Importance of Aflatoxin M_1 Determination

Aflatoxins are highly toxic mycotoxins produced by a variety of molds such as *Aspergillus flavus, A. parasiticus* and *A. nomius.* The toxins most frequently detected are Aflatoxin B_1, B_2, G_1, and G_2. Crops may be contaminated during growth, harvest or storage. These toxins are known carcinogens and can be present in grains, nuts, cottonseed and other foods consumed by humans or in animal feed. When animals are fed contaminated feed, Aflatoxin B_1 is converted to M_1 by hydroxylation and is subsequently secreted into the milk of lactating animals. Aflatoxin M_1 is very stable throughout milk processing methods such as pasteurazation. Human breast milk can also contain Aflatoxin M_1 if a lactating woman has consumed food contaminated with Aflatoxin B_1.

To protect humans, regulatory agencies around the world have imposed regulatory limits regarding the amount of Aflatoxins that are allowable in human and animal foods. The European Union has inacted maximum Aflatoxin M_1 levels as follows:

Milk (raw milk, milk used in the production of milk based products and heat treated milk): 0.05 ppb
Infant formula and infant milk: 0.025 ppb

Performance Data

Sensitivity:

	Detection Limit (cut-off)	
Sample	Direct	Concentrated Extract
Milk	0.5 ppb	0.05 ppb
Dry Milk Powder*	0.5 ppb	0.05 ppb
Infant Formula	0.25 ppb	0.025 ppb
Infant Formula Powder*	0.25 ppb	0.025 ppb

*Note: The Detection Limit (cut-off) for the Dry Milk and Infant Formula Powders are based on the prepared "ready to use" form (see Sample Preparation, Section D), not the concentrated powder form.

Specificity: The Abraxis Aflatoxin M_1 Strip Test was not evaluated for various other Aflatoxins (B_1, B_2, G_1, and G_2) as these mycotoxins are not excreted in milk.

General Limited Warranty: Abraxis, Inc. warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

For ordering or technical assistance contact:

Abraxis, Inc.
124 Railroad Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

R041114

Aflatoxin M_1 Strip Test

A Screening Test for Rapid Detection of Aflatoxin M1
in Milk and Infant Formula Samples

Product No. 53012A

1. General Description

The Abraxis Aflatoxin M_1 Strip Test is designed solely for the preliminary screening of milk and infant formula samples. This test is suitable for the qualitative screening of Aflatoxin M_1 in milk and dry milk powder at 0.5 ppb and infant formula (liquid and powder) at 0.25 ppb (see Sample Preparation, Section D). Sample concentration may be performed for samples requiring a lower limit of detection (0.05 ppb for milk and dry milk powder, 0.025 ppb for liquid and powder infant formula, see Section F, Sample Extraction and Concentration). The Aflatoxin M1 Strip Test provides preliminary qualitative test results only. Other conventional methods, such as ELISA, HPLC, or GC/MS, should be used to obtain quantitative results or to confirm positive samples.

2. Safety Instructions

Discard samples according to local, state and federal regulations.

3. Storage and Stability

The Aflatoxin M_1 Strip Test components and reagents should be stored at room temperature (20-30°C). Protect from light and moisture. Reagents may be used until the expiration date on the box.

4. Test Principle

The Aflatoxin M_1 Test Strip consists of a membrane strip containing an Aflatoxin M_1 conjugate. A Control Line, produced by a different antibody-antigen reaction, is also present on the membrane strip. The microtiter wells contain colloidal gold labeled antibodies, which are preincubated with the milk sample or extract. The colloidal gold labeled antibodies move with the milk sample or extract by capillary action along the membrane. In the absence of Aflatoxin M_1 in the milk sample or extract, the colloidal gold labeled antibody contacts the immobilized Aflatoxin M_1 conjugate on the strip. An antibody-antigen reaction occurs forming a visible line. The Control Line is not influenced by the presence or absence of Aflatoxin M_1 in the milk sample or extract, and therefore, should be present in all reactions. The formation of two visible lines indicates a negative result (below the detection limit or cut-off). If Aflatoxin M_1 is present in the milk sample or extract, it competes with the immobilized Aflatoxin M_1 conjugate in the test area for binding sites on the colloidal gold labeled antibody. If a sufficient amount of Aflatoxin M_1 is present in the milk sample or extract, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the immobilized Aflatoxin M_1 conjugate and, therefore no line will develop. If a colored line is not visible in the Test Line region, or if the Test Line is significantly lighter than the Control Line, the milk sample or extract is positive (above the detection limit or cut-off).

5. Limitations of the Aflatoxin M_1 Strip Test

The Aflatoxin M_1 Strip Test is designed for use with milk and infant formula samples. Powder samples must be prepared as instructed in the Sample Preparation Section (Section D). Samples requiring a lower limit of detection must be extracted and concentrated as instructed in the Sample Extraction and Concentration section (Section F).

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test strip or reagents, inaccurate volumes of sample, extract or reagents, too long or too short incubation times, and extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

The Abraxis Aflatoxin M_1 Strip Test provides preliminary qualitative screening results. Reasonable judgment should be applied to any test results, particularly when preliminary positive results are obtained. Other conventional methods, such as ELISA, HPLC, or GC/MS should be used to obtain quantitative results or to confirm positive samples.

A. Warnings and Precautions

1. Prior to use ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
2. Unused microtiter wells should be resealed in the aluminum pouch with desiccant and stored protected from light and moisture.
3. One dropper is used for each sample or extract for the entire strip test procedure. Do not discard the dropper until the test is complete. Use only the appropriate labeled dropper for the appropriate sample or extract as the use of droppers from other samples or extracts will result in contamination and produce inaccurate results.
4. Use only the dropper provided in the pouch with the strip test cassette. Substitution of other droppers could result in inaccurate sample or extract volumes.
5. Use reasonable judgment when interpreting results.
6. As the sample or extract runs through the test strip, the membrane may become tinted pink. This does not invalidate the test or cause inaccurate results.

B. Reagents and Materials Provided

1. Microtiter wells (5 X 10 strips) coated with colloidal gold labeled antibodies, in a resealable aluminum pouch
2. Strip test cassette in a sealed pouch (50)
3. Droppers (50) in a resealable bag
4. 10X Sample Buffer, must be diluted 1:10 with deionized water before use
5. 12 X 2 microtiter well holder

C. Additional Reagents and Materials (not provided)

1. Timer
2. Dry Milk Dilution Buffer (provided upon request)
3. Vortex mixer

D. Sample Preparation

1. ***Milk*** - Adjust to room temperature. Proceed to Strip Test Procedure, Section G.
2. ***Infant Formula (liquid)*** – Adjust to room temperature. Proceed to Strip Test Procedure, Section G.
3. ***Dry Milk Powder*** – Prepare according to package directions using the ***Dry Milk Dilution Buffer*** instead of water. Cap tightly and vortex until the dry milk powder dissolves. Proceed to Strip Test Procedure, Section G.
4. ***Infant Formula (powder)*** – Prepare according to package directions using ***deionized or distilled water***. Cap tightly and vortex until the infant formula powder dissolves. Proceed to Strip Test Procedure, Section G.

E. Additional Reagents and Materials (for Sample Extraction and Concentration, not provided)

1. Deionized or distilled water
2. Ethyl Acetate, HPLC grade
3. Hexane, HPLC grade
4. 4 mL glass vials with Teflon-lined caps
5. 20 mL glass vials with Teflon-lined caps
6. 50 mL conical centrifuge tubes with caps
7. Serological glass pipettes
8. Pasteur pipettes
9. Balance with 0.01 g accuracy
10. Centrifuge capable of 3000 rpm (1700 x g)
11. Nitrogen
12. Water bath or dry block heater capable of 40-60°C
13. Micropipette (1000 µL) with disposable tips

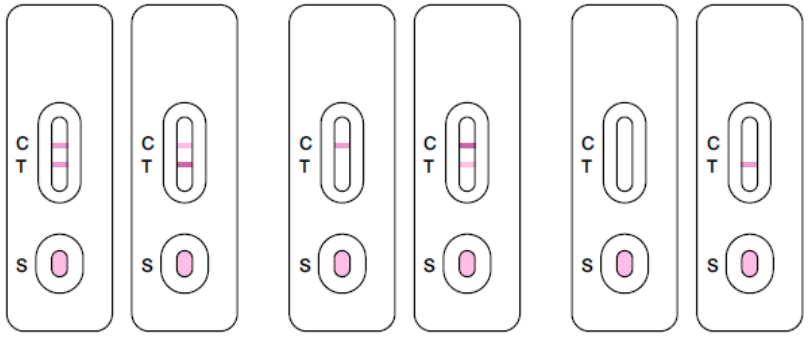
F. Sample Extraction and Concentration (for samples requiring lower detection limits)

1. Prepare 1X Sample Buffer by diluting the 10X Sample Buffer at a ratio of 1:10 with deionized or distilled water (i.e. 1 mL of 10X Sample Buffer into 9 mL of deionized water). Mix thoroughly.
2. Measure 10 mL of prepared sample (see Sample Preparation instructions, Section D) into a 50 mL conical centrifuge tube.
3. ***Carefully*** add 10 mL of Ethyl Acetate to the tube, allowing the Ethyl Acetate to ***slowly run down the side of the conical tube***. *Note: If the Ethyl Acetate and sample mix too rapidly, an emulsion will form and proper separation will not be achieved, requiring the sample to be discarded and re-extracted with a new aliquot.*
4. Cap the conical centrifuge tube tightly. ***Very slowly and gently***, invert the tube repeatedly for 30 seconds. *Note: Do not allow the Ethyl Acetate to mix rapidly with the sample. Do not mix for more than 1 minute. If the Ethyl Acetate and sample mix too rapidly or for too long, an emulsion will form and proper separation will not be achieved, requiring the sample to be discarded and re-extracted with a new aliquot.*
5. Centrifuge the tube at 3000 rpm (1700 x g) for 10 minutes at room temperature. *Note: If proper separation does not occur, discard and re-extract a new aliquot of sample.*
6. Transfer the top Ethyl Acetate layer to a 20 mL glass vial with Teflon-lined cap. Avoid transferring any of the bottom layers.
7. Add 4 mL of the Ethyl Acetate extract (from step 6) to a 4 mL glass vial with Teflon-lined cap.
8. Evaporate to dryness at 40-60°C with a gentle stream of nitrogen.
9. Add 800 µL of Hexane to the 4 mL vial. Cap tightly and vortex.
10. Add 400 µL of 1X Sample Buffer (from step 1) to the reconstituted extract (from step 9). Cap tightly and vortex for 30 seconds.
11. Centrifuge the vial at 3000 rpm (1700 x g) for 10 minutes at room temperature.
12. Remove and discard the top (Hexane) layer. *Note: If the Hexane and 1X Sample Buffer layers remix after centrifuging, discard the sample extract and repeat evaporation (starting with step 7) with a second aliquot of the Ethyl Acetate extract.*
13. Analyze the bottom (1X Sample Buffer) layer with the Aflatoxin M_1 Strip Test. Proceed to Strip Test Procedure, Section G.

G. Strip Test Procedure

1. Prepare sample according to Sample Preparation instructions (Section D) or, for lower detection limits, extract and concentrate according to the Sample Extraction and Concentration instructions (Section F).
2. Open the pouch containing the microtiter well strips. Remove the required number of wells (1 per sample or extract) and place in holder. Place the remaining wells in the pouch and reseal.
3. Open the resealable bag containing the droppers. Remove one dropper per sample or extract and label with the sample id. *Note: One dropper is used for each sample or extract for the entire procedure. Do not discard the dropper until the test is complete. Use only the appropriate labeled dropper for the appropriate sample or extract as the use of droppers from other samples or extracts will result in contamination and produce inaccurate results.*
4. Open the required number of pouches containing the strip test cassette (1 per sample or extract). Remove the cassette being careful not to touch the membrane of the strip test. Label one cassette for each sample or extract.
5. Using the appropriate dropper, place 6 drops (about 200 µL) of the sample or extract into the microtiter well. Incubate for 2 minutes at room temperature.
6. Using the appropriate dropper, carefully mix the sample or extract in the microtiter well by drawing the sample or extract up into the dropper and expelling into the well repeatedly until the reagent coated in the well is dissolved. Incubate for 2 minutes at room temperature.
7. Using the appropriate dropper, transfer the entire contents from the microtiter well to the sample collection region of the test strip cassette. Incubate for 10 minutes at room temperature.
8. Interpret the results ***within 5 minutes*** according to the Interpretation of Results criteria (Section H).

H. Interpretation of Results



1. Negative (below the detection limit or cut-off): T (Test) line is equal to or darker than C (Control) line.
2. Positive (above the detection limit or cut-off): T line is not present or significantly lighter than C line.
3. Invalid Test: C line is not present. Retest using another aliquot of sample or extract with new microtiter well and test strip.

Note: Illustration is for demonstration of test line intensity range only, since overall intensity may vary slightly with different lots of reagents, samples, extracts, etc. Results should be determined ***within 5 minutes*** after completion of the strip test procedure. Interpretation of the results beyond the 5 minutes may produce inaccurate results.