When animals are fed grains and feeds contaminated with aflatoxins, aflatoxin B1 is present in grains and animal feeds. Aflatoxins are carcinogenic and can be present in grains and animal feeds, such as Aspergillus parasiticus and Aspergillus flavus. Aflatoxin M1 is a toxic metabolite of aflatoxin B1, which is produced by molds such as Aspergillus flavus and Aspergillus parasiticus. Aflatoxin M1 is a competitive immunoassay (ELISA) in microwell format for the detection and quantitation of aflatoxin M1 (AFM1) in milk and dairy products.

**ABSTRACT**

Aflatoxins are widely considered to be one of the most potent naturally-occurring carcinogens. Aflatoxin M1 is a toxic metabolite that is secreted in milk by livestock that have consumed feed contaminated with aflatoxin B1. Aflatoxin M1 is present in milk and dairy products. The assay is very sensitive with a limit of detection of 0.1 ppt and a quantitation range from 8-100 ppt. The assay is specific to AFM1 and does not cross-react with any other aflatoxins or aflatoxin M2.

**INTRODUCTION**

Aflatoxin M1 is a toxic metabolite of aflatoxin B1, which is produced by molds such as Aspergillus flavus and Aspergillus parasiticus. Aflatoxins are carcinogenic and can be present in grains and animal feeds.

When animals are fed grains and feeds contaminated with aflatoxins, aflatoxin B1 is converted by hydrolysis to aflatoxin M1, which is subsequently secreted in the milk of lactating cows. Many governments have established regulations for the maximum permissible amounts of aflatoxin M1 in different dairy products. In the EU, the limit for AFM1 in milk and reconstituted milk powder is 0.005 µg/mL (0.05 µg/g) or 50 ppt. In the USA, the Food and Drug Administration (FDA) established an action level of 0.5 µg/mL (0.5 µg/g) in milk.

**SAMPLE PREPARATION AND EXTRACTION**

**LIQUID MILK**

1. Centrifuge milk samples to remove fat for 10 minutes at 3000 g at 10°C (32°F).
2. After centrifugation, carefully separate and discard the upper cream layer from the defatted supernatant.
3. The supernatant (skimmed milk) will be used directly in the test (100 µl per well).

**DRIED MILK POWDER**

1. Weigh 10 g of milk powder in a flask. Add 100 mL of distilled water.
2. Stir to dissolve and homogenize.
3. Extract in a rotary shaker for 30 minutes.
4. Centrifuge milk samples to remove fat for 10 minutes at 3000 g at 10°C (32°F).
5. After centrifugation, carefully separate and discard the upper cream layer from the defatted supernatant.
6. The supernatant (skimmed milk) will be used directly in the test (100 µl per well).

**TEST PROCEDURE**

1. Add 100 µl per well of control or samples in duplicate wells and incubate for 20 minutes with continuous shake.
2. Wash 5 times with PBS-Tween.
3. Add 100 µl per well of aflatoxin conjugate and incubate for 10 minutes with continuous shake.
4. Wash 5 times with PBS-Tween.
5. Add 100 µl per well of ready-to-use substrate and incubate for 15 minutes with continuous shake.
6. Add 100 µl per well of stopping reagent.
7. Read using ELISA reader with a 450 nm filter.
8. Calculate concentration of AFM1 expressed as ppt.

**TEST CROSS-REACTIVITY**

The test is very specific for AFM1. Table 1 shows that there is no cross-reactivity with AFM2 or other aflatoxins (B1, B2, G1 and G2).

**MILK AND MILK POWDER SPIKING RECOVERY**

**CROSS-REACTIVITY**

**Reactivity**

- AFB2: >10,000 <0.40
- AFG2: >10,000 <0.40
- AFB1: 5 - 9 90 12 80 21 84 57 104
- AFG1: 5 - 9 90 12 80 21 84 57 104
- AFM2: 5 - 9 90 12 80 21 84 57 104
- AFM1: 5 - 9 90 12 80 21 84 57 104

**CHEESE AND BUTTER SPIKING RECOVERY**

**Mean recovery**

- Liquid milk: 90%
- Skim milk powder: 94%
- CRM whole milk powder: 94%
- Cheese and butter: 77%

**Recovery**

- Liquid milk: 90%
- Skim milk powder: 94%
- CRM whole milk powder: 94%
- Cheese and butter: 77%