ?

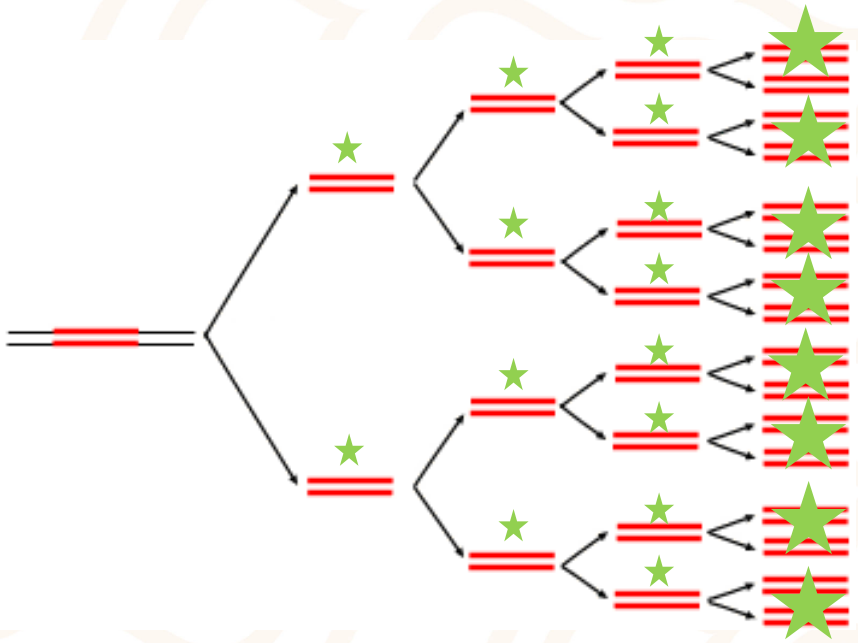
- Technique for amplifying and copying a specific piece of **DNA** multiple times
- Real-time PCR - monitors the DNA amplification during the PCR run using e.g. fluorescent reporter probes
- Highly specific (target specific primers / probe)
- Quantification possible

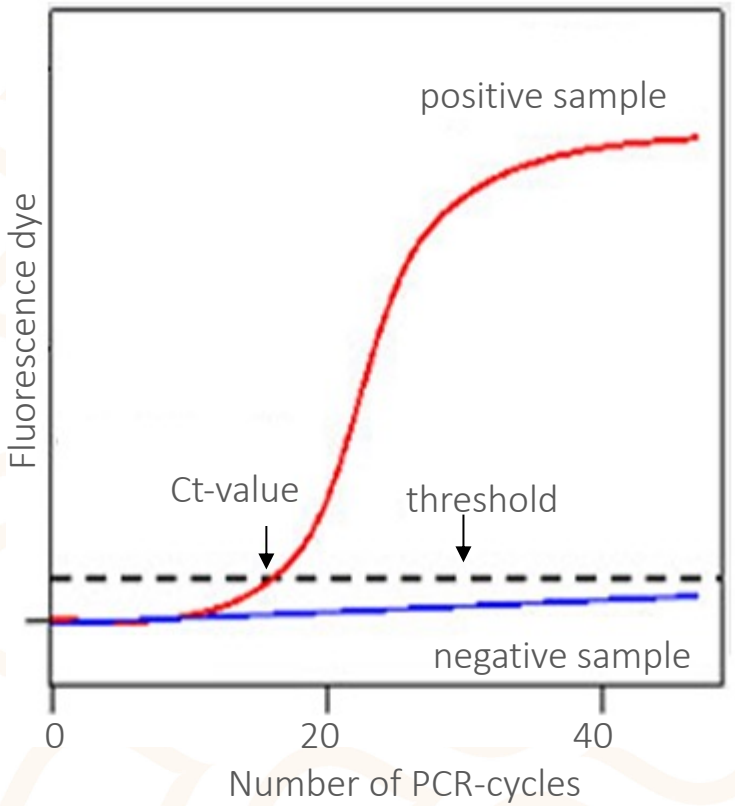
  - no information about allergenic protein expression, presence, (translational) modification, (enzyme) activity

qPCR



Exponentially amplification of the target DNA by PCR





Choose your method. What are customer demands?

Type of testing

- Cleaning efficiency on production lines
- Product testing

Kind of results

- Quantitative
- Qualitative

Number of samples

- One or just a few
- Screening of many samples

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Method comparison

Advantages		
Lateral Flow Assay	**ELISA**	**PCR**
Direct allergen detection	**Direct allergen detection**	**Allergenic species detection**
High specificity	High specificity and sensitivity	High specificity and sensitivity
Best for **non-processed** allergens (raw material, hygiene monitoring)	Best for **non-processed, processed and highly processed** allergens	Best for **non-processed and (highly) processed** allergens
Very fast results (10 min)	**Fast results (30-120 min)**	**Fast results (150-180 min)**
For single samples	For small and high sample numbers	For small and high sample numbers
Easy procedure, no lab equipment	Automated procedure possible	Automated DNA prep possible, RIDA® Cycler available
	Quantitative	Quantitative (nucleic acid)
		Detection of celery
		Multiplex assays
Limitations		
		Indirect allergen quantification
Partially lower sensitive		
Not suited for highly processed food		Low DNA concentrations in highly processed food
Matrix effects by e.g. polyphenols	Matrix effects by e.g. polyphenols	Inhibitors in food may disturb reaction
	Lab equipment needed	**qPCR equipment needed**
	Trained lab staff needed	Trained lab staff needed
		No detection of milk and egg

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Thank You!

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