APPENDIX N BULK MILK TANKER SCREENING TEST FORM

CHARM® SL (Raw Commingled Cow, Sheep, Water Buffalo and Goat Milk), IMS #9-C13
AND
Charm 3 SL3 (Raw Commingled Cow Milk), IMS #9-C15
AND
Charm BL30SEC (raw commingled Cow milk), IMS #9-C20

BETA-LACTAM TESTS

FDA/NCIMS Revision 10/19

[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;
56±1°C 3 min with internal timer – SL3 beta-lactam test;
56±1°C Charm EZ display when message "Add milk to strip and close door"
Required for BL30SEC optional for SL and SL3 _____

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 Reader or Charm Sciences equivalent with print out or download of data; manual available _____

Serial Number: _____ _____

1. ROSA Reader V1.03 or higher _____

a. Calibrators _____

	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 _____

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

b. Maintain records _____

4. Calibrator serial numbers match 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 µL 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 SL3 or BL30SEC beta-lactam test) – mini or equivalent (1200-2000 x g) for frozen controls _____

4. Reagents _____

a. Test Strips (EZ Compatible for Charm EZ) _____

Lot # _____ Exp. Date: _____ _____

QC Date: _____ By: _____ _____

b. Positive Control _____

1. Lyophilized or tablet 5 ppb Penicillin G beta-lactam tests _____

Lot #: _____ Exp. Date: _____ _____

c. Negative Control _____

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

5. Reagent Stability _____

a. SL3 and BL30SEC reagents must be received within 72 hours if shipped non-refrigerated; over 72 hours must be refrigerated. (Not applicable to the SL reagents) _____

b. Store reagents at 0.0-4.5°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 0.0-4.5°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 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 _____

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 (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 0.0-4.5°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 0.0-4.5°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; 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 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.a, 3.b.2.a, or 3.b.3.a _____

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

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

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

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) _____

1. Centrifuge sheep milk sample(s)/controls that have been previously frozen; refer to 5.c.2.a-c and 5.d.2.a-c _____

d. Place strip into appropriate incubator _____

1. EZ reader in incubate and read mode displays appropriate test name on strip insertion and displays “add milk to strip and close door” when in correct temperature range, 55-57C, Follow item 7.i _____

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 SL3 test) of placing first strip in incubator _____

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

1. Using pipettor (item 3.c) with new tip for each sample/control, draw up 300 µL avoiding foam and 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. 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 for SL. For SL test, incubate 8 min not to exceed 9 min. For SL3 test, incubate 3 min not to exceed 3 min and 30 sec _____

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. SLBL slow blink for SL beta-lactam test _____

      2. SLBL solid (no blink) for SL3 beta-lactam test _____

      3. Press ENTER, reading and interpretation appear in 5 sec, read strips within 5 min (3 min with SL3) 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 (3 min with SL3) of completion of incubation. Strips may be held vertically, sample compartment down while waiting to 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 (7.e), add milk (7.f), and re-seal strip (7.g) _____

3. Close door to begin _____

4. Charm EZ automatically prompts for further testing when positive _____

8. 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** _____

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) _____