

**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)**

[Unless otherwise stated all tolerances are ±5%]

**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  $\mu$ L 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  $-15^{\circ}\text{C}$  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  $-15^{\circ}\text{C}$  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 72 hours when stored at  $0.0-4.5^{\circ}\text{C}$

3. Or, aliquot within 24 hours and freeze at  $-15^{\circ}\text{C}$  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

- |  |  |
|--|--|
| <ol style="list-style-type: none"> <li>1. Run six 10 ppb Sulfamethazine</li> </ol> | <ol style="list-style-type: none"> <li>2. Run three Negative Controls</li> </ol> |
|--|--|

Sulfamethazine

Negative Control

- |  |   |
|--|---|
| <ol style="list-style-type: none"> <li>1. _____</li> <li>2. _____</li> <li>3. _____</li> <li>4. _____</li> <li>5. _____</li> <li>6. _____</li> <li>Av. _____</li> <li>+24% _____</li> <li>CP. _____</li> </ol> | <ol style="list-style-type: none"> <li>1. _____</li> <li>2. _____</li> <li>3. _____</li> <li>Av. _____</li> </ol> |
|--|---|



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

- |  |  |
|--|--|
| <ol style="list-style-type: none"> <li>1. Run six 1 ppb chloramphenicol</li> </ol> | <ol style="list-style-type: none"> <li>2. Run three Negative Controls</li> </ol> |
|--|--|

Chloramphenicol

Negative Control

- |  |   |
|--|---|
| <ol style="list-style-type: none"> <li>1. _____</li> <li>2. _____</li> <li>3. _____</li> <li>4. _____</li> <li>5. _____</li> <li>6. _____</li> <li>Av. _____</li> <li>+25% _____</li> <li>CP. _____</li> </ol> | <ol style="list-style-type: none"> <li>1. _____</li> <li>2. _____</li> <li>3. _____</li> <li>Av. _____</li> </ol> |
|--|---|

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  $\pm 30\%$  ( $\pm 20\%$  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 µL 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 15 times in a rise and fall motion in 15 sec (pink tablets do not breakup) \_\_\_\_\_
  - 8. Incubate tubes for 3 min at 85±2°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  $\mu$ 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 or shake 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  $\mu$ 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 µL pipettor immediately add 300 µL 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 or shake 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 µL 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 15 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  $\mu\text{L}$  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 a tube, cap and vortex or shake 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 \_\_\_\_\_