HGCA

PROJECT REPORT No. 212

TESTING CEREALS FOR MYCOTOXINS: REVIEW AND ASSESSMENT OF RAPID TEST KITS

NOVEMBER 1999

Price £5.00

PROJECT REPORT No. 212

TESTING CEREALS FOR MYCOTOXINS: REVIEW AND ASSESSMENT OF RAPID TEST KITS

by

K A SCUDAMORE

KAS Mycotoxins
6 Fern Drive
Taplow
Maidenhead
Berkshire SL6 0JS

This is the final report of a nine month project which started in September 1998. The work was funded by a grant of £11,392 from HGCA (project no 1616).

CONTENTS

	PAGE
General introduction	**1**
Review findings, general	**3**
Review findings, by technique	**4**
Card tests	**4**
ELISA plate tests	**5**
IA column clean-up	**5**
Discussion	**6**
Conclusions and Recommendations	**8**

GENERAL INTRODUCTION

The British consumer demands quality food free from chemical and microbiological contaminants. Mycotoxins are highly toxic chemicals produced by some fungi and can be formed in many food commodities including cereals. If detected they are indicative of reduced quality. Aflatoxins which are formed by certain species of *Aspergillus* are the most important mycotoxins on a world wide scale but are rarely formed in UK-produced grain.

Mycotoxins can be formed both before harvest and in store. However, fungi which infect growing crops are usually different species to those that can grow in stored grain. *Fusarium*, *Alternaria* and *Cladosporium* are 3 important genera of fungi which colonise growing crops while storage fungi include many species of *Penicillium* and *Aspergillus*. As mycotoxins are formed by specific fungi there is the potential for a range of mycotoxins to occur in grain. A number of the important mycotoxins which occur in UK cereals are listed in table 1. In most cases mycotoxins once formed are quite stable and are thus difficult to remove from grain.

Table 1: Important mycotoxins found in UK grain.

Mycotoxin	Main fungal species	Foods infected
Ochratoxin A	*P. verrucosum*, *A. ochraceus*	**Cereals**, coffee beans, field beans, beer, nuts
Citrinin	*P. verrucosum*	**Cereals**
Deoxynivalenol , nivalenol	*F. graminearum, F. culmorum, F. crookwellense*	**Cereals**
T2 toxin HT2 toxin	*F. poae, F sporotrichioides*	**Cereals**
Zearalenone	*F. graminearum, F. culmorum, F. crookwellense*	**Cereals**
Moniliformin	*Fusarium* species	**Cereals**
Alternariol, alternariol monomethyl ether, Tenuazonic acid	*Alternaria alternata, A. tenuis*	**Cereals**, fruit, tomatoes, oil seeds

Ochratoxin A occurs in stored UK cereals and is now regarded as a likely genotoxic carcinogen. As such, it is recommended that the amount in the UK diet is reduced to the minimum that can be achieved. Many food and drink producers now require assurance of freedom from significant amounts of ochratoxin A (and selected other mycotoxins) in cereals and other food ingredients. The EC is currently discussing the introduction of legislation to specify maximum allowed amounts of ochratoxin A in grain and other susceptible commodities. The management of the ochratoxin A problem is thus currently of greatest concern to the UK grain trade.

This situation then presents a number of issues for the cereal industry. These include, how to prevent the formation of mycotoxins such as ochratoxin A, methods for detection of contamination, procedures to handle and dispose of contaminated grain and the need to remain competitive in UK and export markets. To reduce or eliminate mycotoxins in grain requires the means to test consignments at key control points. For this to be effective and economic, tests must be available that are cheap, quick and simple to carry out, reliable and have sufficient sensitivity

Reliable and sensitive analytical methods based on HPLC or GC/MS are available for the mycotoxins which are important in cereals. These methods are vital especially for enforcement purposes. However, their operation requires highly trained technical staff, sophisticated and expensive equipment and laboratory facilities and are not usually suitable for testing where results are required in a few minutes. There are a number test kits commercially available within the UK for detection and measurement of mycotoxins in cereals. The HGCA thus commissioned this review together with a critical appraisal of their performance to establish the extent to which they meet the likely future needs of the grain trade.

REVIEW FINDINGS, general

Rapid test kits are marketed for ochratoxin A, zearalenone, deoxynivalenol, T-2 toxin, citrinin and aflatoxins. No rapid tests exist for moniliformin and other mycotoxins listed in table 1. All tests use antibodies raised to mycotoxins and may cross react to some degree with other closely related compounds present. They may thus not be as specific as HPLC-based methods.

There currently appears to be only a limited requirement for rapid tests for mycotoxins in UK-grown grain. The introduction of legislation for ochratoxin A and possibly other mycotoxins at a later date is likely to add impetus to the need for testing. However, the scale of future testing which will be required is unclear.

The method for obtaining representative samples is of particular concern. Sampling is especially important when testing for mycotoxins by whatever method is used because they are often very unevenly distributed in consignments of cereals. Failure to sample properly may not only provide an invalid result but can lead to costly disputes when grain is tested on more than one occasion and analytical results differ.

Lack of sensitivity was often cited by users. This meant that tests were often operating at their detection limits. Some kits were said to have an unacceptable frequency of false positives or negatives although it was often unclear how the user had assessed the occurrence of false negatives in the absence of checks against other methods. False

negatives are highly undesirable in that this could lead to the acceptance of contaminated consignments or to unknowingly marketing contaminated products. On the other hand, occurrence of false positive results could lead to unnecessary and costly delay of consignments.

There is a considerable reluctance to use rapid test methods especially amongst chemists and laboratories well equipped with instrumentation in use for other analyses. Some of this scepticism is based on the lack validation data or unsatisfactory experience with this type of test.

However, some mycotoxin test kits supplied in the UK have approval from recognised international bodies or have been tested by the supplier. Approval is usually granted following examination of the kits by trained laboratory staff to verify that the claims of the suppliers can be achieved. This leaves open the question of how robust the kits are in the hands of inexperienced or non-technical staff, how suited the kits are to the requirements of the individual users and the need to appreciate any limitations of the test. It is often claimed that these tests can be performed by staff with little or no technical knowledge or training.

REVIEW FINDINGS, by technique

Tests are based on techniques using antibodies raised against each mycotoxin. An antibody is then incorporated into the various formats. These give a series of products: direct competitive ELISA' s which are carried out in test wells, card tests, and immunoaffinity column clean-up. These procedures are combined with a suitable visual or instrumental reading.

Card tests

The simplest format to use and most rapid of the kits currently available is the card test. However, these have been dismissed in their current form by most potential users due to their inherent lack of sensitivity. One feed company visited used card tests to detect aflatoxins, ochratoxin A, zearalenone and T-2 toxin. Sensitivity to aflatoxin was

claimed to be just sufficient to meet the requirements of Animal Feedingstuffs Regulations, while the tests for other mycotoxins provided a useful quality check. These cards were found to be very easy to use although the recommended shelf life of 6 months was considered too short and needed to be strictly observed. When out of date reagents were used false positives sometimes occurred.

Because of the simplicity and rapidity of the test, re-formatting to provide sensitivities in line with current or future legislation would make these tests much more attractive especially when a quick yes/no quality control screen is required. One supplier has recently introduced such a test capable of detecting aflatoxin B_1 at 2 µg/kg

ELISA plate tests

As far as could be ascertained, few tests are currently carried out using ELISA tests other than for flatoxins. These are sought in a range of foods but not usually in cereals other than maize. In a previous comparison of commercial products used to determine ochratoxin A in flour (Personal communication) results were variable and false positives and negatives occurred. However, during preparation of this current review the author had personal experience of using an ELISA-based test kit. Excellent results were obtained using a sample of maize with a pre-checked level of zearalenone (200 µg/kg) and a second sample with a low content. It was clear that familiarity and care with the use of microtitre pipettes is essential to ensure optimum results.

IA column clean-up with detection using fluorescing tips or fluorimetry.

Although relatively new, the OCHRASCAN® test had been used for the determination of ochratoxin A in cereals by more customers identified in this survey than any other product. Sensitivity appears just sufficient to test grain at the 5 µg/kg level although several customers had difficulty in deciding whether or not the silica tip was fluorescing. Experience in its use seemed to increase confidence in recognising a positive sample. In most laboratories any positive result was confirmed using HPLC.

In summary, the main advantages stressed by users were, speed, ability to handle the analysis of a number of samples at the same time and simplicity especially where

instrumentation was not available. Rapid tests were sometimes used as a weeding out procedure with suspected positives then confirmed by other methods. Disadvantages were the lack of sensitivity, difficulty in interpreting end points, unreliability including false negatives and false positives. Current tests were considered too slow for testing grain in lorries at intake. Some users considered that tests were too expensive especially when few samples were processed which meant that the sample capacity of tests was not used or the kits or reagents became out of date.

DISCUSSION

Some of the test kits claim to provide analytical results within 20 minutes, or less. However, this claim usually excludes the time required to sample and also sometimes the time for sample preparation. Any analytical method to determine mycotoxins involves a number of steps. These are sampling, extraction of the mycotoxin, clean-up to remove interfering substances, detection and measurement. An estimate of the time required for each stage would be approximately 3 minutes for weighing, grinding and blending, 3 minutes for clean-up, 5-10 minutes for reactions and 1 minute for the final measurement. The total time excluding sampling would be 12 to 17 minutes although methods using IA clean-up may dispense with the need for chemical reactions to be carried out. These times were borne out by most users interviewed. With current technology it is difficult to see how this can be reduced significantly. However, when a number of samples can collected and analysed together the average time per test will be much reduced. This is particularly true of ELISA based kits if multi-channel pipettes are used.

The time required for sampling must then be added to the test time. Automatic, full-depth samples drawn from several points in a lorry using current in-house procedures can be very quick e.g. about 2 minutes. This is much faster than could be achieved if the procedure recommended by the EC for aflatoxins in cereals were used. Representative sampling from bulk grain would require much longer although the urgency for a result is likely to be much less.

Alternative techniques such as using molecular imprints (in which materials are engineered to produce a form which recognises specific compounds), dip stick tests or lateral flow devices such as employed in pregnancy testing do not appear to have been explored. The relatively small market for mycotoxin tests have probably discouraged investment into development of these techniques.

The importance of validation and testing of rapid test kits by commercial users or by suppliers is often given limited consideration. Reasons for this may be that some customers use rapid tests because they have limited access to sophisticated laboratory facilities, require only qualitative or semi-quantitative results or sometimes have insufficient analytical expertise to judge the need for this.

Four ways of validation testing can be envisaged. These are: examination by internationally respected bodies such as the AOAC, inter-laboratory trials organised by industrial or government bodies, *ad hoc* testing or comparison with established methods by the kit supplier or customer or use with test samples containing known concentrations such as those provided by the UK MAFF FAPAS.

Suitably structured inter comparison trials are perhaps the ideal method for validation of methods although they are expensive to carry out. These can be targeted precisely to the need of a particular industry or association, e. g. ochratoxin A in wheat and barley on behalf of the grain trade. It is recommended that the performance of rapid kits should be assessed alongside established methodology. It is clearly important to define the intended role of the test kit and design appropriate validation tests.

Several suppliers have their own in-house analytical facilities and carry out validation testing for specific applications. This is welcomed and such data should be readily made available to customers whenever possible. Further testing of the products by customers should be encouraged and may be facilitated by subsidised supply of test kits and materials. This often happens in practice although such collaboration may be of a confidential nature and results not passed beyond the supplier and his customer or may be relevant only to that particular customer.

CONCLUSIONS AND RECOMMENDATIONS

- The majority of tests which are carried out on grain in the UK are for aflatoxins in maize or in cereal-based animal feeds.
- A number of companies use test kits on a regular basis for quality control.
- Test kits are frequently cited as insensitive, imprecise and unreliable and positive responses often need confirmation using other techniques.
- The future requirement for rapid test kits in the grain trade is unclear.
- Legislation for ochratoxin A is expected to lead to increased monitoring.
- A reliable rapid test for ochratoxin A would make it feasible to check each lorry load of grain before acceptance. No test currently available would appear to fulfil all the requirements necessary.
- A validation study of candidate test kits currently marketed for ochratoxin A in cereals should be carried out. The feasibility of adapting current kits to the needs of the grain trade should be investigated.
- A new rapid test for ochratoxin A should be developed. Alternative technologies used in other fields should be investigated.
- Similar tests for other mycotoxins such as deoxynivalenol should be developed.
- There is an urgent need to develop protocols for sampling grain for ochratoxin A

ABSTRACT

The British consumer demands quality food free from chemical and microbiological contaminants. Mycotoxins are highly toxic chemicals produced by some fungi and can be formed in many food commodities including cereals and are indicative of reduced quality. Ochratoxin A occurs in stored UK cereals and is now regarded as a likely genotoxic carcinogen. As such, it is recommended that the amount in the UK diet is reduced to the minimum that can be achieved. The European Commission is currently discussing the introduction of legislation to specify maximum allowed amounts of ochratoxin A in grain and other susceptible commodities. To detect mycotoxins in grain in order to reduce or eliminate them from the food chain requires tests which are cheap, quick and simple to carry out, reliable and with sufficient sensitivity. The HGCA commissioned a review of test kits currently available for detecting mycotoxins in cereals together with a critical appraisal of their performance to establish whether current commercial kits meet the likely future needs of the grain trade.

Discussion with suppliers and users suggest that test kits are useful in certain situations for detecting contaminated grain, especially in the absence of sophisticated analytical facilities. A positive sample is often rechecked using HPLC. However, test kits lack either the sensitivity, speed or reliability required in situations where a rapid result is essential, e.g. testing a lorry load of grain before acceptance. There is considerable scepticism, particularly amongst analysts, about their effectiveness and their failure to be universally accepted within the UK is not helped by the lack of publicly available validation data.

It is clear that the current scale of testing grain for mycotoxins by all available methods is very small in the UK. In addition, it is concluded that test kits currently on the market have only limited application for quality control of mycotoxins such as ochratoxin A. It is unclear what scale of testing will be required by the grain trade following legislation for ochratoxin A. In the interim, proper validation studies need to be carried out with selected kits. It is recommended that new tests are then developed if necessary either by modification of current products or by using alternative technology such as has proved highly successful in other fields. There is currently little guidance on sampling grain for mycotoxins other than that laid down in the EC directive for aflatoxins and there is an urgent need to develop simple and soundly-based sampling protocols for ochratoxin A.

CONTENTS

	PAGE
ABSTRACT	**1**
GLOSSARY OF TERMS	**4**
OBJECTIVE	**5**
GENERAL INTRODUCTION	**5**
The mycotoxin problem	**5**
Background information on mycotoxins	**6**
Legislation for mycotoxins	**9**
Mycotoxins occurring in UK grain	**9**
Field derived mycotoxins	**10**
Storage derived mycotoxins	**11**
Rapid test kits for mycotoxins in cereals	**12**
REQUIREMENTS FOR THE DESIGN AND OPERATION OF RAPID TEST KITS	**13**
Sampling	**13**
Extraction	**15**
Clean-up	**15**
Detection and measurement	**16**
Test time	**16**
Sampling time	**17**
PRINCIPLE AND OPERATION OF KITS	**17**
General	**17**
Direct competitive ELISA methods	**18**
Immunoaffinity column clean-up methods	**19**

RAPID TEST KITS COMMERCIALLY AVAILABLE WITHIN THE UK 19

PERFORMANCE AND VALIDATION DATA AVAILABLE FOR TEST KITS 23

Introduction 23

Validation methods 24

The suppliers view 26

The customers view 26

REVIEW FINDINGS, BY TECHNIQUE 28

Card tests 28

ELISA plate tests 29

IA column clean-up with detection using fluorescing tips or fluorimetry 30

GENERAL FINDINGS 30

The overall view 31

Current and future developments 32

CONCLUSIONS 33

RECOMMENDATIONS 34

ACKNOWLEDGEMENTS 35

REFERENCES 35

APPENDIX 1 COMMERCIAL SUPPLIERS 38

APPENDIX 2 LISTING OF AVAILABLE RAPID TEST KITS AND CLAIMED PERFORMANCE 39

APPENDIX 3 SELECTED INFORMATION AND VALIDATION DATA 52

GLOSSARY OF TERMS

AOAC	Association of Official Analytical Chemists
COM	Committee on Mutagenicity*
COT	Committee on Toxicology*
DON	Deoxynivalenol (vomitoxin)
ELISA	Enzyme Linked Immunosorbent Assay
EC	European Community
F2	Zearalenone
FAC	Food Advisory Committee
FAPAS®	Food Analysis Performance Scheme
FGIS	Federal Grain Inspection Service
GC or GLC	Gas chromatography or Gas liquid chromatography
HPLC	High Performance Liquid Chromatography
IA	Immunoaffinity**
IUPAC	International Union of Pure and Applied Chemistry
MS	Mass Spectrometry
OA	Ochratoxin A
ppb	Parts per billion = µg/kg
TLC	Thin Layer Chromatography
USDA	United States Department of Agriculture

* of Chemicals in Food, Consumer Products and the Environment

** = This term relates to the affinity that an antibody has towards the compound used to raise that antibody in a mammalian species.

OBJECTIVE

To review the test kits available within the UK for detection and measurement of mycotoxins in cereals and assess their performance and suitability as alternative, or complementary methods
to HPLC, GLC, MS or TLC.

This is achieved by:

1. Considering sampling procedures for sampling and sample preparation.
2. Listing the kits available for mycotoxins.
3. Determining the requirements of the cereal industry for mycotoxin analysis.
4. Assessing how well these kits perform in terms of the claims of the suppliers.
5. Assessing how far the kits fulfil the needs of the industry.

GENERAL INTRODUCTION

The mycotoxin problem

The British consumer demands quality food free from chemical and microbiological contaminants. Mycotoxins are highly toxic chemicals produced by some fungi and can be formed in many food commodities including cereals. If detected they are indicative of reduced quality. Aflatoxins which are formed by certain species of *Aspergillus* are the most important mycotoxins on a world wide scale but are rarely formed in UK-produced grain.

Ochratoxin A is known to occur in stored UK cereals and is now regarded as a likely genotoxic carcinogen. As such, it is recommended that the amount in the UK diet is reduced to the minimum that can be achieved. Many food and drink producers now require assurance of freedom from significant amounts of ochratoxin A (and selected other mycotoxins) in cereals and other food ingredients. The EC is currently discussing the introduction of legislation to specify maximum allowed amounts of ochratoxin A in grain and other susceptible commodities. The Codex Committee on Food Additives and Contaminants have estimated that 54% of dietary exposure to

ochratoxin A in the "European diet" comes from cereals. (CCFAC position paper CX/FAC99/14)

This situation then presents a number of issues for the UK grain trade. These include, how to prevent the formation of mycotoxins, methods for detection of contamination, how to handle and dispose of contaminated grain and the need to remain competitive in UK and export markets.

To reduce or eliminate mycotoxins in grain requires the means to test consignments at key control points. For this to be effective and economic, tests must be available that are cheap, quick and simple to carry out, reliable and with sufficient sensitivity.

Background information on mycotoxins

Mycotoxins are toxic secondary metabolites produced by certain species of fungi. They have a range of diverse chemical and physical properties and toxicological effects on man and animals. While many hundreds of such products have been identified, only 20 to 30 have been shown to be contaminants of human or animal food (Watson 1985). Many reviews of the subject have been published during the last 30 years (e. g. Scudamore 1993 a, b, Smith *et al.* 1994; Miller 1995, Dutton 1996, Moss 1996). The most important mycotoxins found in food are listed in table 1 together with the principle fungal species responsible for their production. It is clear that raw cereals are at risk from contamination by a range of different toxins.

The presence of mycotoxins in raw commodities such as cereals is only of concern for human health if they survive storage, processing and preparation of the food product as eaten by the consumer. Similarly, their occurrence in animal feeding stuffs, in which cereals are often the major component, only presents a risk for human health if they, or a toxic metabolite, are transferred to meat or animal products such as eggs milk or dairy products, although they may impair animal health and affect productivity. A comprehensive reference work addressing the subject of mycotoxins in animal feeds is that by Smith and Hendersen (1991).

Table 1: Important mycotoxins found in some food commodities and the fungi responsible. Those mycotoxins which are likely to occur in UK grain are highlighted.

Mycotoxin	Main fungal species	Foods infected
Aflatoxins B_1, B_2, G_1, G_2	*A. flavus, A. parasiticus*[a] [a] = aflatoxin B_1 and B_2 only	**Cereals,** nuts, figs, dried fruit, spices, rice bran, maize
Aflatoxins M_1, M_2	Metabolic products of B_1 and B_2	Milk and dairy products
Sterigmatocystin	*A. versicolor, A. nidulans*	**Cereals**, cheese
Cyclopiazonic acid	*A. flavus, P. commune*	**Cereals**, pulses, nuts, cheese
Ochratoxin A	*P. verrucosum,* *A. ochraceus*	**Cereals**, coffee beans, field beans, beer, nuts
Citrinin	*P. verrucosum*	**Cereals**
Patulin	*P. expansum,*	Apple juice, fruits
Deoxynivalenol , nivalenol	*F. graminearum, F. culmorum, F. crookwellense*	**Cereals**
T2 toxin HT2 toxin	*F. poae, F sporotrichioides*	**Cereals**
Fumonisins B_1, B_2, B_3	*F. moniliforme, F. proliferatum*	Maize, maize products
Zearalenone	*F. graminearum, F. culmorum, F. crookwellense*	**Cereals**
Moniliformin	*Fusarium* species	**Cereals**
Alternariol , alternariol monomethyl ether, Tenuazonic acid	*Alternaria alternata, A. tenuis*	**Cereals**, fruit, tomatoes, oil seeds

It is little surprise when the diversity of chemical structures is considered, that mycotoxins exhibit a wide range of acute and chronic toxicological effects. The scourge of the middle ages in Europe, St Anthony's Fire, was caused by ergot alkaloids in cereals and many other animal and human diseases have been attributed to the presence of mycotoxins in food or the environment, although, conclusive evidence

for such associations is often difficult to obtain. Some mycotoxins are proven or suspected carcinogens, mutagens or teratogens, while others have been shown to challenge the immune systems of man and animals. This raises the possibility of increased susceptibility to other diseases, without suspicion of mycotoxin involvement. In view of the highly toxic nature of many of the mycotoxins, it is fortunate that their occurrence in food is spasmodic and often only in small amounts, at least in developed areas of the world. However, the potential for wide-scale problems exist should the appropriate circumstances arise. Low level contamination of cereals by highly biologically active chemicals presents a major difficulty in assessing their true risk for man. Accumulation of toxicological data, information of occurrence and food consumption have enabled Risk Assessment to be carried out for the most important mycotoxins and led in some instances to recommended acceptable total daily intake values.

The detection of minute amounts (parts per billion) of fungal products within a substrate composed of many natural products having similar chemical or physical properties, also presents a major challenge to the analytical chemist. Hence specific and precise analytical methods have required considerable development. Antibodies have been raised to most of the important mycotoxins and a range of immunologically-based methods now complement, or have been incorporated into, chemically based methods such as TLC, HPLC and GC. Antibodies bound to activated Sepherose are commercially available as highly selective clean-up columns (IA columns). There is also a need for rapid, reliable and cheap methods of testing to control mycotoxins in the food chain. Food processors and retailers are increasingly requiring assurance that supplies are low in, or free from, mycotoxins before purchase. This results from a better awareness of the problem and the introduction of, and proposals for, statutory maximum limits for some mycotoxins.

Fungi and mycotoxins, intimately associated with growing crops and stored foods, are difficult to prevent, especially when they develop prior to harvest. In addition most mycotoxins once formed are chemically stable and are difficult to remove. Attention to good farming and storage practice are important factors in reducing the occurrence and amounts of mycotoxins.

In the production of food crops and consumer food products, the aim should be to reduce levels of mycotoxins to the lowest that can be technologically achieved within the economic constraints existing and with regard to Risk Assessment where this has been carried out. The existence of legal or recommended maximum limits requires structured sampling and analysis at agreed key points in trading and marketing of grain and ways of pinpointing contaminated batches. The effective policing of such limits requires rapid, simple, cheap and reliable tests.

Legislation for mycotoxins

Legislation world-wide has been mainly restricted to the aflatoxins, of which aflatoxin B_1 is accepted as a potent liver carcinogen. Limits have usually been set on a national basis. Individual countries have introduced limits for other selected mycotoxins (FAO 1997). From 1 January 1999 the EC have set limits for aflatoxins in a range of food commodities including cereals and are currently discussing limits for ochratoxin A in cereals and some other food commodities. However, for a number of administrative and legal reasons it was not possible for the UK Statutory Instrument to come into force by that date. During the intervening period, sufficient enforcement measures remained in place via the existing 1992 UK Regulations and the main provisions of the Food Safety Act 1990 until 30 June 1999 to ensure that an appropriate level of consumer protection is maintained. Whether legislation will be eventually extended beyond aflatoxins and ochratoxin A is currently unclear.

Mycotoxins occurring in UK grain

Despite the number of mycotoxins listed in table 1, only a few are likely to occur in UK home-grown cereals and these are highlighted. Internationally, aflatoxins are probably the most widespread and the most hazardous mycotoxins in foods. However, the UK climate does not favour the development of aflatoxins hence they rarely found except under special circumstances such as been when wet barley has been stored using a mould inhibitor applied at an insufficient concentration. (Note however, that UK grain is still subject to the EC aflatoxin regulations which now include maximum permissible amounts for aflatoxins in cereals and for which suitable analyses or tests must be

available). As discussed earlier the management of the ochratoxin A problem is currently of greatest concern to the UK grain trade.

Field derived mycotoxins

The most commonly found moulds occurring before harvest are *Cladosporium*, *Fusarium* and *Alternaria*, although many other fungi may be present (Scudamore and Wilkin 1999). No mycotoxins are known to be produced by *Cladosporium* and so this does not present a problem other than by affecting the quality of grain.

Species of *Alternaria* are widespread in grain. However, a very high water activity appears to be necessary to allow the formation of mycotoxins (Magan *et al.* 1984) and it seems unlikely that *Alternaria* mycotoxins are a major problem in cereals although there is little information about this. Few studies or surveys have been carried out in the UK although Nawaz *et al.* (1997) have shown that alternariol monomethyl ether, alternariol and particularly tenuazonic acid can develop in UK and EC grown oil seed rape. Wheat and barley were not examined in that study.

By far the most important mycotoxins which develop in grain in the field are those from *Fusarium*. A previous HGCA funded projects (Polley *et al.* 1991) reviewed these. There are a several species of *Fusarium* which are known to form one or more mycotoxins in cereals and these include *F. culmorum. F. graminearum, F poea. F avenaceum, F. sporotrichioides,* and *F. equesiti*. These mycotoxins include deoxynivalenol, nivalenol, HT-2 toxin, T-2 toxin and related compounds (collectively known as trichothecenes) and also zearalenone.

The trichothecenes are group of more than 100 related compounds although only about 20 have been confirmed as occurring naturally in cereals. The highest amounts reported are usually of deoxynivalenol and nivalenol. However, these are not the most toxic of the trichothecenes and the smaller amounts of related compounds such as HT-2 toxin and T-2 toxin which also occur may be equally important toxicologically. Zearalenone has oestrogenic properties and related compounds may also occur. Some of these are more oestrogenic than zearalenone but have been far less studied.

Currently the main cause for concern about these mycotoxins in the UK are their effect on the health and productivity of livestock.

The FAC (Food Advisory Committee) has recommended that periodic surveillance in UK foods is carried out for moniliformin which is another *Fusarium* mycotoxin. This is mainly found in maize although it may occur in other cereals although little information is available on this.

It appears from information gathered in producing this review suggests that testing for these field-formed mycotoxins takes place only on a very limited scale within the UK at present and is restricted to deoxynivalenol, T-2 toxin and zearalenone principally because of their potential effects on animals.

Storage derived mycotoxins

Field fungi are gradually replaced by storage fungi after harvest, although this change can take several weeks or more. This aspect was investigated as part of a recent HGCA-funded project to study ambient air drying of cereals (Scudamore and Wilkin 1999). It was found that if grain is not dried rapidly, there is potential for further growth of field fungi in store and hence the formation of F*usarium* toxins may not cease at harvest. Further studies are required to investigate this possibility.

If grain remains at a high enough moisture during storage (>16%) or is not dried quickly enough, mycotoxins such as ochratoxin A may be formed. Surveys funded by MAFF (e.g. Scudamore *et al.* 1999) have shown a small but regular occurrence of ochratoxin A in stored grain. This is important because the COT has advised that it should be considered a potential human genotoxic carcinogen and the FAC has recommended that amounts in foods should be reduced to the lowest levels possible. It is also nephratoxic and can effect particularly pigs and poultry. It is also quite stable during processing.

Citrinin also occurs in UK-stored cereals but is less often sought. It is not considered to be a carcinogen. It is less stable than ochratoxin A, and hence appears to degrade during food production. Its effects are most likely to be noticed in livestock to which

it is nephratoxic. As it often co-occurs with ochratoxin A its presence is often not recognised especially as analytical methods are less satisfactory than those for ochratoxin A.

Sterigmatocystin is formed by several species of *Aspergillus* but is rarely found in cereals. In addition there are a range of other mycotoxins which can form in cereals most of which either have not been sought or only occur in badly moulded cereals. Examples of these are *A. fumigatus* mycotoxins, *A clavatus* mycotoxins (Flannigan 1986), napthoquinones compounds such as xanthomegnin and viomellein, gliotoxin (Scudamore *et al.*,1986) and penicillic acid.

Current knowledge would suggest that the need to test UK grain for storage mycotoxins will not go beyond ochratoxin A and aflatoxins in the foreseeable future

Rapid test kits for mycotoxins in cereals

Reliable and sensitive analytical methods based on HPLC or GC/MS are available for the mycotoxins which are important in cereals. These methods are vital especially for enforcement purposes. However, their operation requires highly trained technical staff, sophisticated and expensive equipment and laboratory facilities and are not usually suitable for testing where results are required in a few minutes. This review aims to examine the extent to which test kits currently available within the UK meet the requirements of the rapid turn round situation in the grain trade.

Rapid test kits for ochratoxin A, citrinin, zearalenone, deoxynivalenol, T-2 toxin and aflatoxins have been identified. Several tests kits are marketed for aflatoxins in foods including cereals and cereal products. No rapid tests exist for, moniliformin and the other mycotoxins briefly reviewed earlier because it is considered that there is currently little justification for monitoring these mycotoxins on a large scale. The antibodies used for producing the tests for these mycotoxins may cross react to some degree with other related compounds present. They may thus not be as specific as HPLC-based methods although several aflatoxin B_1-specific antibodies have been raised and used in test kits.

REQUIREMENTS FOR THE DESIGN AND OPERATION OF RAPID TEST KITS

The steps required to determine mycotoxins in cereals are similar whether using sophisticated instrumentation or simple test procedures and are: obtaining the sample, extracting the toxin from the cereal, purification of the extract and detection and measurement of the amount present. These factors influence the ultimate performance of rapid tests and are discussed below.

Sampling

It is fundamental in any trace analysis that the sample obtained is representative of the bulk from which it has been drawn. Failure to achieve this may invalidate results from any method used.

Representative sampling is especially important for mycotoxins as they are often very unevenly distributed in consignments of cereals. This occurs because fungi tend to grow in isolated pockets of damp or broken grains and, if conditions are favourable, very high concentrations may occur at these sites while the rest of the consignment is unaffected. Failure to sample properly may not only provide an invalid result but can lead to costly disputes when grain is tested on more than one occasion and analytical results differ. Although such differences often lead to lengthy argument, the cause is most likely due to poor sampling rather than the shortcomings of the analytical laboratories.

The EC regulations relating to aflatoxins lay down methods for sampling and analysis of mycotoxins (Directive 98/53/EC). While ochratoxin A is not currently subject to a regulatory limit this is being discussed as a priority and maximum permissible levels are likely to be introduced within the next few years. Because establishing sampling protocols based on a sound statistical basis is extremely difficult it is likely that the sampling methods to be proscribed for ochratoxin A in cereals taken for official enforcement purposes will be modelled on those for aflatoxins.

An excerpt from the Community aflatoxin regulations showing the size and number of samples required for sampling cereals is given in Appendix 3, table 4. For example; bulks of grain greater than 1500 tonnes need to be treated as multiples of 500 tonne lots. From each 500 tonne sample, 100 sub samples each of 300 g must be taken giving a total 30 kg bulk sample. Alternatively, a typical lorry load of 25 tonnes will require 100 sub samples each of 100 g to be taken which when combined provides a 10 kg bulk sample. Within the UK grain trade and elsewhere in Europe there is much concern about the practicalities and difficulties involved in applying these procedures, as well as the inherent labour and material costs. In addition, the analyst must handle a much larger sample requiring larger storage, laboratory mills and laboratory handling facilities than are currently normally needed and hence result in much higher running costs.

After considering the difficulties expressed by many parties, the Commission has agreed that 'for non-enforcement purposes other sampling methods may be acceptable'. Thus, in the absence of authoritative guidance, grain traders most likely will use the sampling procedures currently in place for other quality control purposes. In deciding whether such procedures are satisfactory it should be recognised that these may have been designed to measure parameters for which the values are more evenly distributed within the material. Little if any data showing the distribution of ochratoxin A or other mycotoxins in grain are available and it is difficult to conclude whether the adoption of current sampling procedures would be entirely satisfactory.

However, large scale testing for mycotoxins in cereals is only likely to happen after legislation is introduced or when strict quality requirements for mycotoxins are placed on cereals by grain end users. If rapid screening methods are to be used successfully on a large scale for testing and controlling mycotoxins in cereals entering the food chain, such sampling procedures will need validating to provide the necessary assurance to all parties involved, to avoid unnecessary rejection of consignments, inadvertent acceptance of contaminated loads or trade disputes.

Extraction

Because mycotoxins develop as the result of fungi colonising the seeds they are often firmly held within the structure of the whole cereal grains. To obtain a true analytical result, as much as possible of the mycotoxin present must be released from the seed structure prior to analysis. This is usually achieved by finely grinding the grain together with the use of the most suitable solvent. Water alone is unsuitable for most mycotoxins, and an organic solvent such as methanol, acetonitrile or chloroform containing a proportion of water is normally used. However, chlorinated solvents in the work place are undesirable on safety grounds and their use should be avoided as far as possible when developing methods for use with test kits.

There are two normal methods for extraction of mycotoxins. These are either to shake a finely milled sample for about 30 minutes with the appropriate solvent or to blend at high speed with a solvent for 1-2 minutes. There has been much discussion about which technique is most effective for releasing mycotoxins from the cereal structure. However, it is clear that for the development of very rapid test methods, high speed blending must be the choice. This necessitates the purchase of a suitable blender. If qualitative results are acceptable, soaking and shaking for a few minutes can be effective but the technique should be properly assessed and the user should be aware that the sensitivity of the method is likely to be reduced.

Clean-up

The amount of mycotoxin present is normally extremely small and will be present within the grain along with many other natural constituents. As extraction systems must be very efficient in removing the toxins, many other constituents will also be extracted by the solvents used. If these are not then removed from the analytical solution, they may interfere with the final detection and measurement of the mycotoxin. In HPLC and TLC methods co-eluted substances may mask the peak or spot or affect quantitation while in ELISA-based methods the optical density curve may be different from that given by standards. Some rapid tests rely on detecting fluorescence but this may also occur due to co-extracted components even in the absence of mycotoxins.

Clean-up is achieved by passing solutions through solid phase or immunoaffinity columns which selectively retain mycotoxins followed by washing the column and eluting using carefully selected solvents. This stage may take typically 3 to 10 minutes or even longer. However, it may be possible to omit a clean-up stage with a highly selective detection system but overall performance will usually be affected as a result.

Detection and measurement

Detection may be by direct visual comparison of a colour change against a standard or observation of fluorescence under UV irradiation. The sensitivity and reliability of rapid tests is usually limited by the inability of the operator to recognise small differences in colour or fluorescence between a sample and the corresponding standard. It is usually easier to measure these changes using a calibrated instrument. The reading is then likely to be more reliable and repeatable and will not be subject to operator performance or bias but will require an initial outlay to purchase the detector. Time required for measurement will be short, < 1 minute.

Test time

Some rapid test kits are claimed to provide analytical results within 20 minutes, or less. These claims may or may not include the time required to sample and for sample preparation.

With current technology the approximate test time which should be achievable, excluding time for sampling, might be composed as follows:

weighing, grinding and blending	3 minutes
extract clean-up	2-3
reaction (e.g. ELISA)	3-10
visualisation/measurement	1
total	**9-17 minutes**

The scope for reducing test time with current technology seems limited although it may be possible to exclude clean-up or reaction stages in some tests. Total time for sampling and testing is thus difficult to reduce to less than 10 minutes but this might be

achievable. However in those situations when a number of samples can collected and analysed together the average time per test will be much reduced. This is particularly true of ELISA based kits using multi-channel pipettes.

Sampling time

Automatic, full-depth samples drawn from several points in a lorry using current in-house procedures is rapid e.g. about 2 minutes. This is much quicker than could be achieved if the EC procedure recommended for aflatoxins in cereals were adopted. No comparison of the effectiveness of the 2 methods has been carried out

Representative sampling of bulk grain would take longer, depending on the method adopted. However, the urgency to obtain a result will be much less than when testing lorry loads of grain, This then allows the tester to have a wider choice of test kits. In this situation, samples could be accumulated until a batch was obtained and then tested simultaneously.

PRINCIPLE AND OPERATION OF KITS

General

Mycotoxin test kits supplied in the UK may have approval from recognised international bodies or have been tested by the supplier. Approval is usually granted following examination of the kits by trained laboratory staff to verify that the claims of the suppliers can be achieved. This leaves the question of how robust the kits are in the hands of inexperienced or non-technical staff, how suited the kits are to the requirements of the users and the need for the users to appreciate the limitations of the test. It is often claimed that these tests can be performed by staff with little or no technical knowledge or training. These claims and the experiences of some users will be discussed later.

The method of sampling and the effectiveness of extraction procedures are usually considered as issues unrelated to the performance of the kits. However, most suppliers

provide guidance and instructions with their literature for the whole procedure and may also offer a back-up advisory service.

The different principles used for production of rapid tests are discussed in the next section. These are based on direct competitive ELISA' s carried out in test wells, card tests, and immunoaffinity column clean-up or solid phase clean-up. These procedures are combined with a suitable visual or instrumental reading.

Direct competitive ELISA methods

Antibodies to each mycotoxin are produced from established antibody cell lines. After production, purification and testing of the antibody it is incorporated into the test by the manufacturer. The antibodies are usually immobilised on the surface of the container used for the test. In kits microtitre glass or plastic wells are used to carry out the reactions. These may be in the form of 96 well plates or in strips. In 'card' tests, reactions are carried out by dropping the appropriate solutions onto absorbent pads or membrane supports bound with the antibodies.

In these tests the key material is the conjugate which is a pre-prepared reagent in which the mycotoxin is bound to an enzyme. A solution of this conjugate is then mixed with the solvent extract from the sample in the wells or on the test card. The free mycotoxin and the conjugate also containing the mycotoxin then compete for the free sites on the antibody. Unbound conjugate or mycotoxins are removed by washing with water or special buffer solutions. Addition of enzyme substrate to the bound mixture results in colour formation, the extent of which is proportional to the amount of bound enzyme present. Thus the more free toxin in the sample, the less the conjugate will have bound to the antibodies and the less will be the colour development. **More colour means less toxin. Less colour means higher amounts of toxin**. The amount of colour can be determined by comparison with standards of known toxin concentrations. These may be coloured solutions or calibration cards. Alternatively the change in optical density can be read in a spectrophotometer which is normally a plate- or strip-reader.

Immunoaffinity columns clean-up methods

These also use antibodies raised against a specific mycotoxin or mycotoxins. The antibodies are bound to sepherose after purification and characterisation. This product is then packed into a column which is supplied as part of the kit. When a solution containing the specific mycotoxin is added, the toxin binds to the sites on the antibody in the column which recognise the mycotoxin. Other contaminants and co-extractives pass through unaffected. The mycotoxin is then released by changing the solvent composition and is collected for examination The analyte (mycotoxin) present may fluoresce naturally under UV light or can be reacted using a suitable reagent to give a fluorescent product. The degree of fluorescence can be measured by comparison with standards or cards supplied, or in a calibrated fluorimeter. Once advantage of this method is that mycotoxins can be concentrated on the IA column thus improving detection limits.

RAPID TEST KITS COMMERCIALLY AVAILABLE WITHIN THE UK

Details of the UK Companies identified during this review are given in Appendix 1. The test kits identified which appear to fall within the remit of this study are listed in table 2. Details of the performance claimed for most of the kits is given in Appendix 2.

Philip Harris are included in table 2 although it is understood that they no longer market their products. These therefore have not been assessed. ADGEN Ltd, Cortecs Diagnostics Ltd, Digen Ltd, Labtech International Ltd and Rhône-diagnostics technologies Ltd each market 2 or more products. The food diagnostics section of Cortecs Diagnostics Ltd have been bought be Tepnel Life Sciences during the preparation of this review. All companies have recently introduced (or are about to) new or improved tests, several being announced during the progress of this review.

The main objective of this review is to consider the effectiveness and scope of rapid test kits and assess how well they meet the needs of the grain trade. The tests are assessed on the basis of the underlying principle rather than the merits of individual products presented by the suppliers or users interviewed.

Table 2: Kits, suppliers and manufacturers of rapid test kits available in the UK for determination of mycotoxins in cereals.

Kit Supplier	Manufacturer	Product Name	Mycotoxin
ADGEN Ltd	Neogen	Veratox®	Ochratoxin A Deoxynivalenol Zearalenone T-2 Toxin Aflatoxins Fumonisin*
ADGEN Ltd	Neogen	Agri-Screen®	Ochratoxin A Deoxynivalenol Zearalenone T-2 Toxin Aflatoxins Fumonisin*
Cortecs Diagnostics Ltd[$]	Biokits	Ochratoxin assay Aflatoxin assay	Ochratoxin A Aflatoxins
Digen Ltd	R-Biopharm GmbH	RIDASCREEN®:	Ochratoxin A[+] Citrinin DON (Ac-DON)[++] Zearalenone[+] T-2 Toxin[+] Fumonisin*[+] Aflatoxins[+]/aflatoxin B_1
Labtech International Ltd	Vicam	OchraTest™ DONtestTAG™ ZearalaTest™ FumoniTest™ AflaTest® AflaB™ AflaTip™	Ochratoxin A Deoxynivalenol Zearalenone Fumonisins* Aflatoxins
Philip Harris		No longer supporting	kits
Rhône-diagnostics technologies Ltd	Rhône-diagnostics technologies Ltd	OCHRASCAN® OCHRAPREP® AFLASCAN® AFLAPREP® EASY-EXTRACT® EASY-ASSAY® EASY-EXTRACT®	Ochratoxin A Aflatoxins Zearalenone Fumonisins[&]*
Rhône-diagnostics technologies Ltd	Rhône-diagnostics technologies Ltd	AFLAPLATE®	Aflatoxin B_1
Rhône-diagnostics technologies Ltd	Rhône-diagnostics technologies Ltd Med-Tox diagnostics	AFLACARD2ppb OCHRACARD EZ-Screen card	Aflatoxin B_1 Ochratoxin A[&] Ochratoxin A Trichothecene (T-2 toxin) Zearalenone Aflatoxins
Tepnel Life Sciences PLC[$]	-		Ochratoxin A[&], Aflatoxins[&]

*= not found in wheat and barley
[&] = under development
[+] = fast versions now available
[++] = fast and express versions now available
[$] = Cortecs Food Diagnostics was acquired by Tepnel Life Sciences PLC on 21 June 1999

Because there are a considerable number of tests and variations available it is impossible to make specific recommendations for individual products based on the experience of the limited number of users identified. In a few instances the same advantage or problem has been voiced by more than one user and these are used to illustrate the current status of testing and should not be taken as a specific recommendation or criticism of that product.

In summary:

ADGEN LTD

All kits marketed are produced and tested by Neogen Corporation of Lansing USA. These are subjected to independent testing by internationally renowned bodies such as AOAC. These bodies issue certificates confirming that the performance of kits meets the claims of the supplier. There are 2 different series of tests. The Veratox series is claimed to provide quantitative results while Agri-Screen kits are designed for rapid qualitative results. The company has its own laboratory facilities and expert scientific staff in Scotland.

CORTECS FOOD DIAGNOSTICS LTD

This company was acquired by Tepnel Life Sciences PLC on 21 June 1999 and incorporated into a new company, Tepnel Biosystems Ltd. It is understood that the products marketed by Cortecs Diagnostics Ltd are to be phased out and replaced by new kits. No current users were identified and products were not appraised.

DIGEN LTD

The products supplied are manufactured and tested in their laboratories by R-Biopharm GmbH, Darmstadt, Germany and marketed under the name RIDASCREEN®, RIDASCREEN® FAST, RIDASCREEN® EXPRESS and RIDA® (IA columns). The RIDASCREEN®, and RIDASCREEN® FAST tests are for quantitative analysis whereas the RIDASCREEN® EXPRESS tests are for

semiquantative/screening. The IA columns are used in clean-up procedures for certain matrices prior to ELISA or HPLC.

LAB TECH INTERNATIONAL LTD

Kits offered are produced by Vicam in the USA. These are subjected to independent testing by internationally renowned bodies such as AOAC. These bodies issue certificates confirming that the performance of kits meets the claims of the supplier. In addition to rapid test kits, a range of immunoaffinity columns are marketed for use with instrumental techniques.

RHÔNE DIAGNOSTICS TECHNOLOGIES LTD

Products are produced in Glasgow and are tested internally, by internationally renowned bodies such as AOAC and the EC and in conjunction with customers. There are 3 different series of tests: OCHRASCAN® and AFLASCAN® which use IA column clean-up (semi quantitative screening tests), card tests (for qualitative analysis) and an ELISA test for quantitative analysis of aflatoxin B_1. In addition to rapid test kits, a range of immunoaffinity columns are marketed for use with instrumental techniques.
This provides a comprehensive range of rapid tests. The company has its own laboratory facilities and expert scientific staff in Scotland. The company is the only ISO9002 manufacturer of test kits in the UK.

TEPNEL BIOSYSTEMS LTD

Kits for ochratoxin A and aflatoxins are understood to be offered shortly.

PERFORMANCE AND VALIDATION DATA AVAILABLE FOR TEST KITS

Introduction

Before rapid test kits gain universal acceptance as reliable and appropriate means for quality control and testing for mycotoxins in cereals and other foods, it is essential to have proper validation. This needs to be carried out under the conditions in which the tests are ultimately to be carried out.

Official 'approval' or 'certification' is often considered sufficient. An example of these approvals is shown in Appendix 3, figure 1 which lists the approval status for some of the Agri-Screen and Veratox products. Similar approvals have been granted for the kits of other suppliers. While such testing should be strongly encouraged, these approvals are often based on tests for relatively few commodities and are in some cases carried out using much higher mycotoxin levels than are often sought in the UK. In addition, expert scientific staff usually from a limited number of laboratories carry out these trials. In contrast.sales literature often portray that these kits can be used by staff with limited technical training. While this may sometimes be true, without suitable in-house quality control, or an understanding of the scope of validation tests, problems which arise may easily pass unnoticed.

A simple example which could cause problems in the absence of suitable training is that it is crucial with most ELISA-based procedures to take great care in washing cells between adding reagents. It is suggested that this may be one significant source of variability found with these tests. Other factors such as room temperature may significantly change the reaction times of reagents and hence optical densities and colour development. It is thus essential that the user should be aware of the limitations of the conditions and concentration range covered by these approvals and be suitably trained in the use of the kit.

Lack of proper appraisal of the scope and intended use of tests has lead to dismissal of such methods by some analysts and quality managers, disenchantment by some users and at the worst, blissful ignorance by others.

Validation methods

Often, limited consideration appears to be given to the importance of validation and testing of rapid test kits by commercial users or by suppliers. Sometimes there is a naive acceptance of their infallibility and an insufficiently critical approach. One reason for this is that some customers use rapid tests because they have limited access to sophisticated laboratory facilities, require qualitative or semi-quantitative results or sometimes have little or no analytical expertise. Conversely, large organisations or trade associations are usually fully equipped in-house with HPLC or GC run by experienced analysts and see no, or only a limited, role for such tests.

Four ways of testing can be envisaged. These are: examination by internationally respected bodies such as the AOAC Research Institute, inter-laboratory trials organised by industrial or government bodies, *ad hoc* testing or comparison with established methods by the kit supplier or customer or use with test samples containing known concentrations such as those provided by the UK MAFF FAPAS.

Suitably structured inter comparison trials are perhaps the ideal method for validation of methods although they are expensive to carry out. These can be targeted precisely to the need of a particular industry or association, e. g. ochratoxin A in wheat and barley on behalf of the grain trade. It is recommended that the performance of rapid kits should be assessed alongside established methodology. It is clearly important to define the intended role of the test kit and design appropriate validation tests.

Several of the suppliers have their own in-house analytical facilities and carry out validation testing for specific applications. This is welcomed and such data should be readily made available to customers whenever possible. Further testing of the products by customers should be encouraged and may be facilitated by subsidised supply of test kits and materials. This often happens in practice although such collaboration may be of a confidential nature and results not passed beyond the supplier and his customer or may be relevant only to that particular customer.

FAPAS is a scheme run by MAFF which supplies homogenous test materials to laboratories for test analyses. Results are scored and performance based on Z-scores. A value between + and - 2 is considered satisfactory although this range in itself allows quite a spread of result. Examination of Reports from FAPAS exercises show that a few laboratories are using rapid test methods in conjunction with these samples. Results relevant to this study are given in table 3 although few samples have been tested for grain. From 11 results, 4 were outside the range considered acceptable by FAPAS. However concentrations of ochratoxin tested were very low and near the limits of detection claimed for most kits. On 2 occasions when the same kit has been used by more than one laboratory for the same test material there are large differences in results, eg when RIDASCREEN® was used for coffee and AFLASCAN® for maize.

Table 3: Results obtained by rapid test kits identified as being used for the analysis of relevant FAPAS test samples

			Concentration, µg/kg			
KIT	Mycotoxin	Commodity	Mean value	Value found	Z score	Acceptable result
ELISA	OA	Flour	4.41	6.8	3.0	No
ELISA	OA	Flour	4.41	6.3	2.4	No
RIDASCREEN	OA	Green coffee	1.70	1.8	0.1	Yes
RIDASCREEN	OA	Green coffee	1.70	7.5	8.1	No
OCHRASCAN	OA	Currents	7.0	8.0	0.4	Yes
AFLASCAN	Aflatoxin	Maize	33.0	12.0	-2.4	No
AFLASCAN	Aflatoxin	Maize	33.0	32.0	-0.1	Yes
AFLASCAN	Aflatoxin	Maize	33.0	17	-1.8	Yes
AFLAPLATE	Aflatoxin	Maize	33.0	18.5	-1.6	Yes
RIDASCREEN	Aflatoxin	Maize	33.0	36.4	0.4	Yes
'IN HOUSE'	Aflatoxin	Maize	33.0	36.0	0.3	Yes

It is unclear whether these differences are due to the operators or inherent variability in the test kits although similar variability is also met with other methods. These results, although few, are encouraging and suggest that these methods can compete with

instrumental methods and should be applicable for at least semi-quantitative analysis given proper controls.

The suppliers view

Five companies ADGEN Ltd, Cortecs Diagnostics Ltd, Digen Ltd, Labtech International Ltd, and Rhône-diagnostics technologies Ltd, were initially identified as supplying test kits for mycotoxins while it was understood that Phillips still marketed a limited range at the time but were not intending to support these in the future. Cortecs Diagnostics Ltd have since been bought by Tepnel Life Sciences PLC. All suppliers have improved products on the market, or under development. Each company co-operated by supplying literature and information about their products and ADGEN Ltd and Rhône-diagnostics technologies Ltd further assisted by initiating contacts with users of their products so that their views on various aspects of the kits could be sought

It is clear that the extent of testing for mycotoxins within the UK is currently on a small scale when compared with that, for example, in the USA. In addition, by far the greatest number of tests are performed for aflatoxins. These mycotoxins have been controlled in the UK by the Animal Feedingstuffs Regulations in animal feeds since 1980, by the Nuts, Nut Products, Dried Figs and Dried Fig Products since 1992 and by EC regulation since 30 June 1999 for an extended range of products including cereals. The number of tests using rapid methods for mycotoxins such as ochratoxin A in cereals and cereal products is thus very small in a highly competitive market. The current limited potential market for testing cereals for mycotoxins discourages commissioning of costly validation tests for these products in grain. Development of new products is only likely where there is a clear new market.

The customers view

Users interviewed during this review included quality control staff, analysts and management as a result of introductions arranged by the kit retailers, other sources through personal contacts and independent introductions and a number of research laboratories, analytical laboratories and industrial research groups. Each contact involved one or more of the following actions: a personal visit, completion of a short questionnaire, or a telephone discussion.

Some advantages or disadvantages were commonly mentioned although there were many views expressed on the value or need for rapid testing. Such views can only be condensed and summarised here. A majority of those interviewed used or had used kits supplied by ADGEN Ltd or Rhône-diagnostics technologies Ltd. Few users of any of the other rapid test kits were identified. In the absence of positive feedback, the appraisal of these other products is restricted to a list of claimed performance and any validation data found or supplied.

The number of organisations testing for mycotoxins other than aflatoxins by any method (instrumental or test kit) appeared small and confirmed the information given by the suppliers. However ochratoxin A, vomitoxin, zearalenone and T-2 toxin were sought on a regular basis by one or more user using test kits although tests were usually not carried out in any quantity. Not unexpectedly, those companies using rapid test kits were reasonably satisfied that these were providing a useful service. However other organisations had tried test kits and had decided to use HPLC methods or had contracted out analyses.

In summary, the main advantages stressed were, speed, ability to handle the analysis of a number of samples at the same time and simplicity especially where instrumentation was not available. Rapid tests were sometimes used as a weeding out procedure with suspected positives confirmed by other methods. Disadvantages were lack of sensitivity, difficulty in interpreting end points, unreliability including false negatives and false positives. The conscensus view was that tests were considered not quite quick enough for testing grain in lorries at intake. However, some of these tests are used in at least Mexico and Argentina for this purpose although turn round times are believed to be slower than are normally acceptable in the UK. Cost was also mentioned especially when few samples were processed which meant that the sample capacity of tests was not used or the kits or reagents became out of date.

REVIEW FINDINGS, BY TECHNIQUE

Card tests

The simplest format and perhaps most rapid of all the kits currently available is the card test. These have been dismissed by most potential users in their current form due to lack of sensitivity. However, the AFLACARD®2bb recently introduced is specifically designed for use by the grain and cereal industry and the availability of a similar product for ochratoxin A now under development will merit serious consideration by the UK grain trade.

One feed company visited used card tests to detect aflatoxins, ochratoxin, zearalenone and T-2 toxin. Sensitivity to aflatoxin was claimed to be just sufficient to meet the requirements of Animal Feedingstuffs Regulations, while sensitivity to the other mycotoxins tested provided a useful quality check. These cards were found to be very easy to use although the recommended shelf life of 6 months was considered too short and needed to be strictly observed. Kits often had to be disposed of before fully used which meant wastage and increased cost. When out of date reagents were used false positives sometimes occurred. However, the supplier emphasised that it is not possible to mix and match kit reagents. Batches of reagents are quality controlled together according to ISO to ensure that customers do not use expired reagents and risk compromising the accuracy of their results.

Detection limits for ochratoxin A, zearalenone and T-2 toxin were in line with those claimed by the supplier. However the cards were unsuitable for detecting ochratoxin A at the 5 µg/kg level. Time for testing is 20 minutes or less. Legislation for mycotoxins in animal feed exists for aflatoxin B_1 only and the maximum permissible levels range from 5 to 50 µg/kg depending on the animal feed. The cards can be used down this level for aflatoxin B_1. Any aflatoxin positives were checked by the use of other procedures as necessary.

Because of the simplicity and rapidity of the test, re-formatting to provide sensitivities in line with current or future legislation could make these tests much more attractive

especially when a yes/no quality control screen is required. Longer shelf life and ability to purchase individual items that make up the kits would also be advantageous. The test procedure itself can be as short as 5 minutes, hence if sample preparation time could be reduced eg by high speed blending, a test time of 10 minutes should be achievable.

ELISA plate tests

It proved very difficult to gauge the scale of use of this type of test. It is concluded that few tests are currently carried out commercially other than for aflatoxins in a range of foods. Several products commercially available were compared for determination of ochratoxin A in flour (Personal communication). Results were variable and false positives and negatives occurred. This suggested that use in practice at the best would be restricted to semi-quantitative determination. It is believed that at least one product tested at the time was a prototype and has since been modified.

During this review the author had personal experience of using an ELISA based test kit (ADGEN Ltd). Using a sample of maize with a pre-checked level of zearalenone (200 µg/kg) and a second sample with a low content, excellent results were obtained. It was clear that familiarity with the use of microtitre pipettes is essential to ensure optimum results. Care is also required in pipetting the correct reagent into the right vial in sequence. The laboratory temperature on the day was high and this increased the speed of reactions taking place. This effected the shape of the standard optical density curve although samples were run under the same conditions. Visual comparison of the sample with standards provided a semi-qualitative result while use of a simple spectrophotometer provided a result which was close to that previously obtained by HPLC. The method is simple but a methodical and careful approach is required. Time required was about 25 minutes although several samples can be run together.

The ADGEN Ltd, Digen Ltd and Rhône-diagnostics technologies Ltd products are supplied in a strip format which avoids waste when only a few samples are to be tested.

IA column clean-up with detection using fluorescing tips or fluorimetry.

Although relatively new, the OCHRASCAN® test had been used for the determination of ochratoxin A in cereals by more customers identified in this survey than any other product. Sensitivity appears just sufficient to test grain at the 2-5 µg/kg level although several customers had difficulty in deciding whether the silica tip was fluorescing. Experience in its use seemed to increase confidence in recognising a positive sample. In most laboratories any positive result was confirmed using HPLC.

The analagous product for aflatoxins was used more widely but not usually for cereals. Some customers presented limited data comparing the use of OCHRASCAN® or AFLASCAN® with HPLC and these results are given in tables 5 and 6.

IA column clean-up was used by many customers. However, most used HPLC for separation and measurement. Those mycotoxins such as ochratoxin A which fluoresce under UV light can be measured in a fluorimeter although no users of this technique were identified.

GENERAL FINDINGS

Commonly mentioned was the lack of sensitivity which meant that when used,kits were often operating at their limits. The effect of this was for example that the change in optical density was often small or visual assessment of the fluorescence in silica tips was difficult. Kit suppliers were generally aware of this problem and at least in part were making attempts to improve this.

Some kits had an unacceptable frequency of false positives or negatives although it was often unclear how the user had assessed the occurrence of false negatives in the absence of checks against other methods. False negatives are highly undesirable in that this could lead to the acceptance of contaminated consignments or to unknowingly marketing contaminated products. Conversely, in some organisations it was common practice to check positive results where they are close to set limits by other

procedures. However frequent occurrence of false positive results could lead to the unnecessary delay of consignments, which is economically undesirable.

The overall view

Testing for mycotoxins in cereal products other than for maize is considerably less than for mycotoxins in products such as nuts and dried fruit while the use of rapid test methods in the cereal industry is currently on a small scale.

Analytical methods for mycotoxins have progressed from the original methods which relied heavily on TLC to the more sophisticated methods using HPLC, GC or Mass Spectrometry. They can give accurate and sensitive results if operated with appropriate controls. The main drawback of the instrumental techniques is the high capital cost of the equipment, the need to use highly trained analysts, the limited number of analyses which can be performed per day and the time to obtain results. Personnel in laboratories equipped with HPLC and GC equipment are often dismissive about test kits.

There is a considerable reluctance to use rapid test methods especially amongst chemists and laboratories well equipped with instrumentation in use for other analyses. Some of this scepticism is based on lack of information or appreciation of the purpose of the rapid test while some is founded on a previous bad experience or lack of sensitivity. Publicly available validation data is generally limited and performance has often not been assessed against accepted standard methods. Where a company has developed in-house or other information, such data may be provided to a customer.

Until January the only regulation for mycotoxins in cereals in the UK was the animal feedings stuffs regulation which proscribes maximum permissible levels for aflatoxin in six animal feed ingredients (of which only maize is a cereal) and in compound feedingstuffs in which wheat and/or barley is usually the major component. In January 1999, the EC introduced limits for aflatoxin in a range of commodities including cereals and cereal products. However, flatoxins, rarely and only under exceptional conditions, occur in UK grown wheat or barley, so that there is little pressure to test for this group of mycotoxins.

Concern over mycotoxins in cereals is currently centred on ochratoxin A for which there is no regulatory limit. In the absence of such controls the main incentive to test is to supply assurance or certification to purchasers of grain that consignments are free from or low in ochratoxin A or other mycotoxins, or where a 'due diligence' approach is adopted.

The likelihood is that requirement to test cereals or mycotoxins will increase as the number of mycotoxins subject to legislation increases. It seems certain that ochratoxin A will be the next mycotoxin subject to EC regulation although the time-scale for this is unclear.

The total time required for any test is increased when that required for proper representative sampling is included. This added time can however be quite short eg where automated sampling of lorries is carried out.

Demanding analytical performance data are laid down Nationally and within the EC for collection of information from surveys. Satisfactory validation data is unavailable for test kits to allow their use for this purpose.

However, where the incidence of mycotoxin contamination is low, rapid screening methods with suitable sensitivity could be useful in identifying positive samples which can then be re-analysed using instrumental methods if full quantitation was required.

Current and future developments

Several suppliers are currently developing or have marketed new kits in response to regulatory or customers needs. However, some of these improvements appear to be modification of established techniques. For example, ELISA based methods have been made faster by reducing reaction times and adjusting the amount of antibodies bound to test components.

Alternative techniques such as using molecular imprints (in which materials are engineered to produce a form which recognises specific compounds), dip stick tests or

lateral flow devices such as employed in pregnancy testing do not appear to have been explored. The relatively small market for mycotoxin tests have probably discouraged investment into development of these techniques.

CONCLUSIONS

The majority of tests which are carried on grain in the UK are for aflatoxins in maize or in cereal-based animal feeds. The reason for this is the existence of legislation for aflatoxins in animal feed and in all cereals from 30 June 1999. There is no current legal obligation to test for other mycotoxins such as ochratoxin A.

There currently appears to be only a limited requirement for rapid tests for mycotoxins in UK-grown grain. The UK Food Advisory committee recommend reducing ochratoxin A in the food to the lowest level technically possible. As cereals are the main dietary source of ochratoxin A this implies the need for a large increase in testing if this recommendation is to be followed. The introduction of legislation for ochratoxin A and possibly other mycotoxins at a later date is likely to add impetus to the need for testing. The long term market for rapid test kits for UK grown grain will ultimately depend on the approach to the control of ochratoxin A in the food chain. However, legislation for ochratoxin A is expected to lead to an increase in monitoring.

Proper storage of grain in purpose built stores should prevent development of further toxins and hence a logical critical point for controlling ochratoxin A would be at intake to stores. A reliable rapid test would enable each lorry load or grain to be checked. The test would need to be simple, reliable and very rapid (about 5 minutes). No test currently available would appear to fully satisfy all the requirements for sensitivity, reliability, speed and cost although a card test for ochratoxin A is understood to be under development.

Such a test might also be applicable to quality testing at other stages in the food process, particularly when speed is essential.

Organisations with limited or no laboratory facilities prefer to contract out analysis to contract laboratories.

While rapid test kits should be ideally suited to screening and quality control, lack of sensitivity and unreliability is commonly cited as a reason for not using test kits. There is a considerable scepticism amongst analysts about the performance of test kits especially in well equipped laboratories..

There is a reluctance to use solvents such as chloroform and a desire to keep handling of mycotoxin-containing standards to a minimum in field or on-site situations.

Some suppliers of mycotoxin tests market items for use with HPLC methods as well as rapid test kits. Hence improving test kits sales may merely reduce sales of products for use with HPLC methodology. This could lead to a conflict of interests in a limited market although this is strongly disputed by at least one of the companies involved who consider that a wider availability of choice encourages testing.

RECOMMENDATIONS

The future need of the grain trade for testing for mycotoxins needs to be assessed.

A validation study of candidate test kits currently marketed (or under development) for ochratoxin A in cereals should be carried out. Tests should have a limit of detection of at least 1μg/kg and offer semi- or fully quantitative results at 5μg/kg. Full co-operation of the suppliers should be encouraged. The design of the study should be critically appraised before proceeding. Performance should be appraised against a fully validated HPLC method.

No test kit appears to currently offer the speed and reliability necessary to test for ochratoxin A in grain delivered by lorry. The development of a suitable rapid test for ochratoxin A for use by the grain trade should be supported. New formats based on

current products and technology should be explored concentrating on simplicity and reliability. There appears to be scope for improving card tests or rapid dip-stick type tests. Exploration of techniques applied for testing in other fields but not yet applied to mycotoxins should be encouraged. Extension of such development to other mycotoxins such as deoxynivalenol should be considered.

Users of test kits should be probably trained and should have a basic understanding of the principles involved.

There is an urgent need to agree sampling protocols which are soundly based while remaining simple and economic to carry out. The distribution of ochratoxin A in bulk grain and in lorries needs to be determined as the basis for recommending such sampling protocols.

ACKNOWLEDGEMENTS

The author would like to thank everyone who assisted in supplying information which made the production of this Report possible.

REFERENCES

BENNETT, G. A., NELSEN, T., and MILLER, B. M., 1994, Enzyme-Linked Immunosorbent Assay for detection of zearalenone in corn, wheat and pig feed: Collaborative study. *Journal of the Association of Official Analytical Chemists International*, **77**, 1500-1508.

BOSCH, H., TESSIN, N., BELLMER, H-G., and BÄSSLER, H. M. S., 1994, Assessment of the suitability of enzyme immunoassays for mycotoxin analysis in the brewing industry. *Monatsschrift für Brauwissenschraft,* **47**, 196-202. (In German)

DUTTON, M., 1996, Fumonisins, Mycotoxins of Increasing Importance: Their Nature and Their Effects. *Pharmacology and Therapeutics,* **70**, 137-161.

FLANNIGAN, B., 1986, *Aspergillus clavatus*- an allergenic, toxigenic, deteriorgen of cereals and cereal products. *International Biodeterioration,* **22**, 79-89.

FOOD AND AGRICULTURE ORGANISATION OF THE UNITED NATIONS, 1997, *Worldwide Regulations for Mycotoxins,* 1995, A compendium. FAO Food and Nutrition Paper. (Rome: Food and Agriculture Organisation).

MAGAN, N., CAYLEY, G. R., and LACEY, J., 1984, Effect of water activity and temperature on mycotoxin production by *Alternaria alternata* in culture and on wheat grain. *Applied and Environmental Microbiology*, **47**, 1113-1117.

MILLER, J. D., 1995, Fungi and Mycotoxins in Grain: Implications for Stored Product Research. *Journal of stored Product Research,* **31**, 1-16.

MOSS, M. O., 1996, Mycotoxins-Centenary Review. *Mycological Research,* **100**, 513-523.

NAWAZ, S., SCUDAMORE, K. A., and RAINBIRD, S. C., 1997, Mycotoxins in Ingredients of Animal Feeding Stuffs: I. Determination of *Alternaria* mycotoxins in oilseed rape meal and sunflower seed meal. *.Food Additives and Contaminants*, **14** 249-262.

PARK, D. L., MILLER, B. N., NESHEIM, S., TRUCKSESS, M. M., VEKICH, A., BIDIGARE, B., MCVEY, J. L., and BROWN, L. H., 1989, Visual and semi-quantitative spectrophotometric ELISA screening method for aflatoxin B1 in corn and peanut products: Follow up collaborative study. *Journal of the Association of Official Analytical Chemists*, **72**, 638-643.

POLLEY, R. W., TURNER, J. A., COCKERELL, V., ROBB, J., SCUDAMORE, K. A., SANDERS, M. F., and MAGAN, N., 1991, Survey of *Fusarium* species infecting Winter wheat in England, Wales and Scotland, 1989 and 1990. *Home Grown Cereals Authority Project Report No. 39,* 100 pages

SCUDAMORE, K. A., 1993a, Occurrence and significance of mycotoxins in cereals grown and stored in the United Kingdom. *Aspects of Applied Biology,* **36**, 361-374.

SCUDAMORE, K. A., 1993b, The occurrence of mycotoxins in food and animal feedstuffs in the United Kingdom. In: *Proceedings of the United Kingdom Workshop on Occurrence and Significance of Mycotoxins*, edited by K. A.

Scudamore, (Slough: Central Science Laboratory, MAFF), 21-23 April 1993, pp. 172-185.

SCUDAMORE, K. A., CLARKE, J. H., and ATKIN, P. M., 1986, Natural occurrence of fungal naphthoquinones in cereals and animal feedstuffs. Proceedings of the Biodeterioration Society Meeting on spoilage and mycotoxins of cereals and animal feedstuffs. *International Biodeterioration*, **22** (supplement), 71-81.

SCUDAMORE, K. A., and MACDONALD, S. J., 1998, A collaborative study of an HPLC method for the determination of ochratoxin A in wheat using immunoaffinity column clean-up. *Food Additives and Contaminants*, **15** 401-410.

SCUDAMORE, K. A., and WILKIN, D. R., 1999, A study to determine whether on-floor ambient drying systems are conducive to the formation of ochratoxin A in grain. *Home Grown Cereals Authority Project Report No. 1613,* x pages

SCUDAMORE, K. A., PATEL, S., and BREEZE, V., 1999,Surveillance of stored grain from the 1997 United Kingdom harvest for ochratoxin A. *Food Additives and Contaminants*, **16**, 281-290.

SMITH, J. E., and HENDERSON, R. S., 1991, *Mycotoxins and animal foods*, (Boca, Raton USA: CRC Press).

SMITH, J. E., LEWIS, C. W., ANDERSON, J. G., and SOLOMONS, G. L., 1994, Mycotoxins in human health and nutrition. A literature review carried out on behalf of the Agro-industrial division, E2, of the European Commission Directorate-General XII for scientific research and development. *Mycotoxins in Human Nutrition and Health,* 300 pages.

WATSON, D. H., 1985, Toxic Fungal Metabolites in Food. *CRC Critical Reviews in Food Science and Nutrition,* **22**, 177-198.

WEDDELING, K., BÄSSLER, H. M. S.,DOERK, H., and BARON, G., 1994, Preliminary tests to determine the suitability of enzyme immunological methods for the detection of deoxynivalenol. ochratoxin A and zearalenone in malting barley, malt and beer. *Monatsschrift für Brauwissenschraft,* **47**, 94-98. (In German)

APPENDIX 1 COMMERCIAL SUPPLIERS

ADGEN Ltd
Watson Peat Building
Auchincruive
Ayr, KA6 5HW
Scotland

TEL: 01292 525275
FAX: 01292 521080
e-mail: Adgen@au.sac.ac.uk

Digen Ltd
65 High Street
Wheatley
Oxford, OX33 1XT

TEL: 01865 872156
FAX: 01865 872413
e-mail: digen@clara.net

Labtech International Ltd
1 Acorn House
The Broyle
Ringmer
East Sussex, BN8 5NW

TEL: 01273 814 888
FAX: 01273 814 999
e-mail: labtech@labtech.co.uk

Rhône-diagnostics Technology Ltd
West of Scotland Science Park
3.06 Kelvin Campus
Maryhill Road
Glasgow, G20 0SP
Scotland

TEL: 0141 945 2924
FAX: 0141 945 2925
e-mail: RDT@rhone-diagnostics.co.uk

Tepnel BioSystems Ltd
Newtech Square
Deeside Industrial Park
Deeside, CH5 2NT

TEL: 01244 833555
FAX: 01244 281908

APPENDIX 2 Listing of available rapid test kits and claimed performance

1. ADGEN LTD

Kits produced by Neogen Corporation, Lancing, USA.

The Company has its own UK laboratory and expert staff and offers customer support and advice.

Veratox®

Principle Direct competitive ELISA. Colour development in the final solution is used as the indicator for the presence or absence of the mycotoxin and is monitored using a strip or plate reader or a spectrophotometer.

Item	Claimed performance
Mycotoxin tests available	**Ochratoxin A, vomitoxin (deoxynivalenol), T-2 toxin, zearalenone**, aflatoxins and fumonisins
Test time	Within 20 minutes
Use	Quantitative, in-line testing
Sensitivity*, µg/kg	**Ochratoxin A** 3 **Zearalenone** 100 **Vomitoxin** 300 **T-2 toxin** 50 Total aflatoxins 2
Test products	Cereals, many other products
Safety	No hazardous solvents
Approval/Validation	AOAC, IUPAC, USDA-FIGIS

* = Sensitivity might be increased by adjusting protocols after reference to the suppliers.

Procedure Sample is ground and extracted in 70% methanol. After filtering the sample is ready for testing. Test sample and standard(s) are added to mixing wells with reagent (conjugate solution). Samples are then transferred to the antibody-coated wells and left to incubate for 5-15

minutes depending on the toxin under test. The wells are then washed with water and reagent (substrate) is added and incubated for a further 5-15 minutes. The reaction is stopped by addition of a further reagent. A positive result occurs if the sample is lighter blue or pink compared to the standard.

Equipment required Test kits, mycotoxin control standards, pipettors, strip or plate reader or spectrophotometer.

Validation data identified

The Veratox and Agri-Screen kits have been quite widely evaluated by the AOAC and some are recognised as official test kits. A copy of an approval list from 1996 is shown in Appendix 3, figure 1. Levels of mycotoxins tested were set with US legislation in mind and are generally higher than would now be appropriate for the UK and EC.

The most comprehensive testing has been for aflatoxins in non-cereal products. The Veratox AST aflatoxin kit has AOAC approval for testing corn, cornmeal, popcorn, gluten meal, corn germ meal, corn/soy blend, wheat, soybean meal, rice, and milo for aflatoxins between 5 and 320 ppb. Certificate 931201.

The Veratox kit for vomitoxin has been jointly evaluated by the AOAC and USDA-GIPSA. The AOAC test involved a comparison of the method using 40 samples of naturally contaminated wheat and corn with an GC-ECD method. The USDA-GIPSA tested the kit using one naturally contaminated sample of wheat and wheat spiked at 1 and 5 ppb using HPLC as the comparative method. Further comparisons were carried out with flour, middlings, bran, barley, malted barley, oats and corn. The minimum amounts that could be quantified were between 250 and 500 µg/kg. The standard deviation obtained for wheat at 0.500 µg/kg was 0.113

Two relevant papers describing collaborative studies for Agriscreen products have been found. One describes a study on visual and semi-quantitative spectrometric ELISA method for aflatoxin B_1 in corn and peanut products (Park *et al.* 1989) and the other a study of an ELISA method for zearalenone in corn, wheat and pig feed (Bennett *et al.* 1994).

Cross reactivity

For zearalenone	∝-zearalenol	107%	β-zearalenol	29%
	∝-zearalanol	35%	β-zearalanol	25%
For aflatoxin B_1	aflatoxin B_2	25%	aflatoxin G_1	31%
	aflatoxin G_2	12%		
For vomitoxin, T-2 toxin and ochratoxin A	no information found			

Agri Screen®

Principle Direct competitive ELISA. Colour development in the final solution is used as the indicator for the presence or absence of the mycotoxin and is monitored by eye or by spectrophotometer if available.

Item	Claimed performance	
Mycotoxin tests available	**Ochratoxin A, vomitoxin (deoxynivalenol), T-2 toxin, zearalenone**, aflatoxins and fumonisins	
Test time	10-20 minutes	
Use	Qualitative, in-line testing	
Sensitivity*, µg/kg	**Ochratoxin A**	5
	Zearalenone	250
	Vomitoxin	500
	T-2 toxin	50
	Total aflatoxins	4
Test products	Cereals, many other products	
Safety	No hazardous solvents	
Approval/Validation	AOAC, IUPAC, USDA-FIGIS	

Procedure As for Veratox products, but fewer standards included. Measurement is by eye and qualitative

Equipment required Test kits, mycotoxin control standards, pipettors

Validation data identified

The Agri-Screen kit has been evaluated for zearalenone in wheat. The kit was evaluated in an international collaborative study by 22 laboratories and results published (Bennett and Nelsen, 1994). Values found fell within acceptable accuracy limits for wheat, barley, oats, maize and various cereal products at a level of 1000 ppb upwards. The kit was awarded Certificate 950702, 25 July 1995.

Agri-Screen for vomitoxin

Approved for qualitative testing of wheat and grain at ≥ 1000ppb. USDA-FGIS Official Test Kit

Agri-Screen for aflatoxin

Approved for qualitative testing of corn, roasted peanuts, mixed feeds and cottonseed products ≥ 15 ppb.

Note: Methodologies of test kit approved, but not the kit itself.

2. CORTECS DIAGNOSTICS LTD

Biokits® ochratoxin A and aflatoxin assays

These kits are being phased out. New kits are to be launched in 1999 and marketed by Tepnel Biosystems Ltd.

Item	Claimed performance
Mycotoxin tests available	**Ochratoxin A** and aflatoxins
Test time	Within minutes
Use	Accurate, semi-quantitative, minimal training required
Sensitivity*, µg/kg	**Ochratoxin A** 1-300 Total aflatoxins 2-20, 2-200
Test products	**Ochratoxin A** -cereals, kidneys and serum, Aflatoxins -cereals, nuts, dried fruit, animal feeds
Safety	No hazardous solvents
Approval/Validation	AOAC

3. DIGEN LTD

Kits produced by R-Biopharm GmbH, Darmstadt, Germany

Note: These competitive ELISA tests have been marketed for some time in kit form. Tests have taken between 1-4 hours to perform. Recently (1998), 'fast' (quantitative) or 'express' (qualitative/semi-quantitative) versions of these have been introduced and the table following lists performance data for the fast versions and the procedure for the express DON.

R-Biopharm RIDASCREEN® FAST versions

Principle Direct competitive ELISA. Colour development in the final solution is used as the indicator for the presence or absence of the mycotoxin and is monitored by eye or by using a strip or plate reader or a spectrophotometer.

R-Biopharm RIDASCREEN® EXPRESS DON

It is understood that EXPRESS formats may be produced for other mycotoxins.

Procedure for DON Express Sample is ground and extracted by shaking with 10% methanol. After filtering sample is ready for testing. Test sample and standard(s) are added to mixing wells coated with antibodies with reagent (conjugate solution). DON antibody-is then added and left to incubate for 3 minutes. The wells are then washed well with water and reagent (substrate) is added and incubated for a further 2-3 minutes. The reaction is stopped by addition of a further reagent. Compare sample colour with standard within 10 minutes by eye or using a spectrophotometer

Item	Claimed performance
Mycotoxin tests available	**Ochratoxin A, vomitoxin (deoxynivalenol, DON), T-2 toxin, zearalenone**, citrinin, aflatoxins, fumonisins
Test time	About 30 minutes regardless of the number of samples*.
Use	Quantitative
Sensitivity, µg/kg	**Ochratoxin A** 5 **DON** 111 (express version 500) **T-2 toxin** 50 **Zearalenone** 50 **Citrinin** 15 Aflatoxin 5
Test products	Cereals, etc.
Safety	No hazardous solvents
Approval/Validation	see below

* RIDASCREEN DON EXPRESS about 10 minutes

Equipment required Test kit, micropipettes, glassware, microtitre plate spectrophotometer reader (optional)

Validation data identified

Two scientific papers (Weddling *et al.* 1994, Bosch *et al.* 1994) report evaluation of the original kits for the determination of ochratoxin A, zearalenone and deoxynivalenol in the brewing industry. Samples included barley, malted barley, beer and cereals. 7 laboratories took part in the second trial and were sent four naturally contaminated samples of cereals and one beer. No independent analyses were available and results were statistical assessed on the values reported for each laboratory. Limits of

detection were very low compared with some of the other kits evaluated elsewhere, 0.4 ppb for ochratoxin A cereals and 0.1 ppb in beer, and 1.25 ppb for zearalenone and deoxynivalenol in barley and malt. Full details are available in the papers. It was concluded that the kits were suitable for the detection of these mycotoxins in the brewing industry. However, the results showed considerable variation in the results from different laboratories, some of these discrepancies being suggested as due to unfamiliarity with the methods. In another evaluation of several test kits for ochratoxin A (personal communication), this product performed satisfactorily for qualitative detection at the 5 ppb level.

No validation data for the new rapid kits was available although it is understood that AOAC and FIGIS approvals are being sought.

Cross reactivity

For ochratoxin A	ochratoxin C	44%	ochratoxin B	14
	ochratoxin α	<0.1%		
For deoxynivalenol	- and 15- acetyl derivatives all 100%			
	9 other trichothecenes all <0.255			
For T-2 toxin	acetyl T-2 toxin	114%	HT-2 toxin	7%
	iso-T-2 toxin	2%		
For zearalenone	zearanol	27.7%	α- zearalenone	42%
	β-zearalenone	13.8%		
For aflatoxin total	aflatoxin B_2	200%		
	aflatoxin G_1	15%	aflatoxin G_2	16%
Aflatoxin B_1 specific	aflatoxin B_2	0.2%		
	aflatoxin G_1	1.1%	aflatoxin G_2	<0.1%

4. LAB TECH INTERNATIONAL LTD

Kits produced by Vicam, Waterdown, USA

OchraTest™

DONtestTAG™

ZearalaTest™

AflaTest™ (total aflatoxins) or **Afla B™** (specific for aflatoxins $B_1 + B_2$)

AflaTip™

Principle Samples are extracted with solvent. The extract is purified using an immunoaffinity column. Mycotoxins are detected by their fluorescence, after reaction if necessary

Item	Claimed performance
Mycotoxin tests available	**Ochratoxin A, vomitoxin (deoxynivalenol, DON), zearalenone**, aflatoxins, fumonisins
Test time	Within 10 minutes (15 minutes for deoxynivalenol)
Use	Quantitative, no special skills required, 1 year shelf life
Sensitivity*, µg/kg	**Ochratoxin A** 1-100 **Deoxynivalenol** 500-5000 **Zearalenone** 200 upwards Aflatoxins 1-500
Test products	Cereals and other commodities
Safety	Require less toxic materials than conventional methods
Approval/Validation	AOAC, USDA, FGIS

Procedures

OchraTest™ The sample is ground and weighed and then blended with salt and a methanol/water mixture. The samples is then added to an OchraTest IA column. Ochratoxin A is absorbed and the column washed with wash solution followed by water. Ochratoxin A is then eluted with eluting solution into a cuvette which is then placed in a calibrated fluorimeter and the results are read in ppb. (The column extract can alternatively be used for HPLC or TLC).

DONtestTAG™

The sample is ground, weighed and blended with PEG (polyethylene glycol) /salt water mixture and filtered. Developer and resin (containing monoclonal antibody to DON) are added to the solution and incubated for 10 minutes. The slurry mixture is passed into an empty column to pack. The column is washed with water and deoxynivalenol is eluted from the column with methanol into a cuvette. This is placed in a calibrated fluorometer and the results are read in ppm. (The column extract can alternatively be used for HPLC).

ZearalaTest™ The sample is prepared as for ochratoxin A and then added to a ZearalaTest IA column. Zearalenone is absorbed and the column washed twice with water and then eluted with methanol into a cuvette. Developer solution is added and the cuvette placed in a calibrated fluorometer and the results are read in ppm. (The column extract can alternatively be used for HPLC or TLC).

AflaTest™ (total aflatoxins) or **Afla B™** (specific for aflatoxins $B_1 + B_2$)

In principle as for **OchraTest™**

AflaTip™ The sample is ground and weighed and blended with salt and a methanol/water mixture. The extract is filtered and passed through a IA column. Aflatoxins are absorbed and the column washed with water. Aflatoxins are then eluted with methanol into the tip which is viewed under UV light in a viewing box. The fluorescence is matched with the value on a comparator card.

Equipment required IA columns, solvents, developer solutions, fluorimeter and cuvettes or Aflatip kit including comparator card, UV viewing box

Validation data identified

Vicam operate a high quality assurance programme. Although immunoaffinity columns and kits have been validated by the AOAC for a number of years, there appears to be much less readily available data for the rapid tests which are the subject of this review. An extract from an evaluation of the DONtestTAG™ kit is given as Appendix 3, table 7. Excellent results were obtained for barley, malted barley, corn and oats using spiked samples from 0.5 ppm to 5 ppm. This leaves the question of how the kit would perform with naturally contaminated grain in which other closely related compounds might be present. Levels of mycotoxins tested were set with US legislation in mind and are generally higher than would now be appropriate for the UK and EC.

The UK Mycotoxin Analytical Panel evaluated a method for the determination of ochratoxin A using the OchraTest™ IA column and a second commercial IA column with HPLC detection (Scudamore and MacDonald 1998). Excellent results were obtained with samples of wheat at 3 and 6 ppb. While this does not evaluate the fluorescent end point detection used in rapid tests it provides excellent assurance of the operation of the method prior to this final step. While these data do not apply to detection in rapid tests where fluorescent is the end point they provide excellent assurance of the operation of the method prior to this final step. In a separate study using a mixture of naturally contaminated and spiked samples, a good correspondence between fluorimeter readings and HPLC results were obtained, Appendix 3, table 8.

Cross reactivity 'Does not detect related compounds'.

5. RHÔNE DIAGNOSTICS TECHNOLOGIES LTD

Rhône-diagnostics products are developed in-house except for the EZ-card tests.

The Company has its own UK laboratory and expert staff and offers customer support and advice.

EZ-screen tests

Principle Sequential competitive enzyme immunoassay carried out in absorbent wells contained in a small card format.

Procedure 1 drop of the sample extract is added to the sample site on the card and the negative control to a second site. Enzyme and a further solution are added successively to each site. After removing excess liquid, 2 drops of developer are added to each site. Results are interpreted after a further 5 minutes.

Item	Claimed performance
Mycotoxin tests available	**Ochratoxin A, vomitoxin (deoxynivalenol), T-2 toxin, zearalenone**, aflatoxins
Test time	Within 15 minutes
Use	Positive/negative at a pre-set level. Very simple; no expertise required
Sensitivity*, µg/kg	**Ochratoxin A** 20 **Zearalenone** 100 **T-2 toxin** 100 Total aflatoxins 5
Test products	Cereals, many other products
Safety	No hazardous solvents
Approval/Validation	USDA/FGIS as a qualitative test

Equipment required Test kit

Validation data identified

Tests have been based on % of times the correct result has been obtained using standards from 0 to the action level. This does not take into account interference from real samples.

OCHRASCAN®

AFLASCAN®

Principle (cereals)

Sample extract is filtered and added to an IA column containing monoclonal antibodies. After elution, this solution is cleaned up further by adding chloroform and buffer. The chloroform layer is passed through a florisil tip. The fluorescence under uv is compared with that on a card supplied.

Procedure (OCHRASCAN®)

10g of ground cereal is blended with 200 ml of 1% sodium bicarbonate for 2 minutes. After filtering, the extract is applied to an IA column. After washing, ochratoxin A is eluted and mixed with buffer solution and chloroform. The chloroform layer is passed through the florisil tip. The fluorescence intensity on the tip is compared to that on the comparitor card

Item	Claimed performance
Mycotoxin tests available	**Ochratoxin A,** and aflatoxins
Test time	Within 25 minutes
Use	Easy to use; minimal training required. Accurate, semi-quantitative
Sensitivity*, µg/kg	**Ochratoxin A** 2 Total aflatoxins 1
Test products	Cereals, coffee (OA) +many other products
Safety	Uses non-toxic standards
Approval/Validation	In-house and customer evaluation, AOAC, EC

Equipment required OCHRASCAN® kit containing IA columns

Validation data identified

The products are tested thoroughly in-house and development is carried out with close attention to and support for customers needs and problems. They are tested in collaboration with customers. Immunoaffinity columns for ochratoxin A, zearalenone and aflatoxin used with HPLC have been subject of method inter-comparisons. IA column-based methods have also been evaluated within the Standard Measurement and Testing Programme of the EC.

The UK Mycotoxin Analytical Panel evaluated a method for the determination of ochratoxin A using the OCHRAPREP® IA column and a second commercial IA column with HPLC detection (Scudamore and MacDonald 1998). Excellent results were obtained with samples of wheat at 3 and 6 ppb. While this does not evaluate the fluorescent end point detection used in rapid tests it provides excellent assurance of the operation of the method prior to this final step. While these data do not apply to detection in rapid tests where fluorescent is the end point they provide excellent assurance of the operation of the method prior to this final step.

Cross reactivity no data obtained

APPENDIX 3 SELECTED INFORMATION AND VALIDATION DATA

Table 4; Summary excerpt (cereals) from EC Directive on 'Methods of sampling for the official control of the levels for flatoxins in certain foodstuffs'

Lot size, tonnes	Size or number of sublots	No. of incremental samples	Aggregate sample size, kg
≥1500	500 tonne	100*	30
>300 and<1500	3 sublots	100*	30
≥50 and ≤300	100 tonne	100*	30
>20 and ≤50	-	100$	10
>10 and ≤20	-	60$	6
>3 and ≤10	-	40$	4
>1 and ≤3	-	20$	2
≤1	-	10$	1

incremental sample size: * = 300g $ = 100g

Table 5: Comparison of results obtained for AFLASCAN® and OCHRASCAN® test kits with HPLC, customer data (agreed release)

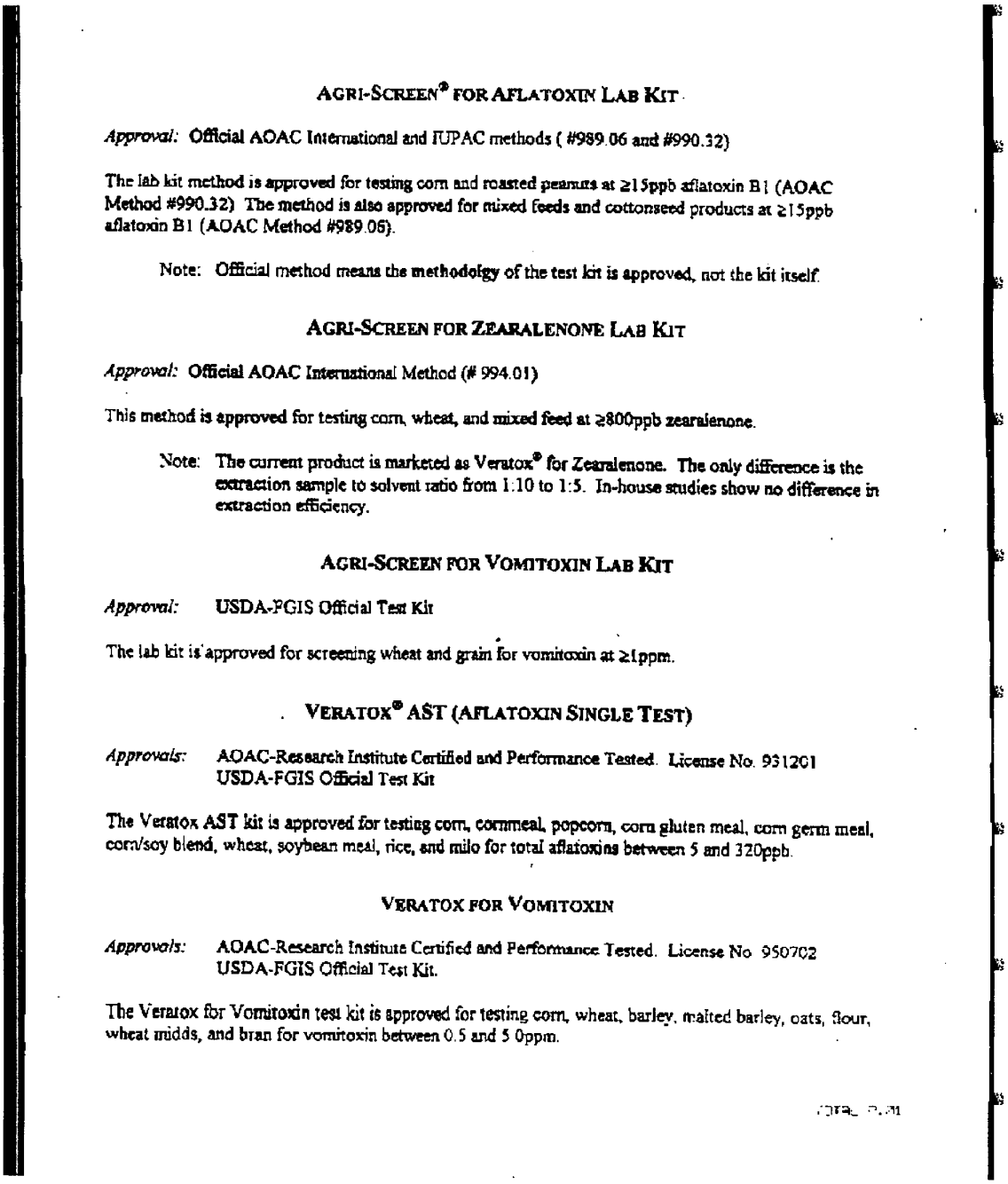
	Mycotoxin, µg/kg	
Commodity	Test kit	HPLC
AFLASCAN® -total aflatoxins		
Not specified	30-35*	22
"	12-15*	6.2
"	15-20*	9.7
"	10-15*	9.2
"	25*	45
"	25-30*	22 (TLC Result)
"	35*	24
OCHRASCAN® - ochratoxin A		
Dried fruit	6	7
Wholemeal flour	8	10

* = modified procedure, not as specified by the supplier

Table 6 : Comparison of results obtained for OCHRASCAN® test kits with HPLC, for coffee, customer data (agreed release)

Ochratoxin A, µg/kg	
Test kit	HPLC
>10	6.0
not detected	0.2
not detected	0.2
4	3.1
4-10	7.0
>10	28

Figure 1: Example of approvals issued by International bodies such as AOAC International



AGRI-SCREEN® FOR AFLATOXIN LAB KIT

Approval: Official AOAC International and IUPAC methods (#989.06 and #990.32)

The lab kit method is approved for testing corn and roasted peanuts at ≥15ppb aflatoxin B1 (AOAC Method #990.32) The method is also approved for mixed feeds and cottonseed products at ≥15ppb aflatoxin B1 (AOAC Method #989.06).

Note: Official method means the methodolgy of the test kit is approved, not the kit itself.

AGRI-SCREEN FOR ZEARALENONE LAB KIT

Approval: Official AOAC International Method (# 994.01)

This method is approved for testing corn, wheat, and mixed feed at ≥800ppb zearalenone.

Note: The current product is marketed as Veratox® for Zearalenone. The only difference is the extraction sample to solvent ratio from 1:10 to 1:5. In-house studies show no difference in extraction efficiency.

AGRI-SCREEN FOR VOMITOXIN LAB KIT

Approval: USDA-FGIS Official Test Kit

The lab kit is approved for screening wheat and grain for vomitoxin at ≥1ppm.

VERATOX® AST (AFLATOXIN SINGLE TEST)

Approvals: AOAC-Research Institute Certified and Performance Tested. License No. 931201
USDA-FGIS Official Test Kit

The Veratox AST kit is approved for testing corn, cornmeal, popcorn, corn gluten meal, corn germ meal, corn/soy blend, wheat, soybean meal, rice, and milo for total aflatoxins between 5 and 320ppb.

VERATOX FOR VOMITOXIN

Approvals: AOAC-Research Institute Certified and Performance Tested. License No. 950702
USDA-FGIS Official Test Kit.

The Veratox for Vomitoxin test kit is approved for testing corn, wheat, barley, malted barley, oats, flour, wheat midds, and bran for vomitoxin between 0.5 and 5.0ppm.

Table 7: Test performance for DON kit

Your Silent Partner
In Food Safety

USDA-FGIS

Report on Data Requested in "Design Criteria and Test Performance Specifications for Quantitative Deoxynivalenol (DON) Test Kits"

By

Scott Kruger
Brian McAlice

With
Barb Kohn, Ph.D.
Nancy Zabe
Michelangelo Pascale
Lisa Cahill
Jesse LoMonaco
for VICAM
313 Pleasant Street
Watertown, MA 02172
Tel: 800-338-4381
Fax: 617-923-8055

Table7 (continued): Test performance for DON kit

3. RESULTS AND PERFORMANCE SPECIFICATIONS FOR WHEAT

A. Time Required for Completion of Analysis

The time required to run DONtest TAG™ is less than thirty minutes, with room temperature incubation accounting for one third of that time. Shorter incubations are also possible, if one wishes for a faster, less precise test.

B. Identified Commodities in Addition to Wheat: Barley, Malted Barley, Oats and Corn

Data on these commodities is included with this report.

C. Avoidance of Toxic or Hazardous Substances

DONtest TAG™ uses methanol to elute DON from affinity columns. There are trace amounts of toxic chemicals in the DONtest TAG™ Developer Solution and DONtest TAG™ Internal Calibrator Solution, but since they are nonvolatile and users will not come into contact with the reagents under normal use, there will be no exposure of toxic or hazardous substances.

D. Accuracy Testing of Fortified Wheat Samples

A summary of data is presented in Table 1. Table 2A contains the data on analysis of wheat by GC/ECD to verify that wheat used had a DON level of < 0.1 ppm. Tables 2B-F then contain tabulated data from each operator. Raw data for all DONtest TAG™ analyses can be found in Appendix A.

TABLE 1. Assay of Fortified Wheat*

Fortification (ppm)	Acceptable Accuracy Limit (ppm)	Accuracy of DONtest TAG™ Mean (ppm)	Acceptable Precision Limit (SD)#	Precision of DONtest TAG™ (SD)#
0	± 0.2	0.01	0.36	0.05
0.5	± 0.3	0.48	0.40	0.37
1.0	± 0.4	0.90	0.42	0.27
2.5	± 0.7	2.4	0.46	0.30
5.0	± 1.2	4.6	0.54	0.54

*Wheat contains less than 0.1 ppm DON by GC/ECD (Table 2A)

#SD Standard Deviation

Wheat samples were spiked as directed, dried and extracted. Multiple extracts were made at each spike level and pooled to produce a sufficient amount of five different extracts for three operators to run analyses in triplicate. Sometimes, more than three operators were assigned to perform testing, since all operators had additional business responsibilities and it was not possible to designate three operators to be solely available for running DONtest TAG™. The presentation of data in the body of the report, and the raw data in Appendix A, list operators for each sample.

Table 2A. Assay of Unfortified Wheat Samples by GC/ECD*

No.	Sample ID	(DON) ppm
1	Wheat A	< 0.1
2	Wheat B	< 0.1
3	Wheat C	< 0.1
4	Wheat D	< 0.1

*GC/ECD analyses were performed as described in " Determination of Deoxynivalenol in Wheat, Barley, and Malt by Column Cleanup and Gas Chromatography with Electron Capture Detection." Journal of AOAC International Vol. 79, No.2, 1996 (p. 472-475).

Table 7 (continued): Test performance for DON kit

4. RESULTS AND PERFORMANCE SPECIFICATIONS FOR COMMODITIES OTHER THAN WHEAT

A. Accuracy Testing of Fortified Commodity Samples

A summary of data is presented in Table 8. Tables 9, 10, 11 and 12 contains data from individual operators, the LOD determinations for commodities, and the data on analysis of clean commodities by the reference method, which was done to determine that the commodities used are substantially free of DON contamination. Some methods used for determination of commodity cleanliness, such as GC/ECD, report undetectable levels of DON as <0.1 ppm.

TABLE 8. Assay of Fortified Commodities*

Fortification (ppm)	Acceptable Accuracy Limit (ppm)	Accuracy of DONtest Mean (ppm)	Acceptable Precision Limit (SD)'	Precision of DONtest (SD)'
BARLEY				
0.5	±0.3	0.43	0.40	0.29
2.5	±0.7	2.4	0.46	0.28
5.0	±1.2	5.6	0.54	0.33
MALTED BARLEY				
0.5	±0.3	0.41	0.40	0.25
2.5	±0.7	2.1	0.46	0.27
5.0	±1.2	5.1	0.54	0.37
CORN				
0.5	±0.3	0.76	0.40	0.36
2.5	±0.7	2.9	0.46	0.21
5.0	±1.2	4.8	0.54	0.47
OATS				
0.5	±0.3	0.62	0.40	0.26
2.5	±0.7	2.3	0.46	0.29
5.0	±1.2	5.6	0.54	0.50

'SD Standard Deviation

*Commodities contain less than 0.1 ppm DON by GC/ECD (Dr. Howard Casper, North Dakota State University). Commodity samples were spiked as directed, dried and extracted. Multiple extracts were made at each spike level and pooled to produce a sufficient amount of extracts for operators to run analyses in triplicate. Sometimes, more than three operators were assigned to perform testing, since all operators had additional business responsibilities and it was not possible to designate three operators to be solely available for running DONtest. The presentation of data in the body of the report, and the raw data in the appendix, list operators for each sample.

Table 8: Comparison of ochratest using a fluorimeter with HPLC results

⇒ In order to compare of the fluorometer and HPLC methods, a study was performed using 24 samples (9 unspiked and 15 spiked). The table below shows the results of this study.

Sample	Spike level	Fluorometer	HPLC (values corrected for 70% recovery)
96RM33012	0 ppb	0.24	0
	5 ppb	4.9	5.3
	20 ppb	24	21.1
96RM26006	0 ppb	0.63	0
	5 ppb	4.0	4.9
	20 ppb	19	18.5
96RM33010	0 ppb	0.64	0
	5 ppb	5.0	5.6
	20 ppb	23	20.1
96RM28029	0 ppb	1.6	0.32
96RM33003	0 ppb	0.7	0
96RM33011	0 ppb	0.4	0
96RM33006	0 ppb	0.64	0
	10 ppb	13.5	11.8
	30 ppb	30	27.9
	50 ppb	55	53.5
96RM33011	0 ppb	0.53	0
	10 ppb	10	10.2
	30 ppb	26	27.6
	50 ppb	52	51.1
96RM33014	0 ppb	0.28	0
	10 ppb	10	10
	30 ppb	26	23.4
	50 ppb	47	41.9

⇒ The results indicate a very good correlation between the two different methods. When analysed by linear regression, the results showed r=0.997.