Importance of Melamine Determination

Melamine is an organic base with the chemical formula $C_3H_6N_6$, and the IUPAC name 1,3,5-triazine-2,4,6-triamine. Melamine is a trimer of cyanamide. Like cyanamide, it is 66% nitrogen (by mass) and provides flame retardant properties to resin formulas by releasing nitrogen when burned or charred. Dicyandiamide (or cyanoguanidine), the dimer of cyanamide, is also used as a flame retardant. Melamine is also a metabolite of cyromazine, a pesticide. It is formed in the bodies of mammals who have ingested cyromazine. Cyromazine is also converted to melamine in plants.

Melamine is used in combination with formaldehyde to produce melamine resin, a very durable thermosetting plastic, and melamine foam, a polymeric cleaning product. The end products containing melamine include countertops, fabrics, glues and flame retardants. Melamine is one of the major components in Pigment Yellow 150, a colorant in inks and plastics. Melamine is also used to make fertilizers.

Ingestion of melamine may lead to reproductive damage as well as bladder or kidney stones, which can lead to bladder cancer. A study in 1953 reported that dogs fed 3% melamine for a year had the following changes in their urine: (1) reduced specific gravity, (2) increased output, (3) melamine crystalluria, and (4) protein and occult blood.

The practice of adding "melamine scrap" to animal feed in order to give the appearance of increased protein content is reported to be widespread in various countries. Melamine has also been intentionally added as a binding agent in fish and livestock feed. This practice can potentially contaminate animal products intended for human consumption such as meat and dairy products. Melamine has also been directly added to foods intended for human consumption. Recently, several companies and individuals were implicated in a scandal involving milk and infant formula which had been adulterated with melamine, leading to kidney stones and renal failure, causing four known infant deaths and sickening nearly 53,000 infants.

The Melamine ELISA allows the rapid and on-site determination of melamine contamination of samples. Less than 1 mL of sample or sample extract is required. The test can be performed in less than 20 minutes. All reagents and equipment needed for the performance of the test are included in the kit.

Performance Data

Test sensitivity: The Abraxis Melamine Strip Test for milk samples will detect Melamine at 250 ng/mL or higher. At this level, the test line exhibits a lesser intensity than that of the control line. When compared with samples of known Melamine concentration, it is possible to obtain a semi-quantitative result.

Samples: A sample correlation between the Abraxis Strip Test and ELISA methods showed a good correlation.

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Melamine Strip Test

(Milk Samples)
Immunochromatographic Strip Test for the Detection of Melamine in Contaminated Milk Samples

Product No. 50005SM (50 Test)

1. General Description

The Abraxis Melamine Strip Test is a rapid immunochromatographic test designed solely for use in the qualitative screening of Melamine in milk at ≥ 250 ppb. Animal feed and other contaminated samples can be tested using the Melamine Feed Strip Test (PN 50005SF). For milk samples, a dilution is performed prior to analysis using the sample buffer provided. The Abraxis Melamine Strip Test provides only preliminary qualitative test results. Samples requiring regulatory action should be confirmed by ELISA, HPLC or other conventional methods.

2. Safety Instructions

Discard samples according to local, state and federal regulations.

3. Storage and Stability

The Melamine Strip Test Kit should be stored between 4–30°C. The test strips, test vials, sample buffer and samples to be analyzed should be at room temperature before use.

4. Test Principle

The test is based on the specific immuno-chemical reaction between an antigen and an antibody. Melamine, if present in a sample, will compete with a melamine conjugate immobilized on the dipstick membrane (test line) for the antibody binding sites of anti-melamine antibodies coupled to gold nanoparticles. If Melamine is present in a sample at a quantity above the limit of detection, it will saturate the antibody binding sites, preventing the antibody from binding with the melamine conjugate immobilized on the test line. Consequently, a test line which is lighter than the control line (or no test line) will occur, indicating a positive result. Conversely, if there is no Melamine present (or Melamine is present below the limit of detection of the test), a test line of equal or greater intensity to the control line will be generated. The control line is not influenced by the presence or absence of Melamine in the sample, as it is produced by a different antibody/antigen reaction, and therefore should be present in all reactions.

Semi-quantitative results can be obtained by comparing the test line intensity of samples to those produced by milk solutions spiked with known amounts of Melamine (control solutions).

5. Limitations of the Melamine Strip Test, Possible Test Interference

Due to the high variability of compounds that might be found in milk, test interferences caused by matrix effects can not be completely excluded.

For best test performance, use fresh milk samples (< 48 hours old), older samples tend to agglutinate and may cause flow problems with the dipstick. If improper flow occurs, filter or centrifuge the sample and re-test.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be:
Inadequate storage conditions of the test strip, too long or too short incubation times, and/or extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The Melamine Strip Test provides only preliminary qualitative test results. Use another, more quantitative, analytical method such as ELISA or instrumental analysis to obtain a confirmed quantitative analytical result. Apply good judgment to any test result, particularly when preliminary positive results are observed.

6. Warnings and Precautions

-Use reasonable judgment when interpreting the test results.
-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.
-For test strips packaged in a desiccant vial, the vial should be kept completely closed except for opening to remove test strips. When re-closing, snap lid firmly.
-Avoid touching or bending the membrane on the dipstick.
-Store Test Kit between 4-30˚C. Do not freeze.
-Avoid cross-contamination of samples by using a new conical vial and disposable pipette for each sample.
-Use only the Melamine Test Strip reagents from one kit lot, as they have been adjusted in combination.

7. Sample Collection and Handling

Collect milk samples in glass containers and test within 48 hours. Milk samples held for longer periods may agglutinate, affecting results. If samples are held more than 48 hours from the time of collection or are agglutinating (as evidenced by reduced flow up the membrane), samples will need to be filtered using a glass fiber filter (available from Abraxis) or centrifuged at 2000 x g for 10 minutes. The middle layer of the centrifuged milk sample will then be removed and used for testing.

Please refer to specific Technical Bulletins for other sample matrices.

A. Materials Provided

1. Melamine Test Strips in a desiccated container
2. Conical test vials
3. Disposable transfer pipettes
4. Sample buffer in dropper bottles (2)
5. User's guide

B. Additional Materials (not provided with the test kit)

1. Timer

C. Test Preparation

1. Adjust the test strip, sample buffer and sample to room temperature before use.
2. Remove the number of test strips required from the package. The remaining strips are stored in the desiccated container, tightly closed.

D. Assay Controls

It is good laboratory practice to use positive and negative controls to ensure proper test performance. Samples which do not contain Melamine (negative controls) as well as samples containing known quantities of Melamine (positive controls) should be analyzed with each lot of test strips to provide a reference for line intensity to be expected when a semi-quantitative result is needed.

E. Assay Procedure (Milk Samples)

1. Test strip(s), sample buffer and sample(s) should be at room temperature before conducting any testing.
2. Label conical test vials for each sample to be tested.
3. Add 3 drops (approximately 100 µL) of the sample buffer to the previously labeled conical test vial(s).
4. Using a separate clean disposable transfer pipette for each sample, transfer 3 drops of the milk sample to the appropriate labeled conical vial.
5. Close the conical test vial tightly and shake for 10-20 seconds to mix.
6. Insert test strip (arrows down), into the conical vial containing the milk sample/buffer mixture.
7. Allow the test to develop for 10 minutes.
8. Remove the test strip. Lay it flat and allow it to dry for 5 minutes. Read the results visually as explained in section F (Interpretation of Results). Results may be interpreted for up to 15 minutes. Beyond 15 minutes, line intensities may be affected and incorrect interpretation may result.

F. Interpretation of Results

Control Line	***Test Line***	***Interpretation***
No control line present	No test line present	Invalid result
Control line present	Test line of equal or greater intensity present	< 250 ng/mL (ppb)
Control line present	Moderate intensity (lesser intensity than control line) or no test line present	> 250 ng/mL (ppb)

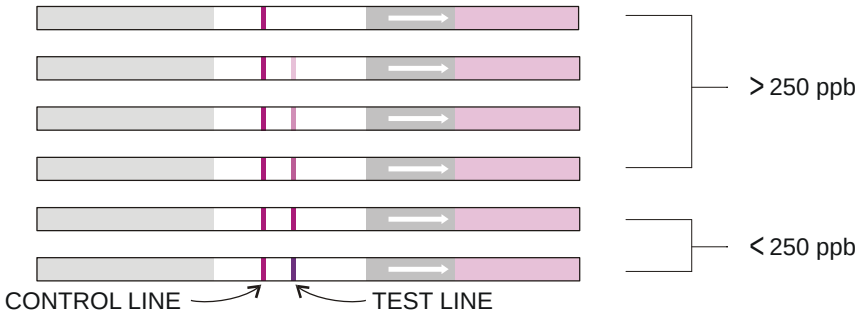

Illustration is for demonstration of test line intensity range only, as overall intensity may vary slightly with different lots of reagents, etc. To obtain semi-quantitative results, solutions of known Melamine concentration must be tested concurrently with samples. Sample test line intensities can then be compared with the test line intensities of those solutions, yielding approximate sample concentrations. Do not use strips run previously to determine semi-quantitative sample concentrations, as test line intensities may vary once strips are completely dry.

G. Additional Analysis

If necessary, positive samples can be confirmed by ELISA, HPLC or other conventional methods.