APPENDIX N BULK MILK TANKER SCREENING TEST FORM

CHARM® ROSA TETRACYCLINE-SL TEST (DILUTION CONFIRMATION)
(Raw Commingled Cow Milk)
IMS #9-C17

[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. Charm Sciences Strip Incubator:
      56±1°C 8 min timer – same incubator as SL beta-lactam test;
      56±1°C Charm EZ display when message “Add milk to strip and close door”

      1. Clean, properly maintained and located on a level surface
      2. Check temperature daily (day of use); maintain records
         a. Charm EZ printout acceptable for daily temperature check (annual accuracy check required); maintain records
      3. Temperature measuring device for each incubator (App N. GR item 3)
      4. Lid closed (slightly sprung so that timer not active)-when not running tests
      5. Incubator Temperature: ______________
      6. Timer if not included in incubator
         Incubation Time of internal timer: __________

   b. ROSA® Reader, ROSA Pearl Reader (with or without ROSA Barcode option),
      Charm EZ or Charm Sciences equivalent with print out or download of data;
      manual available

      Serial Number: ______________
1. ROSA Reader V1.03 or higher (or if ROSA Pearl Reader or Charm EZ, see 3.b.2)
   a. Calibrators - 2 line for ROSA Tetracycline-SL Performance on SL Beta-lactam channel acceptable
      
      Two Line Range(s): Result
      
      Low: ________________________ ________ ________
      High: ________________________ ________ ________
   b. Maintain records

2. ROSA Pearl Reader V3.00 or higher or Charm EZ
   a. Calibrators - Low and High for use in all assay channels
      
      Solid Color Ranges: Result
      
      Low: ________________________ ________ ________
       (darker magenta)
      
      High: ________________________ ________ ________
       (lighter pink)
   b. Maintain records

3. Calibrator serial numbers match reader SN

4. **Do not proceed if out of range.** Manufacturer should be contacted for corrective actions

5. Printer or computer link for hardcopy download

   c. Pipettors
      
      1. 300 µL and disposable tips (see App. N GR item 7)
      2. 1000 µL and disposable tips (see App. N GR item 7)

   d. **FOR SCREENING ONLY** - Single use 300 µL ROSA-pipet with overflow bulb to accurately measure amount of sample, supplied by manufacturer

   e. Vials capable of holding 2.0 mL with adequate headspace
4. **Test Strips**

   a. Test Strips (EZ Compatible for Charm EZ)

   Lot #: __________   Exp. Date: __________

   QC Date: __________   By: __________

5. **Sample and control agitation and dilution**

   a. 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)

   b. Determine if sample is to be run diluted or undiluted

      1. Initial screening of tanker samples and initial testing of producer samples for traceback and re-instatement MUST be run Undiluted

      2. Verification of Initial Positive Tanker Samples, Confirmation of Presumptive Positive Tanker Samples, Confirmation of Producer Traceback on a Confirmed Positive Tanker Samples, and Confirmation of Positive Producer Re-Instatement Samples are all run Diluted

         a. Dilute the sample using 1.0 mL (one 1000 µL dispense) of Tetracycline Dilution Buffer (item 6.c) and adding 1.0 mL (one 1000 µL dispense) of sample and mix before testing

6. **Reagent Stability and Preparation**

   a. ROSA- Tetracycline reagents must be received within 72 hours if shipped non-refrigerated; over 72 hours must be refrigerated

   b. Store reagents at 0.0-4.5°C, desiccant blue, maintain no longer than manufacturers expiration date

   c. Tetracycline Dilution Buffer

      1. Commercially supplied by manufacturer

      Lot #: __________   Exp. Date: __________

   d. Negative Control

      1. Previously tested tetracycline negative raw milk

      2. Milk can be screened (previously tested) by the testing location making and or using the controls

      3. Must be undiluted milk
4. Negative control must test -600 or more negative
   Sample ID: ___________ Test Value: __________ Date tested: ________

5. Use within 72 hours when maintained at 0.0-4.5°C

6. 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 slowly overnight in refrigerator or more rapidly in cold water. Mix well until sample is homogeneous
   1. Do not use if there is visible protein precipitation
   b. Store at 0.0-4.5°C and use within 24 hours; do not refreeze

7. Day of use must produce -600 or more negative; maintain records
   Do not proceed if out of range

   e. Positive Control
      1. Manufacturer supplied, do not use after manufacturer’s expiration date
      2. Lyophilized or tablet 100 ppb Oxytetracycline
         Lot # ___________ Exp. Date: ________
      3. Reconstitute 3 tablets with 5 mL Negative Control (raw milk) item 6.d.1&.2
      4. Tested +400 or more positive, used within 48 hours when maintained at 0.0-4.5°C
         Lab Prep. Date: ________ Lab Exp. Date: ________
      5. 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 1 month
         Lab Prep. Date: ________ Lab Exp. Date: ________
a. Thaw slowly overnight in refrigerator or more rapidly in cold water.
   Mix well until sample is homogeneous

1. **Do not use if there is visible protein precipitation**

b. Store at 0.0-4.5°C and use within 24 hours; do not refreeze

6. Day of use, must produce +400 or greater reading; maintain records
   
   Test Value: ________
   
   **Do not proceed if out of range**

**TECHNIQUE**

7. **Daily Performance and Operation Check**

   a. See App. N GR items 10.b-d

   b. If using ROSA reader Versions 1.05 and higher, or ROSA-Pearl, use ESC 5
      reader function to enter performance monitoring mode of reader; if using
      Charm EZ, use Menu to enter Performance Monitoring mode and “Perf Mon”
      to enter daily performance check; refer to manual for directions

   c. Check Calibrators; items 3.b.1 or 3.b.2

   d. Positive and negative controls must give appropriate readings prior to any
      sample analysis (see App. N GR item 10.a)

   e. Controls in-range when in performance monitoring mode, ROSA reader
      version 1.05 and higher, ROSA Pearl or Charm EZ

   f. **Do not proceed if out of range**

8. **Initial Test Procedure (First screening sample run UNDILUTED)**

   a. Set out required number of test strips and place them in a dry labeled
      container at room temperature, or take out strips as needed

      1. Discard unused test strips at the end of the day

   b. Label test strips, one for each test sample and each control. Avoid crushing
      sample compartment(s)

   c. Mix milk sample(s)/control(s) (See Item 5 a)

   d. Place strip into incubator
e. While holding strip flat, peel back plastic (to 'peel to here' line) to expose sample pad compartment. Avoid lifting the wick and sponge under tape

1. For multiple samples, complete steps 8.d-g for each sample/control, before starting test of next sample

2. Complete all samples within 2 min of placing first strip in incubator

f. Add 300 µL of mixed sample/control to corresponding strip

1. Using pipettor (item 3.c.1) with new tip for each sample/control, draw up 300 µL avoiding foam or bubbles
   a. Remove tip from liquid
   b. While holding the pipettor vertically, expel test portion slowly into either side well of appropriate strip

2. **FOR SCREENING ONLY** - Using new manufacturer-provided ROSA-pipet (item 3.d) for each sample/control
   a. Squeeze top bulb while holding vertically with bulb and overflow reservoir side pointing down, draw up test portion avoiding foam and bubbles. Sample should completely fill pipet shaft and overflow into the bottom half of the overflow reservoir
   b. Remove tip from liquid
   c. While holding the ROSA-pipet vertically, expel test portion slowly into either side well of appropriate strip. Excess portion should remain in reservoir

g. Re-seal plastic firmly around sample pad compartment

h. ROSA Reader and Charm EZ (read only mode)

1. Close lid and latch ROSA incubator to start automatic timer in the incubator. If no automatic timer in incubator, set external timer for 8 min. Incubate 8 min not to exceed 9 min.

2. At end of incubation visually inspect C (Control) line. An absent C line, a partial C line or an indistinct C line indicates an invalid test; and the sample/control must be re-tested

3. Insert only valid test(s) in reader
   a. ROSA reader set to appropriate channel
      1. TETRA slow blink for ROSA Tetracycline test
2. Press ENTER, reading and interpretation appear in 5 sec, read strips within 5 min of completion of incubation. Strips may be held vertically, sample compartment down while waiting to be read

   b. Charm EZ automatically sets channel when color coded strip inserted

1. Close door; reading and interpretation appear in 5 sec, read strips within 5 min of completion of incubation. Strips may be held vertically, sample compartment down while waiting to be read

   i. Charm EZ (incubate and read mode)

      1. Charm EZ automatically sets channel and incubator temperature when color coded strip inserted. Optionally enter sample ID

         2. Peel strip (8.e) and add milk (8.f)

         3. Close door to begin

         4. Charm EZ automatically prompts for further testing when positive

9. Interpretation with Reader

   a. If there is a negative or zero reading on the reader, sample is a **Negative (NF)**

   b. If there is a positive reading on the reader, sample is an **Initial Positive**

10. Verification of Initial (SCREENING UNDILUTED SAMPLE) Positive Tanker Samples done at the Same Testing Facility using DILUTION Confirmation Procedure (see App. N GR item 11)

   a. Set out four test strips and label as negative control, positive control, and two strips with the initial positive sample ID. Avoid crushing sample compartment(s)

   b. Label three vials with same sample identities, one for each control, and one for the initial positive sample. Using 1000 µL pipet (Item 3.c.2) add 1.0 mL (one 1000 µL dispense) of Tetracycline Dilution Buffer (item 6.c) to each vial

   c. Add 1.0 mL (one 1000 µL dispense) of each sample/control to the appropriately labeled vial containing Tetracycline Dilution Buffer

   d. 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)

   e. Place a labeled strip into incubator
f. While holding strip flat, peel back plastic (to ‘peel to here’ line) to expose sample pad compartment. Avoid lifting the wick and sponge under tape _______

1. Complete steps 10.e-h for each sample/control, before starting test of next sample/control _______

2. Complete pipetting all samples within 2 min of placing first strip in incubator _______

g. Add 300 µL of Tetracycline Dilution Buffer mixed sample/control to corresponding strip _______

1. Using pipettor (item 3.c.1) with new tip for each sample/control, draw up 300 µL avoiding foam or bubbles _______
   a. Remove tip from liquid _______
   b. While holding the pipettor vertically, expel test portion slowly into either side well of appropriate strip _______

h. Re-seal plastic firmly around sample pad compartment _______

i. ROSA Reader and Charm EZ (must confirm in read only mode) _______

1. Close lid and latch ROSA incubator to start automatic timer in the incubator. If no automatic timer in incubator, set external timer for 8 min. Incubate 8 min not to exceed 9 min _______

2. At end of incubation remove strips from incubator. Strips may be held vertically with sample compartment down. Visually inspect each strip C (Control) line. An absent C line, a partial C line or an indistinct C line indicates an invalid test; and the sample/control must be re-tested _______

3. Insert only valid test(s) in reader _______
   a. ROSA reader set to appropriate channel _______

1. TETRA slow blink for ROSA Tetracycline test _______

2. Press ENTER, reading and interpretation appear in 5 sec, read strips within 5 min of completion of incubation. Strips may be held vertically, sample compartment down while waiting to be read _______

3. Diluted positive and negative controls must give appropriate readings prior to any sample analysis (see items 6. d-e) _______
   a. Controls in-range when in performance monitoring mode, ROSA reader version 1.05 and higher, ROSA Pearl _______
b. Charm EZ automatically sets channel when color coded strip inserted

1. From initial positive screen, press Continue or Confirm Later
   a. If press Confirm Later, then call the confirmation routine back by pressing Main Menu
   b. Select Perf Mon. then from next menu select CNFM-Later button
   c. Select initial positive sample ID and click ok

2. Follow Prompts to insert negative and then positive control. Click Continue Confirmation upon successful completion of positive control.

3. Follow prompts to enter the first duplicate sample. Insert the strip and then close door; reading and interpretation appear in 5 sec. Follow prompts to enter the second reading and interpretation appear in 5 sec. Read strips within 5 min of completion of incubation. Strips may be held vertically, sample compartment down while waiting to read

4. Charm EZ automatically determines Presumptive Positive or Not Found.

11. Confirmation of Presumptive Positive Tanker Samples (see App. N GR item 12) [Only in an accredited laboratory or by a CIS]
   a. If performing confirmation on the Charm ROSA Tetracycline SL test, perform testing technique as outlined in Item 10 (Dilution Confirmation)
   b. If performing confirmation on an equivalent test follow confirmation procedure for that test (refer to M-a-85 current revision for a listing of test kits to assure equivalence)

12. Traceback of Producer(s) on a Confirmed Positive Tanker (see App. N GR item 13) [Only in an accredited laboratory or by a CIS (refer to M-a-85 current revision for a listing of test kits to assure equivalence)]
   a. Initial test on producer samples is run on undiluted sample
   b. Any producer sample that is positive must be re-tested
   c. Duplicate testing of producer is performed following the testing technique as outlined in Item 10 (Dilution Confirmation)
13. Re-instatement of Producer(s) [Only in an accredited laboratory or by a CIS (refer to M-a-85 current revision for a listing of test kits to assure equivalence)]

a. Initial test on producer samples is run on undiluted sample

b. Any producer sample that is positive must be re-tested

c. Duplicate testing of producer is performed following the testing technique as outlined in Item 10 (Dilution Confirmation)

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