UNIVERSITY OF KENTUCKY

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From: Darrell Johnson, Director of Regulatory Services
Cynthia Gaskill, Veterinary Clinical Toxicologist, UK Veterinary Diagnostic Lab

RE: Aflatoxin analysis

Many producers are concerned about mycotoxins in their corn or corn silage crops due to drought damage. Of special concern to dairy producers is aflatoxin at greater than 20 ppb. This is the maximum allowable in feed for lactating dairy cattle, as it may pass into the milk and is a known carcinogen. However, other classes of livestock can be fed higher levels of aflatoxin. Other mycotoxins may be of concern as well. Below is a list of laboratories that will run aflatoxin or complete mycotoxin screens. I have included prices when possible. It is advisable to contact the lab prior to submitting samples to determine whether they are doing quantitative or qualitative tests, using approved methods, and if you are planning on testing forages such as silage and TMR rations, make sure the laboratory can analyze these complex feeds. See pages 3 - 4 for more information on various analytical methods.

A sampling of laboratories that provide mycotoxin analyses include:

UK Veterinary Diagnostic Lab
1490 Bull Lea Road
Lexington, KY 40511
(859) 257-8283
Analysis with HPLC for accurate quantification
$30.00 for aflatoxin only or $100 for complete mycotoxin panel
www.vdl.uky.edu

Breathitt Veterinary Diagnostic Laboratory
P.O. Box 2000 – 715 North Drive
Hopkinsville, KY 42241-2000
(270) 886-3959
www.breathitt.murraystate.edu
$44.50 for mycotoxin panel (TLC)

A & L Labs
2790 Whitlen Road
Memphis, TN 38133
(800) 264-4522
www.allabs.com
$55.00 for qualitative aflatoxin at 20 ppb

Barrow-Agee Lab
1555 Three Place

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Memphis, TN 38116-3507
(901) 332-1590
www.balabs.com
$75.00 for aflatoxin on HPLC

Cumberland Valley Analytical Lab
P. O. Box 669
Maugansville, MD 21767
(800)CVASLAB
www.foragelab.com
$25.00 for aflatoxin (ELISA method)

Dalryland Laboratories
217 E. Main Street
Arcadia, WI 54612
(608) 323-2123
www.dairylandlabs.com
2012 special of $21.00 for aflatoxin
Normal 48 hour turnaround

Dairy One Lab
730 Warren Road
Ithaca, NY 14850
(800) 496-3344
www.dairyone.com
$65.00 for complete mycotoxin panel

Eurofins
2200 Rittenhouse St. Suite 175
Des Moines, IA 50321
(515) 280-8378
www.eurofins.com
Contact for analyses and pricing

Holmes Laboratory, Inc.
3559 US 62
Millersburg, OH 44654-8834
(800) 344-1101
www.holmeslab.com
Aflatoxin $35.00 in addition to forage
Analysis; $53.00 for aflatoxin only

Midwest Laboratories
13611B Street

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Omaha, NE 68144
(402) 334-7770
www.midwestlabs.com
$35.00 aflatoxin (LC/MS)

Romer Labs
1301 Stylemaster Drive
Union, MO 63084-1156
(635) 583-8600
www.romerlabs.com
Contact for analyses and pricing

Information for feed mills or others looking to initiate on-site mycotoxin analyses:
Commercial kits can be purchased from a number of manufacturers. See the a list of methods, manufacturers, and instrumentation requirements from the USDA Department of Agriculture Grain Inspection, Packers and Stockyards Handbook at http://www.gipsa.usda.gov/publications/fgis/handbooks/aflatoxin/aflatoxin-ch01.pdf

A partial list of manufacturers includes:
Neogen Corporation (www.neogen.com)
Pickering labs (www.pickeringlabs.com)
R-Biopharm Rhone Ltd (www.r-biopharmrhone.com)
Romer Labs (www.romerlabs.com)
Vicam (www.vicam.com)
Tepnel Biosystems (www.tepnel.com)

MYCOTOXIN TESTING METHODS

Methods for mycotoxin analyses fall into two main categories: **(1) rapid screening methods**; and **(2) conventional confirmatory methods**. The most appropriate method depends on the intended use of the results – for example, is a "yes/no" result sufficient, or is exact quantitation needed? Brief summaries of the various testing methods are provided below.

1. The benefits of **rapid screening methods** are generally lower cost (often < $30 per mycotoxin), faster results (often same day), less skilled technical requirements, portability, and more rapid through-put of large numbers of samples. The downside of rapid screening methods is generally increased cross-reactivity and matrix interference, co-extraction of other substances from the sample, and considerably greater chance of false positive results or false negatives. Also, many methods provide a "yes/no" result, indicating the presence or absence of a mycotoxin above a predetermined value, but cannot give an actual concentration. Most rapid screening methods also require some degree of instrumentation for detection of results, such as a spectrophotometer or fluorometer, but these are less expensive than the instrumentation required for more specific confirmatory testing. And lastly, most rapid screening methods are only valid for specific sample types. Any positive results generated by a rapid screening method should be confirmed with a more selective/specific confirmatory method.

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Examples of direct rapid screening methods available include immunoassay-based methods (eg, enzyme immunoassay [ELISA], fluorescence immunoassay [FIA], flow-injection liposome immunoanalysis, and lateral flow devices); sensors and biosensors such as molecularly imprinted polymers; and thin-layer chromatography (TLC). Other indirect screening methods include Fourier transform infrared spectroscopy (FTIR), near-infrared spectroscopy (NIR), and detection of volatile metabolites of fungi by "electronic noses". Other new emerging rapid methods are becoming more available.

2. The standard **confirmatory methods** provide accurate, selective, and sensitive analyses and generally involve separation methods such as chromatography or electrophoresis. The benefits of these methods are high specificity, high sensitivity, and hence much less risk of false positive or false negative results. Also, these methods can provide actual concentrations of the different mycotoxins, so that the suitability of various feeds for different species of animal can be determined. The downside of these methods includes more expensive instrumentation, much more skilled technical requirements, higher cost per analysis (typically $30 or greater per mycotoxin, or $100 or more for a full panel), and a longer run time for analyses (typically several days to a week). The standard confirmatory methods include high performance liquid chromatography (HPLC) with various detection methods such as fluorescence or mass spectrometric (LC-MS or LC-MS/MS); and gas chromatography for a few select mycotoxins. Other analytical methods are available but much less common.

Sample preparation considerations: Regardless if the method is a rapid screening method or standard confirmatory analysis, most methods require drying and milling of the sample, solvent extraction of mycotoxins from the sample, various "clean-up" methods to remove interfering substances from the extract, and quality control measures such as concurrent analysis of positive and negative controls with known amounts of mycotoxins. Note: Sample collection methods are the single most important factor in variability of results. See other sources for information regarding proper sampling techniques.

Note on black light tests for mycotoxins: The black light test looks for the presence of a fluorescent green/yellow color when a sample is evaluated under a black light. Fluorescence is caused by a substance co-produced by the fungus that produces aflatoxin - not by aflatoxin itself. This is only a presumptive screen, and false positives and false negatives are very common. Many other compounds in grains can fluoresce, and any positive result should be confirmed with a conventional method. This test is inappropriate for any mycotoxin other than aflatoxin. Because of the inaccuracy of this test, it is generally not recommended.

Example of when to choose a rapid screening method: When testing a load of shell corn intended for human food production or for undeclared interstate transport. In such case, the aflatoxin level must be < 20 ppb.
Example of when to choose a standard conventional method: When testing corn for on-farm use, where higher levels of aflatoxin can be fed depending on the species of animal, and determining concentration of aflatoxin greater than 20 ppb is important. Additionally, any sample positive for mycotoxins using a rapid screening method should be confirmed with a standard conventional method.