



# Egg Allergen (Ovomucoid) Detection and Quantification Using the AlerTox® ELISA Egg Kit

Summary of Results from Seven Fapas® Proficiency Testing Studies

## Allergenic Egg Proteins

Egg allergies are a common childhood food allergy that often develops during infancy and usually resolves before adolescence. Because of the prevalence of egg allergy and the risks to children, egg is one of the “Big 9” food allergens that require product labeling.

The most common egg allergens are egg white proteins. Egg whites contain 9 – 11% protein, with four allergenic proteins comprising approximately 80% of those proteins. The dominant allergen, ovomucoid (11% of egg white protein), is heat stable. Ovalbumin (54% of egg white protein) and lysozyme (~3% of egg white protein) are not heat stable. Lastly, the weakest egg white allergen, ovotransferrin/conalbumin (12% of egg white protein), is also not heat stable. Egg yolk proteins, primarily  $\alpha$ -levitin and vitellogenin-1 fragments, may be allergenic but at lower levels than egg white proteins.

## Description of the AlerTox ELISA Egg Kit

AlerTox® ELISA Egg Kits are immunosorbent assays designed for the quantitative determination of ovomucoid, the major allergenic egg white protein, in raw materials and final products. The kits are 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 the kits to precisely quantify allergens in all types of food and drinks. The limit of detection (LOD) is 0.05 ppm (mg of egg white protein/kg of food sample), and the limit of quantification (LOQ) is 0.4 ppm (mg of egg white protein/kg of food sample).

AlerTox ELISA Kits that target other allergenic egg proteins (ovalbumin and lysozyme) are available but are typically used to test for wine fining agents. The same sample preparation can be used with AlerTox ELISA tests for egg (ovomucoid), ovalbumin and lysozyme.

## Proficiency Testing Procedure and Test Materials

Proficiency testing (PT) is an essential part of laboratory quality procedures, and participation is required for ISO 17025 accreditation for testing and calibration laboratories. As the developer of allergen tests and kits, Hygiena® regularly participates in proficiency testing to provide an independent assessment of test performance and to compare results to those of laboratories worldwide. Completing 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.

From 2022 to 2024, Hygiena participated in seven Fapas® PT studies (Fera Science), including detecting and quantifying egg allergens (Table 1). As with all Fapas studies, laboratories ran tests without knowing the allergenic protein levels in the samples.

Although most of these studies involved multiple allergens, this summary focuses on the data for egg allergens using the AlerTox ELISA Egg Kit. For Studies 1 and 2, samples were spiked with egg white powder, while for all other studies, samples were spiked with whole egg powder.

Some kits, like the Hygiena AlerTox ELISA Egg Kit and the Romer® AgraQuant® Egg White Kit, provide results as egg white protein. Other kits provide results as whole egg, which cannot be directly compared to egg white protein measurements. For some comparisons, results have been adjusted using conversion factors based on kit manufacturer specifications or literature references.

**Table 1. Overview of Fapas Food Chemistry Proficiency Testing Studies.**

Study	Study Material	Number of Participants Submitting Results	Sample	
			Matrix*	Egg Spike from the Organizer
1	27314	97 of 107 (91%)	Cooked biscuit	Egg white powder (amount not specified)
2	27382	81 of 84 (96%)	Cooked biscuit	Egg white powder (amount not specified)
3	27331A	156 of 163 (96%)	Cake mix	15 mg/kg whole egg powder
	27331B			20 mg/kg whole egg powder
4	27364A	115 of 126 (91%)	Cake mix	18 mg/kg whole egg powder
	27364B			39 mg/kg whole egg powder
5	27342A	31 of 37 (84%)	Cake mix	20 mg/kg whole egg powder
	27342B			40 mg/kg whole egg powder
6	27377A	63 of 65 (97%)	Cake mix	40 mg/kg whole egg powder
	27377B			50 mg/kg whole egg powder
7	27393A	34 of 37 (92%)	Cake mix	None
	27393B			25 mg/kg whole egg powder

\* This technical bulletin will only focus on results from egg testing. Before spiking, all matrices were allergen-free (i.e., egg-, gluten- and/or milk-free). For Studies 1 – 4, the analytes were egg, gluten and milk. For Studies 5 – 6, the analytes were egg and gluten. For Study 7, the analyte was egg.

### Overview of Study Analysis and Reports

All participating labs, including Hygiena’s, indicated qualitative and quantitative results for the study analytes as well as the limits of detection and quantification for their methods. Results for egg detection and quantification were submitted from mass spectrometry and at least 14 different commercial or in-house kits.

Fera Science then summarized the data and provided a report to all participants. The results were segregated into subsets according to the method used. If enough results from the same method 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  $\leq 2$  is considered a satisfactory or “passing” value, meaning the submitted result is similar to the assigned value in the study. Similarly, for this technical bulletin, we can calculate a z-score to compare the AlerTox ELISA Egg Kit results to the assigned values of other commercial kits.

## Results and Discussion

### Cooked Biscuit: Testing Results (Studies 1 – 2)

The amounts of egg white powder in the final study materials were not specified in the reports for Studies 1 and 2 from the organizer. However, egg protein was detected by the AlerTox ELISA Kit and all other methods in both study materials (Table 2 and data not shown).



The results from the r-Biopharm® and Neogen® kits are in units of whole egg. To compare these results with those from the AlerTox ELISA Kit, the assigned values from those kits were converted to the equivalent amount of egg white protein, and a z-score was calculated (Table 2). Based on satisfactory, passing z-scores, the AlerTox ELISA Egg Kit results were consistent with those from the two r-Biopharm kits and Neogen Veratox® kit, although the r-Biopharm Ridascreen® Egg Kit results appeared to be much higher.

**Table 2. Comparison of Results from Cooked Biscuit Testing Using the AlerTox ELISA Egg Kit or Other Commercial Kits.**

Study	Hygiena’s AlerTox ELISA Egg Kit (ppm)*	r-Biopharm’s Ridascreen Egg Kit: Converted ppm <sup>†</sup> (z-score, Assigned ppm)	r-Biopharm’s Ridascreen Fast Egg Kit: Converted ppm <sup>‡</sup> (z-score, Assigned ppm)	Neogen’s Veratox for Egg: Converted ppm <sup>§</sup> (z-score, Assigned ppm)
1	<b>8.82</b>	69.12 <sup>¶</sup> (-1.68, 144)	5.7 (0.58, 21.8)	12.479 (-0.29, 50.4)
2	<b>12.21</b>	167.0 (-1.78, 348)	15.99 (0.24, 61.5)	25.998 <sup>¶</sup> (-0.526, 105)

\* Data from the Hygiena laboratory. ppm = mg allergen/kg sample.

† The results were converted from whole egg to egg white protein for this table [0.25 mg/kg (ppm) whole egg powder is equivalent to 0.12 mg/kg (ppm) egg white protein]. For Study 1, the main mode of the multimodal distribution of submitted values was chosen as the assigned value. For Study 2, the median of the submitted values was chosen as the assigned value because of the low number of data points.

‡ The results were converted from whole egg to egg white protein for this table [0.5 mg/kg (ppm) whole egg powder is equivalent to 0.13 mg/kg (ppm) egg white protein]. For Study 1, the mean of the submitted values was chosen as the assigned value. For Study 2, the median of the submitted values was chosen as the assigned value because of the low number of data points.

§ The results were converted from whole egg to egg white protein for this table using a conversion factor of 0.2476 [1,2]. For Studies 1 and 2, the median of the submitted values was chosen as the assigned value because of the low number of data points.

¶ For information only based on uncertainty associated with the data.

### Cake Mix: Hygiena Testing Results (Studies 3 – 7)

Considering the amount of spiked egg white protein contained in whole egg powder spike, the AlerTox ELISA Egg Kit detected egg white protein in all egg-containing samples at slightly lower levels than the spiked amount (Table 3). The AlerTox ELISA Egg Kit did not detect egg white protein in the only egg-free sample (Table 3, Study 7, Study Material 27393A). Two labs that were not affiliated with Hygiena submitted the same or similar results as the Hygiena lab (Table 3, Studies 3 and 7, Labs 48 and 33, respectively).

**Table 3. Submitted Results for Cake Mix Testing Using the AlerTox ELISA Egg Kit.**

Study	Study Material	Egg Spike from the Organizer (ppm whole egg powder)	Equivalent Egg White Protein Spike [1,2] (ppm egg white protein)	AlerTox ELISA Egg (ppm egg white protein)*
3	27331A	15	3.71	1.91 1.8 (Lab 48)
	27331B	20	4.95	2.3 2.3 (Lab 48)
4	27364A	18	4.46	3.18
	27364B	39	9.66	6.34
5	27342A	20	4.95	3.84
	27342B	40	9.90	7.59
6	27377A	40	9.90	8.69
	27377B	50	12.38	11.50
7	27393A	0	0	<LOQ <LOQ (Lab 33)
	27393B	25	6.175	5.20 5.6 (Lab 33)

\* All results were from the Hygiena laboratory, except for those submitted by Lab 48 (Study 3) and Lab 33 (Study 7).

### Cake Mix: Comparison of the AlerTox ELISA Egg Kit to Other Commercial Kits (Studies 3 – 7)

The results from Hygiena’s AlerTox ELISA Egg Kit, Romer’s AgraQuant Egg White Kit and Neogen’s BioKits Egg Assay Kit can be directly compared because they are all in units of egg white protein (Table 4). When the whole egg powder spike-in amount was adjusted to egg white protein, most of the results from these kits were slightly lower than the spike-in amount, and based on satisfactory (passing) z-scores, the AlerTox ELISA Egg Kit results were similar to those of the other commercial kits.

**Table 4. Comparison of Results for Cake Mix Testing Using the AlerTox ELISA Kit or Other Commercial Kits That Provide Results as Egg White Protein.**

Study	Converted Spike-in Amount (ppm)*	Hygiena’s AlerTox ELISA Egg Kit <sup>†</sup> (ppm) (Hygiena Lab)	Romer’s AgraQuant Egg White Kit <sup>‡</sup> (ppm) (z-score)	Neogen’s BioKits Egg Assay Kit <sup>‡</sup> (ppm) (z-score)
3	<b>3.71</b>	<b>1.91</b>	1.94 (–0.06)	—
3	<b>4.95</b>	<b>2.3</b>	2.29 (0.02)	—
4	<b>4.46</b>	<b>3.18</b>	4.19 <sup>§</sup> (–0.96)	—
5	<b>4.95</b>	<b>3.84</b>	4.02 <sup>§</sup> (–0.18)	—
5	<b>9.90</b>	<b>7.59</b>	8.89 <sup>§</sup> (–0.59)	—
7	<b>6.18</b>	<b>5.20</b>	6.13 (–0.607)	6.96 (1.01)

\* Equivalent egg white protein spike from Table 3 (ppm: mg egg white protein/kg sample).

<sup>†</sup> Data from the Hygiena laboratory.

<sup>‡</sup> The median of the submitted values was chosen as the assigned value because of the low number of data points.

<sup>§</sup> For information only based on uncertainty associated with the data.

The results from r-Biopharm’s Ridascreen and Ridascreen Fast, Neogen’s Veratox and Morinaga’s ELISA II egg kits are in units of whole egg. To compare these results with those from the AlerTox ELISA Kit, the assigned values from those kits were converted to the equivalent amount of egg white protein, and a z-score was calculated. Except for the results from the r-Biopharm’s Ridascreen Egg Kit, most of the AlerTox ELISA Egg Kit and the other commercial kit results were slightly lower than the spike-in amount of egg allergen in the study materials. Based on satisfactory, passing z-scores, the results from these kits were similar to those from the AlerTox ELISA Egg Kit (Table 5).

**Table 5. Comparison of Results For Cake Mix Testing Using the AlerTox ELISA Egg Kit or Other Commercial Kits\*, Adjusting Assigned Results from Whole Egg Powder to Egg White Protein.**

Study	Converted Spike-in Amount <sup>†</sup>	Hygiena's AlerTox ELISA Egg Kit <sup>‡</sup>	r-Biopharm's Ridascreen Egg Kit: Converted <sup>§</sup> (z-score, Assigned)	r-Biopharm's Ridascreen Fast Egg Kit: Converted <sup>¶</sup> (z-score, Assigned)	Neogen's Veratox for Egg: Converted <sup>**</sup> (z-score, Assigned)	Morinaga's Egg ELISA Kit II: Converted <sup>**</sup> (z-score, Assigned)
3	<b>3.71</b>	<b>1.91</b>	4.63 (-1.13, 9.64)	1.5 (0.31, 5.66)	1.99 (-0.0398, 8.05)	1.139 (0.67, 4.60)
	<b>4.95</b>	<b>2.3</b>	6.38 (-1.22, 13.3)	1.9 (0.24, 7.16)	2.476 (-0.07, 10.0)	1.46 (0.56, 5.90)
4	<b>4.46</b>	<b>3.18</b>	9.94 <sup>††</sup> (-1.30, 20.7)	4.58 (-0.32, 17.6)	5.447 (-0.41, 22.0)	1.72 (0.84, 6.95)
	<b>9.66</b>	<b>6.34</b>	—	8.74 (-0.28, 33.6)	11.389 (-0.439, 46.0)	3.39 (0.86, 13.7)
5	<b>4.95</b>	<b>3.84</b>	10.656 <sup>††</sup> (-1.23, 22.2)	—	—	—
	<b>9.90</b>	<b>7.59</b>	21.98 <sup>††</sup> (-1.25, 45.8)	7.0 (0.08, 27.0)	—	—
6	<b>9.90</b>	<b>8.69</b>	—	7.62 (0.146, 29.3)	—	3.34 (1.58, 13.5)
	<b>12.38</b>	<b>11.50</b>	—	9.386 (0.23, 36.1)	—	3.86 (1.96, 15.6)
7	<b>6.175</b>	<b>5.20</b>	—	4.186 (0.25, 16.1)	5.249 <sup>††</sup> (-0.009, 21.2)	1.91 <sup>††</sup> (1.70, 7.70)

\* Results from the ELISA Systems' Enhanced Egg Residue Kit were not included in this table because it was only evaluated in Study 4.

† The sample spike was whole egg powder.

‡ Data from the Hygiena laboratory.

§ The results were converted from whole egg to egg white protein for this table [0.25 mg/kg (ppm) whole egg powder is equivalent to 0.12 mg/kg (ppm) egg white protein]. For Studies 3 – 7, the median of the submitted values was chosen as the assigned value because of the low number of data points.

¶ The results were converted from whole egg to egg white protein for this table [0.5 mg/kg (ppm) whole egg powder is equivalent to 0.13 mg/kg (ppm) egg white protein]. For Studies 3 and 4, the mean of the submitted values was chosen as the assigned value. For Studies 5 – 7, the median of the submitted values was chosen as the assigned value because of the low number of data points.

\*\* The results were converted from whole egg to egg white protein for this table using a conversion factor of 0.2476 [1,2]. For Studies 3 – 7, the median of the submitted values was chosen as the assigned value because of the low number of data points.

†† For information only based on uncertainty associated with the data.

## Conclusions

Proficiency testing results showed that the Hygiena AlerTox ELISA Egg Kit was fit for the purpose of detecting and quantifying egg allergens in food. After adjusting the amount of whole egg protein spikes to levels of egg white protein, the kit results were slightly lower than the spiked amount. Qualitative and quantitative results from two independent labs using the AlerTox ELISA Egg Kit were similar to those from the Hygiena lab.

The AlerTox ELISA Egg Kit performed equally well as the other commercial kits used in the seven PT studies described in this summary. Based on z-score analysis, the AlerTox ELISA Egg Kit results were consistent (satisfactory, passing z-score) with those from the other commercial kits used in these studies. Although the RidaScreen Egg Kit tended to report higher levels of egg allergen (sometimes with high standard deviation values), most commercial kits obtained results that were slightly below the amount of egg spiked into the samples in the studies that included the amount of egg allergen spiked into the matrix.

ELISA, PCR and mass spectrometry (MS) are commonly used techniques for allergen detection and quantification. However, ELISA is the fastest and least expensive technique and requires the least amount of technical expertise. With only 60 minutes of incubation time, our easy-to-use sandwich ELISA test has the sensitivity and specificity that make it a useful part of a testing program for egg allergens in food matrices. If needed, a single sample extract made with this kit can be used to determine the content of 16 other allergens by using other AlerTox ELISA tests, including ones for two other egg proteins (i.e., ovalbumin and lysozyme).

## Reference

1. National Institute of Standards and Technology. (2020) Standard Reference Material® 1845a, Whole Egg Powder. <https://tsapps.nist.gov/srmext/certificates/1845a.pdf>.
2. Rehaul-Godbert S, Guyot N and Nys Y (2019) The Golden Egg: Nutritional Value, Bioactivities, and Emerging Benefits for Human Health. *Nutrients* 11(3):684 doi: [10.3390/nu11030684](https://doi.org/10.3390/nu11030684).

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