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°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°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 ±10% of ZCS _____

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

2. Use within 72 hours when stored at 0.0-4.5°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°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

<u>Sulfamethazine</u>

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

Av. _____
+24% _____
CP. _____

2. Run three Negative Controls

<u>Negative Control</u>

1. _____
2. _____
3. _____

Av. _____

_____

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

1. Run six 1 ppb chloramphenicol

<u>Chloramphenicol</u>

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

Av. _____
+25% _____
CP. _____

2. Run three Negative Controls

<u>Negative Control</u>

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

_____

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 ±24% _____

2. For Tetracycline Assay cannot deviate by more than ±23% _____

3. For Chloramphenicol Assay cannot deviate by more than ±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 ±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 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 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 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 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 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±2°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 _____