



Setting the Record Straight: Dispelling Gluten-Free Manufacturing and Production Myths

Featuring:

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Original Webinar Presentation Sponsored by Frito-Lay

Important Note:

The following presentation is an abridged version of a presentation Tricia Thompson gave during NFCA's webinar on Thursday, July 26, 2012. The following information presented in this abridged version specifically discusses the assays used to test foods for gluten and why testing is needed to ensure that foods labeled gluten-free are truly gluten-free. NFCA has abridged this presentation because the original presentation focused on the FDA's proposed gluten-free labeling rule and on August 2, 2013 the FDA announced the final gluten-free labeling rule. The testing information presented here remains accurate and up-to-date.



Welcome!

Tricia Thompson, MS, RD



- Founder of glutenfreedietitian.com and glutenfreewatchdog.org
- Internationally recognized nutritional consultant, researcher, and writer on celiac disease and the gluten-free diet
- Has written for numerous publications including *Gluten-Free Living* magazine, *The Journal of the American Dietetic Association*, and *The New England Journal of Medicine*
- Author of a variety of books and book chapters including *The Gluten-Free Nutrition Guide* and *The Complete Idiot's Guide To Gluten-Free Eating*



Testing Food for Gluten

- State-of-the-art testing of finished gluten-free products is the only way manufacturers can know if their labeled gluten-free foods are safe for individuals with celiac disease and non-celiac gluten sensitivity



Sandwich R5 ELISA

- The standard sandwich R5 ELISA (R7001 Ridascreen Gliadin by R-Biopharm) with cocktail extraction (Mendez method) is widely regarded as the best available validated ELISA for assessing final food product for gluten
 - The competitive R5 ELISA (R7021 Ridascreen Gliadin Competitive) also should be used if the food product contains hydrolyzed or fermented ingredients



Sandwich R5 ELISA, cont.

- One of only two commercially available ELISAs formally validated at the levels used for regulatory purposes and official governmental methods (the other is the Morinaga Wheat Protein ELISA)
- The R5 ELISA (along with the Morinaga) is included in the FDA's proposed gluten-free labeling rule as a possible method for rule enforcement

Note: In the FDA's online Q&A document addressing the final gluten-free labeling rule, the FDA has named the specific scientifically valid methods they intend to use as a pair when determining compliance with the rule: Sandwich ELISA R5-Mendez Method and Morinaga Wheat Protein ELISA Kit (Gliadin). These are the same tests addressed in this presentation.



Sandwich vs. Competitive ELISAs: Overview

- Sandwich ELISAs cannot accurately quantify gluten that has been highly hydrolyzed
- Sandwich ELISAs require two epitopes or antibody binding sites
- When a protein is hydrolyzed, the various fragments may not contain two epitopes
- As a result, these fragments will not be measured



ELISA Overview, cont.

- A Competitive ELISA may be used to help assess gluten content of hydrolyzed foods
- Competitive ELISAs require one epitope or antibody binding site



ELISAs: A Bit More Detail

- Sandwich R5 ELISA is based on the R5 monoclonal antibody to the epitope QQPFP (*amino acid sequence glutamine-glutamine-proline-phenylalanine-proline*)
- For the sandwich R5 ELISA to work, two QQPFP epitopes are required

Source: Thompson, Mendez *J Am Diet Assoc.* 2008; 108: 1682-1687.



ELISA Details, cont.

- When gluten protein has been hydrolyzed or broken into smaller protein fragments, the resulting peptides may no longer contain two epitopes or antibody binding sites
- If sandwich R5 ELISA is used to assess the gluten content of a product containing hydrolyzed or fermented gluten, gluten content may be underestimated



ELISA Details, cont.

- For example:
 - “QQPFP” represents the epitope and “a” represents other amino acids:
 - **aaaaaQQPFPaaaaaaaaaaaaaaaaQQPFPaaaQQPFPaaaaaaQQPFP**
 - If this protein undergoes hydrolysis, the following three fragments may result:
 1. **aaaaaQQPFP**
 2. **aaaaaaaaaaaaaaaaQQPFPaaaQQPFP**
 3. **aaaaaaQQPFP**
 - Only the second protein fragment would be measured by the sandwich R5 ELISA



Dedicated Facilities and Testing

- Using a dedicated facility is NOT a legitimate reason for not testing food products for gluten
- Grains, flours and other ingredients can arrive at a dedicated gluten-free manufacturing facility already contaminated with gluten
- Contamination can occur in the field, during transport and at the processing plant



Dedicated Facilities, cont.

- Finished products can only be as “clean” as the raw ingredients used to make them
- Ingredient vendors should provide a Certificate of Analysis that includes gluten
 - Manufacturers should also test at-risk ingredients; there is no such thing as too much testing!



Contamination: Gluten-Free Grains

- U.S. Grain Standards Act allows contamination:
 - Grains with standards are allowed to contain a certain percentage of other grains for which standards have been established
 - Grains with standards include barley, corn, flaxseed, oats, rye, sorghum, soybeans, sunflower seeds, triticale and wheat
 - Corn, flaxseed, oats, sorghum, soybeans and sunflower seeds are allowed to contain wheat, barley and rye

Source: www.gipsa.gov



Grain Contamination Study

- 22 single ingredient “naturally gluten-free” grains, flours, and seeds not labeled gluten-free assessed for gluten contamination
- Samples homogenized and tested in duplicate (to evenly distribute the contaminant) using the sandwich R5 ELISA

Source: Thompson, Lee, Grace. JADA. 2010;110; 937-940



Study, cont.

- 13 of 22 products contained $<$ limit of quantification (LOQ) for gluten (5 ppm)
 - Basmati rice, brown rice, enriched cornmeal, polenta, hulled buckwheat, buckwheat groats, amaranth flour, flaxseed, amaranth seed
- 9 of 22 products contained \geq the LOQ for gluten (range 8.5 to 2,925 ppm)
- 7 of 22 products contained \geq 20 ppm gluten



Study, cont.

<u><i>Grain/Flour</i></u>	<u><i>Mean Part Per Million Gluten</i></u>
– <i>Millet flour</i>	305.0
– <i>Millet flour</i>	327.0
– <i>Millet grain</i>	14.0
– <i>Millet grain</i>	25.0
– <i>White rice flour</i>	8.5
– <i>Buckwheat flour</i>	65.0
– <i>Sorghum flour</i>	234.0
– <i>Soy flour</i>	2,925.0
– <i>Soy flour</i>	92.0



Study, cont.

- Labeled Gluten-Free Flours (mean of 6 extractions)
 - Buckwheat flour brand A: <5 ppm gluten
 - Buckwheat flour brand B: 8 ppm gluten
 - Corn meal: <5 ppm gluten
 - Millet flour: 15.5 ppm gluten
 - Rice flour (brown): <5 ppm gluten
 - Sorghum flour: <5 ppm gluten (1 extraction tested at 7 ppm gluten)
 - Soy flour: <5 ppm gluten (1 extraction tested at 6 ppm gluten)

Source: *Gluten Free Watchdog*, www.glutenfreewatchdog.org



Sourcing Gluten-Free Oats

- Particularly important for manufacturers to source oats that are certified gluten-free
- Well established that “regular” commercial oat products may be contaminated with gluten-containing grains
- Producers of specially-manufactured gluten-free oats take extraordinary steps to ensure product is gluten-free



Gluten Content of Oats Not Labeled Gluten-Free

Brand	Mean ppm gluten
McCann's Steel Cut Irish Oats (4 different lot numbers tested in duplicate)	< 3, 12, 23, 725
Country Choice Organic Oats (4 different lot numbers tested in duplicate)	< 3, 120, 131, 210
Quaker Old Fashioned Oats (4 different lot numbers tested in duplicate)	338, 364, 971, 1807




Source: Thompson. NEJM. 351;19:2021-22



Testing Samples

- How is a food tested when it arrives at a laboratory?
- Kit instructions from R-Biopharm for the R5 ELISA say to:
 - Homogenize a sufficient amount of sample by grinding it thoroughly to powder
 - Add 0.25 grams from the homogenized sample to the cocktail solution for testing

Source: www.r-biopharm.com

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Testing Samples, cont.

- To visualize 0.25 grams:
 - Think of a 1 oz. packet of instant oatmeal, which is approximately 28 grams
 - 0.25 grams is 1/112 of the packet



Testing Samples, cont.

- No uniform standards used by labs regarding:
 - What constitutes a “sufficient” amount of sample to homogenize
 - How to make sure the sample taken to homogenize is representative of the larger sample
 - What particle size is considered “powder”



Testing Samples, cont.

- One way to help make sure the 0.25 grams tested is representative of the larger homogenized sample is to test each homogenized sample in duplicate -- two extractions
- The results of the two extractions should be similar
- If they are not, the sample is not homogenized
- If the lab tests only one extraction, there is no way to know if the sample is sufficiently homogenized



Test Results: Gluten-Free Watchdog



<i>Labeled Gluten-Free Product</i>	<i>Extraction One</i>	<i>Extraction Two</i>	<i>Mean</i>
Flavor A	9 ppm gluten	9 ppm gluten	9 ppm gluten
Flavor B	15 ppm gluten	19 ppm gluten	17 ppm gluten
Flavor C	7 ppm gluten	7 ppm gluten	7 ppm gluten

Source: www.glutenfreewatchdog.org

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Test Results: Gluten-Free Watchdog



Labeled Gluten-Free Product	Extraction One	Extraction Two	Mean	Notes
First Round Testing	17 ppm gluten	68 ppm gluten	42.5 ppm gluten	Sample NOT homogenized Further grinding required
Second Round Testing	30 ppm gluten	28 ppm gluten	29 ppm gluten	Sample homogenized

Source: www.glutenfreewatchdog.org



Uniform Standards for Food Testing Laboratories are Desperately Needed!!!