

Protocol for testing imports of *Zea mays* seed for sowing for the presence of genetically modified seed

Ministry of Agriculture and Forestry (MAF), 21 March 2005.

This document is also available on the MAF website
<http://www.biosecurity.govt.nz/imports/plants/genetically-modified-organisms.htm>

Introduction

1. This protocol aims to prevent **genetically modified (GM) varieties and sub-species of *Zea mays*** unapproved for import, development, field-testing or release under the Hazardous Substances and New Organisms Act 1996 (HSNO Act) being released in the New Zealand environment through seed imported for sowing. The protocol applies only to varieties and sub-species of *Zea mays* seed for sowing that have forms that have been genetically modified elsewhere (i.e. maize - *Zea mays* subsp. *indentata* and sweet corn - *Zea mays* subsp. *saccharata*). It does not apply to seeds imported for processing.
2. Unapproved GM organisms, including viable seeds, are new organisms under the HSNO Act. The Act prohibits the importation, development, field-testing or release of any new organism without approval from the Environmental Risk Management Authority. To date, no GM organisms have been approved for release in New Zealand. The HSNO Act is enforced at the border through section 28 of the Biosecurity Act 1993.
3. The Import Health Standard *Zea mays*, Commodity Sub-Class: Seed for Sowing¹ will specify the mandatory requirements associated with this protocol and the phytosanitary requirements. Where this protocol refers to biosecurity clearance, it relates only to requirements to ensure that biosecurity clearance is not given for unapproved GM seeds. Seed consignments must meet other phytosanitary requirements to receive biosecurity clearance, as detailed in the import health standard.
4. All costs associated with sampling, approving laboratories and testing will be borne by the importer and all associated MAF activities will be charged on a user pays basis.

Options for importers of *Zea mays* seed

5. It is illegal to import GM organisms into New Zealand without approval. Importers are made aware of this requirement in the Import Health Standard *Zea mays*, Commodity Sub-Class: Seed for Sowing¹ and must take appropriate precautions to ensure that they are complying with the law. Such precautions might include isolation of non-GM seed from GM types and a testing protocol such as that described in this document.
6. Unless otherwise stated (e.g. seed imported from a country that MAF has granted area freedom from commercial GM production), every consignment must be tested for the presence of unapproved GM seeds. Importers can either:
 - have the consignment sampled and tested at the border, or
 - provide certification that all seed lines/varieties in the consignment have been tested individually prior to shipping.

In both cases, each seed line must be sampled as per sections 9-12 and tested as described in sections 13-23 by an organisation fully approved according to MAF Biosecurity New Zealand Standard “*Approval of Laboratories for Genetically Modified Organism Testing*”. The consignment will only be given biosecurity clearance if no GM seeds are detected.

Testing must be performed by a MAF approved laboratory, and only current testing certificates will be accepted (i.e. testing has been done in the current year of importation or in one year prior). Older seed would need to be re-tested by a MAF approved laboratory. This ensures that the testing procedure continues to keep pace with changes made to this protocol.

7. If requested, MAF will consider the option of area freedom from commercial GM production (on a crop:country basis). MAF will grant area freedom if the country can demonstrate that it has sufficient systems in place to provide a level of assurance equivalent to testing every seed line.

8. In addition to the above, importers of small quantities (defined as those weighing less than 5 kg per line) of seed for cultivar trials and/or multiplication will have three further or modified options:

A. Test samples can be collected by either taking some seed from a number of randomly selected small packets of seed, or taking a random selection of whole packets of seed. If GM material is detected, none of the seed lines included in the composite test will be given biosecurity clearance. Composite testing cannot be extended to include bulk lines which may be imported in the same consignment.

B. Untested seed may be imported into, and grown in a quarantine facility (this may be Level 1, though there may be a phytosanitary requirement that may require the seed to be grown in a Level 2 quarantine facility) registered and operated according to MAF Biosecurity Authority Standard PBC-NZ-TRA-PQCON *Specification for the Registration of a Plant Quarantine or Containment Facility, and Operator*. During growth and before pollen is produced, MAF will test leaf disc samples for GM material. If unapproved GM plants are detected then the consignment will be destroyed.

C. Untested seed may be imported into and grown in an appropriate quarantine facility registered and operated according to MAF Biosecurity Authority Standard PBC-NZ-TRA-PQCON *Specification for the Registration of a Plant Quarantine or Containment Facility, and Operator* (if in a level 1 quarantine facility, the crop must be isolated by at least 200m from other domestic *Zea mays* crops). The importer must sign a declaration that the seeds have been produced under a quality assurance system to avoid adventitious presence of GM seeds and are not known to contain GM seeds. The plants will not be tested and will not receive biosecurity clearance. Once the trial is complete, all harvested seeds must be exported out of New Zealand and the remaining vegetative material destroyed, including any eventual emerging volunteer plants.

Sampling

9. The sampling procedure is designed to collect a representative sample. Several assumptions have been made including:

- 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 described in section 10 will be reduced).
- Any GM seeds present are randomly dispersed throughout the seed line.
- The sample will be ground and analysed as a whole, seeds will not be analysed individually.

- The laboratory will correctly identify the presence or absence of GM material in 99% of samples.

10. MAF requires a high level of confidence (95%) that the inadvertent presence of 1 GM seed in 1000 seeds will be detected. In order to achieve this a sample (weight basis) drawn from each seed line for testing must contain at least 3200 seeds (the weight of the sample size can be calculated by multiplying the standard 100 seed weight by 32 and rounding up to the nearest 50 grams).

Testing laboratories must have validated PCR methods capable of detecting GM seed in the seed sample at the lowest reliable limit of detection. This is currently accepted to be 0.01% GM presence.

11. The sample will be collected using either the standard International Seed Testing Association (ISTA) or Association of Official Seed Analysts (AOSA) methodology. The ISTA methodology is summarised in the following tables:

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

Containers per seed line	1-4	5-8	9-15	16-30	31-59	> 59
Number of sub-samples	3 per container	2 per container	1 per container	15 total, taken at random	20 total, taken at random	30 total, taken at random

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

Weight of line (kg)	100-500	501-3,000	3001-20,000	> 20,000
Number of sub-samples	5	1 per 300 kg, not less than 5	1 per 500 kg, not less than 10	1 per 700 kg, not less than 40

Combine the sub-samples evenly to form one uniform collection then reduce it to get a sample of not less than 3200 seeds.

12. Samples taken at the border will be collected under controlled conditions by MAF staff, or organisations approved by MAF (at the border or industry premises). The sample will be held under MAF supervision until it can be sent to an approved testing facility. The rest of the consignment will be held in a MAF-approved transitional facility until testing is completed.

Testing method

13. This test is designed to indicate the presence or absence of GM seeds, but not the concentration of GM seeds in the consignment. Presence of GM material, regardless of concentration, will prevent import of the seed line unless an appropriate approval under the Hazardous Substances and New Organisms Act (1996) exists. A **qualitative PCR** test must be performed to determine whether GM seeds are present or absent in the sample. Quantitative PCR test results are not acceptable by themselves, and will only be accepted if a negative result at the limit of detection is also clearly reported on the certificate. The test uses the polymerase chain reaction (PCR) to screen for two gene sequences commonly used in GM plants: the nos 3'

terminator sequence derived from the soil bacterium *Agrobacterium tumefaciens* and the 35S promoter sequence derived from *Cauliflower mosaic virus* (CaMV).

14. PCR procedures are specific for the combination of equipment and reagents used. Conditions for thermocycling equipment should be based upon manufacturers' recommendations and optimised for a strong signal for each target sequence. When the PCR testing procedures are being validated, GM negative controls, i.e. DNA from certified non-GM *Zea mays*, must be included to ensure there is no cross reaction with DNA from such plants.

15. DNA will be extracted from a sub-sample of the ground seed. There are several methods for extracting and purifying DNA from a sample. All methods will be optimised in the laboratory and evidence should be provided that the extracted DNA is of PCR quality. Laboratory manuals will contain the detailed steps for extracting, purifying and checking the quality of nucleic acid. Soil and extraneous plant tissue will be removed from samples to reduce the risk of false positive results. Details of any seed cleaning procedures to remove seed dressings that may interfere with the PCR method and any soil particles will be recorded.

16. Procedures should include the following quality assurance samples:

- template-free controls
- sample replicates
- sample preparation controls
- positive PCR controls for sample DNA extraction quality
- positive controls of GM DNA from certified or validated standards

17. Test results must clearly indicate how the testing was performed. The analyst will record the number of seeds ground for analysis and the weight of ground material used for individual PCR tests.

18. All testing will be carried out by organisations fully approved according to the MAF Biosecurity New Zealand Standard "*Approval of Laboratories for Genetically Modified Organism Testing*".

19. Records of sampling and testing done at the border will be kept by MAF staff and forwarded to the Senior Adviser (Plants, Operational Standards) for reference. This information is subject to the Official Information Act 1982 and will be reported to the Environmental Risk Management Authority and made available to the public.

Interpreting test results

20. Overseas there are currently 18 constructs of GM *Zea mays* approved for commercial production, involving four suites of characteristics:

- herbicide tolerance to glyphosate or glufosinate
- herbicide tolerance to glufosinate and male sterility
- insect resistance
- herbicide tolerance to glyphosate or glufosinate and insect resistance

21. Ten of these 18 constructs contain both the 35S promoter sequence and the nos 3' terminator sequence. A further seven contain the 35S promoter sequence but not the nos 3' terminator sequence and the remaining construct contains the nos 3' terminator sequence but not 35S promoter sequence.

22. A sample that is positive for both the 35S and nos 3' sequences will be considered to indicate the presence of GM material.

A sample that is positive for only one of the 35S or nos 3' sequences will be considered to indicate the possible presence of GM material.

A sample that is negative for both the nos 3' and 35S sequences will be considered to have no detectable GM material.

False positive or negative results are also possible but the majority will be excluded by the use of appropriate controls. However, the nos 3' sequence is derived from a common soil bacterium (*Agrobacterium tumefaciens*) and a test may give a false positive result if the bacterium or a close relative is present in the sample. Similarly, the 35S sequence is derived from CaMV, a virus that naturally infects cruciferous plants. Soil and extraneous plant material should be removed from samples to reduce the risk of false positive results from these sources. In the event of unclear or ambiguous results, further testing may be appropriate to confirm the presence or absence of GM *Zea mays*. In cases where uncertainty cannot be resolved by further testing, MAF will make a judgement in consultation with other relevant agencies, including the Environmental Risk Management Authority, and appropriately qualified experts outside MAF.

23. PCR will be performed in duplicate for each sample tested. For each duplicate pair, results can be positive (the sequence is present +/+), negative (the sequence is not present -/-), or ambiguous (uncertain results +/-). Tests giving ambiguous results will be repeated by PCR. If this also gives an ambiguous result (+/-), a second DNA extract will be isolated and PCR testing repeated. If this is also an ambiguous result (+/-), the result will be reported as +/- and may indicate a positive result at the limit of detection. In such cases, the importer has the option of further testing for specific GM genes to demonstrate whether the sample contains GM material.

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

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