RIDASCREEN® Total Gluten Development and Validation

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Why do we analyze gluten in food?

- 1% of the population suffers from **Celiac Disease**
- inflammatory intestinal immune disorder (differs from IgE-mediated food allergies)
- symptoms may appear at any time from early childhood to senior years.
- Celiac disease is caused by a reaction to gluten
- Treatment requires a strict, life-long gluten-free diet to allow the intestine to recover and to avoid complications.

What is Gluten?

- Storage protein in wheat, rye and barley
- Total protein content of wheat is approx. 10%

Wheat

Rye

Barley

What is Gluten?

Osbourne fractionation (early 20th century)

according to solubility

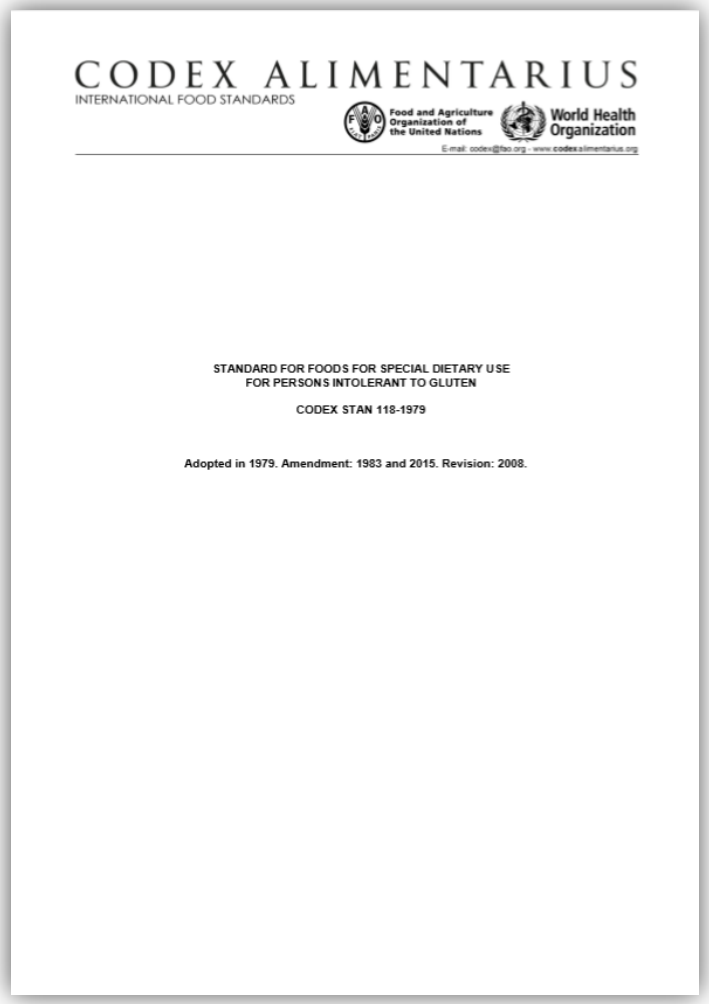
with water	→	albumins
with NaCl solution	→	globulins
with 40 – 70 % Ethanol	→	prolamins
in the residue remain	→	glutelins

→ Gluten

80 % of the proteins in wheat are gluten

Gluten = 50 % Prolamin + 50 % Glutelin

What is Gluten?



CODEX ALIMENTARIUS
INTERNATIONAL FOOD STANDARDS

Food and Agriculture Organization of the United Nations
World Health Organization

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.

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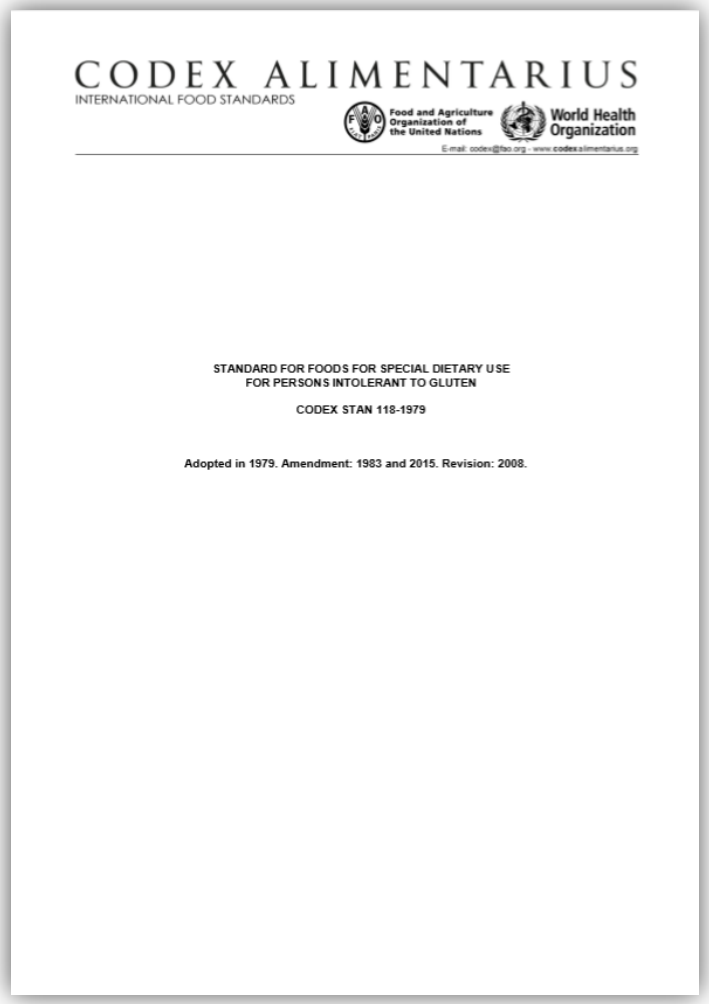
Gluten = 50 % Prolamin x 2

detected by R5 antibody

What is Gluten?



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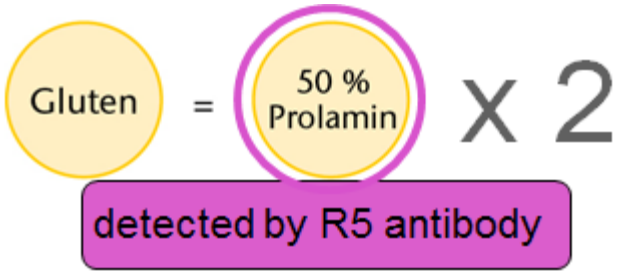
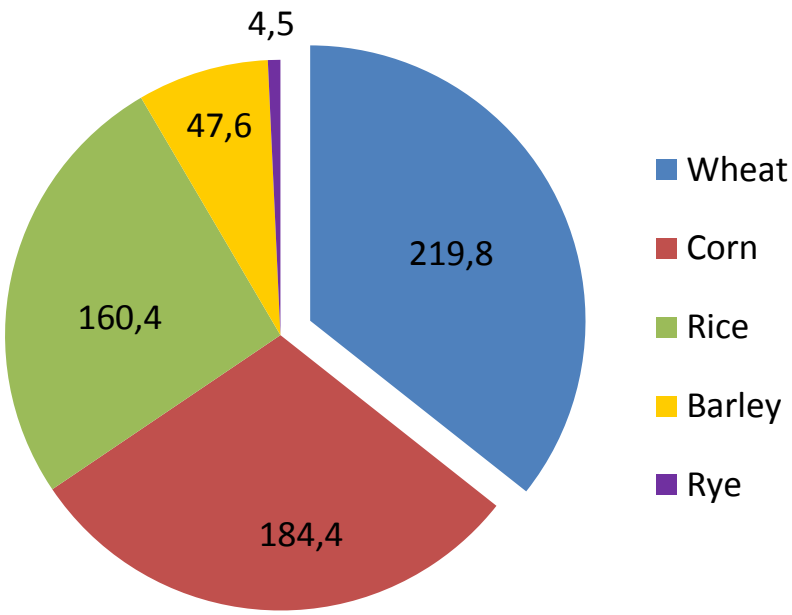


- Measurement of prolamin and calculation of gluten with conversion factor of 2
- Conversion factor is based on wheat calibrator
- R5 antibody binds more frequently to prolamin in rye and barley
- For rye and barley this factor of 2 was also accepted (Codex, AOAC)

Worldwide grain production

- Wheat is by far the most commonly used gluten containing cereal worldwide

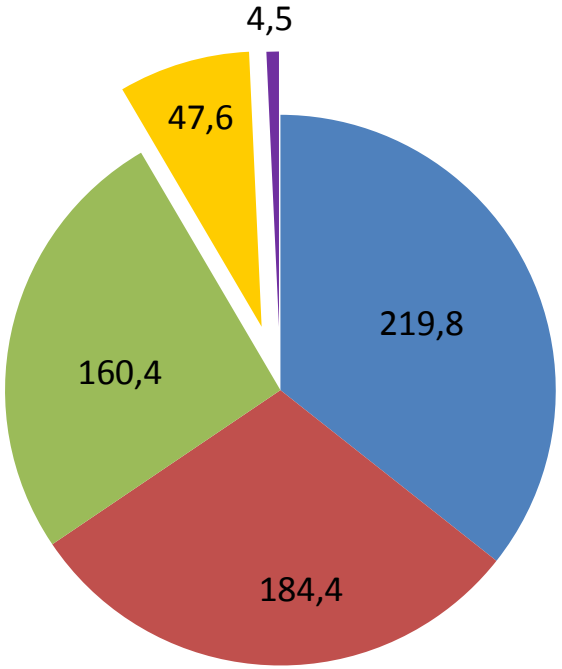


Worlwide grain production in million hectar

Worldwide grain production

- **BUT:** Rye and barley may also be a source of gluten contamination in food…

…**especially in oats**



To address the "oat issue", AOAC published an SMPR for the quantification of gluten in oats

AOAC SMPR® 2017.021

Standard Method Performance Requirements **(SMPRs) for Quantitation of Wheat, Rye, and Barley Gluten in Oats**

Intended Use: Quantitation of Gluten in the Context of Food Manufacturing.

1 Purpose

AOAC *Standard Method Performance Requirements* (SMPRs) describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site laboratory collaborative study. SMPRs are written and adopted by AOAC stakeholder panels composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC expert review panels (ERPs) in their evaluation of validation study data for a method(s) being considered to determine if it meets the requirements for *Performance Tested Methods*℠ or AOAC *Official Methods of Analysis*, and can be used as acceptance criteria for verification at user laboratories.

2 Applicability

Quantitation of total wheat, rye, and barley gluten in groats, rolled oats, steel cut oats, oat flour, oat bran, and extruded/cooked/finished oat products.

3 Analytical Technique

Enzyme-linked immunosorbent assay (ELISA) or related binding-based technologies.

4 Definitions

Gluten.—Protein fraction from wheat, rye, barley, or their crossbred varieties and derivatives thereof, to which some persons are intolerant and that is insoluble in water and 0.5 M NaCl.

Enzyme-linked immunosorbent assay (ELISA).—For the purposes of this document, ELISA is defined as "an analytical procedure characterized by the recognition and binding of specific antigens by antibodies" (*Appendix M*) (APPENDIX M OF WHAT?). This definition is not meant to be restrictive, and encompasses other related binding-based technologies.

Limit of detection (LOD; Appendix M).—LOD is defined as the

Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (%RSD_R).

Recovery.—The fraction or percentage of analyte that is recovered when the test sample is analyzed using the entire method.

5 Method Performance Requirements

See Table 1.

6 System Suitability

See antibody information, cross reactivity, and information on calibrators in *Appendix M*.

7 Reference Material(s)

Samples of oat flour spiked with wheat, rye, and barley for validation studies are available from Paul Wehling at General Mills (Paul.Wehling@genmills.com) until a suitable neutral source is established.

Refer to Annex F: *Development and Use of In-House Reference Materials* in *Appendix F: Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA. Available at http://www.eoma.aoac.org/app_f.pdf.

8 Validation Guidance

For all candidate methods, developers must:

(*1*) Provide antibody information, cross reactivity data, and information on calibrators according to *Appendix M*

(*2*) Wherever possible, identify peptide sequences or target epitopes for all antibodies used

Table 1. Method performance requirements

Parameter	Acceptance criteria
Analytical range, ppm	≤5 to ≥15
LOQ, ppm	≤5
LOD, ppm	≤5
Recovery, %[a]	50 to 200%[a]

[a] For validation purposes, individually measured as gluten from wheat, rye, and barley spiked individually in the prepared oat flour test samples, calculated from the slope of the dose response curve.
A sample series shall consist of one sample of unspiked oat flour; two samples spiked with wheat; two samples spiked with rye; and two samples spiked with barley.

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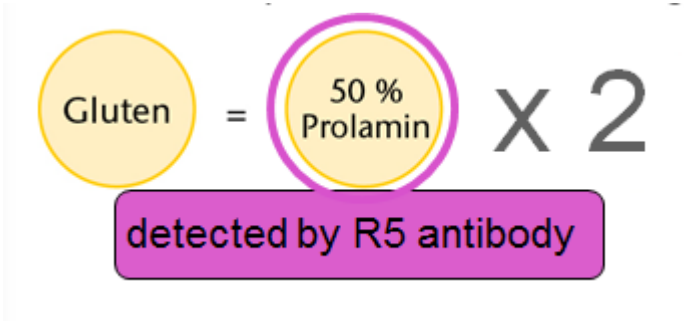
Producing a new Gluten Kit on customer demand

- Oats are often contaminated with rye and barley
- The conversion factor of 2 is not suitable for rye and barley
- Contamination with barley and rye leads to a declaration of gluten in oat products that is “really” below 20 mg/kg Gluten.
- Initiative was founded by General Mills, Quaker Oats, Grain Millers Inc., Neogen Inc., Romer Labs, R-Biopharm, Elution Technologies in 2016
- Acceptance criteria were set by stakeholders by vote in 2017

Development

Gluten in rye and barley: The conversion factor 2 leads to inaccurate results for gluten-quantification in rye and barley



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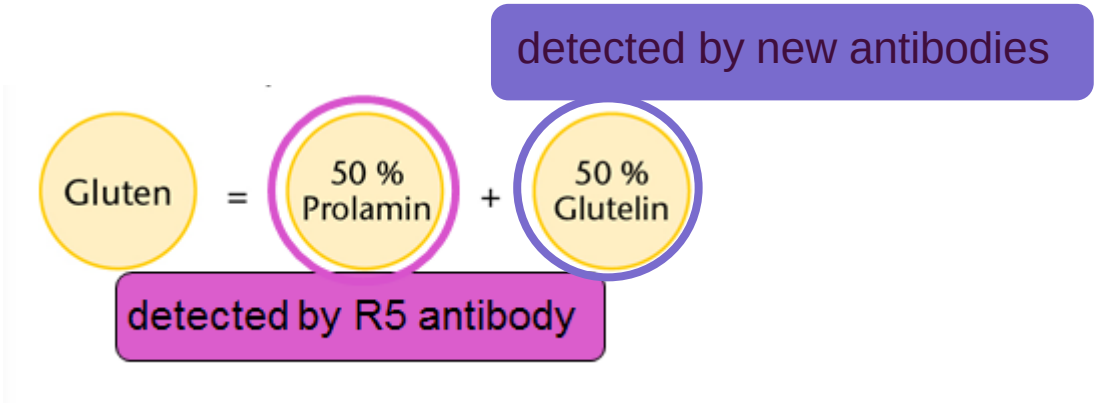
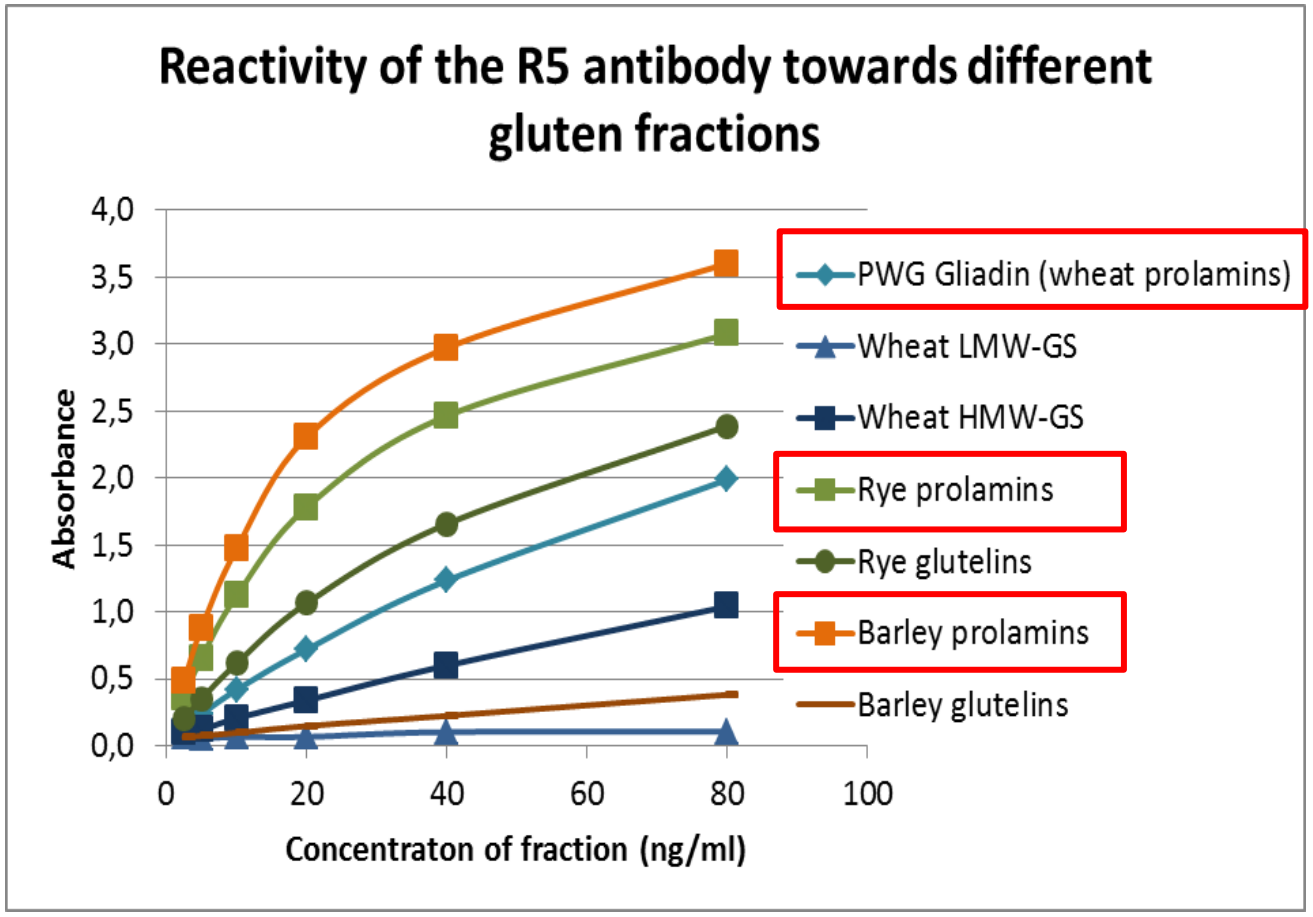
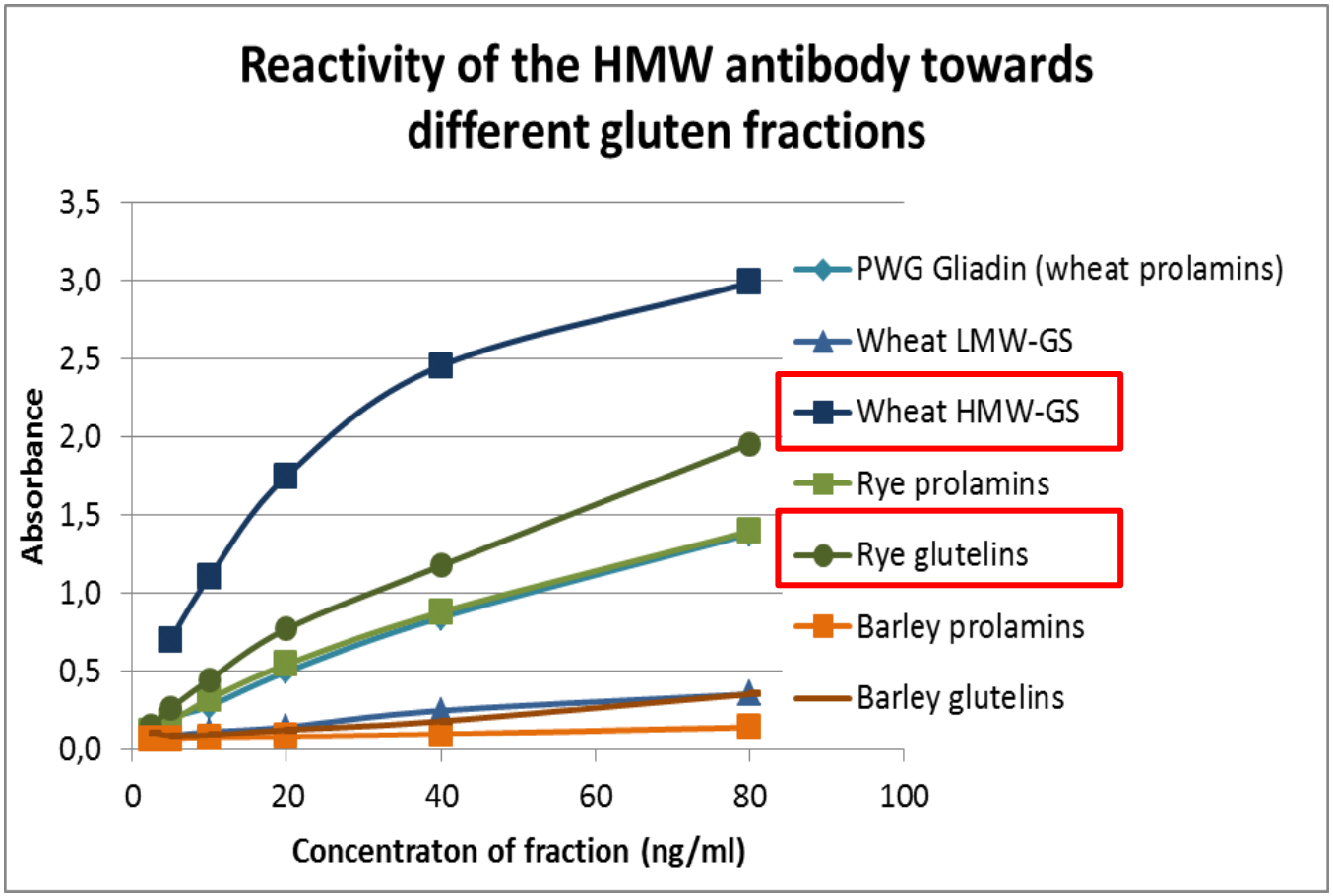


R5 antibody shows main reaction to prolamins from barley, rye and wheat



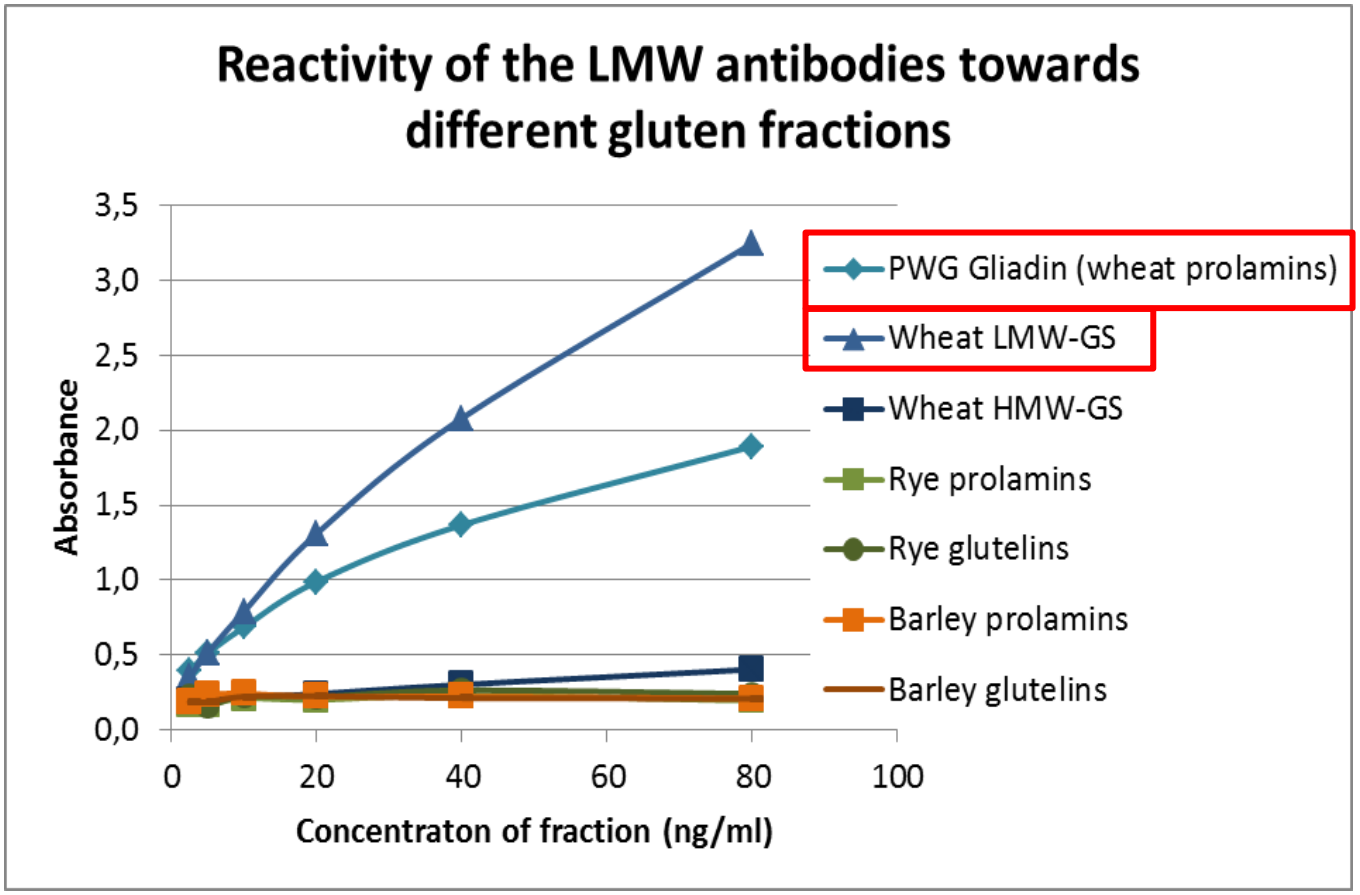
Reactivity against **wheat**, **rye** and **barley** fractions (obtained from Dr. Katharina Scherf, Leibniz-Institute for Food Systems Biology at the Technical University of Munich, Germany)

Glutelin antibody 1 (HMW GS antibody) shows main reaction to glutelins from wheat and rye



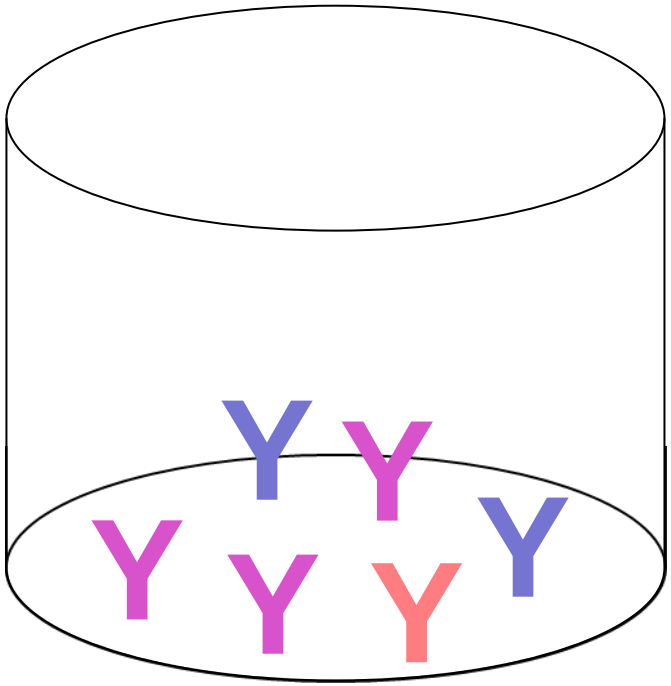
Reactivity against **wheat**, **rye** and **barley** fractions (obtained from Dr. Katharina Scherf)

Glutelin antibody 2 (LMW GS antibodies) show main reaction to glutelins



Reactivity against **wheat**, **rye** and **barley** fractions (obtained from Dr. Katharina Scherf)

Future: The new RIDASCREEN® Total Gluten simultaneously detects prolamins and glutelins



Three different monoclonal antibodies on the microtiter plate in **ONE** well

Y Prolamin antibody (R5)

Y Glutelin antibody 1

Y Glutelin antibody 2

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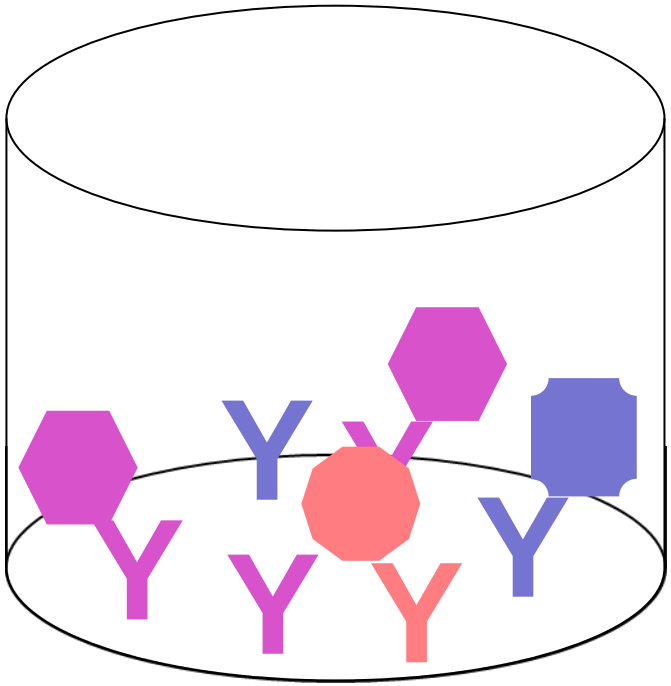

Wheat standard material / samples containing different gluten fractions

Prolamin

Glutelin 1

Glutelin 2

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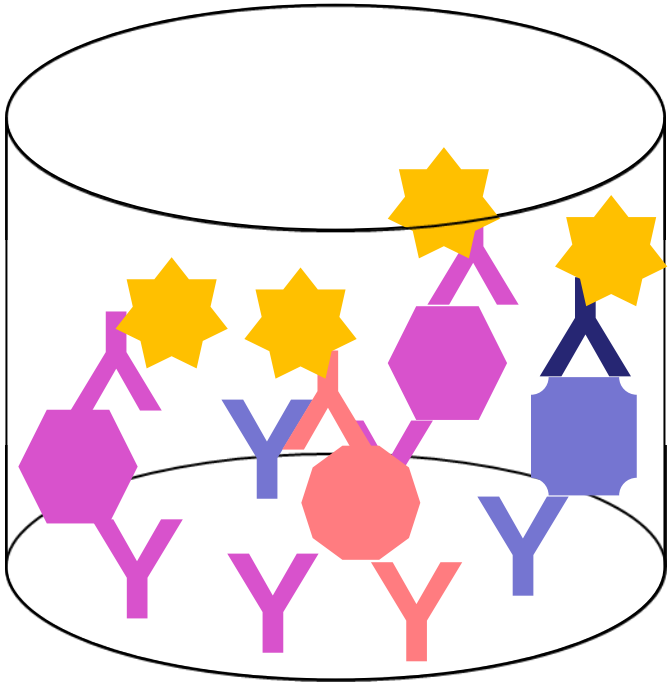

Three different monoclonal antibodies in the conjugate

Conjugated Prolamin antibody (R5)

Conjugated Glutelin antibody 3

Conjugated Glutelin antibody 2

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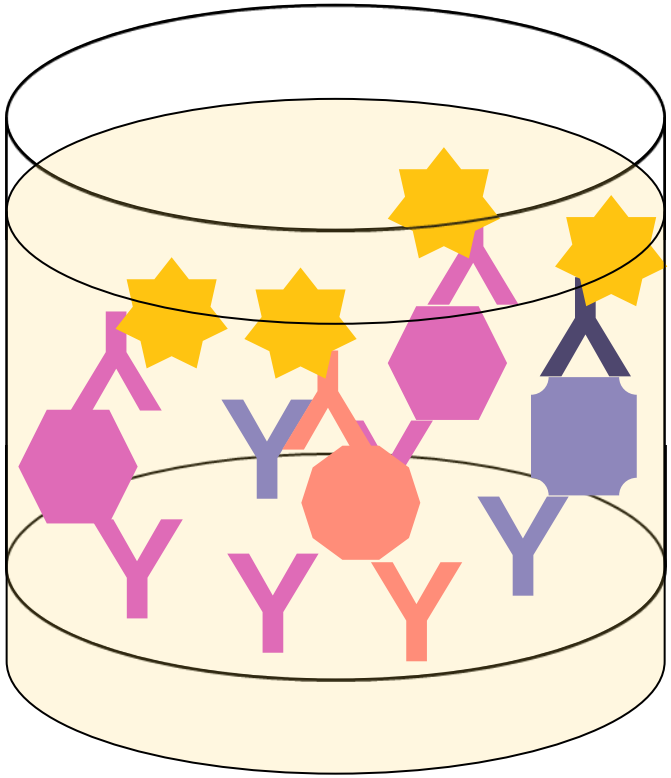
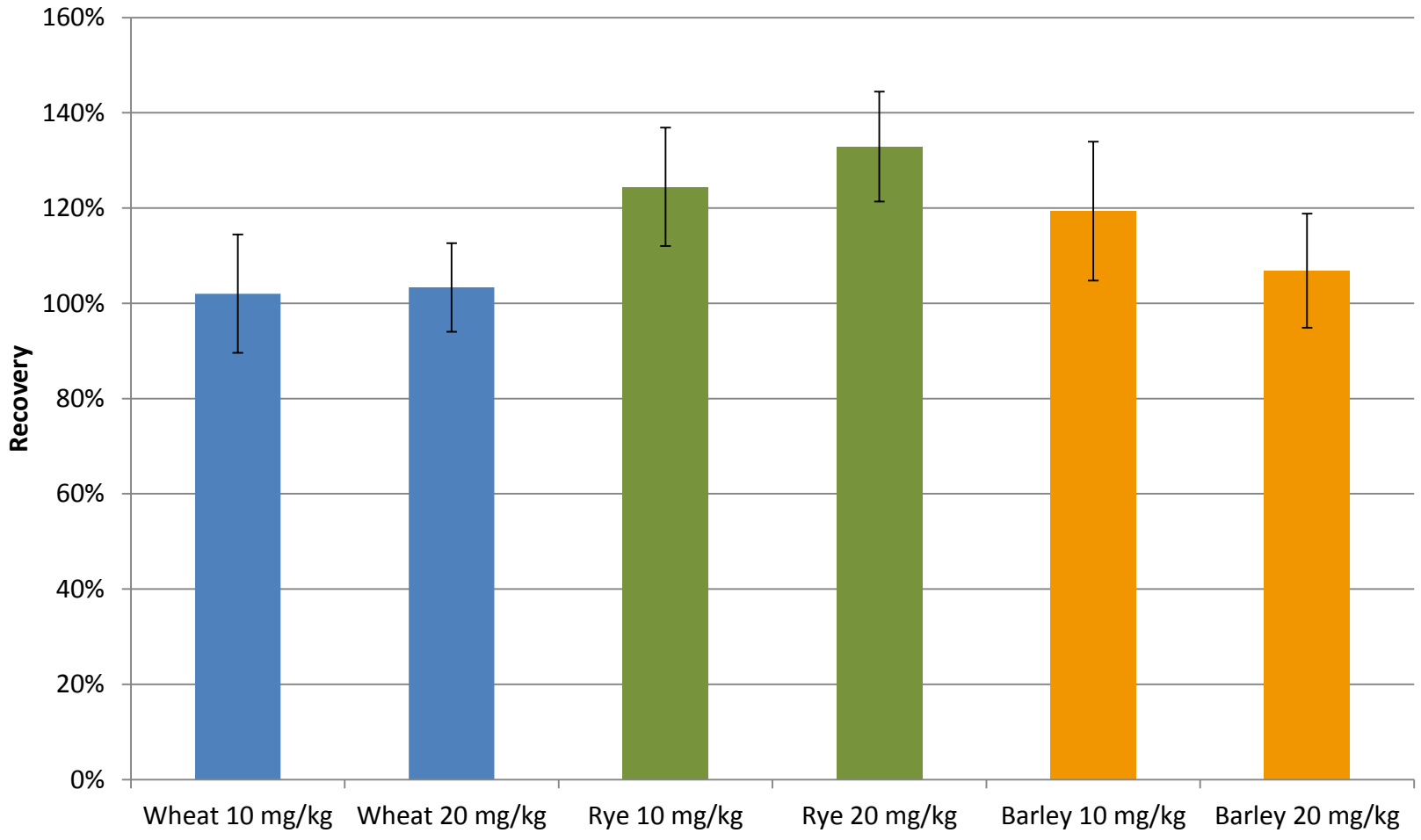


Outcome

Sum result of prolamins and glutelin is given as **TOTAL GLUTEN**

Reactivity of RIDASCREEN® Total Gluten against wheat, rye and barley in oat flours



Validation

In house validation: More than 80 potentially cross reacting food commodities have been assessed – no cross reactivity detected

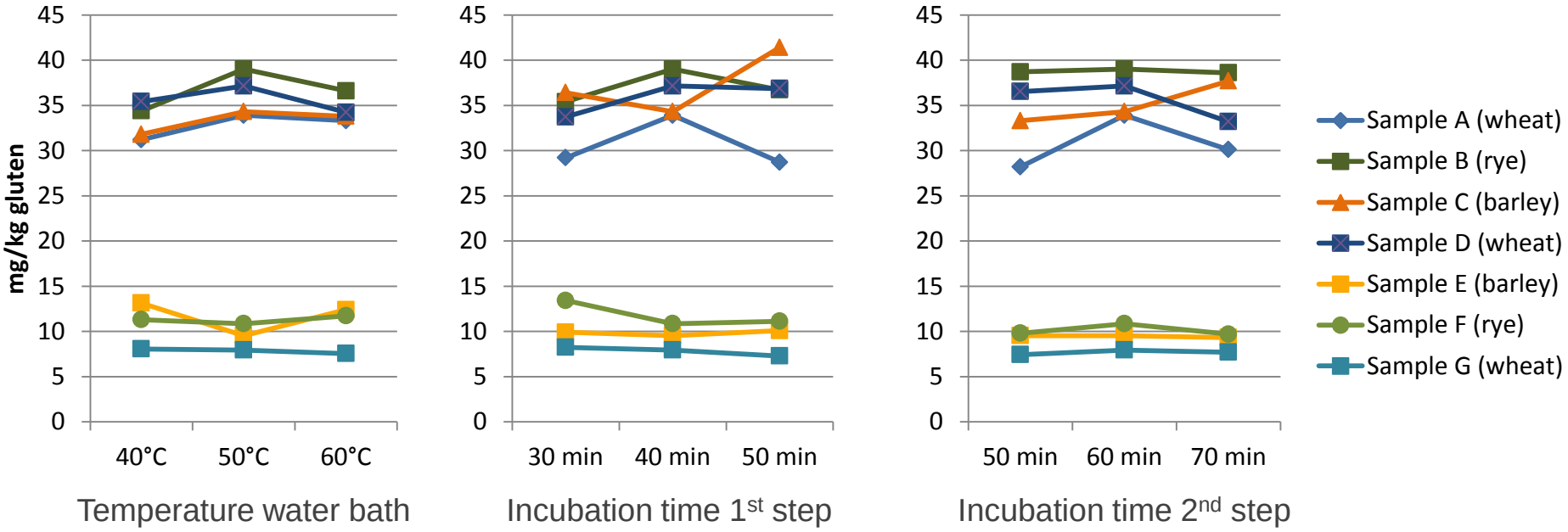
Nuts	
Almond (raw)	<LoQ
Almond (roasted)	<LoQ
Cashew (raw)	<LoQ
Hazelnut (raw)	<LoQ
Hazelnut (roasted)	<LoQ
Macadamia raw	<LoQ
Peanut (roasted)	<LoQ
Peanut (raw)	<LoQ
Walnut (raw)	<LoQ
Meat	
Beef and pork hash	< LoQ
Chicken	< LoQ
Sausage	< LoQ
Turkey hen	< LoQ

Spices	
Anise[1]	< LoQ
Basil[1]	< LoQ
Cacao[1]	< LoQ
Caraway[1]	< LoQ
Cinnamon[1]	< LoQ
Cloves[1]	< LoQ
Coriander[1]	< LoQ
Cumin[1]	< LoQ
Curcuma[1]	< LoQ
Curry[1]	< LoQ
Fennel[1]	< LoQ
Garlic[1]	< LoQ
Ginger[1]	< LoQ
Marjoram[1]	< LoQ
Mustard powder[1]	< LoQ
Mustard[1]	< LoQ
Nutmeg[1]	< LoQ
Paprika[1]	< LoQ
Pepper[1]	< LoQ
Salt[1]	< LoQ

Extract from the validation report for RIDASCREEN® Total Gluten (in preparation)

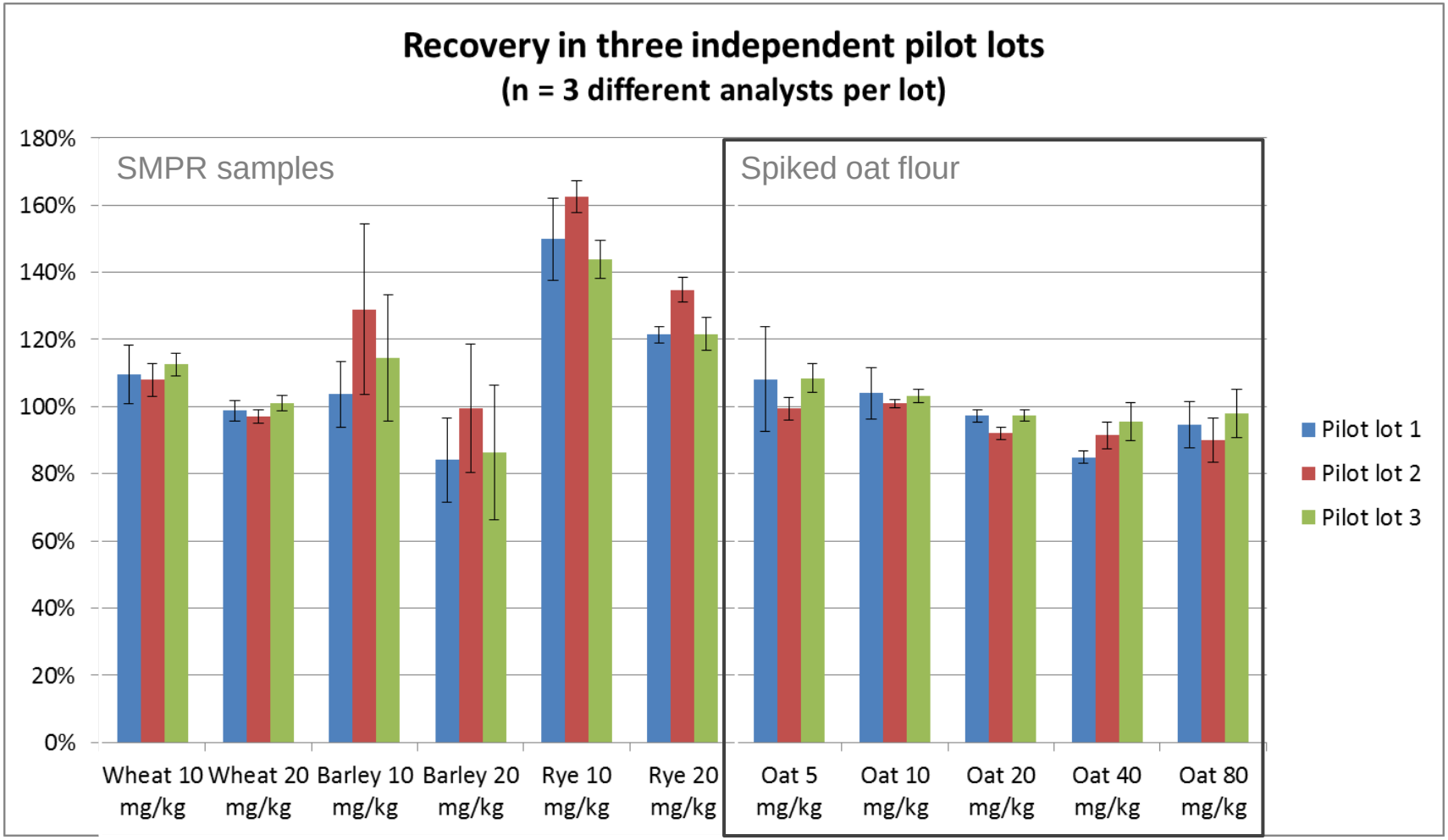
Ruggedness testing included ELISA procedure and extraction procedure – no differences were observed

- water bath temperature for first incubation step
- extraction time at 50°C (first step in water bath)
- incubation time for extraction for the second incubation step at room temperature



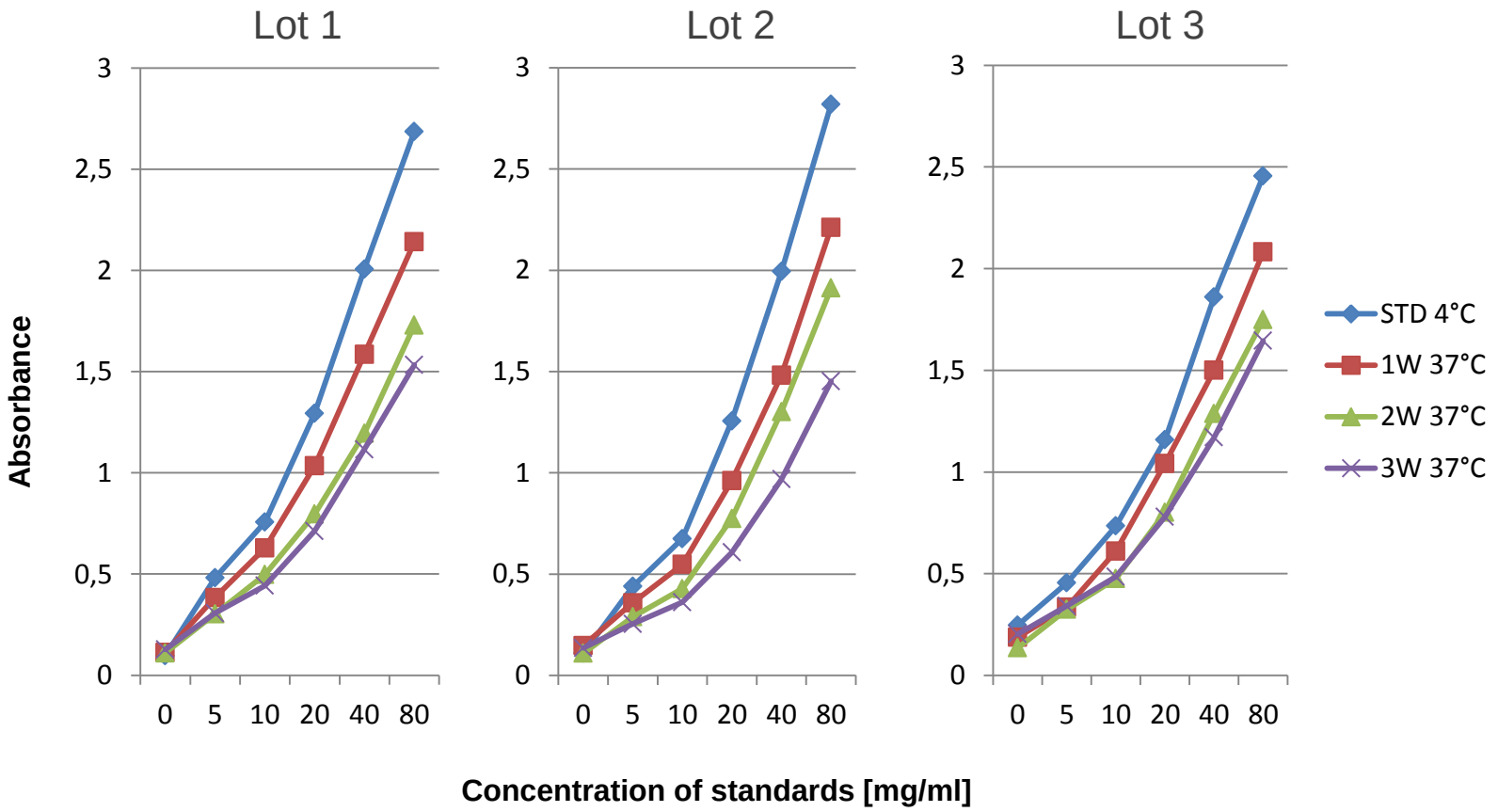
Temperature water bath | Incubation time 1st step | Incubation time 2nd step

Production of three pilot lots showed very good reproducibility of the new ELISA



Stability Study: Accelerated stability testing over 3 weeks at 37°C did not show a decrease in ELISA performance

In addition, real time stability testing is performed at R-Biopharm (storage at 4°C)



Analysis of incurred material – preparation of incurred cookies

Cookies were baked at 175°C/347°F (upper/lower heat) for 21 minutes.

External Validation

AOAC appoved methods according to guidelines
Performance Tested Methods
Official Methods of Analysis

Collaborative test:

- One method
- More than 8 participating labs
- Blind-coded samples in duplicate (blank and spiked)
- Participants perform analysis including extraction
- Calculation of recovery and reproducibility
- Calculation of LoD
- Publication of results

Results from the AOAC collaborative study

Results in mg/kg gluten						
Sample	**expected**	**mean**	**s_r**	**s_R**	**RSD_r**	**RSD_R**
Wheat flour in oat	10	10.8	2.3	2.3	21.1	21.1
Barley flour in oat	10	11	1.4	2.0	12.7	17.8
Rye flour in oat	10	13.7	1.9	2.1	13.7	15

n = 19

Summary

- RIDASCREEN® Total Gluten is the **first** ELISA testing gluten in **oats**
- RIDASCREEN® Total Gluten is the first ELISA detects gluten in total (targeting glutelins and prolamins)
- New ELISA had been in-house validated comprehensively
- AOAC OMA “First Action” status will be available soon

Thank you very much for your attention!