

ELMA Statement

Uncertainties of peanut testing

In response to the feedback of several renowned laboratories performing Peanut analyses on Lecithins, ELMA would like to clarify the question on possible differences of Peanut protein results for the same sample using different laboratories.

Factors to consider for variation on Peanut results with ELISA:

1) **Different test kits**

There are different ELISA kits and brands to perform an ELISA Peanut analysis (examples of brands: R-Biopharm, Morinaga, Römer Labs, ELISA Systems, Qiagen). Each brand has then several kits. Hence, laboratories use different test kits.

2) **Protein Conversion Factor**

Documented levels of protein in peanut vary between 22 and 27%. For ELISA testing, therefore, a peanut-containing calibrator with a specified peanut protein content must be used. Most of the test kits use a NIST Standard Reference Material containing 22.2g protein/100g. If a calibrator with a different protein level is used, this will result in a different reported peanut protein content.

3) **Different source and composition of calibration material**

Peanut kernels contain over 50 different types of proteins, 19 of which can cause allergic reactions in sensitized individuals, although the three most commonly associated with allergenic responses are: Ara h1, Ara h2, Ara h3.¹

For peanut protein testing by ELISA, laboratory test methods may be based on one or more allergenic proteins as the target for the antigen; this is method/test kit dependent. Different peanut protein calibrator substances may also be used. Typically, these are roast and raw peanut, or peanut butter.

4) **Results, LODs, LOQs and Measurement Uncertainty**

Laboratories may report test results, Limits of Detection (LOD), and Limits of Quantification (LOQ) in mg/kg of peanut, or in mg/kg of peanut protein, or both. Reported values, LODs, LOQs, and Measurement Uncertainties (MU) will differ between laboratories, for different test kits and depending on the peanut-to-peanut protein conversion factor that has been used (22% or 27%). Therefore, it is difficult to compare results between different laboratories or when different test kits have been used. ELMA would recommend that the level of uncertainty is included on the laboratory reports.

¹ Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes, EFSA Journal 2014;12(11):3894: <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3894>

5) Cross-reactivity with other allergens

Cross-reactivity with other allergenic proteins is not reported for peanut detection in lecithin by ELISA methods, however, some substances, such as tannins or phenolic chemicals may interact with the peanut proteins and decrease extraction efficiency. Also, the processing that the food material has undergone can change the structure of the peanut protein, which can reduce recognition by the antibody and give a lower result.

Additional reasons for variation in peanut results could be: the experience of the laboratory personnel, lab material used, experience with the matrix lecithin, number of analyses performed, sampling (homogeneity), *etc.*

Conclusion: this statement was created to raise awareness, that for peanut analysis there are no ideal laboratories or methods, and that the same sample can have different Peanut protein results, depending on different factors listed above in this statement. Therefore, ELMA has identified three most suitable testing kits for detection of peanuts in the lecithin matrix. These are:

- ELISA peanut AOAC 112102, test kit RIDACSCREEN® – R-Biopharm R6811
- MIOBS M2120:2020-09, PV 01540, ELISA test kit II (code JKEPN-10) - (Morinaga Institute of Biological Science)
- AgraQuant® Plus Peanut, ELISAFast® Peanut

It is important to carry-out the analysis in an ISO17025 accredited laboratory for peanut protein analysis, using a validated method for lecithin. The above-mentioned kits were identified at the time of the creation of the statement. Since then, other ELISA suitable testing kits may have been developed.

Brussels, 10th January 2023²

² Updated June/2023