Gluten-Free Certification Program (GFCP)
SUMMARY OF TEST METHODS FOR GLUTEN
February 6, 2015

Test Methods for Gluten-Free Products/Ingredients - Not Hydrolyzed or Fermented

- **Omega-gliadin ELISA** is a sandwich enzyme-linked immunosorbent assay based on monoclonal antibodies sensitive to the omega-gliadin fraction of wheat. It can be used to assess the gluten content of heated and unheated food but its major drawback is that it greatly underestimates the hordein content in foods contaminated with barley.

- **Standard R5 ELISA (R7001 Ridascreen Gliadin)** is a sandwich enzyme-linked immunosorbent test method based on the R5 monoclonal antibody designed to detect the epitope (i.e., antibody-binding site) QQPF (glutamine-glutamine-proline-phenylalanine-proline). This method has been validated by the Prolamin Working Group of the Codex Alimentarius Commission. It can be used to assess the gluten content of heated and unheated food. Although it overestimates hordein content in barley contaminated foods, it is probably the most recommended test for multi-purpose use.

- **Fast R5 ELISA (R7002 Ridascreen Fast Gliadin)** is a sandwich enzyme-linked immunosorbent test based on the R5 monoclonal antibody to measure the epitope, QQPF (glutamine-glutamine-proline-phenylalanine-proline). This assay is a less sensitive version of the standard sandwich R5 ELISA.

- **Quick R5 ELISA (R7003 Ridascreen Quick Gliadin)** is an immunochromatographic test using the assay based on the R5 monoclonal antibody for the qualitative detection of gliadin/gluten contamination on surfaces (swab test for the hygiene control in production areas and in laboratories) as well as in gluten-free declared raw materials. The test should only be used for the detection of small amounts of gluten (contaminations) and due to its lack of sensitivity is best used for screening purposes.

- **EZ Gluten (lateral flow sold by ELISA)** is a rapid test to detect gluten at 10 ppm or above. It is very suitable in screening products/ingredients and swabbing for qualitative detection to verify cleanliness of equipment or utensils.

- **Aller-Tek (lateral flow sold by ELISA)** uses a monoclonal antibody which recognizes both the gliadin and glutenin fractions of gliadins/gluten with a limit of quantification of 5ppm.

- **Veratox Quantitative Gliadin R5 (Neogen, 8510)** is used for the detection and quantification of ingredients, clean-in-place and finished food products intended to be gluten-free for the presence of gliadin and prolamins found in wheat, barley and rye. Quantitation with Veratox Gliadin R5 ranges from 2.5-40 ppm gliadin (5-80 ppm gluten) and can be extended by dilution of positive extracts. Veratox Gliadin R5 is AOAC approved (AOAC-RI 061201).
• **AgraQuant® ELISA Gluten G12 (Romer)** uses a monoclonal antibody (G12) to develop a sandwich enzyme-linked immunosorbent assay. The G12 methods specifically measure the most immunotoxic proteins for those intolerant to gluten.

• **GlutenTox Pro (ELISA)** Designed especially for commercial kitchens, GlutenTox Pro is a user-friendly gluten detection kit for foods, drinks and surfaces. GlutenTox Pro contains the G12 anti-gliadin antibody that specifically recognizes the 33-mer peptide, the gluten fraction that triggers a reaction in those with celiac disease.

**Test Methods for Gluten-Free Products/Ingredients - Hydrolyzed or Fermented**

The regular sandwich ELISA test methods are not suitable for products or ingredients that have been hydrolyzed or fermented. This is due to the fact that these processes break the harmful gluten protein fraction into smaller protein fragments which can create single epitopes rendering them virtually undetectable, giving a false negative or underestimated result when using regular sandwich test methods. Added to this, incomplete hydrolysis or fermentation as well as ingredients that have blended with both regular or rendered from hydrolyzed or fermented sources make testing and assessing the significance of results extremely complicated. The following methods are suitable for these products/ingredients which have been hydrolyzed or fermented.

• **Competitive R5 ELISA (R7011)** is a competitive enzyme-linked immunosorbent assay based on the R5 monoclonal antibody based on epitope, QQPFP (glutamine-glutamine-proline-phenylalanine-proline). The competitive ELISA requires only one epitope to work. Unfortunately, the results are an expression of gluten peptides versus gliadins/gluten. Unfortunately, this makes it difficult to quantify the peptide concentration in terms of parts per million of gluten.

• **Competitive R5 ELISA (R7021)** is a competitive enzyme-linked immunosorbent assay based on the R5 monoclonal antibody based on epitope, QQPFP (glutamine-glutamine-proline-phenylalanine-proline). The competitive ELISA requires only one epitope to work. Again, the results are an expression of gluten peptides versus gliadin/gluten making it difficult to quantify the peptide concentration in terms of parts per million of gluten.

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