



Disclosure

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Testing Food for Hydrolyzed Gluten & Why This is So Difficult

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**Learning Outcomes:
Testing Food for Hydrolyzed Gluten**

At the end of this presentation you should be able to:

- State the differences between the two main types of ELISAs used to test food for gluten
- Discuss the limitations associated with testing food containing hydrolyzed gluten
- Name hydrolyzed foods and ingredients that are difficult to accurately assess for gluten

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Tests Used to Assess Food for Gluten

–**Solid food (e.g. starch and starch syrup):** weigh 1 g of a representative, homogeneous sample and add 10 ml 60 % ethanol solution (see 5.2.)

–**Polyphenol-containing solid food (e.g. malt and hops):** weigh 1 g of a representative, homogeneous sample and add 10 ml 60 % ethanol solution, containing 10 % fish gelatin (see 5.2.)

–**Liquid food (e.g. soy sauce):** mix 1 ml of the sample with 9 ml 60 % ethanol solution (see 5.2.)

–**Beer:** mix 1 ml of the sample with 9 ml 60 % ethanol solution, containing 10 % fish gelatine (see 5.2.)

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Testing for Gluten: ELISAs


- An enzyme-linked immunosorbent assay (ELISA) is commonly used to assess food for gluten
 - This assay involves an antigen-antibody reaction
 - Antibodies in the assay will react with antigens (e.g., gluten) in the food sample
- Two types of ELISAs currently used¹
 - Sandwich --Competitive
- Sandwich ELISAs require **two** epitopes or antibody binding sites on a continuous sequence of amino acids
- Competitive ELISAs require only **one** epitope or antibody binding site on a continuous sequence of amino acids

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Sandwich R5 ELISA

- Sandwich R5 ELISA (Ridascreen Gliadin R7001) used worldwide to test food for gluten
 - Based on the R5 antibody
 - Recognizes the pentapeptide sequence QQPFP found repeatedly in gluten protein
 - Codex Alimentarius Type 1 Method
 - Definition: "A method which determines a value that can only be arrived at in terms of the method per se and serves by definition as the only method for establishing the accepted value of the item measured"²
 - Used to detect and quantify **intact** gluten proteins from wheat, barley, and rye

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Competitive R5 ELISA

- Competitive R5 ELISA (Ridascreen Gliadin Competitive R7021) is a modification of the sandwich R5 ELISA
 - Used to test food for the presence of **hydrolyzed gluten**-- Gluten that has been broken down into smaller gluten peptide fragments

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Detecting QQPFP Hydrolyzed Gluten

- Question: Why can't the sandwich R5 ELISA be used to accurately assess food for the presence of hydrolyzed gluten?
- Answer: Hydrolysis may result in gluten peptide fragments that do not contain the two QQPFP epitopes necessary for the sandwich R5 ELISA to work
- Example: A gluten protein containing the repeating epitope QQPFP* (with "a" representing other amino acids) might appear as:
QQPFPaaaaaaaa**QQPFP**aaaaaaaa**QQPFP**aaaaaaa**Q**
QPFPaaaaaaaa**QQPFP**aaaaaaaa**QQPFP**
- *glutamine-glutamine-proline-phenylalanine-proline

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Detecting QQPFP, cont

If this protein is hydrolyzed it may break apart into protein fragments:

1. **QQPFP**aaaaaaaa**QQPFP**aa
Sandwich R5 ELISA **CAN** detect
2. aaaaaaaaa**QQPFP**aa
Sandwich R5 ELISA can **NOT** detect
Competitive R5 ELISA **CAN** detect
3. aaaaa**QQPFP**aaaa
Sandwich R5 ELISA can **NOT** detect
competitive R5 ELISA **CAN** detect
4. aaa**QQPFP**aaaaaaaa**QQPFP**
Sandwich R5 ELISA **CAN** detect

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Detecting QQPFP, cont

- Competitive R5 ELISA is not a universally approved method of analysis by all regulatory agencies
- One the one hand...
 - Approved by the Association of European Coeliac Societies³ and AACC International⁴
- On the other hand...
 - Questions have been raised by the FDA and testing experts⁵

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Competitive R5 ELISA: Questions

- Is the assay more **qualitative** than quantitative?
 - In other words, the peptide fragments can be detected
 - BUT at what point do the number of peptide fragments become harmful?
 - AND can the peptide fragments be quantified into intact gluten protein?

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Questions, cont

- FDA questions (in the proposed rule) the ability of a competitive ELISA to quantify gluten peptides into intact gluten protein:
 - "There is uncertainty about whether these methods can quantify the amount of protein from which those fragments were generated by hydrolysis. **This uncertainty creates problems in equating these test results to an equivalent amount of intact gluten in the fermented or hydrolyzed product.**"⁶

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Questions, cont

- Is the R5 epitope found in all "toxic" sequences?
 - **R5 epitope itself is not toxic**—it is used as a marker protein for longer sequences of amino acids that are toxic and contain the QQPFPP epitope
 - Might be the case that the R5 epitope occurs in a fragment that is not toxic while it does not occur in a sequence that is toxic

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Difficult to Test Products

- ✓ Barley malt/malt extract/malt syrup
- ✓ Beer
- ✓ Probiotic supplements
- All difficult to assess for gluten due to the possible presence of hydrolyzed gluten

NOT Complete List

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Malt Ingredients



Malt Ingredients: Bottom Line

- Malt, malt extract, malt syrup, and malt vinegar can **not** be included in foods labeled gluten-free and sold in the US
- Products sold in the US and labeled gluten-free sometimes (wrongly) contain malt ingredients⁷
 - Both imported and domestic products
 - Malt ingredients may be included in gluten-free foods in the UK and EU depending upon gluten levels⁸

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Malt Ingredients: Definitions

- **Malt:** According to 21CFR184.1443a, "Malt is an enzyme preparation obtained from **barley**..."⁹
- **Malt extract/malt syrup:** According to 21CFR184.1445, "Malt syrup and malt extract are interchangeable terms for a viscous concentrate of water extract of germinated **barley** grain..."¹⁰
- Common or usual name for "barley malt" is "malt"
- Common or usual name for "barley malt extract" is "malt extract"
- Malt ingredients can not be assessed for gluten using a sandwich ELISA because they are **naturally hydrolyzed** by enzymes present in barley grain

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Definitions, cont

- **Malt vinegar:** According to CPG Sec. 525.825, Malt vinegar is "The product made by the alcoholic and subsequent acetous fermentations, without distillation, of an infusion of barley..."¹¹
- Malt vinegar can not be assessed for gluten using a sandwich ELISA because it is a **fermented** product

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Malt Ingredients: Gluten-Free Foods

- FDA clarified their position on the use of malt ingredients in foods labeled gluten-free during an Academy webinar:¹²
 - "Malt extract and malt syrup are ingredients derived from gluten containing grains and containing gluten therefore we would consider them **as ingredients not processed to remove gluten and they would not be permitted in foods bearing the claim gluten-free.**"
 - "... if malt syrup or malt extract are further processed in some way to remove proteins we believe that would change its nature to such an extent and alter the characteristics of the ingredient so that they would no longer bear the same common or usual name."

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Gluten-Free Foods, cont

- FDA clarified their stance on malt vinegar in the proposed rule:⁶
 - "... some malt vinegars are the product of fermentation, without distillation, of an infusion of barley malt or cereals whose starch has been converted to malt. **Because these types of malt vinegar are derived from gluten-containing grains that have not been distilled or otherwise processed to remove gluten, they may not be used as ingredients in a food bearing a "gluten-free" claim or bear such a claim themselves ..."**

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Regardless...

- Of labeling rules...
 - In 2016, Gluten Free Watchdog has dealt directly with **12** manufacturers of foods labeled gluten-free yet containing malt ingredients from barley⁷
 - Ingredients include "gluten-free" malt, "gluten-free" malt extract, dried malt vinegar and malt vinegar powder

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Manufacturer Response

- Based on conversations with manufacturers most believe they are in compliance with the FDA's gluten-free rule because:
 - Products test below 20 ppm gluten using the sandwich R5 ELISA (and sometimes the competitive R5 ELISA)
 - They are under the impression the test result alone means the ingredients have been "processed to remove gluten"

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Product Label

puffed rice (rice, sugar, edible salt, gluten free barley malt), potato starch, 3% coconut, 2.5% hazelnuts, 1.7% almonds, 1.5% honey, 1.5% puffed amaranth, flavor, edible salt, antioxidant (ascorbyl palmitate, tocopherol-rich extract (natural), sunflower lecithin).
 Contains hazelnuts, almonds, coconut and soya.
 May contain traces of milk, sesame, peanuts and other nuts

MANUFACTURED BY:
 Emco spol. s r. o.



Malt & Gluten-Free Foods: What to Tell Consumers

- Consumers should be advised to:
 - Trust the gluten-free label
 - BUT if they come across a product labeled gluten-free that includes malt, malt extract, or malt vinegar they should avoid it
 - Notify their local FDA Consumer Safety Officer about potentially mislabeled products <http://www.fda.gov/Safety/ReportaProblem/ConsumerComplaintCoordinators/ucm2008077.htm>

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Beer

Gluten-Free Beers

CAN PREMIUM

Light
Banquet
Lite
Genuine Draft
High Life
Pilsner
Light
Job Ultra
3oz. Can)

GLUTEN FREE

Omission
IMPORT
Amstel Light
Corona Extra
Corona Light
Heineken
Heineken Light
Modelo Especial
Negra Modelo
CRAFT
New Belgium Slow Ride IPA
Dogfish Head 60 Minute IPA
Lagunitas

SPECIALITY MALT BEVERAGES

Coney Island Hard R Beer
Henry's Hard Ginger Ale
Henry's Hard Orange Soda
Redd's Apple Ale
Smirnoff Ice
NON-ALCOHOLIC
O'Doul's
Buckler



Beer: Bottom Line

- Beer is regulated by BOTH the TTB and FDA depending upon whether the definition of a malt beverage is met
- Beers regulated by the TTB are considered malt beverages and under TTB rules can **NOT** be labeled gluten-free
 - Can be labeled "processed to remove gluten"
- Beers regulated by the FDA are not considered malt beverages and under FDA rules may be labeled gluten-free if criteria are met
- Beers regulated by the TTB and labeled "processed to remove gluten" are sometimes promoted by restaurants, bars, and package stores as gluten-free

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Beer: Definitions of Malt Beverage

- "Malt beverage" is defined as "a beverage made by the alcoholic fermentation... of **malted barley with hops**..."¹³
- In other words, beer regulated by the TTB contains **BOTH** malted barley and hops
- Beer can not be assessed for gluten using a sandwich ELISA because it is a **fermented** beverage

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Definition of Malt Beverage, cont

- Beer regulated by the FDA is not made from BOTH malted barley and hops
- FDA-regulated beer is NOT considered a malt beverage
 - It is made from either:
 - Hops and a substitute for malted barley
 - OR
 - Malted barley but not hops

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Beer: TTB Rules & Gluten-Free Labeling

- Beer regulated by the TTB:¹⁴
 - Can **NOT** be labeled gluten-free
 - Can include the claim "Processed or treated or crafted to remove gluten" **IF** processed to remove all or some of the gluten **AND**
 - The following statement is also included on the product label:
 - "Product fermented from grains containing gluten and [processed or treated or crafted] to remove gluten. The gluten content of this product cannot be verified, and this product may contain gluten."
- Examples of "gluten-removed" beer:
 - Omission & Daura

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Beer: FDA Rules & Gluten-Free Labeling

- Beer regulated by the FDA can be labeled gluten-free **IF** it is made from a gluten-free substitute for malted barley
- Beer regulated by the FDA and made from a gluten-containing grain can **NOT** be labeled gluten-free **UNLESS** (and quoting from the proposed rule):
 - "the gluten-containing grain has been processed to remove gluten in accordance with the provisions in the "gluten-free" definition prior to making beer"⁶
- **BUT** FDA also states in the proposed rule, "we are not aware of any scientifically valid way to evaluate such a claim."⁶

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Beer: Testing

- Gluten-removed beer is subject to the same testing limitations as other fermented products with added constraints⁵
 - This type of beer may be treated with a prolidase enzyme such as Brewer's Clarex (designed to prevent chill haze)
 - This enzyme breaks apart proteins at the amino acid **proline**
- In the opinion of some experts this makes the competitive R5 ELISA useless for measuring the gluten content of beers treated with this enzyme
 - Remember the R5 ELISA recognizes the pentapeptide **QQPFP** (the Ps represent proline)

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Testing, cont

- According to the FDA (from the proposed rule), "... adding these haze control enzymes may generate peptides that are not detectable using the commercially available competitive ELISA-based methods that rely on the presence of proline in the epitopes. However, it is uncertain that cleavage at proline residues totally eliminates the concern for people with celiac disease because there may be immunopathogenic protein fragments still present."⁶

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Regardless...

- Of testing constraints and TTB labeling rules...
 - Not uncommon for beers labeled as "processed to remove gluten" to be listed as gluten-free on restaurant menus

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Menu Blackboard



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Beer and Gluten-Free Labeling: What To Tell Consumers

- Consumers should be advised that:
 - It may be prudent at this time to avoid gluten-removed beer
 - They may not be able to trust the gluten-free designation for beer on menus and shelf tags; they should look at the actual product label
 - It may be helpful to familiarize themselves with the names of gluten-free beers and gluten-removed beers

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Probiotics: Bottom Line

- Probiotics may contain bacterial strains grown on gluten-containing fermentation media
- It is unclear at this time whether use of gluten-containing fermentation media disqualifies products from being labeled gluten-free
 - It is hoped that the FDA's gluten-free labeling rule on fermented and hydrolyzed foods will specifically address this issue

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Probiotics: Regulations

- Probiotics are generally regulated as supplements
 - If they are labeled gluten-free they must comply with FDA rules
- An ingredients list is required on the label of supplements
 - Probiotics typically include the names of bacterial strains in the ingredients list
 - Composition of growth media is not generally included in the ingredients list
- Probiotics can not be assessed for gluten using a sandwich ELISA because residual gluten from fermentation media may be in the form of gluten peptide fragments

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Probiotics: Residual Gluten

- Gluten Free Watchdog test results
 - Manufacturer of a probiotic labeled gluten-free was using a barley-containing growth medium--yeast protein from spent brewer's yeast--for one of the bacterial strains
 - Probiotic tested well above 20 ppm using the competitive R5 ELISA
 - Manufacturer changed the growth medium for this bacterial strain to molasses
 - "New" formulation tested below 10 ppm using the competitive R5 ELISA

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Probiotics: What to Tell Consumers

- Consumers should be advised to:
 - Use only those probiotics labeled gluten-free
 - Contact manufacturers if they have any concerns about the growth medium
 - If the growth medium does include a gluten-containing grain a sandwich R5 ELISA is not sufficient for assessing gluten content

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Summary

- Sandwich ELISAs require **two** epitopes and Competitive ELISAs require only **one**
- Gluten protein that has been hydrolyzed may **not** be detected by a sandwich ELISA
- Ingredients that can **not** be accurately assessed for gluten using only a sandwich ELISA include malt, beer, and probiotics

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Thank You!

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