



# Market Requirement Information Package

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## Grain Assay

### Assay Methods

The most precise methods are based on high performance liquid chromatography (HPLC), which can measure individual mycotoxins, but these require expensive equipment and highly skilled staff, so they are a little slower and more costly than other methods. HPLC can be used for most mycotoxins, although different mycotoxins require different extraction processes.

Immunoassay methods such as enzyme-linked-immuno-assays (ELISA) are highly specific, sensitive and robust, but less accurate and precise (more variable) than HPLC. These are the most commonly used methods because less equipment is required. They might not measure individual mycotoxins. For example, aflatoxin test kits usually measure an approximation of 'total' aflatoxins B1, B2, G1 & G2, since not all are detected with equal sensitivity. These are very suitable for first-line testing of largely negative samples, but not as satisfactory for precise determination of concentrations in positive samples. Assays must be routinely run in duplicate because of variations across ELISA plates. Care has to be taken to ensure that sample extracts remain in the quantifiable range. ELISA kits are available for all mycotoxins of interest.

Thin layer chromatography (TLC) (and variations) is still used for screening large numbers of mainly negative samples. Highly specific in skilled hands, but less precise than HPLC. Skill is required, but less expensive equipment than HPLC. Individual mycotoxins (aflatoxins, ochratoxin, zearalenone) can be assayed, often in a single operation. Fumonisin and trichothecenes are not as well suited to assay by TLC.

**To summarise**, ELISA can provide the fastest result for routine screening of large numbers of mainly negative samples, but HPLC is the method of choice when the highest accuracy and precision are required. It is very important to discuss with the chosen laboratory which method will be used and the 'confidence limits' of that method. These 'confidence limits' show the normal variability of the method itself, which is separate to the sampling variability, discussed above.

### Assay Interpretation

Some countries specify 'negative' for certain mycotoxins. This usually relates to the sensitivity of the method they regard as standard. Find out what this is!

How will the importing authority deal with a positive test? Often, it is the practice to assay a duplicate sample as a check on the method. Other authorities and laboratories might take several



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additional samples for testing. Will multiple test results be averaged, or will one sample exceeding the standard cause rejection? The latter criterion might be impossible to meet, given normal variation in aflatoxin content within bulk maize. In addition, it is important to agree on what the 'batch' consists of, that the testing purports to represent – is it the entire container, parts of it, or several containers?

Will a duplicate sample be retained for an independent assay if case of dispute? Is the regulatory agency or end-user prepared to use different methods to test the result?

### **Use a laboratory that is NATA certified for mycotoxin assay**

Obtaining an accurate result in any laboratory depends on the interaction of many factors: effective management with knowledge of client's needs, skilled and well-trained staff, regularly maintained and calibrated equipment, validated methods and techniques, and regular practice on reference samples of known mycotoxin content. Any of these factors can and do go awry at times. Certification with the National Association of Testing Authorities (NATA) provides a safeguard that the operation of the laboratory is regularly checked by other experts in laboratory practice and also that its analytical performance is satisfactory in comparison to other laboratories.

A list of laboratories certified for aflatoxin and other mycotoxin assays can be obtained by searching the NATA website (<http://www.nata.com.au/nata/>). Costs of NATA accreditation in terms of additional check sample assays, extensive documentation, calibration, staff training, and fees can add 30-40% to the costs of assays, but can pay off in increased reliability of the results.

As the client, you need to discuss your needs with the chosen laboratory, and to ascertain if the assay method to be used has sufficient accuracy and precision for your purpose. You can also ask for details of their certification, and evidence of method validation. Ensure that the chosen laboratory is aware of these protocols, and will abide by your requirements, particularly in regard to grinding of large samples before sub-samples are drawn for assay.

### **Assays and Contracts**

Decide on a plan to meet the client's requirements. When will mycotoxin tests be performed, how often and at what cost? The contract price must include the costs of all mycotoxin assays.

There is much maize available on the world market. Aflatoxin contamination is a serious trade barrier for maize grown in most countries of south-east Asia. Developed importing countries expect to pay a premium for a guarantee that Australian maize is free of aflatoxin and other mycotoxins. You must be able to provide that guarantee.