



Detection and Quantification of Peanut Allergen in Snacks and Spices Using the AlerTox® ELISA Peanut Kit

Highlights from Six Fapas® Proficiency Testing Studies

Peanuts and Peanut Cross-Contamination

Peanuts are considered a major food allergen that requires product labeling in the Americas, Europe, much of Asia and the Middle East as well as parts of Africa. While many types of food contain peanuts as an ingredient, there also is a need for producers and manufacturers to test for allergenic peanut residue on equipment, raw ingredients and final products to minimize or detect cross-contamination before products are released to consumers.

Description of the AlerTox® ELISA Peanut Kit

The AlerTox ELISA Peanut Kit is an immunosorbent assay designed for the quantitative determination of peanuts in fresh and processed food products with results measured as whole peanut and not as peanut proteins. 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 peanut presence in many types of solid and liquid foods. The limit of detection is 0.3 ppm (i.e., 0.3 mg of whole peanut per kg of sample), and the limit of quantification is 1 ppm (i.e., 1 mg of whole peanut per kg of sample).

Proficiency Testing Procedure and Test Material Preparation

Proficiency testing is an essential part of laboratory quality procedures, and participation is required for ISO 17025 accreditation for testing and/or 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, product brands and technical skills of the staff, allowing the opportunity to improve or confirm the delivery of quality testing results.

Between 2019 and 2023, Hygiena participated in six Fapas® proficiency studies for peanut testing organized by Fera Science (Tables 1 and 2). In addition, the cooked biscuit studies included Hygiena Kit results from four independent laboratories that were not affiliated with Hygiena.

- Chocolate Study

Chocolate is often mixed with peanuts in various snacks and desserts, so cross-contamination issues may need to be monitored in some production processes.

This PT study focused on nut-free chocolate with and without a spike of a known amount of peanut powder.

- Cooked Biscuit Studies

Peanuts and peanut flour are often used in baked goods, so cross-contamination issues may need to be monitored in some production processes.

These three studies focused on biscuits that contained peanut flour in the cookie mix. After cooking, samples were freeze-dried and frozen until dispatch for testing. The final peanut content was not provided in the PT report.

Although these studies were designed to assess peanut and hazelnut allergens, this overview will focus only on the results of peanut testing.

- Garlic Powder Studies

Because of where some garlic and peanuts are grown, peanuts can be a common adulterant of garlic powder.

The samples from Study 5 included nut-free garlic powder with and without a spike of a known amount of peanut flour. The samples from Study 6 included garlic powder with and without a spike of peanut, but the amount of peanut in these samples was not provided in the PT report.

Table 1. Overview of Fapas Food Chemistry Proficiency Testing Studies for Peanut Allergens.

Study Number	Number of Participants Submitting Results	Matrix	Study Analytes*	
1	27263	45 of 51 (88%)	Chocolate	Peanut
2	27242	80 of 85 (94%)	Cooked biscuit	Peanut and hazelnut
3	27266	96 of 106 (91%)	Cooked biscuit	Peanut and hazelnut
4	27353	85 of 89 (96%)	Cooked biscuit	Peanut and hazelnut
5	27246	39 of 44 (89%)	Garlic powder	Peanut
6	27358	37 of 44 (84%)	Garlic powder	Peanut

* This technical bulletin will focus on results from peanut testing.

Table 2. Overview of Test Materials.

Study Number	Study Material	Sample*	Consensus of Submitted Qualitative Results from All Participants (%)
1	27263A	Nut-free chocolate + 16 mg/kg of peanut powder	100
	27263B	Nut-free chocolate	100
2	27242	Freeze-dried biscuits containing peanut flour	98
3	27266	Freeze-dried biscuits containing peanut flour	95
4	27353	Freeze-dried biscuits containing peanut flour	93
5	27246A	Nut-free garlic powder	95
	27246B	Nut-free garlic powder + ~20 mg/kg of peanut flour	83
6	27358A	Garlic powder + peanut	100
	27358B	Garlic powder	96

* The final amount of peanut or peanut flour was not specified in some PT reports.

Overview of Data and Analysis Methods

All labs, including Hygiena, indicated both qualitative and quantitative results of peanut presence as well as the limit of detection (LOD) and limit of quantification (LOQ). 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. Overall, results for peanut detection and quantification were submitted from at least nine 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. 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 for the same kit. A kit with a higher percentage of z-scores ≤ 2 indicates more consistent allergen quantification in the study.

Results

Qualitative Results

In agreement with test results from most study participants, the AlerTox ELISA Peanut Kit detected peanuts in samples containing peanuts and did not detect peanuts in samples without peanuts.

The consensus from all participants for the detection of peanuts in the six samples containing peanuts ranged from 83% to 100% (Table 2). The consensus from all participants for no detection of peanuts in the three samples that did not contain peanuts ranged from 96% to 100% (Table 2).

Quantitative Results

Within Studies 2 – 4, the AlerTox ELISA Peanut Kit performed similarly in different laboratories (Table 3). Only the Hygiena Laboratory used the AlerTox ELISA Peanut Kit in the other studies.

Table 3. Submitted Results from the AlerTox ELISA Peanut Kit.

Material*	Lab Number	Submitted Result from the AlerTox Kit (mg/kg)
Study 2 (Cooked Biscuit)		
27242	46 (Hygiena)	12.46
	50	15
	70	13.8 [†]
Study 3 (Cooked Biscuit)		
27266	95 (Hygiena)	22.48
	88	16.7
Study 4 (Cooked Biscuit)		
27353	26 (Hygiena)	21.39
	77	22.4 [†]

* The amount of peanuts in the material was not specified in the PT report.

[†] LOQ = 3 mg/kg (LOQ = 1 mg/kg for all other submitted values from the AlerTox ELISA Peanut Kit)

The AlerTox ELISA Peanut Kit performed as well as other kits. Using the information in the reports, Hygiena scientists calculated z-scores to compare measurements from our laboratory results to those from other kits that were statistically analyzed. In most cases, the submitted values from the AlerTox Kit were similar to the assigned value obtained from other commercial kits (i.e., |z| value ≤2) (Table 4).

Overall, there were four kits that had large z-scores, indicating high variability compared to the AlerTox kit results. Four results did not have acceptable z-scores ($z > 2$): compared to the AlerTox Kit, the Neogen® Veratox® Peanut Kit (in two studies), BioFront MonoTrace® Peanut Kit and ELISA Systems Peanut Kit had lower peanut measurements and high variability (Table 4, red font). In addition, the z-score for the Ridascreen® Fast Peanut Kit ($z = 1.99$) was acceptable but approaching the threshold in Study 2 (Table 4).

Also, there were four kits where analysis was performed with the caveat that questionable uncertainty was associated with their results, so their results were provided for information only (Table 4, gray shading).



Table 4. Quantitative Results (mg/kg) from Samples Containing Peanut Allergens.

Material (peanut amount)	Submitted Result, AlerTox Kit (Hygiena lab)	Assigned Values (z-scores, number of labs submitting results)*							
		Ridascreen Fast Peanut Kit	Ridascreen Peanut Kit	Neogen Veratox Peanut Allergen Kit	Romer Labs® AgraQuant® Peanut Kit	SENSISpec ELISA Peanut Kit	BioFront MonoTrace Peanut Kit	ELISA Systems Peanut Kit	ProGnosis Allergen-Shield Peanut Kit
Study 1 (Chocolate)									
27263A (16 mg/kg)	71.2	89.9 (z = -0.83, n = 18)	ND	60.6 (z = 0.70, n = 4)	62.9 (z = 0.53, n = 4)	ND	ND	ND	ND
Study 2 (Cooked Biscuit)									
27242 (unknown)	12.46	8.33 (z = 1.99, n=31)	ND	8.0 (z = 2.3, n = 14)	12.6 (z = -0.04, n = 14)	9.32 (z = 1.35, n = 4)	5.8 (z = 4.69, n = 3)	ND	ND
Study 3 (Cooked Biscuit)									
27266 (unknown)	22.48	24.3 (z = -0.30, n = 33)	ND	19.3 (z = 0.66, n = 14)	25.1 (z = -0.42, n = 11)	ND	ND	ND	ND
Study 4 (Cooked Biscuit)									
27353 (unknown)	21.39	26.3 (z = -0.75, n = 3)	25.6 (z = -0.66, n = 14)	23.3 (z = -0.33, n = 10)	22.8 (z = -0.25, n = 12)	20.3 (z = 0.21, n = 6)	ND	0.97 (z = 84.03, n = 3)	15.3 (z = 1.59, n = 6)
Study 5 (Garlic Powder)									
27246B (20 mg/kg)	6.39	4.65 (z = 1.50, n = 12)	ND	3.80 (z = 2.73, n = 7)	5.18 (z = 0.93, n = 4)	ND	ND	ND	ND
Study 6 (Garlic Powder)									
27358A (unknown)	11.04	ND	15.1 (z = -1.07, n = 14)	13.6 (z = -0.75, n = 8)	ND	ND	ND	ND	ND

* ND = not done (either no or not enough results were submitted for statistical analysis in the PT report).

Light gray shading: The PT report specified that the assigned value and z-scores are for information only because of the questionable uncertainty associated with the results.

Red text: |z| value >2 is an unsatisfactory score that indicates high variability between the AlerTox submitted result and the assigned value for the other kit.



Discussion

The AlerTox ELISA Peanut Kit consistently detects peanuts in a wide variety of food matrices. The PT testing described here included chocolate, cooked biscuits and garlic powder, but in-house testing has also been done with cornflakes, ice cream and hazelnut. See the *AlerTox ELISA Peanut Claim Support* and the *AlerTox ELISA Instructions* for additional information, including lists of matrices with no cross-reactivity [1 – 2].

Based on z-scores, quantitative results from the AlerTox ELISA Peanut Kit are similar to most other commercial kits used in these studies. However, the Neogen Veratox Kit tended to report lower levels of peanut with higher variability compared to the AlerTox ELISA Kit.

Only two of the six study reports included the spiked amount of peanut content in the sample material. In Study 1 (chocolate), all the submitted results for the AlerTox Kits and assigned results for other kits were consistently higher than the spiked value. In contrast, in Study 5 (garlic powder), all the submitted and assigned results were consistently lower than the spiked value. Additional PT studies that include known spiked amounts of peanuts in similar sample types may help clarify these observations.

Conclusions

Qualitative and quantitative results showed that the Hygiena AlerTox ELISA Peanut Kit was fit for purpose for detecting and quantifying peanut proteins in food. The AlerTox ELISA Peanut Kit performed equally well or better than the commercial kits used in six PT studies described in this overview.

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 peanut allergens in a wide range of food matrices. And if needed, a single sample extract made with this kit can be used to determine the content of 15 other allergens using other AlerTox ELISA Kits [2].

References

1. Hygiena (2014) AlerTox ELISA Peanut Claim Support. Available at www.hygiena.com/documents. Accessed 16 October 2023.
2. Hygiena (2020) AlerTox ELISA Allergen Instructions. Available at www.hygiena.com/documents. Accessed 16 October 2023.