

APPENDIX N BULK MILK TANKER SCREENING TEST FORM

**CHARM® FLUSLBL (Raw Commingled Cow Milk)
FLUNIXIN and BETA-LACTAM TEST
IMS #9-C16**

[Unless otherwise stated all tolerances are $\pm 5\%$]

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 \pm 1°C 8 min timer
56 \pm 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: _____

- 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: _____

1. ROSA Reader V1.07 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. 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: _____
(darker magenta) _____

High: _____
(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 μ L and disposable tips (see App. N GR item 7) _____

d. Or single use 300 μ L ROSA-pipet with overflow bulb to accurately measure amount of sample; supplied by manufacturer **(screening only)** _____

4. Reagents _____

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

Lot #: _____ Exp. Date: _____

QC Date: _____ By: _____

b. Positive Control (labeled as 2 ppb Flunixin and 5 ppb Penicillin G Standard) _____

1. 5 ppb Penicillin G Standard _____

Lot #: _____ Exp. Date: _____ _____

2. 2 ppb Lyophilized Flunixin _____

Lot #: _____ Exp. Date: _____ _____

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 0.0-4.5°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. Reconstituted Control (4.b.4), tested +400 or more positive; use 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 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 0.0-4.5°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 0.0-4.5°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 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 0.0-4.5°C and use within 24 hours; do not refreeze _____

3. Day of use must produce –400 or more negative with FLUSLBL 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 ROSA reader Version 1.07 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 & 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 1.07 and higher, ROSA Pearl or Charm EZ
 - 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 μ L of mixed sample/control to corresponding strip _____
- 1. Using pipettor (item 3.c) with new tip for each control/sample, 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 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. 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 not to exceed 9 min _____
 - 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. Press ENTER; reading and interpretation appear in 5 sec, read strips within 5 min 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 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 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)
