

APPENDIX N BULK MILK TANKER SCREENING TEST FORM

**CHARM[®] SL (raw commingled cow, sheep, water buffalo and goat milk),
SL-6[®] (raw commingled cow milk) AND
Charm 3 SL-3 (raw commingled cow milk)
BETA-LACTAM TESTS**

[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 - SL beta-lactam test;
45±2°C 8 min timer - SL-6 beta-lactam test;
56±1°C 3 min with internal timer - SL-3 beta-lactam test _____

1. Clean and level. Check temperature daily (day of use);
maintain records _____

2. Temperature measuring device for each incubator
(App N. GR item 3) _____

3. Lid closed (slightly sprung so that timer not active)
when not running tests _____

4. Incubator Temperature: _____

5. Timer if not included in incubator
Incubation Time of internal timer: _____

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

Serial Number: _____

1. SL beta-lactam test - ROSA Reader V1.03 or higher (or if ROSA Pearl Reader see 3.b.3) _____
 - a. Calibrators - 2 line for SL beta-lactam _____

Two Line Range(s):	Result	
Low: _____	_____	_____
High: _____	_____	_____
 - b. Maintain records _____

 2. SL-6 beta-lactam test - ROSA Reader V1.07 or higher (or if ROSA Pearl Reader see 3.b.3) _____
 - a. Calibrators - 3 lines for SL-6 beta-lactam _____

Three Line Range(s):	Result	
Low: _____	_____	_____
High: _____	_____	_____
 - b. Maintain records _____

 3. SL-3 beta-lactam test - ROSA Pearl Reader V3.00 or higher _____
 - a. Calibrators - Low and High for use in all assay channels _____

Range(s)		
Solid Color Ranges:	Result	
Low: _____ (darker magenta)	_____	_____
High: _____ (lighter pink)	_____	_____
 - b. Maintain records _____

 4. Calibrator serial numbers match ROSA reader SN _____
 5. **Do not proceed if out of range.** Manufacturer should be contacted for corrective actions _____
 6. Printer or computer link for hardcopy download _____
- c. Pipettor - 300 μ L and disposable tips (see App. N GR item 7) _____

- d. Or single use 300 µL ROSA-pipet with overflow bulb to accurately measure amount of sample, supplied by manufacturer (**screening only**) _____
- e. Optional Centrifuge (Not applicable to SL-6 or SL-3 beta-lactam test) - mini or equivalent (1200-2000 x g) for frozen controls _____

4. Reagents _____

- a. Test Strips _____

Lot #: _____ Exp. Date: _____

QC Date: _____ By: _____

- b. Positive Control _____

- 1. Lyophilized or tablet 5 ppb Penicillin G for SL and SL-3 beta-lactam tests _____

Lot #: _____ Exp Date: _____

- 2. Lyophilized or tablet 5ppb Penicillin G/10ppb Cloxacillin for SL-6 beta-lactam test _____

Lot #: _____ Exp. Date: _____

- c. Negative Control _____

- 1. Previously negative tested raw milk (item 5.d) _____

5. Reagent stability _____

- a. SL-6 reagents received refrigerated; SL-3 reagents must be received within 72 hours if shipped non-refrigerated _____

- b. Store reagents at 0.0-4.4°C, desiccant blue, maintain no longer than manufacturer's expiration date _____

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

- c. Positive Control - Manufacturer supplied, maintain no longer than manufacturer's expiration date _____

- 1. Reconstitute with Negative Control (raw milk), tested +400 or more positive, used within 48 hours when maintained at 0.0-4.4°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 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 $0.0\text{-}4.4^{\circ}\text{C}$ and use within 24 hours; do not refreeze

- c. For SL **ONLY**, centrifuge 3 min and cool

1. Test portion below fat layer without mixing

3. Day of use, must produce +400 or greater reading; maintain records

Test Value: _____

Do not proceed if out of range

- d. Negative Control - raw milk tested -600 or more negative with SL and SL-3 beta-lactam tests or -400 or more negative with SL-6 beta-lactam (SL Test Negative Control can be any of the approved species milk)

Sample ID: _____ Test Value: _____

Date tested: _____

1. Use within 72 hours when maintained at $0.0\text{-}4.4^{\circ}\text{C}$

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 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 $0.0\text{-}4.4^{\circ}\text{C}$ and use within 24 hours; do not refreeze

c. For SL **ONLY**, centrifuge 3 min and cool _____

1. Test portion below fat layer without mixing _____

3. Day of use must produce –600 or more negative with SL and SL-3 beta-lactam Tests or –400 or more negative with SL-6 beta-lactam test; maintain records _____

Do not proceed if out of range _____

TECHNIQUE

6. Daily Performance and Operation Check _____

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

b. If using reader Versions 1.05 and higher, or ROSA-Pearl, use ESC 5 reader function to enter performance monitor mode of reader; refer to manual for directions _____

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

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, reader version 1.05 and higher _____

1. If out of range, manufacturer should be contacted for corrective action, 800-343-2170 _____

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

7. Test Procedure _____

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) 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) _____

d. Place strip into appropriate 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 7.d-g for each sample/control, before starting test of next sample _____
 - 2. Complete all samples within 2 min (1 min 15 sec for SL-3 test) of placing first strip in incubator _____
- f. Add 300 μ L of mixed sample/control to corresponding strip _____
 - 1. Using pipettor (item 3.c) with new tip for each sample/control, draw up 300 μ L 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. Using new manufacturer-provided ROSA-pipet (item 3.d) for each sample/control **[Screening only]** _____
 - 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. Close and latch incubator cover to start automatic timer in the incubator. If no automatic timer in incubator, set timer for 8 min. For SL and SL-6 tests, incubate 8 min not to exceed 9 min. For SL-3 test, incubate 3 min not to exceed 3 min and 30 sec _____
- i. 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 _____
- j. Insert only valid test(s) in reader (set to appropriate channel) _____
 - 1. SLBL slow blink for SL beta-lactam test _____

2. SLBL rapid blink for SL-6 beta-lactam test _____

3. SLBL solid (no blink) for SL-3 beta-lactam test _____

k. Press ENTER, reading and interpretation appear in 5 sec, read strips within 5 min (3 min with SL-3) of completion of incubation _____

8. Interpretation with ROSA 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** _____

9. Verification of Initial Positive Tanker Samples (see App. N GR item 11); Confirmation of Presumptive Positive Tanker Samples (see App. N GR item 12); and Traceback of Producer(s) on a Confirmed Positive Tanker (see App. N GR item 13) _____

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