NATIONAL ASSOCIATION OF TESTING AUTHORITIES, AUSTRALIA

CRITERIA FOR ACCREDITATION OF LABORATORIES TESTING GENETICALLY MODIFIED ORGANISMS

GUIDELINES FOR ASSESSMENT

ACCREDITATION FOR TESTING FOR GENETICALLY MODIFIED ORGANISMS

This annex details criteria for accreditation of laboratories testing for the presence of Genetically Modified Organisms (GMOs) through the use of DNA extraction and Polymerase Chain Reaction (PCR) methods of detection. Testing for the presence of genetically modified sequences in foodstuffs or whole grain and related plant materials relies on the ability to test for the specific DNA sequences associated with modifications and/or the promoting and terminating sequences associated with the inserted sequences. The scope of this document is currently restricted to DNA detection methods. Protein testing methods for detecting GMOs are not covered in this document presently, but are still in development for later editions. At this time also, the document does not deal with accreditation of quantitative PCR testing of GMOs. This will be developed as the reliability of DNA quantitative methods is shown to be applicable and reliable.

The PCR detection methods in these guidelines deal with the PCR techniques for detection of genes associated with genetic modifications. In particular, they deal with the requirements for applying PCR techniques for the detection of "events" which for a GM "event", is a term referring to " the position in the genome which demarcates the site for integration of DNA foreign to that organism". The process is essentially random and so the event itself can be considered to be unique. PCR detection methods will be accredited which indicate the detection of specific events eg the presence of Roundup® ready soy inserted genes, rather than just screening methods detecting the insertion and termination sequences (eg Cauliflower Mosaic Virus (CMV) and NOS terminator) associated with gene insertion. While these sequences may be used as part of PCR detection of GMOs, eg in screening tests, they may lead to erroneous positive GM results if not associated with more specific event testing. Detection of any specific DNA sequences using PCR methods may be accredited under other PCR guidelines but is not within the scope of this document. The scope of this document is the guidelines associated with meaningful GMO detection and reporting.

The guidelines detail the requirements for the accreditation of a laboratory and its personnel and infrastructure. It includes the PCR assay and conditions associated necessary for PCR testing, the scope of interpretation of PCR results, the technical requirements of the facility and personnel conducting GM testing and the reporting of results to give unambiguous and meaningful results to clients requesting analyses of GM testing laboratories.

Accreditation is granted under the classes:

8.81 Qualitative analysis of GMO
.01 Detection by DNA
.02 Detection by Protein

8.82 Quantitative analysis of GMO
.01 Detection by DNA
.02 Detection by Protein (currently under development)

Currently quantitative detection cannot meet validated levels of accuracy and therefore accreditation is not possible.

4.6.2 **Assuring the quality of reagents and consumables**

The quality of the standard samples and reagents must be adequate for the procedure used.

It must be ensured that the following critical reagents have been tested in a standard assay and found to perform optimally, prior to their use in routine testing.

- Oligonucleotide primers
- Taq polymerase enzymes
- Deoxynucleotide triphospates
- Enzyme buffers
- Magnesium solutions
- Sterile water
- Kits and related reagents

Batch numbers of all critical reagents are to be recorded.

Standards and reagents must be labelled with:

- name of the reagent/standard;
- concentration where appropriate;
- preparation date;
- identity of preparer.

Where necessary, the following must be included on the labels:

- expiry date;
- storage conditions
- hazard warnings

5.2.1 **Staff competence and technical control**

NATA signatories must hold a Bachelor in a relevant discipline and have two years relevant experience. Signatories must be able to evaluate and interpret test results and have the ability to communicate orally the technical aspects of GMO testing to a lay audience.

Technicians are permitted to prepare test reports but not to sign test reports.

5.3 **Accommodation**

Nucleic acid amplification techniques are highly sensitive and thus highly susceptible to contaminations that can give rise to false positive results. Furthermore, false negative results can be the consequence of a poorly designed system for detecting the GM content of a sample due to a lack of overall sensitivity. Therefore, extra precautions are required to minimise the risk of false positive and false negative results within a laboratory when using nucleic acid amplification for the detection of GM food. The greatest protection a laboratory has against such results arises from:

- the competency of its staff at performing the test (refer to section 5.2.1);
- the design of the laboratory; and
- the routine use of controls.

Laboratory design

In order to reduce the risk of false positive results via cross-contamination or carry-over contamination of samples and reagents by other samples in the laboratory or by amplified material, four physically separate and contained areas with known air conditioning/ventilation airflows are required within a laboratory undertaking nucleic acid amplification for the detection of GM food. The four laboratory work areas are:

Sample Preparation
DNA Extraction
Reagent Preparation
Product Analysis.

The Sample Preparation area

This area is set aside for the purpose of preparing samples for nucleic acid extraction.

Conditions for this area are:

(i) The Sample Preparation area must be contained from the DNA Extraction work area, the Reagent Preparation work area and the Product Analysis work area.

(ii) The normal airflow from the Sample Preparation area should not come from the DNA Extraction and Product Analysis areas.

(iii) If sample preparation is performed inside an exhaust system capable of removing any airborne contaminants generated, the outlet for such an exhaust system shall not contaminate other areas.

(iv) Any apparatus and equipment that come in contact with the sample shall be cleaned by washing in 10% hypochlorite (or a product at least as effective) and rinsed thoroughly with doubly distilled water.

The DNA Extraction area

This area is set aside for the purpose of extracting nucleic acids from samples and for the addition of sample DNA to tubes containing master mix prior (previously dispensed in the Reagent Preparation area) to amplification.

Conditions for this area are:

(v) The extraction work area must be contained from the Sample Preparation work area, the Reagent Preparation work area and the Product Analysis work area.

(vi) The normal airflow from the DNA Extraction area should not come from the Sample Preparation and Product Analysis areas.

(vii) The exhaust air from the DNA Extraction area must not flow into the Sample Preparation, the Reagent Preparation and Product Analysis areas.

(viii) Normal airflow patterns in the DNA Extraction area are to achieve a slight positive pressure so that air flows out of the area in order to avoid contamination of the samples. This is not necessary if the DNA Extraction area is distantly separated from the Product Analysis area and has no airflow connections with the Sample Preparation and the Reagent Preparation areas.

The Reagent Preparation area

This area must be physically separated from all other regions of the laboratory. This work area is where the primer systems used to detect the GMO varieties are stored and dispensed.

Conditions for this area are:

(i) Air coming into this area must not come from the Sample Preparation or the Product Analysis areas.

(ii) Samples for analysis are never to be taken into the Reagent Preparation area.

(iii) Reagents must be stored separately from samples.

(iv) Normal airflow patterns in the Reagent Preparation area are to achieve a slight positive pressure so that air flows out of this area. If this cannot be achieved, then reagent preparation must be carried out in a dedicated PCR workstation within the contained area.

Note - Where the areas for Sample Preparation and Reagent Preparation cannot be separated there must be the provision of a PCR workstation for reagent preparation and a Class II Biological Safety Cabinet (BSC) for specimen preparation.The air outflow from the sample preparation BSC must have a HEPA filter and must be directed way from the reagent preparation area. Wide separation of these activities is to be maintained and appropriate procedures and controls are to be implemented to detect contamination.

The Product Analysis area

This area is only for amplification and product detection from the sample to be analysed. This area is where phenomenal amounts of nucleic acid sequences are amplified that are the specific sequences used to identify GMO varieties. Hence, this region is a potential source of contamination that will lead to false positive results.

Conditions for this area are:

(i) This area must be physically separated from the other two regions. Under no circumstances should Product Analysis activities be incorporated into areas where Sample Preparation and Reagent Preparation occur.

(ii) The normal airflow pattern from the Product Analysis area must not pass into the Sample Preparation or Reagent Preparation areas.

(iii) Normal airflow patterns in the Product Analysis area are to achieve a slight negative pressure so that air flows in to the area, or it is self contained.

Other conditions of laboratory design:

(i) The movement of samples and in limited cases, equipment, must be unidirectional from the sample preparation area to the product analysis area. It is recommended that items be marked to clearly indicate to which area they belong and must be retained in these areas unless decontaminated. Equipment that must pass from other areas to the reagent preparation area must be decontaminated in 2-10% sodium hypochloride or another non-corroding disinfectant agent for 4 h prior to this.

(ii) Laboratory coats and gloves are to be changed, and preferably hands should be washed when going to and from each area, or if they become soiled.

(iii) Aerosol resistant pipette tips or positive displacement pipettes must be used to minimise contamination.

(iv) Work surfaces must be decontaminated with 2-10% sodium hypochloride or another non-corroding disinfectant agent.

5.4 Methods DNA/PCR only

All test methods and related procedures must be documented and readily available to staff. Laboratories must have documented policies for the interpretation of data for each method of DNA analysis.

Documentation of methods and procedures must include where appropriate:

- description of the sample to be tested;
- parameters or quantities to be determined;
- procedure required;
- descriptions of sample preparation methods, controls, standards and calibration procedures;

- a discussion of precautions, possible sources of error or limitations of the procedure;
- criteria for the rejection of results;
- data/observations to be recorded and method of analysis and presentation;
- literature references.

a) Whenever possible, a portion of the original sample must be retained as established by laboratory policy.

i) When destructive tests are necessary, procedures ensure that as much material as possible is retained for reanalysis if necessary.

ii) In instances where there may be only one attempt at GMO testing (eg due to insufficient sample), it must be ensured that the following have been tested prior to use:
- Taq DNA Polymerase
- Kits and related reagents

b) The DNA isolation procedure must protect against sample contamination.

c) An appropriate procedure must be used for estimating the quality (extent of DNA degradation) and/or quantity of DNA recovered from samples.

d) Laboratories must have documented policies for the interpretation of data for each method of analysis.

Nucleic acid amplification controls
To ensure the accuracy, sensitivity, and fidelity of GM content in the analyses, results provided by nucleic acid amplification techniques should be evaluated in conjunction with appropriate controls. Controls that are to be incorporated into the GM test system must address:

Inhibition
An inhibition control is used to monitor samples that may contain inhibitory substances, thus also acts as the positive GMO control and is included with every sample undergoing amplification analyses.

Sensitivity
This control can be part of the inhibition monitoring system, to determine whether the sensitivity claimed is achieved by the primer system used in the evaluation process.

It should be recognised that the DNA from suitable reference materials at appropriate concentrations should be used to ensure that the control material and its concentration is appropriate for the determination of sensitivity.

Contamination
These are the negative controls used throughout the whole process. An extraction control will monitor the DNA extraction process to evaluate the presence of contaminating molecules throughout the procedure that can give a false positive result (usually one control for up to 15 samples in the sample extraction process). A negative PCR reagent control is used on a sample known as not containing the DNA target and its amplification products and must be included with every extraction series. It is sufficient that the extraction control can also act as the PCR reagent control since the PCR step is the extension of the extraction process.

Control data must be recorded in such a way that trends in analysis can be readily evaluated.

5.4.5 **Method validation**

GMO methodology must be validated for each group of products (see Appendix 1) that are tested. The data so generated will be assessed by NATA's technical assessors. It expected that suitable statistical analysis will be applied and that both the results of this analysis and the raw data we be avail able for review.

Validation is the developmental process used to acquire the necessary information to assess the ability of a procedure to obtain a result reliably, to determine the conditions under which such results can be obtained and to determine the limitations of the procedure. The validation process identifies critical aspects of a procedure that must be carefully controlled and monitored. Validation studies can be conducted by the scientific community (as in the case of a standard or published method) or by the laboratory itself (as in the case of methods developed in-house or where significant modifications are made to previously validated methods). As a minimum, the method must be tested using known samples (eg proficiency test samples, samples from an external agency.)

Methods must be validated by comparison with other methods using certified reference materials (where available) or materials of known characteristics. In validating test methods, the following issues (among others) may need to be determined, as appropriate:

- matrix effects
- interferences
- sample homogeneity
- concentration ranges
- specificity
- stability of measured compounds]
- linearity range
- precision
- measurement uncertainty

a) Test/examination methods and procedures used must be generally accepted in the field or supported by data generated and recorded in a scientific manner.

Since a variety of scientific procedures may be validly applied to a given problem, standards and criteria for assessing need to remain flexible. The important point is that the procedures used be demonstrably capable of producing valid results.

b) Prior to implementing a new DNA analysis procedure or an existing procedure developed by another laboratory that meets the developmental criteria, the laboratory must first demonstrate the reliability of the procedure in-house against any documented performance characteristics of that procedure.

   i. The method must be tested using known samples (eg proficiency test samples, samples from an external agency). At least ten samples must be tested.

   ii. Where appropriate, precision (eg measurement of fragment lengths) must be determined by repetitive analysis to establish for matching.

   iii. If a subsequent significant modification is made to an analytical procedure, the modified procedure must be compared to the original using known samples.

   iv. Records of performance verification must be maintained for future reference and available for review at assessment.

   v. It is recommended that the method also be tested using non-probative samples.

c) Where appropriate, the degree of correlation between the methods must be established and documented.

d) Where possible, an appropriate procedure must be used for estimating the quality (extent of DNA degradation) and /or quality of DNA recovered from specimens.

e) The following must be included in the validation process:

   i. Investigate the ability of the system to detect the components of mixed specimens and define the limitations of the system.

   ii. Where appropriate, establish the quantity of DNA needed to obtain a result.

f) The validation of PCR based DNA procedures must include the following:

i. The basic characteristics of the GMO must be determined and documented.

ii. Conditions and measures necessary to protect preamplification samples from contamination by post PCR materials and to detect such contamination must be determined.

iii. The reaction conditions such as thermocycling parameters (including the number of cycles necessary to give specific amplification) and critical reagent concentrations (primers, polymerase and salts) needed to provide the required degree of specificity must be determined.

iv. The potential for differential amplification must be assessed.

v. When a PCR product is characterised directly, the reference standards necessary to discriminate the products shall be established.

vi. When PCR product is characterised by direct sequencing, the reference standards necessary to determine the sequence shall be established.

vii. When a PCR product is characterised with hybridisation, hybridisation and stringency wash conditions necessary to provide the desired degree of specificity must be determined.

NATA Technical Note 17 provides guidance on procedures for validation and verification of analytical test methods.

5.5 **Equipment**

Nucleic acid detection techniques require some specialised equipment such as the thermal cycler for PCR and nucleic acid analysers (spectrophotometers, enzyme immunoassay microtiter plate readers, fluorometers, luminometers, microscopes) capable of detecting labelled DNA fragments.

5.6 **Measurement traceability**

Many pieces of equipment are common to all laboratories please refer to sections 3 and 4 of the ISO/IEC 17025 Application document for Biological Testing for details of equipment calibration.

Instrument manufacturers have developed the recommendations for maintaining and assessing the reliability of thermal cyclers and nucleic acid analysers. Many instruments have internal diagnostic checks built into them. Minimally, the recommendations from manufacturers should be followed; otherwise, calibrate at least twice a year. Detection based instruments should be calibrated and checked within the ranges used for nucleic acid detection.

Further assurance checks include monitoring the internal temperature of the wells of the thermal cycler. Placing a thermocouple in a reaction tube during a typical run program does this. The temperature of each well (where appropriate) must be checked annually.

In addition to temperature checks, printing out the amplification program with each run is recommended. The printout will show that the correct program was used for the assay, provide a record that the proper number of cycles were used and record any anomalies in the cycling. If the printout reveals that the wrong program was used or the program was altered, reject results. Examination of the times recorded to obtain each of the temperature plateaus may provide early warning of instrument malfunction.

Performing quarterly functional amplification checks can also assist in determining equipment reliability. Amplifying a low level target in each of the wells of the thermal cycler and observing the results accomplish this. Wells not amplifying the target should be temperature checked.

5.8 Sample handling

Procedures for sample handling must ensure that sample integrity is maintained. Staff are to be aware that minor degrees of cross-contamination, which would not be significant for other tests, may result in erroneous results by nucleic acid amplification.

The laboratory must have a documented sample control system. This must include procedures for the receipt, recording, handling, protection and storage of samples.

Wherever possible a separate sample should be collected for nucleic acid analyses and used exclusively for that purpose. If a sample is unable to be wholly dedicated to nucleic acid detection then it is to be aliqouted prior to any other form of analysis. The technique used to aliquot a sample must prevent contamination of the sample.

Each specimen must be marked with a unique designator for identification. Should the item not lend itself to marking, its proximal container must be marked.

Because the quality of the nucleic acid prepared from the sample has a major effect on the probability of successfully performing the diagnostic test, care needs to be taken to ensure the nucleic acids remain intact during sample storage, transport and preparation.

5.9 Assuring the quality of test and calibration items

An accredited laboratory must have in place an internal staff assurance program which demonstrates the ability of staff to perform adequate testing in an infrequently analysed matrix.

It is expected that a test be performed by all analyst's in a matrix that is not usually tested at least every three months.

Proficiency test samples must be representative of items examined in normal laboratory operations. A proficiency test sample may be apportioned among analyst's if doing so does not alter the character of the testing. It is essential that proficiency tests be properly designed, appropriately administered and fairly evaluated. The testing process must be well understood by all participants.

The proficiency of analysts is tested only if they complete the testing unaware of the results expected.

If available laboratories are encouraged to participate in relevant proficiency programs.

Participation in proficiency testing programs is defined as analysis of sample(s), submission of results by due dates and assessment of results against the performance criteria issued by the test provider.

Proficiency test records must detail the extent of participation by individuals.

When participating in proficiency testing programs, the laboratory's routine test procedures must be used

Performance in proficiency testing programs must be reviewed by the quality manager and relevant supervisory staff.

Feedback must be provided to all relevant staff.

Where necessary, corrective action must be taken.

Proficiency testing records must include:

- full details of the analyses/examinations undertaken and the results and conclusions obtained;
- an indication that performance has been reviewed; and
- details of the corrective action undertaken, where necessary.

5.10 **Reporting the results**

Requirements for reporting test results are detailed in ISO/IEC 17025 clause 5.10 and section 4 of the ISO/IEC 17025 Application document for Biological Testing.

The following additional information specific to GMO testing is required:

i. The size of the sample.

ii. The method of preparation
iii. The limitation of the particular detection method used for the particular sample or sample type.

Reporting of the results of GMO detection must be sufficiently descriptive to allow the reader a clear understanding of what tests have been conducted and to what accuracy.

No affirmation shall be made stating that there is no GMO present in the sample analysed as determined from test samples.

The calculation mode shall thus be per species the ratio of the sum of each GMO quantity to the quantity of the corresponding species.

An example of a negative report for GMOs would therefore indicate:

A 2Kg sample of soy beans was ground up and mixed and 10g of this was extracted for DNA using method no XYZ123. At a detection limit of 0.01% for this test in this matrix, no genetic sequences for the presence of Roundup Ready Soy genes were detected. Suitable positive and negative controls and standards were included in the detection.

And a positive report would be similar with slightly altered wording

…..genetic sequences for the presence of Roundup Ready Soy were detected and identified……

Appendix 1

Currently approved/pending/non approved GM crops as of 9 January 2002.

Laboratories are advised to check for changes to the following lists on the ANZFA website at WWW.anzfa.gov.au or WWW.anzfa.gov.nz.

GM testing on foods

.01 Particle representation
- grains, seeds, and nuts
- animal feed
- vegetable and fruits

.02 Products
- grains, seeds, and nuts
- vegetable and fruits
- composite foods
- processed foods
- pet foods

.03 Edible fats and oils
.04 Sugar products, honey and confectionary
.05 Fermented products
.06 Gelatine and other gums
.07 Additives, nutritional supplements, and herbs and spices
.99 Other foods

GM tests approved

.01 Canola
- Roundup Ready (GT73)

.02 Corn
- MON810
- GA21
- BT176
- Bt11

.03 Cotton
- Bollgard/Ingard
- Roundup Ready

.04 Potato
- New Leaf - S
- New Leaf - Y
- New Leaf - Plus

.05 Soybean
- Roundup Ready
- High Oleic Acid

GM tests Pending

.01 Canola
- Liberty Link
- Oxy235

.02 Corn
- Liberty Link
- Bt-Xtra
- NK603
- Herculex

.03 Cotton
- Bollgard II
- BXN

.04 Sugarbeet
- Roundup Ready (GTSB77)

GM tests Non approved

.01 Canola
- SeedLink
- Laurical
- Phytaseed

.02 Corn
- MON802
- MON809
- MON RR
- Pioneer MS
- MaxGard
- MS6
- StarLink
- Herculex I

.03 Cotton
- SU
- BXN-IR

.04 Potato
- New Leaf - A
- New Leaf - RB

.05 Soybean
- AgrEvo
- Event GU262

.06 Sugarbeet
- Liberty Link

.07 all Tomato
.08 all Tobacco
.09 all Squash
.10 Flax
.11 Papaya
.12 Radicchio
.13 all Rice
.14 Cantaloupe