

DEPARTMENT OF HEALTH AND HUMAN SERVICES FOOD AND DRUG ADMINISTRATION MILK LABORATORY EVALUATION RECORD	Laboratory	
	Location	
Laboratory Evaluation Officer	Lab Number	Date (mm/dd/yyyy)

List of acceptable entries in columns: **Blank** = No deviations observed; **RO** = Item has been Reviewed or Observed (this entry is for temporary use only and must be replaced with another entry on final record); **X** = Deviation; **N** = Note; **O** = Not Used; **NA** = Not Applicable; **U** = Undetermined

APPENDIX N BULK MILK TANKER SCREENING TEST PROCEDURES
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
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
 - 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”
 - 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: _____

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

Range(s)	Result
Low: _____	_____
High: _____	_____
 - b. Maintain records _____
2. SL3 beta-lactam test - ROSA Pearl Reader V3.00 or higher or Charm EZ _____
 - a. Calibrators - Low and High for use in all assay channels _____

Range(s)	Result
Low: _____ <i>(darker magenta)</i>	_____
High: _____ <i>(lighter pink)</i>	_____
 - 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. 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 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 _____

Lot #: _____ Exp. Date: _____
 - c. Negative Control _____
 1. Previously tested negative raw milk (item 5.d) _____

- 5. Reagent stability** _____
- a. SL3 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** _____

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- 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 _____
 - 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; (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

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- 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** _____

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 pad 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 _____
- 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 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. ROSA Reader and Charm EZ (read only mode) _____

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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) and add milk (7.f)
 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)**

Notes
