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Application Note - Mycotoxins

Title: Break the mould, cover all your mycotoxin screening needs simultaneously using revolutionary Biochip Array Technology with the option to customise your test array

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Aim

Multiplex screening of a broad range of mycotoxins from a single sample of animal feed with detection values complying with regulatory limits.

Background

Mycotoxins are a group of naturally occurring toxins produced by certain fungi, commonly known as moulds, which are harmful to humans, domestic animals and livestock. Ochratoxin A for instance is a nephrotoxic and nephrocarcinogenic compound, zearalenone has an estrogenic action and is significantly toxic to the reproductive system of animals.¹ Some mycotoxins are also immunosuppressive, reducing resistance to infectious disease.

Mycotoxins are found in a wide range of foods and feeds, particularly in areas with climates of high temperature and humidity. Mycotoxins can enter the food or feed chain through contaminated crops, in particular cereals, but also nuts, beans, spices, dried fruit, oilseeds, coffee and cocoa, poultry meat and kidneys, pig kidneys and pork sausages.² Contamination may also occur post-harvest during storage, transport, and processing stages of the food or feed supply chain.

Factors of both a scientific and socio-economic nature largely influence the establishment of mycotoxin limits and regulations, therefore various mycotoxin limits and regulations in feed have been set by multiple food agencies worldwide.^{3, 4}

Currently chromatographic, spectrometric and immunoassay based techniques are used for the detection of these toxins. Biochip Array Technology (BAT) enables simultaneous quantitative determination of multiple analytes from a single sample.^{5,6}

With BAT from Radox Food Diagnostics, multiple mycotoxins can be screened from a single feed sample, as multiple immunoassays take place at the same time in discrete test sites on the biochip surface. This increases the output of test results. Furthermore this methodology is flexible, with MycoFlex the user can customise the mycotoxins to be screened according to the most prevalent in a particular geographical region for example.

Simple sample preparation

The feed sample preparation for the Mycotoxins biochip arrays is simple, fast, highly robust and generic for all the mycotoxins on the array.

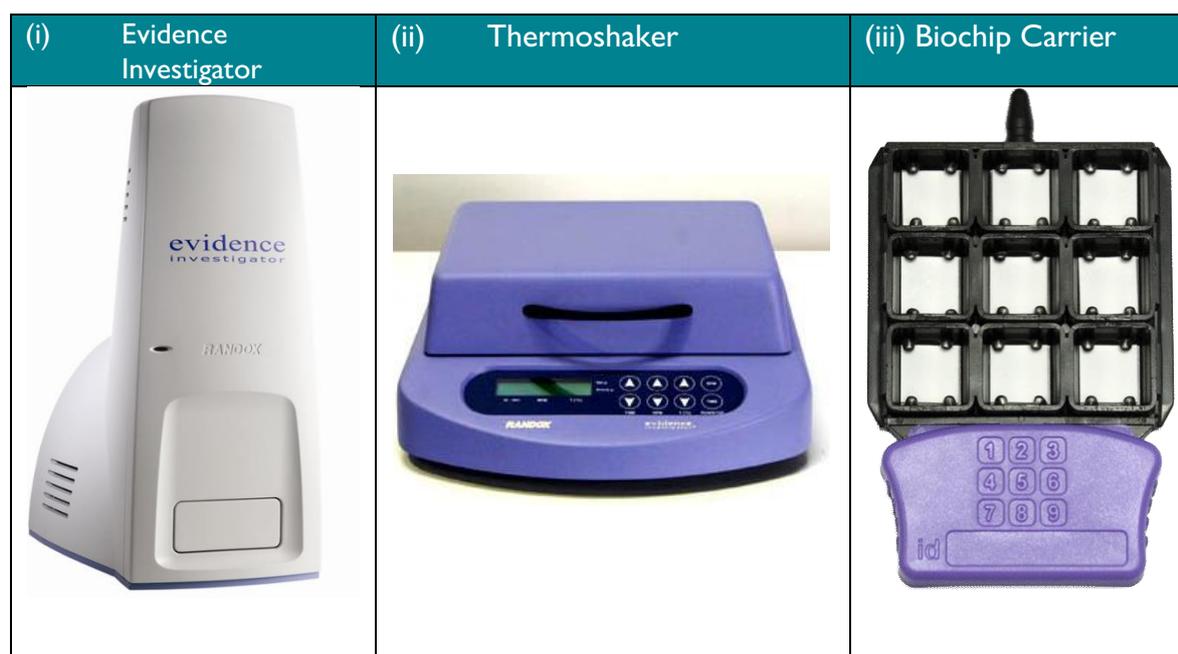
It **does not** require:

- X the use of multiple sample extraction methods - one extraction method is suitable for all of the mycotoxins on the array.
- X the use of immunoaffinity columns for sample clean up
- X the use of Solid Phase Extraction (SPE) for sample clean up
- X the use of filters
- X the use of large volumes of organic solvents

Note: 45 samples can typically be extracted for all mycotoxins within 45 minutes equating to approximately 1 minute per sample.

Simultaneous immunoassays on the biochip platform

For the quantitative detection of mycotoxins (and their cross-reactants), simultaneous competitive chemiluminescent immunoassays, arrayed on the biochip surface, are employed. The biochip (9mm x 9mm) is also the vessel for the immunoassays. The assays are applied to the well established semi-automated Evidence Investigator analyser (i). With this system, the immunoassay steps (i.e. reagent loading and washing) are manually performed under controlled incubation conditions as a customised ThermoShaker (ii) unit is provided. The biochips are supplied in carriers (iii) (3x3 biochips per carrier = 9 reaction vessels per carrier). A handling tray with the capacity to accommodate 6 carriers (54 biochips) is also provided with the system. Once the biochip carrier is inserted in the image station of the analyser the dedicated software processes, reports and archives the data generated for retrospective access. This system enables the quantitative screening of up to 10 mycotoxins (and their cross-reactants) in less than 3 hours for a batch of 45 samples.



Simultaneous detection of a broad range of mycotoxins
Mycotoxins that can be detected simultaneously with Randox Food Diagnostics mycotoxin biochip arrays are shown in Table I.

Table I Mycotoxins detected simultaneously with biochip arrays

Mycotoxins Detected	
Aflatoxin B1 (including B2)	Fumonisin (including B1/B2/B3)
Aflatoxin G1 (including G2)	Ochratoxin A
Deoxynivalenol (including 3-acetyldeoxynivalenol / 15-acetyldeoxynivalenol)	Paxilline
Diacetoxyscirpenol	T-2 toxin (including HT-2 toxin)
Ergot alkaloids (including their related -inines)	Zearalenone (including metabolites)

Optimal analytical performance

With over 2000 participants in over 100 countries, FAPAS^R proficiency testing scheme is the largest and most comprehensive in the food sector. It provides an independent check of a laboratory's procedures ensuring the delivery of quality results and Randox Food Diagnostics is a regular participant in this scheme. The participants' data are analysed statistically providing assigned values to the test sample and individual z-scores for the sample result submitted from each participant (in normal circumstances about 95% of z-scores will lie in the range $-2 \leq z \leq 2$ over time). Test material from the scheme was assessed with Randox Food Diagnostics mycotoxin biochip arrays and table 2 shows data for Aflatoxin B1 (AFBI), Deoxynivalenol (DON), Ochratoxin A (OTA) Zearalenone (ZON) and total T-2 and HT-2. The results from the mycotoxin biochip assays lie comfortably within the range and are fit for purpose when compared with other testing methods.

Table 2. Data from test material from proficiency testing scheme assessed with mycotoxin biochip arrays

Mycotoxin	FAPAS Assigned value (µg/kg)	Randox Food Diagnostics result (µg/kg)	z-score
Ochratoxin A	4.72	3.71	-1.00
Zearalenone	245	192.87	-1.10
Deoxynivalenol	1981	1537.94	-1.60
Aflatoxin B1	9.45	5.90	-1.70
Total T-2 & HT-2	693	496.61	-1.70

Conclusion

The mycotoxin biochip arrays allow for the simultaneous quantitative detection of up to 10 mycotoxins and their cross-reactants from a single sample. With MycoFlex it is possible for the user to customise the number of toxins that are screened as per the user's individual needs. BAT is a labour saving, multi-analytical solution for the accurate quantifiable screening of all the most prevalent mycotoxins to comply with global regulations. Combining confidence with convenience Randox Food Diagnostics is breaking the mould of laborious mycotoxin test methods.

References

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