



Accurate Detection and Quantification of Sesame Allergen in Food Products Using the AlerTox® ELISA Sesame Kit

An Overview of Three Fapas® Proficiency Testing Studies of Cumin and Bread

Overview of Food Labeling Regulations for Sesame Allergens

Preventing exposure to sesame in food products is not as easy as other allergens. Small amounts of sesame seeds, oil or flour are included in dressings and spice mixes and used as natural flavors in many foods. In part because of the large variability in the amount of sesame that can trigger an allergic reaction, standardized thresholds have not been established. Sesame allergies can lead to life-threatening reactions, so detection of very low levels of allergen is desirable for food testing; whereas during environmental testing, setting the value too low can trigger unnecessary, time-consuming recleanings that can cause potential product release delays.

In the EU, sesame seed is one of 14 allergens whose presence must be indicated in foods, according to *Regulation (EU) No. 1169/2011 Annex II* established by the European Food Safety Authority. As the prevalence of food allergies has grown, the US FDA *Food Allergy Safety, Treatment, Education, and Research Act* (FASTER Act of 2021) added sesame as the ninth major food allergen with package labeling required for products manufactured after January 1, 2023. The UK, Australia, New Zealand, Canada and Israel are additional regions where sesame is considered a major food allergen and must be specifically declared on labels.

Description of the AlerTox® ELISA Sesame Kit

The AlerTox® ELISA Sesame Kit is an immunosorbent assay designed for the quantitative determination of sesame proteins (measured as whole sesame seed) in raw materials and final products. The kit is based on the ELISA sandwich technique that is often used to analyze substances found at very low concentrations. This method, combined with the high specificity and sensitivity of the antibody used in these tests, allows this kit to precisely quantify sesame in many types of solid and liquid foods. The limit of detection (LOD) is 0.2 ppm (mg of sesame seeds per kg of food sample), and the limit of quantification (LOQ) is 2 ppm (mg of sesame seeds per kg of food sample).

Proficiency Testing Procedure and Test Material Preparation

Proficiency testing is an essential part of laboratory quality procedure, and participation is required for ISO 17025 accreditation for testing and calibration laboratories. As the developer of allergen tests and kits, Hygiena® participates in proficiency testing (PT) to provide an independent assessment of test performance and to compare results to those of laboratories worldwide. Taking part in proficiency testing gives insight into a laboratory's equipment, methods, brands of products and technical skills of the staff, allowing the opportunity to improve or confirm the delivery of quality testing results.

Cumin

Proficiency studies 27317 (Study 1 from early 2022) and 27351 (Study 2 from early 2023) each involved testing two cumin samples for sesame and gluten. Although this summary will focus on the detection and quantification of sesame, foods with nuts and seeds are often at risk for cross-contact with grains containing gluten.

The original, unspiked samples (test materials 27317B and 27351B) were purchased from retail sources by Fera Science, the organizer of these Fapas® PT studies. In Study 1, a portion of the original sample was artificially contaminated (spiked) with a combination of wheat (gluten) and sesame flours before tumble mixing to produce homogeneous test material 27317A (Table 1). In Study 2, the original sample was naturally contaminated with sesame, and test material 27351A was created by spiking the original sample with gluten and additional sesame (Table 1).

Bread

Proficiency study 27373 (Study 3 from late 2023) involved testing bread for sesame. Bread without (test material 27373A) and with (test material 27373B) sesame flour was prepared for the study and stored at -20°C until dispatched to participants (Table 1).

Table 1. Test Materials Used in Three Fapas Food Chemistry Proficiency Testing Studies.

Material No.	Matrix	Sesame Flour Spike	Wheat Flour (Gluten) Spike
Study 1*			
27317A	Cumin	~22 mg/kg (ppm)	~22 mg/kg (ppm)
27317B	Cumin	None	None
Study 2†			
27351A	Cumin with sesame	Yes‡	Yes‡
27351B	Cumin with sesame	None	None
Study 3§			
27373A	Bread	None	—
27373B	Bread	~20 mg/kg	—

* Study 1: 74 of 80 laboratories (93%) submitted results that they obtained without knowing the actual spiked levels.

† Study 2: 60 of 63 laboratories (95%) submitted results that they obtained without knowing the actual spiked levels.

‡ Study 2: The spiked levels of sesame and wheat flours were not specified in the final Fapas report.

§ Study 3: 38 of 43 laboratories (88%) submitted results that they obtained without knowing the actual spiked levels of sesame flour. This study did not include the detection and quantification of gluten.

Overview of Study Design and Report

All labs, including Hygiena, indicated both qualitative and quantitative results for gluten (Studies 1 and 2) and sesame (Studies 1, 2, 3) as well as the limits of detection and quantification. Fera Science then summarized the data and provided a report to all participants. The results were segregated into subsets according to the ELISA kit used. Results for sesame detection and quantification were submitted from at least seven different commercial or in-house kits.

If enough results from the same type of kit were submitted, Fera Science statistically analyzed the data to provide an assigned value and z-score. For sesame detection and quantification, the final report included statistical analysis for the r-Biopharm's Ridascreen® Fast Sesame Kits in Studies 1, 2 and 3. There were enough samples in Study 2 for analyses of data from two other kits (ELISA Systems' Sesame Kit and Gold Standard Diagnostics' SENSISpec ELISA Sesame Kit). However, no assigned value was set for the SENSISpec Kit for sample 27351B because of the high uncertainty of the assigned value.

The assigned value is typically either the mean or median of the submitted results for a kit. Then, the assigned value for an analyzed kit is used in combination with the standard deviations for proficiency testing to calculate a z-score ($|z|$) for each result. The lower the z-score, the less variability in results. Any $|z|$ value ≤ 2 is considered a satisfactory or "passing" value, meaning the submitted result is similar to the assigned value in the study.

Results

AlerTox Kit: Quantitative Results

Study 1: The AlerTox ELISA Sesame Kit obtained a result of 25.6 mg/kg sesame, which was very close to the spiked value of 22 mg/kg sesame flour, in material 27317A and did not detect sesame in material 27317B, which was consistent with the results from the other laboratories in this study (82% consensus for this material) (Table 2, Hygiena Lab 005; Figure 1).

Study 2: A laboratory affiliated with Hygiena (Lab 029) and a laboratory unaffiliated with Hygiena (Lab 055) submitted results using AlerTox ELISA Sesame Kits (Table 2; Figure 2). The results from the two labs were close to each other. Both labs also reported that the level of sesame detected in the unspiked, naturally contaminated sample (27351B) was lower than that of the further spiked, naturally contaminated sample (27351A).

Study 3: The AlerTox ELISA Sesame Kit obtained a result of 18.0 mg/kg sesame, which was very close to the spiked value of 20 mg/kg sesame flour, in material 27373B and did not detect sesame in material 27373A, which was consistent with the results from the other laboratories in this study (100% consensus for this material) (Table 2, Hygiena Lab 003; Figure 3).

Table 2. Results from the AlerTox ELISA Sesame Kit.

Material	Lab Number	Sesame Flour Spike	Submitted Result (AlerTox Kit)	Limit of Quantitation (LOQ)
Study 1 (Cumin)				
27317A	005	~22 mg/kg	25.6 mg/kg	2
27317B	005	None	<2 mg/kg	2
Study 2 (Cumin)				
27351A	029	Yes (amount unknown)	77.44 mg/kg	2
	055	Yes (amount unknown)	60.1 mg/kg	5
27351B	029	None	44.74 mg/kg	2
	055	None	54.5 mg/kg	5
Study 3 (Bread)				
27373A	003	None	<2 mg/kg	2
27373B	003	~20 mg/kg	18.0 mg/kg	2

A dot plot helps summarize and compare all the results. Figure 1 (Study 1) and Figure 3 (Study 3) include lines to show the spiked value in the sample and the assigned value for the Ridascreen Fast Sesame Kit. Figure 2 from Study 2 shows submitted values for both naturally contaminated samples: additionally spiked (27351A) and unspiked (27351B).

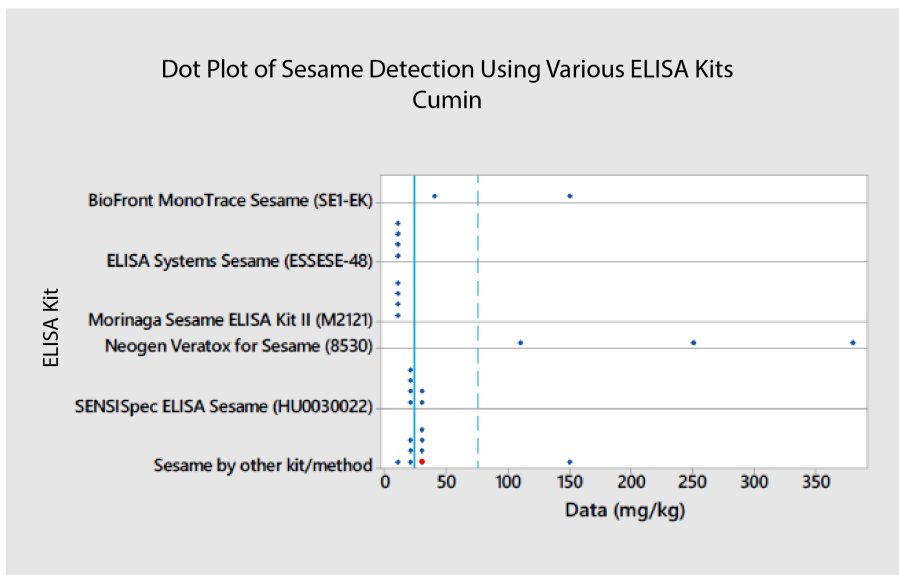


Figure 1. Study 1—Dot Plot of Sesame Quantification in Cumin by Kit. Sample 27317A was spiked with 22 mg/kg of sesame flour (vertical, light blue line). Each dot represents a submitted value from different participants. The AlerTox ELISA Sesame Kit result is shown as a red dot. The assigned value (mean) for the Ridascreen Fast Sesame Kit is shown as a dotted, light-blue line, while the range of submitted values with this kit varied from 25 to 125 mg/kg (data not shown).

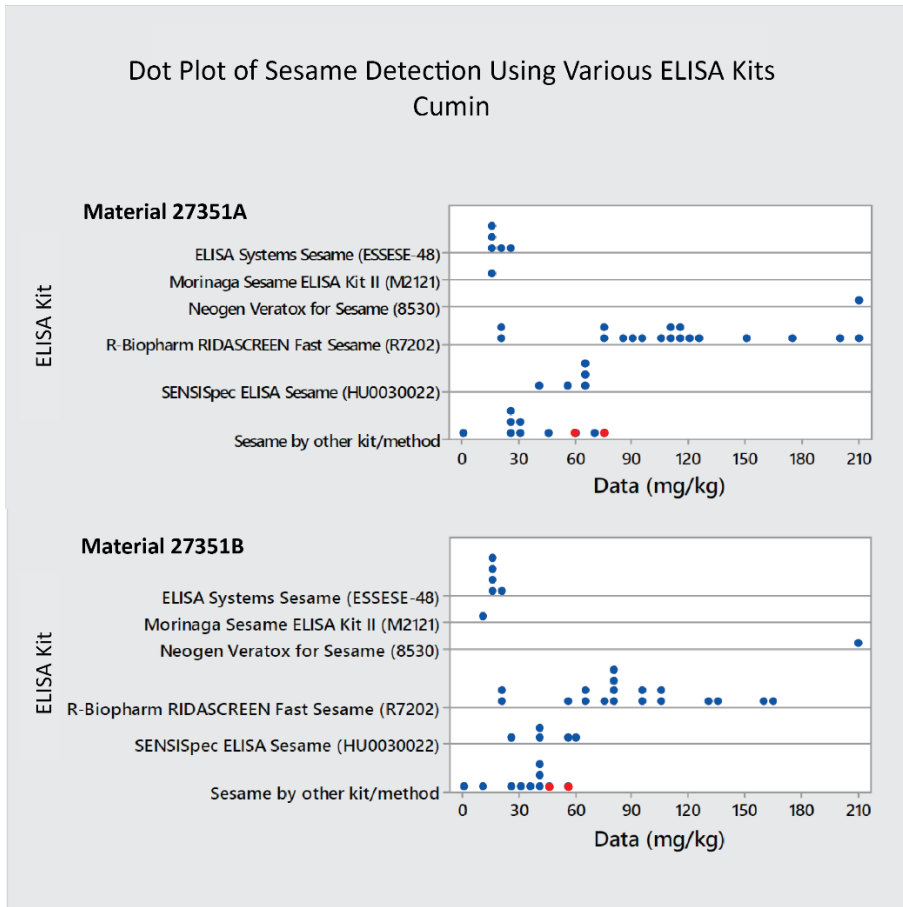


Figure 2. Study 2—Dot Plot of Sesame Quantification in Cumin by Kit. Sample 27351B was naturally contaminated with sesame. Sample 27351A was spiked with additional sesame. Each dot represents a submitted value from different participants. The AlerTox ELISA Sesame Kit results are shown as red dots.

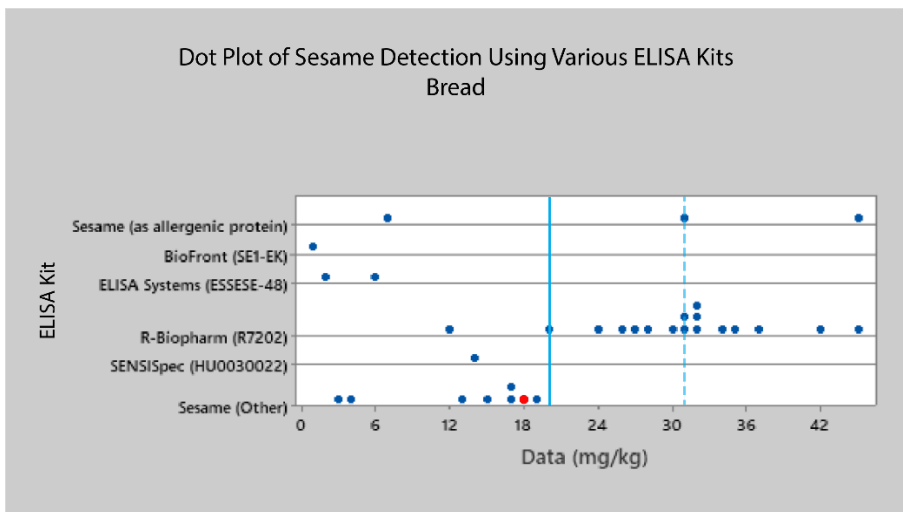


Figure 3. Study 3—Dot Plot of Sesame Quantification in Bread by Kit. Sample 27373B was spiked with 20 mg/kg of sesame flour (vertical, light blue line). Each dot represents a submitted value from different participants. The AlerTox ELISA Sesame Kit result is shown as a red dot. The assigned value (median) for R-Biopharm’s Ridascreen Fast Sesame Kit is shown as a dotted, light-blue line.

Overview of Results from Other Methods

Study 1: The results obtained by most of the participants using non-Hygiena kits were also consistent with the spiked value provided by the organizer. The exceptions were the high results from R-Biopharm’s Ridascreen Fast Sesame Kit and Neogen’s Veratox® for Sesame Kit and the relatively low results from ELISA Systems’ Sesame Kit and Morinaga’s Sesame ELISA Kit II. For the Ridascreen Fast Sesame Kit, the assigned value (the mean value) was 74.0 mg/kg sesame (Table 3), and for the Veratox for Sesame Kit, the submitted values from three participants were more than five times the level of the spiked value (Figure 1). ELISA Systems’ Sesame Kit and Morinaga’s Sesame ELISA Kit II returned results that consistently were less than half of the spiked value (Figure 1).

Study 2: Like Study 1, results from Ridascreen Fast Sesame Kits and Veratox for Sesame Kits were noticeably higher than those from other kits. Also like Study 1, the results submitted from ELISA Systems' Sesame Kit and Morinaga's Sesame ELISA Kit II appeared lower than those from other kits.

It is also worth noting the large variability of the results submitted by participants using the Ridascreen Fast Sesame Kit (Table 3). Two labs (accredited ISO 17025 with this kit) reported results as different as 59 mg/kg and 116 mg/kg of sesame, the latter being an unsatisfactory result (z -score >2) compared to the assigned value.

Study 3: Like Studies 1 and 2, results from the Ridascreen Fast Sesame Kits were noticeably higher than those from other kits, and the results from ELISA Systems' Sesame Kit were lower than those from other kits.

Table 3. Results and Analysis of the Ridascreen Fast Sesame Kit.

Material	Assigned Value*	Data Points	Range of Variability	$ z \leq 2$	Uncertainty	Standard Deviation for Proficiency
Study 1 (Ridascreen Fast Sesame Kit)						
27317A	74.0 mg/kg	22	25 – 125 mg/kg	77%	3.9	18.5
Study 2 (Ridascreen Fast Sesame Kit)						
27351A	112 mg/kg	16	73.18 – 210.8 mg/kg	81%	7	27.9
27351B	87.4 mg/kg	16	54.8 – 166.2 mg/kg	81%	6.8	21.9
Study 3 (Ridascreen Fast Sesame Kit)						
27373B	30.9 mg/kg	17	12.09 – 45 mg/kg	94%	1.3	7.73

* The assigned value for material 27317A was the mean, while the assigned value for materials 27351A, 27351B and 27373B were the medians.

Comparison of Results from the AlerTox Kit

For data from Study 2, we calculated z -scores to compare our results (Lab 029) using the AlerTox ELISA Sesame Kit to those from three other kits with statistical information in the final report (Table 4). Our results were consistent (satisfactory, passing z -score) with the SENSISpec ELISA Sesame Kit and the Ridascreen Fast Sesame Kit, although the latter may have been influenced by the large standard deviation of the Ridascreen kit. In contrast, our results were not consistent with those from ELISA Systems' Sesame Kit, whose results generally were lower than others.

Table 4. Comparison of Results from AlerTox ELISA Sesame Kit with Three Other Kits in Study 2.

Material (Cumin)	Submitted Value	Assigned Value* (z -score [†])		
	Hygiena's AlerTox ELISA Sesame Kit	r-Biopharm's Ridascreen Fast Sesame Kit	Gold Standard Diagnostics' SENSISpec ELISA Sesame Kit	ELISA Systems' Sesame Kit
27351A	77.44 mg/kg (Lab 029)	112 mg/kg ($z = -1.24$)	63.2 mg/kg ($z = 0.90$)	15.0 mg/kg ($z = 16.65$)
27351B	44.74 mg/kg (Lab 029)	87.4 mg/kg ($z = -1.95$)	Not calculated [‡]	14.0 mg/kg ($z = 8.78$)

* The assigned values were the medians due to the low number of data points.

[†] $|z| < 2$ is a satisfactory, passing score that indicates low variability between results.

[‡] Analysis not done due to high uncertainty.

Conclusions

As shown in Fapas PT Study 1, the results using the AlerTox ELISA Sesame Kit (and most of the other kits included in the study) are consistent with the sesame content of the sample. However, r-Biopharm's Ridascreen Fast Sesame Kit and Neogen's Veratox for Sesame Kit tend to overestimate the sesame content in the cumin samples, while ELISA Systems' Sesame Kit and Morinaga's Sesame ELISA Kit II tend to underestimate sesame content in the cumin samples. These trends were supported by the results from Study 2, although the actual amounts of sesame in the cumin samples were not provided for Study 2. These trends were also supported during the analysis of sesame in bread from Study 3, although this study had fewer participants and did not include results from the Veratox for Sesame Kit and Sesame ELISA Kit II.

It is important that Fapas reports include the spiked value of the analytes in the samples as was done for Study 1. Otherwise, the results might be misinterpreted and compared with the assigned result obtained by the most widely used kit. For example, in Study 1, the value of 25.6 mg/kg of sesame obtained with the AlerTox ELISA Sesame kit, which is aligned with the spiked value (22 mg/kg sesame), would have been interpreted as unsatisfactory ($|z\text{-score}| > 2$) if compared with the assigned value from the Ridascreen Fast Sesame kit. The results from Studies 1 and 3 help provide context for the results from Study 2, where the amount of sesame and the sesame spike were not provided.

Food safety and labeling requirements make it important to choose a test that is accurate and sensitive, such as the AlerTox ELISA Sesame Kit. With only 60 minutes of incubation time, this easy-to-use sandwich ELISA test has the sensitivity and specificity that make it a useful part of a testing program for sesame allergens in a wide range of food matrices and beverages.

Additional Resources

1. [“Validation Report: AlerTox ELISA Sesame.”](#) Hygiena Validation Report.
Contains results from validation tests, including matrix and cross-reactivity details
2. [“Beyond the Big 8: Sesame Allergen Testing.”](#) Hygiena White Paper.
Provides an overview of testing for sesame allergens
3. [“Allergens: Validating Label Claims Via Allergen Limits Testing.”](#) Hygiena White Paper
Includes information about and considerations for validating allergen control plans