

**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  $\mu$ L and disposable tips (see App. N GR item 7) \_\_\_\_\_

d. Or single use 300  $\mu$ 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  $\mu$ L of mixed sample/control to corresponding strip \_\_\_\_\_
- 1. Using pipettor (item 3.c) with new tip for each control/sample, draw up 300  $\mu$ 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

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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

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i. Charm EZ (incubate and read mode)

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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

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**8. Interpretation with ROSA Reader**

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- 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**

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**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)**

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

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