

Protocol for Testing Seed Imports for the Presence of Genetically Modified Seed

Ministry of Agriculture and Forestry (MAF) Biosecurity New Zealand, 31 July 2010.

This document is also available on the MAF website:

<http://www.biosecurity.govt.nz/files/regs/imports/plants/gm-seeds/seed-testing-protocol.pdf>

Purpose

1. This protocol describes the seed sampling and DNA testing protocol, using the polymerase chain reaction (PCR), to be used for testing seed imported for sowing into New Zealand for the presence of unapproved **genetically modified (GM)** seeds. Successful testing will mitigate the risk of unapproved GM seeds being released in the New Zealand environment. The protocol applies to the following species:
 - Maize and sweet corn (*Zea mays* subsp. *indentata* and *Zea mays* subsp. *saccharata*),
 - Soybean (*Glycine max*),
 - Oilseed rape (*Brassica napus* var. *oleifera*), and
 - Lucerne/alfalfa (*Medicago sativa*).

NOTES:

- a) This protocol replaces the four previous DNA testing protocols produced by MAF for *Zea mays*, *Glycine max*, *Brassica napus* var. *oleifera* and *Medicago sativa*.
- b) MAF will continue to accept test results using the previous testing protocols for two years following the issue date of this protocol.

Introduction

2. Genetically modified varieties of organisms, including seeds of plants imported for sowing into New Zealand, are defined as new organisms under the Hazardous Substances and New Organisms (HSNO) Act 1996. The purpose of this Act is to prohibit the importation, development, field-testing or release of any new organism without approval from the Environmental Risk Management Authority (ERMA). The Act is enforced at the New Zealand border under section 28 of the Biosecurity Act 1993.
3. As stipulated in the MAF Import Health Standard: *155.02.05: Importation of Seed for Sowing*¹, it is illegal to import unapproved GM seeds into New Zealand. Seed importers must take appropriate precautions to ensure that seed for sowing consignments do not contain unapproved GM seed. Such precautions might include purchasing seed which has been produced under a quality assurance system covering field isolation and other seed production management practices, and testing for GM presence throughout the seed production chain.
4. This MAF testing protocol ensures a high level of confidence (95%) that the inadvertent presence of 1 GM seed in 1000 seeds will be detected through sampling and testing. In order to achieve this, a sample of at least 3200 seeds must be drawn from each seed lot and tested using PCR testing methods.
5. All individual seed lines in a consignment must be tested for the presence of unapproved GM seeds. Importers can either have seed consignments sampled and tested at the New Zealand

¹ <http://www.maf.govt.nz/biosecurity/imports/plants/seeds-sowing.htm>

border or offshore prior to importation. A current seed testing certificate² must accompany each seed line in a consignment.

6. Seed must be sampled as per sections 12-16 and tested as described in sections 17-21 of this protocol.
7. Testing must be performed by a MAF-approved laboratory, approved to the MAF Biosecurity New Zealand Standard: *Approval of Laboratories for Genetically Modified Organism Testing*³.

8. Seed lines in a consignment will only be given biosecurity clearance if no unapproved GM seeds are detected (i.e., the testing result is negative) and all requirements of the import health standard have been met.

9. Only current testing certificates will be accepted (i.e., testing has been done recently, or in the last two years). Seed accompanied by older seed testing certificates must be re-tested. This ensures that the testing procedure continues to keep pace with changes made to this protocol.
10. All seed sampling and testing costs are the responsibility of the importer, as per the Biosecurity (Costs) Regulations 2006⁴.
11. MAF will consider alternatives to a single border PCR test, as described by this protocol, if a level of assurance equivalent to this testing protocol can be demonstrated. For example, equivalence could be demonstrated on a crop-by-company basis, via an accredited quality assurance programme.

Seed Sampling and Testing Options

12. Seed may be sampled for testing either offshore prior to shipping to New Zealand or on arrival at the New Zealand border. Samples taken at the border will be collected under controlled conditions by MAF staff, or organisations approved by MAF. The sample will be held under MAF supervision until it can be sent to the MAF-approved testing facility of the importer's choice. The rest of the consignment will be held in a MAF-approved transitional facility until acceptable results are received and biosecurity clearance is given. Records of sampling and testing done at the border will be kept by MAFBNZ. This information is subject to the Official Information Act 1982.
13. The sampling procedure is designed to collect a representative sample. Several assumptions have been made:
 - Individual seeds are either GM or not GM (if seeds are present which are heterozygous for the GM trait, e.g. due to cross-pollination, the confidence of detection will be reduced),
 - Any GM seeds present are randomly dispersed throughout the seed lot,
 - The sample will be ground and analysed as a whole; seeds will not be analysed individually, and
 - The laboratory will correctly identify the presence of 1 (or more) GM seeds in a 3200 seed sample.
14. Samples for each seed line to be tested must contain at least 3200 seeds. This can be determined by weight in the following way: Count and weigh 100 seeds, multiply this weight by 32 and round up to the nearest 50 grams (or in the case of *Brassica napus* and *Medicago sativa* – rounding to the nearest 5g). This is the weight of seed required to make up the 3200 seed sample. Small quantities of seeds may be sampled and tested as a composite sample (see section 16 a).

² Current – ie. according to the latest protocol, or no older than two years from the date a protocol is re-issued

³ <http://www.biosecurity.govt.nz/files/regs/imports/plants/gm-seeds/labs-gmo-testing.pdf>

⁴ Or any subsequent amendments to these regulations

15. Seed samples must be collected using either the standard International Seed Testing Association (ISTA) or Association of Official Seed Analysts (AOSA) methodology. Sub-samples are collected from each seed container, combined to form one uniform collection, and then reduced to obtain a sample containing no fewer than 3200 seeds. Ideally the seed sample will not contain any broken or part seeds, and will be clean and free of dust and debris that might lead to a false result in testing. If a sample has been in contact with soil or extraneous plant material, it may be washed and dried thoroughly prior to sending to the testing laboratory.

The ISTA methodology for medium-sized and bulk-sized containers is summarised in the following tables:

a) For sacks (i.e. Containers up to and including 100 kg capacity):

No. of containers per seed line	1-4	5-8	9-15	16-30	31-59	> 59
No. of sub-samples to be taken	3 per container	2 per container	1 per container	15 total, taken at random	20 total, taken at random	30 total, taken at random

b) For bulk bins (i.e. Containers greater than 100 kg capacity):

Weight of seed line (kg)	100-500	501-3,000	3001-20,000	> 20,000
No. of sub-samples to be taken	5	1 per 300 kg, not fewer than 5	1 per 500 kg, not fewer than 10	1 per 700 kg, not fewer than 40

Small Quantities of Seed

16. Small quantities of seed are frequently imported for research, education, cultivar trials and/or multiplication. In these cases, the sampling of 3200 seeds from each seed line may be impossible (because there isn't that number of seeds in the consignment) or would significantly affect the purpose of import. Small quantities are defined as:

- less than 5 kg for large seeded species, such as *Zea mays* and *Glycine max*, and
- less than 100g for small seeded species, such as *Brassica napus* var. *oleifera* and *Medicago sativa*.

For small quantities of seeds, the amount of seed sampled and the testing method can be modified in the following three ways:

- a) Composite Sampling:** A composite test sample can be collected by taking a proportionate sample of each small quantity seed line in the consignment, up to a maximum of 50 seed lines. These sub-samples can be pooled together to make up a 3200 seed sample, and tested as one sample. Testing certificates must report the names of each of the lines that formed part of the composite sample, so that they are clearly identifiable for border clearance purposes should they test negative for the presence of GM seeds. If a positive result is found, none of the lines that formed part of the composite sample can be given clearance.

NOTE: Any bulk line imported in the same consignment must be tested separately.

- b) Leaf Disc Sampling:** Living material (such as leaf discs) can be **tested** for GM DNA sequences. Untested seed may be imported into, and grown in an appropriate transitional (quarantine) facility approved to MAF Biosecurity New Zealand standard *PBC-NZ-TRA-PQCON: Specification for the*

Registration of a Plant Quarantine or Containment Facility, and Operator. This may be Level 1, though there may be additional phytosanitary requirements that require the seed to be grown in a Level 2 facility.

During growth and before pollen is produced, leaf samples can be taken and sent for testing at a MAF-approved testing laboratory. The sampling and testing plan must be approved by MAFBNZ. Biosecurity clearance will be given to all rows (or blocks) of plants testing negative for the presence of unapproved GM material. If unapproved GM material is detected in a plant row, then this row must be destroyed, and any remaining unplanted seed of this seed line will not receive biosecurity clearance.

- c) Quality Assurance Declaration:** The importer can sign a declaration that the seeds have been produced under a quality assurance system and are not known to contain GM seeds.

Under this option, untested seed may be imported into, and grown in, a transitional (quarantine) facility approved to MAF Biosecurity Authority Standard *PBC-NZ-TRA-PQCON: Specification for the Registration of a Plant Quarantine or Containment Facility, and Operator*. Plants grown from this seed will not be tested and will not receive biosecurity clearance. All harvested seed must be exported out of New Zealand and the remaining vegetative material destroyed, including any subsequent emerging volunteer plants.

Seed Testing Method Guidelines

17. The GM screening tests required for specific seed species are shown in the following table.

Species	Type of Screen
Maize and sweet corn (<i>Zea mays</i>)	Requires: <ul style="list-style-type: none"> ▪ CaMV p35S AND <ul style="list-style-type: none"> ▪ t-nos screens
Soybean (<i>Glycine max</i>)	Requires: <ul style="list-style-type: none"> ▪ CaMV-p35S AND <ul style="list-style-type: none"> ▪ FMV-35S or CP4 EPSPS or equivalent MON 89788 screen
Oilseed rape (<i>Brassica napus var. oleifera</i>)	Requires: <ul style="list-style-type: none"> ▪ FMV-35S or EPSPS or equivalent screen (Roundup Ready) AND <ul style="list-style-type: none"> ▪ Laurical (thioesterase) or equivalent screen AND <ul style="list-style-type: none"> ▪ Pat or equivalent screen (T45, Topas19/2) AND <ul style="list-style-type: none"> ▪ Bar or equivalent screen (Seedlink/Restorer) AND <ul style="list-style-type: none"> ▪ t-nos or bxn or equivalent screen (Oxy-235)
Alfalfa/Lucerne (<i>Medicago sativa</i>)	Requires: <ul style="list-style-type: none"> ▪ FMV-35S or an EPSPS screen

18. Testing laboratories must have validated methods capable of detecting haploid GM seed in the seed sample at the lowest reliable limit of detection. This limit shall not exceed 0.03% at the 95% confidence level.
19. Both **Qualitative** and **Quantitative PCR tests** can be used to test for GM seed presence. Methodology guidelines are described below.

Qualitative PCR tests are used to screen for the presence of specific gene sequences commonly incorporated into GM plants, and is the recommended PCR test method for this protocol.

Quantitative PCR tests (which estimate the likely concentration of GM seeds in the seed consignment) may also be used, but can only be accepted if a negative result at the limit of detection is **also** clearly reported on the testing certificate.

- a) **Sample preparation:** The seed sample may be washed and thoroughly dried to remove any contaminating particles or debris if requested by the customer.
- b) **Grinding and DNA Extraction:** The seed must be ground to a fine powder. DNA must be extracted from two test portions of the ground seed. There are several methods for extracting and purifying DNA from a test portion, and the methodology must be optimised in each laboratory and evidence must be provided that the extracted DNA is of PCR quality. Laboratory manuals must contain the detailed steps for extracting, purifying and checking the quality of DNA.
- c) **PCR Tests:** The effectiveness of PCR procedures are dependent on the combination of equipment and reagents used. Conditions for thermocycling equipment should be based upon the manufacturers' recommendations and optimised for a strong signal for each target DNA sequence.
- (i) The test should employ a maximum of 40 cycles to avoid possible accumulation of PCR artefacts, which may give rise to false positive results. If more than 40 cycles are performed, the laboratory will be able to justify their cycling protocol through a validation study of the minimum cycle number necessary to amplify a single target DNA copy in the absence of PCR inhibition.
- (ii) The PCR cycling conditions used in routine analysis must be designed to reliably detect the target DNA sequence (in either the test sample or reference material) at the Limit of Detection (LoD).
- (iii) Testing should include the following quality assurance samples:
- a template-free control (negative control),
 - a sample preparation control (laboratory contamination control),
 - a PCR inhibition control (eg. using an internal reference gene), and
 - a positive control, using certified or validated reference material.

Interpreting and Reporting Test Results

20. Test results must clearly indicate how the testing was performed. The number of seeds ground for analysis and the weight of the test portions used for PCR analysis must be recorded.
21. The DNA extracted from each test portion should be analysed at least once, giving two data points for each screening test. Results for test portions can be **positive** (the sequence is present **+/+**), **negative** (the sequence is not present **-/-**), or **ambiguous** (uncertain results **+/-**).

If results are ambiguous, the analysis must be repeated with two new test portions. If this again produces an ambiguous result, the final result must be reported as negative.

Table: Interpretation of Test Results for Each Screening Test

Test Portion 1	Test Portion 2	Combined Test Portion Results	Reported Result
+	+	+/+	Positive
-	-	-/-	Negative
+	-	+/-	Repeat analysis
-	+	-/+	Repeat analysis

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