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THE 2016 GLUTEN-FREE STAKEHOLDER UPDATE & PLANNING SESSION PRESENTED IN COOPERATION WITH:

Agriculture and Agri-Food Canada

Canadian Grain Commission

Growing Forward 2

Prairie Oat Growers Association

Pulse Canada

EVALUATING PROBLEMATIC MATRICES

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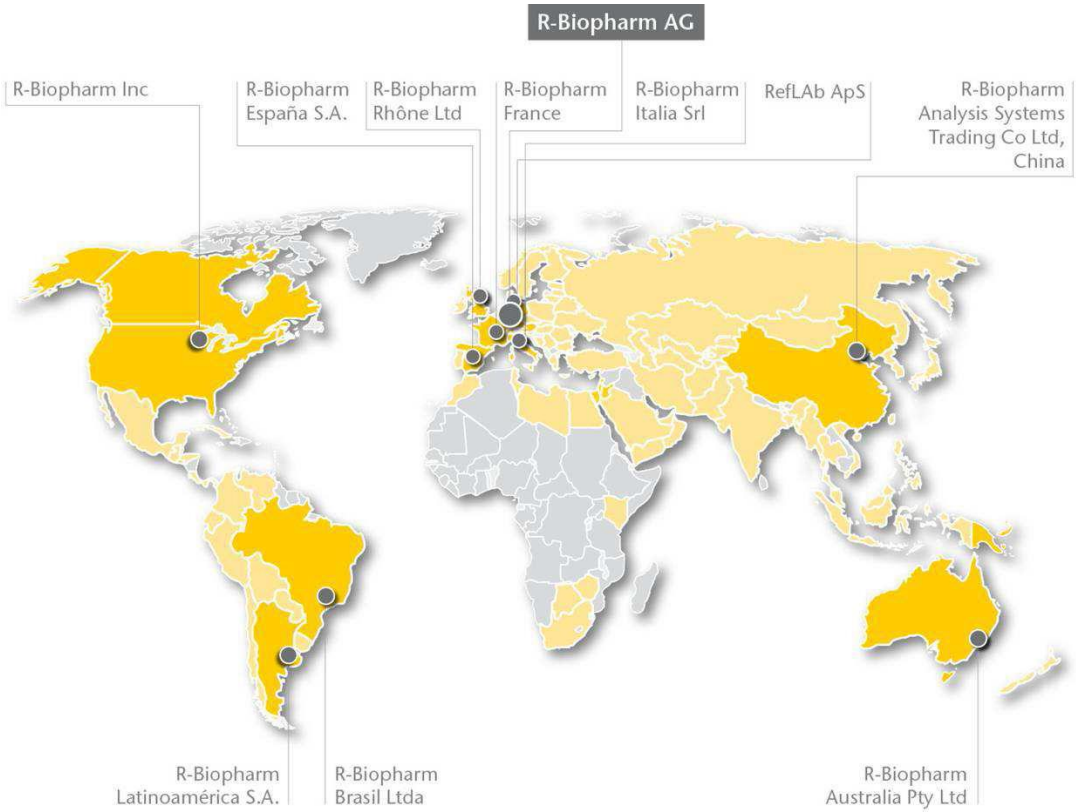
R-BIOPHARM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R-BIOPHARM & GLUTEN, A BRIEF HISTORY

1988	Foundation of Rohm GmbH (later changed to R-Biopharm)
1998	Foundation of R-Biopharm, Inc (USA & Canada)
1999	R5 Sandwich ELISA prototype created
2001	Launch of R-Biopharm RIDASCREEN® Gliadin R5 ELISA test kit
2002	Launch of R-Biopharm RIDA® Quick Gliadin R5 lateral flow test kit
2006	RIDASCREEN® Gliadin R5 ELISA received AOAC-RI approval
2007	Launch of R-Biopharm R5 Gliadin Competitive test kit (Gen1)
2008	Codex Alimentarius: 20ppm threshold and R5 sandwich as Type 1 Method
2009	Launch of R-Biopharm Surefood PCR Gluten
2011	Launch of updated R-Biopharm R5 Gliadin Competitive test kit (Gen2)
2012	RIDASCREEN® Gliadin R5 ELISA is adopted as an AOAC Official Method (2012.01)
2015	RIDASCREEN® Gliadin Competitive is adopted as an AOAC Official Method (2015.05)
2016	RIDA® QUICK Gliadin R5 Lateral Flow is adopted as an AOAC Official Method (2015.16)

CASE 1 - OATS

<u>Question:</u> Does the R5 antibody react with Oats?

Origin: Oat samples occasionally testing positive

CASE 1 - OATS

Experiment

Gather a variety of certified gluten free oat samples

Analyze them with multiple antibodies

Confirm positive results with PCR

CASE 1 - OATS

Oat variety	Antibody				PCR
	R5 Sandwich ELISA (R7001)	Antibody 2 Sandwich ELISA	Antibody 3 Sandwich ELISA	Antibody 4 Sandwich ELISA	PCR (on wheat, rye and barley - multiplex)
Ivory	positive	positive	positive	positive	positive
Scorpio	positive	positive	positive	positive	positive
Zorro	negative	negative	negative	negative	Not tested
Poseidon	negative	negative	negative	negative	Not tested
Symphony	positive	positive	positive	positive	positive
Oberon	negative	negative	negative	negative	Not tested
Aragon	negative	negative	negative	negative	Not tested
Husky	negative	negative	negative	negative	Not tested
Flämingslord	negative	negative	negative	negative	Not tested
Lutz	negative	negative	negative	negative	Not tested

CASE 1 - OATS

Conclusion

Pure gluten free oats do *not* generate a positive response with the R5 antibody

Notes:

The 3 positive varieties were reacquired at a later date and tested negative

This work was done in 2013 and more varieties have since been tested and verified

CASE 2 - HORSERADISH

Question: Does horseradish cause false positives due to horseradish peroxidase (HRP)?

Origin: Certain horseradish powders were tested positive by ELISA

CASE 2 - HORSERADISH

Experiment:

Test "positive" horseradish powder with Antibody and PCR methods to determine contamination…or false positive due to HRP

Samples to be analyzed:

#1 'negative' horseradish powder *(customer supplied)*
#2 'positive' horseradish powder *(customer supplied)*
#3 'very positive' horseradish powder *(customer supplied)*
#4 Raw horseradish *(purchased from grocery store)*

CASE 2 - HORSERADISH

Sample	Method			
	RIDASCREEN®Gliadin (Ethanol prep)	**RIDASCREEN®Gliadin (Cocktail prep)**	**RIDASCREEN® QUICK Gliadin (ethanol prep)**	**SureFood® ALLERGEN ID Gluten PCR**
#1 negative HR powder	<5ppm gluten	<5ppm gluten	<5ppm gluten	Negative
#2 positive HR powder	75ppm	>80ppm	~80ppm (serial dilution)	Positive
#3 very positive HR powder	~1500ppm	>80ppm	>160ppm (serial dilution)	Positive
#4 raw HR	<5ppm gluten	<5ppm gluten	<5ppm gluten	Negative

CASE 2 - HORSERADISH

Conclusion

Horseradish peroxidase contained in horseradish *does not cause false positives* in either RIDASCREEN®Gliadin ELISA or RIDASCREEN®QUICK Gliadin Lateral Flow

Notes:

For sample #2 it is possible that accidental contamination was to blame, however adulteration was likely the cause of the contamination in sample #3

AFTER the science proved contamination, the ingredient broker investigated his supplier in Asia…

And our customer switched brokers

CASE 3 - PROTEASE

Question: Does a protease concentrate cause false negatives in sandwich ELISA and false positives in competitive ELISA?

Origin: An enzyme manufacturer wanted to ensure no carryover from a potential contamination sources during the manufacturing process. Their analysis indicated interference when testing the enzyme concentrate.

CASE 3 - PROTEASE

Experiment:

Conduct a series of spike & recovery analyses to determine root cause

Due to the known interference of enzyme concentrates it is necessary to include a denaturing step in the sample prep.

All experiments were tested with a sandwich ELISA (RIDASCREEN®FAST Gliadin) and competitive ELISA (RIDASCREEN®Gliadin competitive)

Experiment #1	*Test enzyme with normal sample prep*
Experiment #2	*Test enzyme with denaturing sample prep*
Experiment #3	*Spike enzyme before denaturing sample prep*
Experiment #4	*Spike enzyme after denaturing sample prep*

CASE 3 - PROTEASE

Experiment	Method			
	RIDASCREEN®FAST Gliadin		**RIDASCREEN® Gliadin competitive**	
	Result	**% recovery**	**Result**	**% recovery**
Normal prep	<10 ppm		132.4 ppm	
Denatured prep	<10 ppm		43.1 ppm	
Spike *before* denature	<10 ppm		195.6 ppm	316%
Spike *after* denature	23.5 ppm	118%	75.7 ppm	163%
Spike control	35.2	136%	37.5 ppm	187%

CASE 3 - PROTEASE

Conclusion

The untreated enzymes will cause *false negatives* in a sandwich ELISA and *false positives* in a competitive ELISA

It is theorized that the heating process accelerated the enzyme activity causing it to alter the spike before being denatured

Note:

Results from RIDASCREEN® Gliadin competitive indicated that there were still allergenic peptide fragments present in the sample, thus confirming contamination

CONCLUSION

Through 15+ years of servicing the gluten free industry, R-Biopharm has had the opportunity to evaluate many unique samples.

This experience gives us great confidence that we will find a solution for any potentially problematic sample matrix.

Thank You

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