

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 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 72 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 $\pm 30\%$ ($\pm 20\%$ 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 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 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 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 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 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 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 _____