Operator's Manual Supplement:

Charm® II Test for Antimicrobial Drugs or Aflatoxin M1

Addendum for Testing Cheese, Condensed Milk, Cream, Flavored Milk, Individual Cow Milk, Pasteurized Homogenized White Milk, Powdered Milk, and Whey

Precautions

- **IMPORTANT:** Read all instructions before beginning testing. For complete instructions, refer to the appropriate test's Operator's Manual for Charm II Tests in Milk: Beta-lactams, Tetracyclines, Macrolides, Aminoglycosides, Novobiocin, Sulfa Drugs, Chloramphenicol, Amphenicols, or Aflatoxin M1.
- Preservatives, emulsifiers, flavors, and thickeners (agar, gelatin, xanthum gum) may interfere with assay binding and/or pellet stability.
- Products that originate from raw milk of poor microbial quality (greater than 10^6 CFU/ml) and fermented products may contain the microbial by-product PABA (para-aminobenzoic acid) that may interfere with Charm II Sulfa Drug Test.
- All verified positive sulfa drug and tetracycline results need to be validated by HPLC, HPLC-receptorgram, or the Validation Kit for Sulfa Drugs and Tetracyclines (VKIT) available separately from Charm Sciences, Inc.

Cheese

Sample Preparation

1. Weigh 25 g of cheese. Add 50 ml Zero Control Standard (negative milk). Rapidly heat to melt, mix, then rapidly cool OR homogenize in blender or food processor.
2. Adjust pH to 6.80 to 6.90 with 1M HCl or 1M NaOH.
3. Centrifuge two 6 ml portions for 5minutes to remove solids.
4. Draw 3 ml portion of liquid extract from each tube and combine. Use 5 ml of this prepared cheese sample at 4 ± 2 °C for testing.

Assay Procedure

- Standards and Control Points for raw milk may be used. Sensitivity level is approximately 1/3 that of milk level due to dilution.
- Perform test according to Charm II Test Operator's Manual, using this prepared sample in place of milk.
- See **Precautions** for confirmation of suspect samples**.**

Condensed Milk

Sample Preparation

1. Normalize total solids to 10% by diluting condensed milk with distilled or deionized water. Mix well. Approximately 7 ml are needed per test.
2. Adjust pH to 6.80 to 6.90 with 1 M NaOH or 1 M HCl.
3. Centrifuge 7 ml per test for 5 minutes to remove solids.
4. Draw 5 ml of skim portion from below fat layer for testing.

Assay Procedure

- Standards and Control Points for raw milk may be used.
- Perform test according to Charm II Test Operator's Manual, using condensed milk supernatant in place of milk.

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Cream (Light, Medium, Regular) and Individual Cow Milk

Sample Preparation

1. Centrifuge 7 ml of cream or individual cow milk for 5 minutes. Several test tubes (or a single 16x100 mm test tube) are suggested for cream samples.
2. Draw 5 ml of skim portion from below fat layer for testing. For individual cow milk, avoid protein pellet that may form at bottom of test tube.

Assay Procedure

- Standards and Control Points for raw milk may be used.
- Perform test according to Charm II Test Operator's Manual. Use skim portion in place of milk.

Flavored Milk and Whey

Sample Preparation

1. Normalize total solids to 10% by diluting flavored milk or whey with distilled or deionized water. Mix well. Approximately 7 ml are needed per test.
2. Adjust pH to 6.80 to 6.90 with 1 M NaOH or 1 M HCl.
3. Centrifuge 7 ml per test for 5 minutes to remove solids.
4. Draw 5 ml of skim portion from below fat layer for testing.

Assay Procedure

- Perform test according to Charm II Test Operator's Manual, using skim portion from **Sample Preparation** in place of milk.

Preparation and Storage of Standards

- Use flavored milk or whey known to be antimicrobial drug free as a zero control standard. Follow **Sample Preparation** before testing and for Positive Control reconstitution.
- Use prepared zero control flavored milk or whey supernatant (from above) in place of antimicrobial drug-free milk to reconstitute Positive Control standards according to Operator's Manual.

Pasteurized Homogenized Milk (Skim to Whole)

- The procedure and Control Point used for testing raw milk may also be used for pasteurized, homogenized skim to whole white milk.

Powdered Milk

Sample Preparation

1. Normalize total solids to 10% by reconstituting with distilled or deionized water. Use 40°C water to re-suspend powdered milk, mix well, and cool to 2 to 6°C before testing.
2. Adjust pH to 6.80 to 6.90 with 1 M NaOH or 1 M HCl.
3. Centrifuge 7 ml per test for 5 minutes to remove solids.
4. Draw 5 ml of skim portion from below fat layer for testing.

Assay Procedure

- Standards and Control Points for raw milk may be used.
- Perform test according to Charm II Test Operator's Manual, using reconstituted milk supernatant in place of milk.

Warranty Information