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 or EZ Protect+ _____

1. Clean, properly maintained and located on a level surface _____

2. Check temperature daily (day of use); maintain records _____

a. Charm EZ and EZ Protect+ 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 when not running tests _____

a. 4 strip incubator lid closed (slightly sprung so that timer not active) _____

b. 2 strip incubator lid may be open _____

c. Charm EZ- reader lid open _____

d. Charm EZ-Protect+ not applicable _____

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 Reader, EZ-Protect+, or Charm Sciences equivalent with print out or download of data; manual available _____

Serial Number: _____ _____

1. ROSA Reader V1.03 or higher _____

   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 _____

   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. Charm EZ reader _____

   a. Calibrators – Low and High for use in all assay channels _____

   Range(s) Result(s) _____

   Low: _____ _____ _____
   (darker magenta)

   High: _____ _____ _____
   (lighter pink)

   b. Maintain records _____

4. Charm EZ-Protect+ each optics module in base unit _____

a. Calibrators – Low and High for use in all assay channels. Shall be tested in all optics modules used each day attached to EZ Protect+ base with matching serial number _____

b. Low and High Calibrators results – IN RANGE=1, OUT OF RANGE=0 _____

	Result(s)	
Low: _____ (darker magenta)	_____	_____
High: _____ (lighter pink)	_____	_____

c. Maintain records _____

5. Calibrator serial numbers match reader SN or base of EZ Protect+ _____

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

7. 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 _____

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 _____

   1. **Do not use if desiccant indicator is white, pink or purple** _____

c. Tetracycline Dilution Buffer _____

   1. Commercially supplied by manufacturer _____

      Lot #: _____ Exp Date: _____ _____

d. 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 (qualified raw milk) item 6.e.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 _____

e. 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 _____

f. Optional Use of Negative Control Qualifier (NCQ) – Negative Control Qualifier is used if no previously tested Negative Control (qualified raw milk, item 6.e) is available _____

1. Rehydrate NCQ _____

a. Allow bottle to warm at room temperature for 10 minutes _____

b. Reconstitute with 5.0 mL of 45°C potable water. Shake well _____

c. Allow to stand refrigerated or on ice for 15 minutes _____

d. Shake before use _____

e. Use within 48 hours when maintained at 0.0-4.5°C _____

f. DO NOT use NCQ to rehydrate Positive Control _____

2. Qualification of raw milk as Negative Control _____

a. Test prepared NCQ twice _____

1. Both NCQ readings must be -600 or more negative _____

b. Test raw commingled milk twice _____

1. Both raw commingled milk readings must be -600 or more negative _____

c. Average the two NCQ readings and the two raw commingled milk readings _____

1. The average of the two commingled milk readings must be within ±1300 of the average of the two NCQ readings _____

2. If these conditions are met, the raw commingled milk is qualified as a Negative Control, item 6.e _____

d. Proceed to item 7 to run Performance Monitoring _____

TECHNIQUE

7. Daily Performance and Operation Check

a. See App. N GR items 10.b-d. If a reader/optics module is used to test multiple tests, one of those tests shall be performance tested daily _____

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; if using Charm EZ Protect+, use “PM” button and select “Performance Monitoring” to enter daily performance check. Each EZ Protect+ optics module in use must complete Daily Performance and Operation Check, item 7; refer to manual for directions _____

c. Check Calibrators; items 3.b.1, 3.b.2, 3.b.3 or 3.b.4 _____

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

e. Controls in-range when in performance monitoring mode, ROSA reader _____

version 1.05 and higher, ROSA Pearl, Charm EZ or EZ Protect+ _____

f. **Do not proceed if c., d. and e. are not met** _____

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). Re-shape dented sample compartments to fit into incubator _____

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

d. Place strip into incubator _____

1. EZ reader and EZ Protect+ in incubate and read mode displays appropriate test name and message when in correct temperature range, 55-57°C _____

a. EZ reader displays “add milk to strip and close door”. Follow item 8.i _____

b. EZ Protect+ displays “add milk to strip”. Follow item 8.i within 1 minute of message display _____

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. DO NOT press on compartment or sponge in compartment _____

h. ROSA Reader and Charm EZ and EZ Protect+ (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 _____

b. Charm EZ and EZ Protect+ automatically sets channel when color/bar coded strip inserted _____

1. Charm EZ- Close door; reading and interpretation appear in 5 sec after entering required information, read strips within 5 min of completion of incubation _____

2. Charm EZ Protect+ - reading and interpretation appear in 5 sec on strip insertion, detection and entering required information _____

i. Charm EZ and EZ Protect+ (incubate and read mode) _____

1. Charm EZ and EZ Protect+ automatically sets channel and incubator temperature when color/bar coded strip inserted. Optionally enter sample ID and/or operator. Wait for “Add milk to strip” message indicating incubator temperature is in range _____

2. Peel strip (8.e), add milk (8.f), and re-seal strip (8.g) _____

3. Start Reading _____

   a. EZ-Reader - Close door to begin _____

   b. EZ-Protect+- Count starts automatically on strip and milk flow detection and times out if milk not detected in specified time _____

4. Charm EZ and EZ Protect+ 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, Charm EZ and EZ Protect+ (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. 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 _____

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 and EZ Protect+ automatically sets channel when color/bar coded strip inserted _____

1. From initial positive screen, on Charm EZ press Continue or Confirm Later on Charm EZ Protect+ press Verify or Verify Later _____

a. If press Confirm/Verify Later, then call the confirmation routine back by pressing Main Menu _____

b. On Charm EZ- Select Perf Mon. then from next menu select CNFM-Later button. On Charm EZ Protect+ select PM icon from main page. Select Verify/Confirm Positive _____

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 on ROSA and Charm EZ readers 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 _____

4. Charm EZ and Charm EZ Protect+ 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) _____