Testing Method Recommendation:
Two Method Confirmation for Food Allergen Testing with ELISA

7/29/2026

FDA Guidelines for Food Allergen Testing require a "Two Method Confirmation" with ELISA. The use of lateral flow tests is not accepted. Depending on the nature of contamination (homogenous vs. heterogenous), homogenization and analysis of multiple sub-samples without compositing may be required. Please refer to the recent IOM allergen sampling guidance Chapter 4 – Sampling for heterogenous contamination.

See below for details:

1. Two Method Confirmation with ELISA means, testing the one sample with two commercially available validated Food Allergen ELISA kits for the target allergen. Please provide the validation reports for the ELISAs to FDA for review before analyzing the samples.
2. The "one sample" may consist of two individual subs at minimum. Please refer to the recent IOM allergen sampling guidance for heterogenous contamination.
3. Both ELISA kits must be "fit for purpose" for the commodity being tested (check product inserts for validation studies or warnings against usage with certain types of commodities before performing analysis).
4. The samples and controls must be tested in duplicate at minimum (triplicate is recommended), meaning two (or three) separate extracts must be made and tested independently in the ELISA according to the product inserts.
5. Positive controls (allergen reference material spiked into an equivalent allergen-free matrix at minimum two different concentrations) and negative controls (allergen-free matrix) must be included along with the samples in the analysis. Positive controls should show a calculated recovery of 50-150%, and negative control should render a result below the lowest limit of quantitation (LOQ) of the ELISA kit, preferably close to the kit negative control. (Recoveries of 20-49%" will be reviewed by HFP-OCT on a case-by-case basis.)
6. The kit standards (positive and negative) supplied with the ELISA must be analyzed in duplicate at minimum and perform as per the specifications provided in the product inserts.
7. If a sample tests negative, additional testing should be conducted to rule out matrix interference and to prove that a sample is a "true negative". The sample must be "spiked" with the target allergen at two different concentrations above the lowest LOQ stated in the ELISA kit product insert, in duplicate at minimum (two separate extracts for spikes made and tested) that would render a result above the lowest LOQ and/or show calculated recovery of 50-150%. (Recoveries of 20-49%" will be reviewed by HFP-OCT on a case-by-case basis.)
8. Technicians performing analysis must clearly date and initial all steps of the analysis.
9. To demonstrate that "all supporting documentation meets requirements", the lab must

submit documentation for both “quality assurance” information from analysis and “proof of competency” for analysts.

10. “Quality assurance information” includes the lot numbers and expiration dates of all reagents and supplies used in analysis including the ELISA kits, pH strips, etc. which can also be submitted from any documentation provided from the manufacturer. In addition, all information for any equipment used during analysis, including the specific identification of the equipment (name, serial number) and function verifications for the dates used.
11. “Proof of competency” must be submitted for all technicians/analysts who performed the analyses or any part of the analysis. A curriculum vitae must be submitted for each technician/analyst that performed any part of the analysis and if this is a new method for the lab then a Training Report that shows the analyst was able to perform this analysis and get satisfactory results.
12. For additional information about Private Laboratory Review Submissions, please reference FDA’s Private Laboratory Testing website and Laboratory Procedure – Private Laboratory Analytical Packages.