Aflatoxin Test Strip
20 ppb

A Screening Test for Rapid Detection of Aflatoxin in Grain Samples.

ABOUT AFLATOXIN

Aflatoxin is a potent liver toxin known to cause cancer in animals. In swine, aflatoxin can cause reduced weight gain, reduced Abraxislity to resist diseases, hepatitis and death. The Food and Drug Administration (FDA) has established action levels of 20 parts per billion (ppb) for grain and feed products, and 0.5 ppb for milk. Aflatoxins are produced by the fungus *Aspergillus flavus*. This fungus causes a disease in pre-harvest corn known as *Aspergillus* ear rot. Although *Aspergillus flavus* Bis a common fungus growing as a saprophyte on dead plant debris, infection and aflatoxin production in pre-harvest corn occur almost exclusively in years when plants are severely stressed by drought. Aflatoxin production in the field is favored by high grain moisture, temperatures in the range of 80-100º F, severe drought stress, nitrogen deficiency, and significant insect damage. Except in hot, dry years, aflatoxin in the Midwest is almost exclusively associated with improper storage of grain or feed. Aflatoxin production in stored grain should not occur if grain is sufficiently dried to 13-14% moisture and maintained at that level.

Grain, feed, or milk containing aflatoxin at or above these levels cannot be sold for food or feed in interstate sales. Mixing aflatoxin contaminated grain with sound grain for sale is illegal. In the U.S. corn and other grain with less than 20 ppb aflatoxin can be sold as normal grain. Recommended limits in feed are: [i]

- 0.5 ppb for milk
- 20 ppb for dairy animals
- 100 ppb for breeding cattle, breeding swine, and mature poultry
- 300 ppb for finishing cattle and swine.

INTENDED USE

This Rapid Aflatoxin Test is designed solely for use in preliminary screening of grain samples. It is a competitive inhibition immunoassay for the qualitative detection of aflatoxin in samples such as corn, corn meal and rice. The test produces a discernible positive result in grain samples containing aflatoxin in quantities of approx. 5 ppb or higher. The Rapid Aflatoxin Test provides only a preliminary qualitative analytical test result. Other methods must be used to obtain a more confirmed analytical result. Professional judgment should be applied to any test results, particularly when preliminary positive results are used. HPLC or GCMS are recommended as methods of choice for confirmation of positive results obtained with the Rapid Aflatoxin Test.

INTRODUCTION

The Rapid Aflatoxin Test is a qualitative one-step competitive inhibition immunoassay for the detection of aflatoxin. It detects the presence of aflatoxin at 5 ppb or higher in grain samples by utilizing highly specific reactions between antibodies and aflatoxin in grain samples. The need for this product is illustrated by recent literature discussing rapid, on-site tests for aflatoxin.[2,3,4]

TEST PRINCIPLE

The toxin conjugate competes for antibody binding sites with toxins that may be present in the grain sample. The test device consists of a membrane strip to which a conjugate of the toxin of interest is attached. A colloidal gold labeled antibody is located at one end of the membrane. A control line, produced by a different antibody/antigen reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of mycotoxins in the grain sample, and therefore, it should be present in all reactions. In the absence of toxin in the grain sample, the colloidal gold labeled antibody complex moves with the grain sample by capillary action to contact the immobilized aflatoxin conjugate. An antibody-antigen reaction occurs forming a visible line in the 'test' area.

The formation of two visible lines indicates a negative test result. This means the test did not detect the toxin at or below the cut-off point established for the toxin.

If aflatoxin is present in the grain sample, it competes with the immobilized toxin conjugate in the test area for the antibody binding sites on the colloidal gold labeled antibody complex. If a sufficient amount of toxin analyte is present, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the toxin conjugate. If a colored line is not visible in the Test Line region, or Test Line is lighter than negative controls, aflatoxin is present at levels of concern. Available Aflatoxin Controls may be used to approximate the quantity of toxin present in grain samples.

MATERIALS PROVIDED

1. Aflatoxin Test Strips
2. Package insert sheet

MATERIALS REQUIRED BUT NOT SUPPLIED:

3. 50 ml screw cap vial for extracting specimen
4. Graduated cylinder for preparing Extraction Solution (70% Methanol/DI Water)
5. Methanol
6. Deionized Water
7. 1.7 ml microcentrifuge tubes
8. Pipets to deliver 250 ul
9. Timer

WARNINGS AND PRECAUTIONS

1. This test is for *screening* use by professionals only.
2. 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.
3. The desiccant vial containing the test strips must remain completely closed except for opening to remove test strips. When re-closing, snap lid firmly.
4. Avoid cross-contamination of grain samples by using a new container for each specimen.

STORAGE

The Rapid Aflatoxin Test should be stored at room temperature (15° to 30°C) or refrigerated (2° to 8°C). The test strip and grain extract should be at room temperature before use.

SAMPLE COLLECTION AND HANDLING

1. Prepare a solution of 70% methanol in PBS by mixing Methanol and PBS in a ratio of 70:30.
2. Add 10 gm grain to 20 ml 70% methanol/ PBS pH 7.4.
3. Shake mixture for 3 minutes and allow to settle for 2 minutes.
4. Mix 1 ml of extract from above with 1 ml PBS pH 7.4.

ASSAY PROCEDURE

1. Equilibrate test strip and grain extract to room temperature before conducting any testing.

2. Transfer 500 ul of diluted grain extract (from step 3 above) to a small test tube.
3. Remove test strip from the desiccant vial and dip into diluted extract with arrows pointing downward.
4. Allow the test to develop for 10 minutes and read the result as explained below under Interpretation of Results.

INTERPRETATION OF RESULTS
Negative test results may be visible within 2-3 minutes. It is recommended that results be reviewed after 10 minutes when strips are completely developed.

Figure 1: Positive Result – Aflatoxin greater than 20 ppm. Lower aflatoxin levels produce test line of increasing intensity.

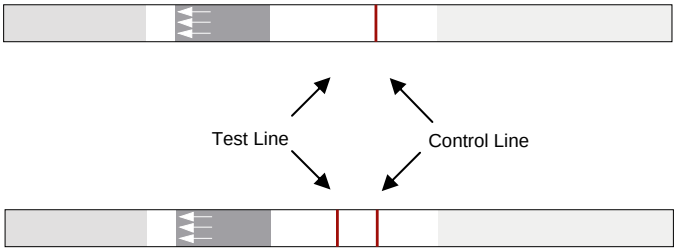

Figure 2: Negative Result – Aflatoxin less than 20 ppm

Control Line	*Test Line*	*Interpretation*
No control line present	No test line present	Invalid result
Control line present	Distinct test line present	0 ppb
Control line present	Moderate intensity test line present	Between 0 and 20 ppb
Control line present	No test line present	>20 ppb

TEST INTERPRETATION

LIMITATIONS OF PROCEDURE
The assay is designed for use with grain extracts. The Rapid Aflatoxin Test provides only a preliminary qualitative test result. Use another more quantitative analytical method to obtain a confirmed quantitative analytical result. Apply professional judgment to any test result, particularly when preliminary positive results are observed.

SENSITIVITY
The ABRAXIS Rapid Aflatoxin Test will detect aflatoxin at 5 ppb or lower. At this level the test line exhibits moderate intensity. At greater than 20 ppb the test line is not visible. When compared with samples of known aflatoxin contamination, it is possible to obtain a semi-quantitative result.

CONTROLS
It is good laboratory practice to use positive and negative controls to ensure proper test performance. Grain samples containing known quantities of Aflatoxin should be run on each lot of test strips to provide a reference for line intensity to be expected.

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