

CHARM[®] II COMPETITIVE ASSAYS

FOR SULFONAMIDES (IMS #9C-10), TETRACYCLINES (IMS #9C-12) AND CHLORAMPHENICOL (IMS #9C-11)

APPENDIX N BULK MILK TANKER SCREENING TEST FORM (Raw Commingled Cow Milk)

GENERAL REQUIREMENTS

1. See Appendix N General Requirements (App. N GR) items 1-8 & 15 _____

SAMPLES

2. See App. N GR item 9 _____

APPARATUS & REAGENTS

3. Equipment _____

- a. Analyzer heater for 13 x 100 mm tubes _____

1. 85±2°C for Sulfonamide Assay _____

2. 35±2°C for Tetracycline Assay _____

3. Check temperature by electronic display, or by placing accuracy checked temperature measuring device in tube containing liquid (bulb submersed) in heating unit; maintain records _____

4. Or, use 6 inch partial immersion thermometer placed directly into small thermometer well in middle of heating unit; maintain records _____

5. Temperature measuring device for each incubator (App. N GR item 3) _____

- b. Ice-water bath, 0.0-4.5°C for Chloramphenicol Assay _____

c. Mixer, Maxi-mixer II or equivalent _____

d. Centrifuge, Whisperfuge[®] or Heraeus[®] (3400 rpm) or equivalent _____

e. Scintillation counter, Charm II or equivalent _____

f. Scintillation fluid dispenser, set to dispense 3 mL _____

1. Checked every six (6) months with Class A graduated cylinder and record; maintain records _____

g. Cotton swabs (not applicable for Chloramphenicol Assay) _____

h. Borosilicate test tubes, 13 x 100 mm _____

- i. Plastic stoppers for tubes _____
- j. Pipettors – Fixed Volume or electronic (see App. N GR item 7) _____
 - 1. 300 µL and appropriate tips _____
 - 2. 5.0 mL and appropriate tips _____
 - 3. 1.0 mL and appropriate tips (not applicable Sulfa Drug Assay) _____
- k. Timer _____

4. Reagents

- a. Scintillation fluid – Optifluor or equivalent supplied by manufacturer of test kits _____
- b. Sulfonamide Assay (Competitive Assay) _____
 - 1. Reagent blister packages: microbial/antibody binder (white) tablet, tracer reagent (pink) tablet _____
 - Lot #: _____ Exp. Date: _____
 - 2. 10 ppb Sulfamethazine standard or multi-standard _____
 - Lot #: _____ Exp. Date: _____
 - 3. Zero control standard _____
 - Lot #: _____ Exp. Date: _____
- c. Chloramphenicol Assay (Chloramphenicol and other Amphenicols) _____
 - 1. Reagent blister packages: reagent (white tablet), tracer reagent (green tablet) and Charcoal (black tablet) _____
 - Lot #: _____ Exp. Date: _____
 - 2. 1 ppb Chloramphenicol standard or multi-standard _____
 - Lot #: _____ Exp. Date: _____
 - 3. Zero control standard _____
 - Lot #: _____ Exp. Date: _____
- d. Tetracycline Assay (Competitive Assay) _____
 - 1. Reagent blister packages: microbial/antibody binder (white) tablet, tracer reagent (orange) tablet _____
 - Lot #: _____ Exp. Date: _____

2. 30 ppb Oxytetracycline standard or multi-standard _____

Lot #: _____ Exp. Date: _____ _____

3. Zero control standard _____

Lot #: _____ Exp. Date: _____ _____

5. Reagent stability _____

a. All tablet reagents stored at -15°C or below _____

b. Positive Control – Lyophilized 10 ppb Sulfamethazine, 30 ppb Oxytetracycline and 1 ppb Chloramphenicol standards _____

1. Reconstitute with 100 mL (measured) Negative Control (allow to sit 15 min prior to use or aliquotting); use within 48 hours at $0.0-4.5^{\circ}\text{C}$ _____

Lab Prep. Date: _____ Lab Exp. Date: _____ _____

2. Or, aliquot within 24 hours and freeze at -15°C or colder in a non-frost-free freezer or in an insulated foam container in a frost-free freezer; use within 2 months _____

Lab Prep. Date: _____ Lab Exp. Date: _____ _____

a. Thaw and use within 24 hours. Store at $0.0-4.5^{\circ}\text{C}$ _____

c. Negative Control – Lyophilized Zero Control Standard (ZCS) or alternatively raw milk qualified to test similar to ZCS _____

1. Reconstitute ZCS according to manufacture instructions. (Allow to sit 15 min prior to use or aliquotting) _____

a. To qualify raw milk, test sample 3 times and average results. Average must be within $\pm 10\%$ of ZCS _____

Lab Prep. Date: _____ Lab Exp. Date: _____ _____

2. Use within 48 hours when stored at $0.0-4.5^{\circ}\text{C}$ _____

3. Or, aliquot within 24 hours and freeze at -15°C or colder in a non-frost-free freezer or in an insulated foam container in a frost-free freezer; use within 2 months _____

Lab Prep. Date: _____ Lab Exp. Date: _____ _____

a. Thaw and use within 24 hours. Store at $0.0-4.5^{\circ}\text{C}$ _____

d. Scintillation fluid expires six (6) months after opening _____

Date opened: _____ Lab Exp. Date: _____ _____

TECHNIQUE

6. Control point and Zero Control Average to be determined for each new lot of reagents _____

a. Sulfonamide Assay Control Point (CP) and Negative Control Average _____

- | | |
|----------------------------------|--------------------------------|
| 1. Run six 10 ppb Sulfamethazine | 2. Run three Negative Controls |
|----------------------------------|--------------------------------|

Sulfamethazine

Negative Control

- 1. _____
- 2. _____
- 3. _____
- 4. _____
- 5. _____
- 6. _____
- Av. _____
- +24% _____
- CP. _____

- 1. _____
- 2. _____
- 3. _____
- Av. _____

b. Chloramphenicol Assay Control Point (CP) and Negative Control Average _____

- | | |
|----------------------------------|--------------------------------|
| 1. Run six 1 ppb chloramphenicol | 2. Run three Negative Controls |
|----------------------------------|--------------------------------|

Chloramphenicol

Negative Control

- 1. _____
- 2. _____
- 3. _____
- 4. _____
- 5. _____
- 6. _____
- Av. _____
- +25% _____
- CP. _____

- 1. _____
- 2. _____
- 3. _____
- Av. _____

c. Tetracycline Assay Control Point (CP) and Negative Control Average _____

1. Run six 30 ppb Oxytetracycline
2. Run three Negative Controls

Oxytetracycline

Negative Control

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
- Av. _____
- +23% _____
- CP. _____

1. _____
2. _____
3. _____
- Av. _____

7. Acceptability of control point determinations _____

- a. If any of the 6 control point determinations deviate from the average, redo that determination _____
 1. For Sulfonamide Assay cannot deviate by more than $\pm 24\%$ _____
 2. For Tetracycline Assay cannot deviate by more than $\pm 23\%$ _____
 3. For Chloramphenicol Assay cannot deviate by more than $\pm 25\%$ _____
- b. If the re-determined value is within the allowed deviation recalculate the average and proceed with testing _____
- c. If the value is not within allowed deviation then another set of 6 standards must be run _____
- d. A common control point for multiple analysts may be used _____
 1. Control point determination performed by one analyst only _____
 2. Control point determination rotated and inclusive of all certified/approved analysts _____
 3. If daily performance check fails and is not resolved by using fresh controls, technique should be reviewed for consistency and corrective action taken as necessary _____

8. Daily Performance and Operation Check (also see App. N GR item 10) _____

- a. The Negative Control tests $\pm 30\%$ (20% Chloramphenicol Assay) established for each new lot of kits _____
- b. The positive control tests less than or equal to the control point _____

- c. If these conditions are not met re-determine control point(s) _____
- 1. Conditions met; proceed with testing _____
- 2. Conditions not met; discontinue testing and seek technical assistance _____

9. Test Procedures _____

- a. Sulfonamide Assay _____
 - 1. Label test tubes, one for each test sample _____
 - 2. Add 1 white tablet to each tube _____
 - 3. Add 300 μ L water to each tube _____
 - 4. Breakup tablets in tubes by vortexing tubes 10 times in a rise and fall motion in 10 sec, white tablets must be completely broken apart or continue vortexing before proceeding _____
 - 5. Mix milk sample(s)/control(s) 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting; use within 3 min (samples/controls must be in appropriate containers to allow the use of vortexing) _____
 - 6. Add 5 mL of mixed sample/control to corresponding tube _____
 - a. Using pipettor (item 3.j.2) with new tip for each sample/control, draw up 5 mL avoiding foam or bubbles _____
 - b. Remove tip from liquid _____
 - c. Expel test portion into appropriate tube _____
 - 7. The following steps must be completed within 40 sec (all sample tubes being assayed) _____
 - a. Add pink tablet to each tube _____
 - b. Vortex tubes 10 times in a rise and fall motion in 10 sec (pink tablets do not breakup) _____
 - 8. Incubate tubes for 3 min at $85\pm 2^{\circ}\text{C}$ _____
 - 9. Remove tubes and centrifuge for 3 min; optionally for 5 min (use same time used to determine control point) _____
 - 10. After centrifugation, immediately pour off milk _____
 - 11. While still draining tubes, remove fat ring with 2 or more cotton swabs, continue until dry, do not touch pellet (do not go much below the fat ring) _____
 - 12. Add 300 μ L of water to tubes and break up pellets using vortex mixer _____

13. Pellets must be completely suspended before proceeding to next step _____
14. Add 3 mL of scintillation fluid to each tube, cap and vortex until uniformly mixed _____
15. Count tubes on scintillation counter for 1 min using [3H] channel _____
16. Record counts as counts per minute (CPM) _____

b. Chloramphenicol Assay _____

1. Label test tubes, one for each test sample _____
2. Add 1 white tablet to each tube _____
3. Add 300 μ L water to each tube _____
4. Breakup tablets in tubes by vortexing tubes 10 times in a rise and fall motion in 10 sec, white tablets must be completely broken apart or continue vortexing before proceeding _____
5. Mix milk sample(s)/control(s) 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting, use within 3 min (samples/controls must be in appropriate containers to allow the use of vortexing) _____
6. Add 1.0 mL of mixed sample/control to corresponding tube _____
 - a. Using pipettor (item 3.j.3) with new tip for each sample/control, draw up 1 mL avoiding foam and bubbles _____
 - b. Remove tip from liquid _____
 - c. Expel test portion into appropriate tube _____
7. The following steps must be completed within 40 sec (all assay tubes being assayed) _____
 - a. Add 1 green tablet to each tube _____
 - b. Vortex tubes as in 4 above _____
 - c. Add black tablet to each tube _____
 - d. Vortex tubes as in 4 above _____
8. Incubate tubes in an ice bath (50% ice, 50% water) at 0.0-4.5°C for 3 min _____
9. Remove tubes and centrifuge for 5 min _____
10. Using 300 μ L pipettor immediately add 300 μ L of centrifuged sample to a new labeled tube (remove by avoiding fat and without disturbing pellet) _____

11. Use fresh tip for each sample _____
12. Add 3 mL of scintillation fluid to each tube, cap and vortex until uniformly mixed _____
13. Count tubes on scintillation counter for 1 min using [3H] channel _____
14. Record counts as counts per minute (CPM) _____

c. Tetracycline Assay _____

1. Label test tubes, one for each test sample _____
2. Add 1 white tablet to each empty tube _____
3. Add 300 μ L water to each tube _____
4. Breakup tablets in tubes by vortexing tubes 10 times in a rise and fall motion in 10 sec, white tablets must be completely broken apart or continue vortexing before proceeding _____
5. Mix sample(s)/control(s) by shaking 25 times in 7 sec through 1 ft movement or vortex for 10 sec at maximum setting; use within 3 min. Dilute 1 mL of sample with 9 mL of Zero Control, repeat mixing.
Controls are not diluted before testing _____
6. Add 5.0 mL diluted milk sample or undiluted control to corresponding tube _____
 - a. Using pipettor (item 3,j,2) with new tip for each sample/control, draw up 5 mL avoiding foam or bubbles _____
 - b. Remove tip from liquid _____
 - c. Expel test portion into appropriate tube _____
7. The following steps must be completed within 40 sec (all sample tubes being assayed) _____
 - a. Add orange tablet to each tube _____
 - b. Vortex tubes 15 times in a rise and fall motion in 20 sec (orange tablets do not breakup) _____
8. Incubate tubes for 3 min at $35\pm 2^{\circ}\text{C}$ _____
9. Remove tubes and centrifuge for 5 min _____
10. After centrifugation immediately pour off milk _____
11. While still draining tubes, remove fat ring with 2 or more cotton swabs, continue until dry, do not touch pellet (do not go much below the fat ring) _____

- 12. Add 300 µL of water to tubes and break up pellets using vortex mixer _____
- 13. Pellets must be completely suspended before proceeding to next step _____
- 14. Add 3 mL of scintillation fluid to a tube, cap and vortex until uniformly mixed. Count tubes on scintillation counter for 1 min using [3H] channel _____
- 15. Repeat step 14 with each tube to be analyzed. _____
- 16. Record counts as counts per minute (CPM) _____

10. Interpretation _____

- a. If the number of the measured activity in the analyzer is greater than the control point, then the sample is Negative (NF) _____
- b. If the number of the measured activity in the analyzer is less than or equal to the control point then the sample is Presumptive Positive _____

11. Verification of Initial Positive Samples (see App. N GR item 11); Confirmation of Presumptive Positive Samples (see App. N GR item 12);and Producer Traceback (see App. N GR item 13) _____

12. Reporting (see App. N GR item 14) _____

13. Handling of Exempt Quantities of Radioactive Materials _____

- a. No mouth pipetting _____
- b. No smoking, eating or use of cosmetics while reagents are being handled _____
- c. Nuclear Regulatory Commission (NRC) licensed facilities must meet requirements as they relate to the use of gloves, other protective measures, and handling of wastes _____
- d. Wash hands thoroughly after handling reagents _____
- e. Wipe up spills immediately and thoroughly _____
- f. Properly dispose of all contaminated waste _____