

APPENDIX N BULK MILK TANKER SCREENING TEST FORM

Charm SL (raw bovine and caprine) and SL-6 (raw bovine)
Beta-Lactam Tests

GENERAL REQUIREMENTS

1. See Appendix N General Requirements form items 1-8 & 15 _____

SAMPLES

2. See Appendix N General Requirements (GR) form item 9 _____

APPARATUS & REAGENTS

3. Equipment _____

a. Strip Incubator, 56±1°C SL Beta-lactam test; _____
45±2°C SL-6 Beta-lactam Test _____

1. Level. Temperature checked daily (day of use), _____
records maintained _____

2. Thermometer, for each incubator (App N GR #3) _____

3. Lid closed (spring ajar), when not running tests _____

4. Incubator Temperature: _____

b. ROSA Reader or equivalent with print out or download _____
of data. ROSA Reader V1.07 or higher for SL-6, or _____
equivalent capable of reading 3 line test, _____
Manual Available Serial Number: _____

1. Calibrators - 2 for each assay- 2 lines for SLBL _____
and/or 3 lines for SL-6. _____

Range(s)
Two Line:
Low: _____
High: _____

Three Line:
Low: _____
High: _____

2. Calibrator serial numbers match ROSA reader SN _____

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

4. Printer or computer link for hardcopy download _____

5. Records maintained _____

- c. Pipettor- 300 μ L and disposable tips, calibrated
(see App. N GR item 7) _____
- d. Or single use 300 μ L poly-pipet with overflow bulb
to accurately measure amount of sample, supplied by
manufacturer (**screening only**) _____
- e. Optional Timer - if not built into Incubator _____
- f. Optional Centrifuge (Not applicable to SL-6
Beta-lactam Test)- mini or equivalent (1200-
2000 x g) for frozen controls _____

4. Reagents _____

- a. Test Strips _____
 Lot #: _____ Exp. Date: _____
 Rec'd: _____ QC Date: _____ by _____

- b. Positive Control _____
 - 1. Lyophilized or tablet 5 ppb Penicillin G for SL
Beta-lactam Test _____
 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 5b) _____

5. Reagent stability _____

- a. SL-6 reagents received refrigerated. _____
- b. Reagents stored at 0-4.4^oC, maintain no longer than
manufacturer's expiration date _____
- c. Negative Control - raw milk tested -600 or more
negative with SL Beta-lactam Test or -400 or more
negative with SL-6 Beta-lactam _____
 Test Value: _____ **Do not proceed if out of range.** _____
 - 1. Used within 72 hours when maintained at 0-4.4^oC _____
 - 2. Test for suitability each time, records
maintained _____

3. Or, frozen immediately (within 6 hours) and stored in a non-frost-free freezer, or in a styrofoam container in a frost free freezer, for no more than 2 months at -15°C or below _____

Lab Date prep: _____ Lab Exp. Date: _____

- a. For SLBL **ONLY**, not applicable to SL-6 thaw, centrifuge 3 minutes, cool and use within 24 hours. Do not refreeze. _____
- d. Positive Control- Manufacturer supplied, maintain no longer than manufacturer's expiration date _____
1. Reconstituted with Negative Control (raw milk), used within 48 hours when maintained at 0-4.4°C _____
2. Test for suitability each time prepared and/or day of use, must produce +400 or greater reading, records maintained. _____

Test Value: _____ **Do not proceed if out of range.** _____

3. Or, frozen immediately (within 6 hours) and stored in a non-frost-free freezer, or in a styrofoam container in a frost-free freezer, for no more than 2 months at -15°C or below _____

Lab Date prep: _____ Lab Exp. Date: _____

- a. Thaw, centrifuge 3 minutes(not applicable to SL-6 Beta-lactam Test), cool and use within 24 hours. Do not refreeze. _____

TECHNIQUE

6. Daily Performance and Operation Check _____

- a. See App. N GR item 10) _____
- b. Reader Versions 1.5 and higher, use Escape 5 reader function to enter performance monitor mode of reader-reference manual for directions _____
- c. Check Calibrators, records maintained _____
- d. Controls give appropriate readings in performance monitoring mode (reader version 1.05 and higher) prior to any sample analysis. If out of range, manufacturer should be contacted for corrective action, 800-343-2170. _____
- e. **Do not proceed if out of range.** _____

7. Test Procedure

- a. Label Test Strips, one for each test sample and each control
- b. Place strip into appropriate incubator, avoid crushing sample compartment. For multiple samples, complete steps b-f for each sample/control, before starting test of next sample.
- c. While holding strip flat, peel (to 'peel to here' line) back plastic to expose sample pad compartment
- d. Mix milk sample or control 25 times in seven seconds with a 1 ft movement or vortex control for 10 seconds when appropriate
- e. Slowly pipette 300 μ L sample to either side of the sample pad compartment (if applicable, use etched line on incubator as location guide). Avoid contact of sponge with pipet tip.
 1. Draw up sample, avoiding foam and bubbles. (with 300ul calibrated pipet only pre-wet tip by expelling sample and drawing up again)
 2. Or for **Screening only** use poly pipet
 - a. While holding poly pipet almost vertically with bulb side pointing down, depress top bulb and insert into sample (below foam).
 - b. Release bulb and sample should completely fill pipet shaft and overflow into bottom $\frac{1}{2}$ of lower bulb
 - c. Pipet sample in pipet shaft by depressing top bulb only into test strip while leaving excess sample in overflow bulb
- f. Re-seal plastic firmly around sample pad compartment
- g. Repeat Step 7b-f for each sample and/or control test strip in incubator
- h. Close and latch incubator cover. If no automatic timer in incubator, set timer for 8 minutes, do not exceed 10 minutes
- i. At end of incubation, visually inspect C (Control) line, an absent C line or 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 ROSA reader (set to appropriate channel). _____

1. SLBL slow blink for SL Beta-lactam Test _____

2. SLBL rapid Blink for SL-6 Beta-lactam Test _____

k. Press ENTER, reading and interpretation appear in 5 seconds, read strips within 10 minutes of completion of incubation _____

8. Interpretation with ROSA Reader _____

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

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

9. 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) _____

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

a. Reader tapes or computer print outs maintained _____