



## Is Barley-Based “Gluten-Removed” Beer Safe for People with Celiac Disease?

A Special Report by Tricia Thompson, MS, RD of Gluten Free Watchdog [www.glutenfreewatchdog.org](http://www.glutenfreewatchdog.org) with a lot of help from the *true* experts

### Introduction

The safety of gluten-removed barley-based beer is a very complicated issue for everyone, including the experts and an emotional one for many. In an effort to stick to the facts and views of the true scientific experts this post is going to be presented largely in Q&A and bullet point format. It also will start out very simply and become more complicated as it progresses. Hopefully this style of presentation will make the information easier to understand.

Some experts consulted for this post asked not to be identified. This request is obviously being honored and all experts consulted will remain anonymous. But rest assured that the individuals providing their insight are among the most knowledgeable in their respective fields--mass spectrometry, gluten protein amino acid sequencing, and ELISA testing.

### Questions and Answers

Q1: What is a malt beverage?

A1: The Alcohol and Tobacco Tax and Trade Bureau’s (TTB) definition of a malt beverage, including beer, is a beverage made with **both** malted barley and hops (i.e., a flavoring agent from the flowers of the hops plant).

Q2: Who regulates the labeling of beer?

A2: The labeling of all beers made with both malted barley and hops is under the jurisdiction of the TTB. If a beer is made without either malted barley or hops its labeling is under the jurisdiction of the Food and Drug Administration (FDA).

Q3: Can beer be labeled gluten-free?

A3: Beer whose labeling is under the jurisdiction of the TTB can NOT be labeled gluten-free. Beer whose labeling is under the jurisdiction of the FDA can be labeled gluten-free provided all gluten-free labeling criteria are met.

Q4: Who regulates barley-based “gluten-removed” beers, such as Omission?

A4: Beer that is made from **both** malted barley and hops, such as Omission is regulated by the TTB.

Q5: Can Omission beer be labeled gluten-free?

A5: No. Under the TTB's 2012 Interim Policy on Gluten Content Statements in the Labeling and Advertising of Wines, Distilled Spirits, and Malt Beverages Omission beer can NOT be labeled gluten-free. However, the TTB will allow the following statement on Omission beer and similar "gluten-removed" malt beverages:

- "Processed to remove gluten" IF the following statement is also included on the product label or in advertising:
  - "Product fermented from grains containing gluten and processed to remove gluten. The gluten content of this product cannot be verified, and this product may contain gluten."

*Note: In the state of Oregon where Omission is brewed, bottled, and sold it MAY be labeled gluten-free. The labels of beer that enter interstate commerce must be preapproved by the TTB. Beer that does NOT enter interstate commerce is under the jurisdiction of the state. The state of Oregon approved Omission's gluten-free label.*

Q6: The TTB states in its interim policy that the gluten content of "gluten-removed" beer cannot be verified. Why is this the case?

A6:

- It is difficult to quantify the amount of gluten in a product, such as beer when the protein has undergone hydrolysis (meaning it has been broken apart into smaller fragments).
- The barley protein (hordein) in beer is broken apart into smaller peptides during the malting process because it is acted upon by many enzymes (a natural part of the malting process).
- Foods and beverages that have not been hydrolyzed are tested for gluten using a sandwich ELISA, such as the sandwich R5 ELISA Mendez Method.
- A sandwich ELISA works well when assessing protein that is relatively intact but not protein that has been hydrolyzed and fragmented.
  - For example, the sandwich R5 ELISA measures the amino acid sequence QQPFP which stands for glutamine-glutamine-proline-phenylalanine-proline BUT the R5 ELISA requires two of these sequences for the protein to be measured.
  - When gluten protein is hydrolyzed, there may not be two of these sequences. As a result some gluten peptides may not be measured when the gluten content is assessed using the sandwich R5 ELISA.
- A competitive ELISA, such as the competitive R5 ELISA, is used when a protein has been hydrolyzed.
  - The competitive R5 ELISA only requires one sequence of QQPFP.
- While the competitive R5 ELISA has been validated in a multi-lab international trial there continue to be concerns among some researchers about this assay:

- Can the competitive R5 ELISA accurately quantify peptide fragments into parts per million gluten protein? In other words, the peptide fragments can be detected but at what point do the number of peptide fragments (assuming they are toxic) become harmful?
  - Another issue to consider (as pointed out by a couple experts) is that the R5 epitope itself is not toxic—it is simply used as a marker for the multiple 9 or 9+ amino acid sequences containing the QQFPF epitope that are toxic. It may 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. This is not such a problem when assessing intact gluten proteins using the sandwich ELISA but it may be a problem when assessing protein fragments with the competitive ELISA.
- Is the extraction solution used with the competitive R5 ELISA—ethanol—ideal? According to the late Dr. Enrique Mendez, ethanol is capable of completely extracting prolamins from foods containing native proteins only. Once proteins have been heated and denatured, ethanol is no longer capable of extracting all prolamin fractions.

*Note: The small amino acid fragments from beer are very soluble, including in alcohol so using an ethanol extraction should not be an issue.*

- There are additional questions concerning the competitive R5 ELISA and its use in measuring the gluten content of barley-based “gluten-removed” beers:
  - These beers are treated with a prolidase enzyme.
    - Omission uses Brewer’s Clarex
      - Brewer’s Clarex was developed by DSM Food Specialties. According to the manufacturer website ([www.dsm.com](http://www.dsm.com)), “Brewers Clarex™ is a pure enzyme containing a proline-specific endo-protease that prevents the formation of chill haze in beer by hydrolyzing the haze-active proteins that cause it.”
  - This enzyme breaks apart proteins at the carboxyl end of the amino acid proline.
  - The competitive R5 ELISA which detects the amino acid sequence QQFPF (where the P is proline) would be destroyed by this enzyme.
    - In the opinion of some experts this makes the competitive R5 ELISA useless for measuring the gluten content of beers treated with this enzyme.
    - As pointed out by one expert this is because the enzyme works to hydrolyze the gluten protein in a systematic way by breaking apart the amino acid sequence QQFPF at the amino acid proline. The competitive R5 ELISA is

nonetheless fairly robust for detecting gluten peptides when hydrolysis in a food or beverage is random and not targeted (as it is with Brewer's Clarex).

- However, in the opinion of another expert:
  - All known peptides toxic to people with celiac disease contain proline.
  - If the enzyme used by barley-based "gluten-removed" beers breaks apart peptides at the prolines and the extent of this breakdown is high than the likelihood of large fragments of toxic gluten protein remaining in the beer is low.
- Regardless, neither the TTB nor Health Canada is allowing barley-based "gluten-removed" beer to be labeled gluten-free based on ELISA testing.
  - Note: Please contact Health Canada regarding their current position on labeling of barley-based gluten-removed beers.
- This brings us to another type of testing called liquid chromatography-mass spectrometry (LC-MS).
  - This testing is not commercially available to manufacturers of gluten-free foods. If manufacturers want this testing performed on their products they must contact a laboratory well-versed in this methodology.

Q7: What is LC-MS testing?

A7: This type of testing first pre digests protein fragments using an enzyme such as trypsin in order to have well defined peptides. Mass spectrometry data is then collected on each of these peptides, which is compared to known data in a protein database in order to determine the amino acid sequence of the peptide. This information can then be used to determine what protein this amino acid sequence has come from and the potential toxicity. In the case of celiac disease, these peptide fragments would be compared to a database of peptides known to be toxic to individuals with celiac disease.

Q8: What are the drawbacks when testing barley-based beer using LC-MS?

A8: In order to identify toxic sequences in barley they have to be in the database of toxic fragments. So the big question becomes--do we know all of the toxic epitopes in barley? The answer to this question is likely no. But the next question becomes—do we know enough of the toxic epitopes in barley. The answer to this question appears open to debate.

Q9: Can LC-MS testing be used to quantify gluten proteins and peptides in beer?

A9: Yes, but according to one expert it is necessary to synthesize or purify each likely peptide or protein identified in beer to serve as a standard for calibration of the mass spec results.

- This has not been done because:
  - It is expensive and time-consuming.
  - The number of suspect proteins and peptides in beer is very large.

- But in order to quantify barley hordeins and barley hordein peptides in beer this is what must be done.

Q10: Are there any good articles on quantifying gluten proteins in beer using LC-MS?

A10: Yes, please see the sources section and the article by Sealey-Voyksner.

Q11: How does Craft Brew Alliance (Omission Beer) test their beer?

A11: Currently they use the competitive R5 ELISA.

Q12: Has Omission conducted LC-MS testing on a sampling of its beers?

A12: Yes. According to Omission, “The results show that Omission beers are devoid of known toxic epitopes, the specific peptide sequences and reactive sites in gluten molecules that cause reactions in the human small intestine.”

*Note: Omission based this information on a review article. Only a few of the epitopes included in the list are from barley. According to one expert, there are numerous suspect proteins and peptides in beer. The majority of epitopes included in the list are from wheat. According to one expert the vast majority of research into the celiac specific epitopes has been done on wheat proteins. While there will be some similar amino acid sequencing, it is unclear whether the epitopes from wheat would be useful in predicting the immunological character of barley. There appears to be some scientific debate on this issue—some experts believe that the toxic amino acid sequences in wheat will turn out to be good predictors of the toxic amino acid sequences in barley. But at this point in time we simply do not know.*

Q13: Has Omission released any information about the LC-MS testing?

A13: Yes, they have released some information in a brochure-like document. Please contact Omission for this information.

Q14: What do the experts say about the data Omission has released?

A14: **The experts consulted for this article all seem to agree that what Omission has released is not detailed enough. The company should publish their data in a peer-reviewed scientific journal so that the results and the methods can be assessed by the scientific community.**

Q15: Is Omission planning to publish their data?

A15: This is the reply I received from a representative of Omission after asking that question in an email, “We are not planning on submitting the sequencing results to any peer reviewed journal because it is not our original work. I believe the scientists who did the research plan to publish, but, I don’t have a concrete view of their intents or schedule.”

Q16: Has there been any independent testing of barley-based “gluten-free” beers using LC-MS testing?

A16: Yes, According to Health Canada, the beers they have tested with LC-MS have all contained residual gluten fragments.

Q17: So, is Omission beer safe for people with celiac disease to drink?

A17: **Experts who I trust immensely agree that they can NOT say for certain at this point in time whether Omission beer is safe for people with celiac disease to drink based on the data that has been released by Omission.**

Q18: What is my opinion on Omission beer?

A18: My answer to these types of issues is always the same...If Omission is confident in the robustness of their data they should figure out a way to publish their findings in a peer-reviewed journal so that the data can be reviewed by the experts. While my opinion on Omission beer has softened somewhat, it remains my belief that individuals with celiac disease should avoid these types of beers until the LC-MS data is released by Omission and reviewed by the *true* experts.

*Note: I was asked by a representative of Omission upon their review of this article to stress that Omission cannot publish their data. In email correspondence they state, “You mention several times that we should and I’ve been very clear that the information does not belong to Omission and we cannot thus submit for peer review.”*

***It appears then that we are at a bit of a stalemate. The experts consulted for this article and I all agree that more data should be released on the LC-MS testing in order to determine safety of Omission beer. However, Omission states it is not in their power to do so. Perhaps they should encourage those who “own” the data to submit the research to a peer-reviewed journal for evaluation.***

## **Acknowledgements**

**Thank you to the experts in this field who answered all my questions and then some.**

**Thank you to Kristin Voorhees, MA for reviewing the document from the perspective of consumers and health care professionals.**

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