

APPENDIX N BULK MILK TANKER SCREENING TEST FORM

**CHARM® FLUSLBL (raw commingled cow milk)
FLUNIXIN and BETA-LACTAM TEST
[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 _____

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: _____

- 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. ROSA Reader V1.07 or higher (or if ROSA Pearl Reader see 3.b.2) _____

- a. Calibrators - 3 lines for SL-6 beta-lactam _____

Three 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 _____

Range(s)	Solid color Ranges:	Result
Low calibrator: _____ (darker magenta)		_____
High Calibrator: _____ (lighter pink)		_____
 - b. Maintain records _____
- 3. Calibrator serial numbers match ROSA 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**) _____

4. Reagents _____

- a. Test Strips _____

Lot #: _____	Exp. Date: _____
QC Date: _____	By: _____
- b. Positive Control _____
 - 1. Positive Control labeled as "2 ppb Flunixin and 5 ppb Penicillin G Standard" _____

Lot #: _____	Exp Date: _____
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 - 2. Lyophilized 2 ppb Flunixin _____

Lot # _____	Exp. Date: _____
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 - 3. Or alternative to 4.b.1-4.b.2, 5 ppb Penicillin G and 2 ppb Flunixin tablet _____

4. Preparation

- a. Add 10.0 mL negative raw milk (item 5.d) to Flunixin control (item 4.b.2), and allow to rehydrate for 5 min _____
- b. Add 8.0 mL of reconstituted Flunixin control (item 4.b.4.a) to Positive Control (item 4.b.1), and allow to rehydrate for 5 min _____
- c. Or, alternative to 4.b.4.a and 4.b.4.b, add 5.0 mL negative milk (item 5.d) to Flunixin and Penicillin G tablet (item 4.b.3), and allow to rehydrate for 5 min _____

c. Negative Control _____

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

5. Reagent stability _____

- a. FLUSLBL reagents received 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. Reconstituted Control (4b4), tested +400 or more positive; use 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 3 weeks _____

Lab Date prep: _____ 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 0.0-4.4°C and use within 24 hours; do not refreeze _____

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 -400 or more negative with FLUSLBL test

Sample ID: _____ Test Value: _____

Date tested: _____

1. Used within 72 hours when maintained at 0.0-4.4°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 3 weeks

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 0.0-4.4°C and use within 24 hours; do not refreeze

3. Day of use must produce -400 or more negative with FLUSLBL test; maintain record

Do not proceed if out of range

TECHNIQUE

6. Daily Performance and Operation Check

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

- 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 _____
 - 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 for samples to be tested in one day 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 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 control/sample, 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 control/sample **[Screening only]** _____

- a. Depress 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 8 min. automatic timer in the incubator. Incubate 8 min, not to exceed 9 min _____

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 reader (set to appropriate channel) _____

1. SLBL rapid blink for FLUSLBL Test _____

k. Press ENTER, reading and interpretation appear in 5 sec, read strips within 5 min 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) _____