# Specific Accreditation Criteria

## ISO/IEC17025 Application Document

## Life Sciences - Annex

## Testing for food allergen proteins and gluten

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# Testing for food allergen proteins and gluten

## Purpose

In addition to the *General Accreditation Criteria: ISO/IEC 17025 Standard Application Document* (SAD) and the accompanying *Life Sciences - Appendix*, this document provides interpretative criteria and recommendations for testing for food allergen proteins and gluten by Enzyme Linked Immunosorbent Assay (ELISA) and lateral flow technology for both applicant and accredited facilities. This document does not cover testing for allergenic proteins and gluten by mass spectrometry or polymerase chain reaction.

Facilities must comply with all relevant documents in the NATA Accreditation Criteria (NAC) package for Food and Beverage (refer to *NATA Procedures for Accreditation).*

The clause numbers in this document follow those of ISO/IEC 17025; however, as not all clauses require interpretation, the numbering may not be consecutive.

## Background introduction

In Australia mandatory allergen declaration requirements for food are set out in the Australia New Zealand Food Standards Code (ANZFSC). These requirements apply to food produced or imported into Australia for sale in Australia or New Zealand. The ANZFSC requires the presence of peanuts, lupin, tree nuts, milk, egg, sesame seeds, fish, crustacea, soybeans as well as cereals containing gluten and added sulphites to be declared on food labels whenever they are present as ingredients or as components of food additives or processing aids.

Further details of the requirements of the ANZFSC in relation to allergens and gluten can be found in Standard 1.2.3 of the ANZFSC.

Allergenic proteins are defined as proteins that can cause an allergic reaction in sensitised consumers. Testing for the presence of proteins from allergenic foods may be undertaken in raw materials, in process samples, finished product and flush solutions and from environmental contact surfaces. Assays generally target one or more soluble marker proteins for the presence of an allergenic food. These marker proteins are not necessarily allergenic themselves but have requisite properties to allow quantitation of an allergenic food in a variety of processed and non-processed samples. It is widely documented that highly processed samples are more challenging to analyse since the detectability of any proteins present may be compromised due to the effects of processing on protein solubility, extraction efficiency and confirmation (refer to references). Such proteins may however still remain allergenic to consumers. Due to such considerations, together with matrix specific interferences, testing for food allergens should only be undertaken by suitably qualified and experienced analysts using validated methods.

## NATA scopes of accreditation

The scopes of accreditation of NATA accredited facilities will identify the matrix under test and the specific protein. Where additives to food or mixed foods are tested, these will be further defined to ensure the scope of accreditation adequately describes the correct range of matrices.

# 6 Resource requirements

## 6.3 Facilities and environmental conditions

**6.3.1** The possibility of cross contamination must be minimised, ideally by the use of dedicated separate areas for sample processing and analysis. If this is not possible, strict house-keeping procedures must be documented and adhered to.

**6.3.3** A program of environmental monitoring must be established to detect potential cross contamination in both dedicated and shared facilities.

# 7 Process requirements

## 7.1 Review of requests, tenders and contracts

**7.1.1** The first time new product types are received by the facility for analysis and particularly when the ingredients and processing are not self-evident, discussion must be had with the customer relating to ingredients and the processing involved in the manufacturing of the product. This is to ensure that the ELISA test kit used is suitable for the detection of the protein(s) in question. Refer to 7.2.1.5.

## 7.2 Selection, verification and validation of methods

Commercial ELISA kits must not be used beyond the scope of their intended use as specified by the manufacturer, or recognised other body such as AOAC, without validation.

Information on method verification and method validation can be found in AOAC International *Appendix M: Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices.*

### 7.2.1 Selection and verification of methods

**7.2.1.5** Kits must be verified for the range of matrices that reflect the different sample types which may be tested. As a minimum, the following must be considered to verify kit performance:

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is defined as the concentration or mass of analyte in a test sample that can be distinguished from a true blank sample at a specified probability level.

LOQ is defined as the lowest level of analyte in a test sample that can be reasonably quantified at a specified level of precision.

Sample matrix

Protein detectability in various processed and non-processed matrices forms part of the manufacturer's validation and verification of assay performance. This is generally performed on a number (≥5) of incurred/spiked positive samples and known negative samples.

Acceptable spike recovery may not be comparable to the protein detectability in samples from highly processed matrices. In such cases, additional evidence of fitness-for-purpose of the ELISA kit is required.

Constituents of the sample matrix may suppress/enhance assay signal. Some of these may already be identified by the manufacturer, for example,

- chocolate (especially high cocoa content) has high levels of polyphenols that inhibit the extraction of protein;
- some balsamic vinegar and other caramel IV containing products may show non-specific binding in numerous ELISAs, producing false positive results (e.g. caramel flour).

  **Note:** False Positive Detection of Peanut Residue in Liquid Caramel Coloring Using Commercial ELISA Kits: T. Stelk; L. Niemann; D. M. Lambrecht; J. L. Baumert; S. L. Taylor. First published: 6 May 2013 https://doi.org/10.1111/1750-3841.12146

Sample matrices that may interfere with the ELISA test kit must be identified and modified testing protocols validated.

The suitability of a matrix can be assessed by spiking experiments, taking into account the following:

- at least three representative sample types containing the matrix of interest;
- at least two independent replicates of each sample type;
- two levels of the target analyte;
  - at or near the level of reporting;
  - around midway on the standard curve;
- recovery should be in the range 50% to 150% of the spike value. Consideration should be given to the suitability of the sample for analysis where matrices exhibit recovery outside 50% to 150%. Where recovery is outside 50% to 150%, consideration should also be given to adjusting the limit of reporting (LOR).

  **Note:** For the purposes of this document, the LOR is defined as the lowest concentration at which an analyte can be detected in a sample and be reported with a reasonable degree of accuracy and precision based on the facility's own investigations. Further information on spike recovery of ELISA can be found in AOAC Appendix M.

Ideally, the spiking material should be a co-extracted quality control (QC) or reference material sample. Where QC material is used, this must be different to that used for QC of the ELISA kit.

Where a pre-extracted protein solution is used as a spiking agent, its fitness-for-purpose must be determined.

Selectivity (cross reactivity)

It is important to document any identified food constituents (ingredients) that cross-react or interfere to elicit a non-specific signal. In such circumstances, the quantitative impact of the constituent should be determined and processes implemented to remove the interference.

### 7.2.2 Validation of methods

Commercial ELISA kits used beyond the scope of their intended use as specified by the manufacturer, or recognised other body such as AOAC, must be validated. Also refer to 7.2.1.5 covering verification of test kits.

As a minimum validation must include:

- different commodity types within food groups (e.g. meat may be raw or processed, have high or low fat, or high or low moisture);
- LOD;
- LOQ;
- LOR;
- linearity (concentration range);
- matrix applicability;
- measurement of uncertainty (MU) (dependent on matrix properties identified).

## 7.6 Evaluation of measurement uncertainty

**7.6.1** Estimation of MU must consider test kit bias. Assessment of bias for each ELISA kit covering different matrices may be determined through use of certified reference materials. However, as accepted certified reference materials for use with food allergen ELISA based methods are unavailable, bias can be determined by recovery of spiked samples.

## 7.7 Ensuring the validity of results

**7.7.1** Acceptance criteria for assay results and standard curves must be established to ensure result variation and kit performance can be monitored.

The procedures in place to monitor the validity of test results must include, but not limited to, the following:

- QC samples as part of the assay run and performed with each batch of samples; and
- a blank or zero standard included with each batch of test samples.

Certain commodities of natural origin may vary significantly from batch to batch, and for these, additional quality measures may be required, for example, wheat, soy flours and milled grains.

Kit performance must be monitored for trends, e.g. by the use of control charts.

Quantitative assays require the whole standard curve to be generated. Qualitative assays require 2 or 3 points on the curve but this must include zero and the first standard.

Facilities should be aware that assay drift is seen in runs with large sample numbers. This is caused by variations in incubation times between wells due to the time required for sample/reagent addition. This can be as much as 20% across a 96 well plate. Facilities must consider the significance of assay drift in each run or monitor and observe test run history (covering a range of wells/strips used). Facilities must establish a process for monitoring and assessing the performance of plate wells with respect to assay drift.

## 7.8 Reporting of results

**7.8.1.2** Results below the LOQ cannot be reported as “Nil” or “Zero”.

Facilities must establish a procedure for reporting results that fall between the LOD and the LOQ. For such results, the customer must be informed that a signal was detected but that it could not be readily quantified and hence, may need to be investigated further.

The report must clearly describe the measurand (i.e. refer to specific protein, total protein, or whole commodity) to avoid any misunderstanding of what has been tested.

Where appropriate, the report must also include reference to the test kit employed as different manufacturers’ kits may detect different measurands for the same proteins tested.

# References

This section lists publications referenced in this document. The year of publication is not included as it is expected that only current versions of the references shall be used.

### Standards

| | |
|---|---|
| ISO/IEC 17025 | General requirements for the competence of testing and calibration laboratories |

### NATA Publications

NATA Accreditation Criteria (NAC) package for Food and Beverage

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|---|---|
| General Accreditation Criteria | ISO/IEC 17025 Application Document Life Sciences - Appendix |
| Specific Accreditation Criteria | ISO/IEC 17025 Standard Application Document |

### Other Publications

AOAC Official Methods of Analysis 2012 *Annex M: Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices*

Clare Mills et al (Risk Management for Food Allergy 2014, Pages 227-251 Chapter fourteen - Effect of Processing on the Allergenicity of Foods https://doi.org/10.1016/B978-0-12-381988-8.00014-2)

Food Research Development Centre, Agriculture and Agri-Food Canada, Quebec (Detection of Allergens in a Multiple Allergen Matrix and Study of the Impact of Thermal Processing Joyce Boye et al)

# Amendment table

The table below provides a summary of changes made to the document with this issue.

| Section or Clause | Amendment |
|---|---|
| | New document. |