APPENDIX N BULK MILK TANKER SCREENING TEST FORM

CHARM® TRIO TEST (Raw Commingled Cow Milk)
BETA-LACTAM, SULFONAMIDE and TETRACYCLINE (BLSTE) TEST
APPROVED FOR BETA LACTAM and SULFONAMIDE ONLY
(Also Detects Tetracycline under Appendix N Section VI)
IMS #9-C19

[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 ROSA Incubator:
56±1°C 3 min timer – same incubator as SL3 beta-lactam test;
56±1°C Charm EZ or EZ Protect+ _____

1. Clean, properly maintained and located on a level surface _____

2. Check temperature daily (day of use); maintain records _____

    a. Charm EZ and EZ Protect+ 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 when not running tests _____

    a. 4 strip incubator lid closed (slightly sprung so that timer not active) _____

    b. 2 strip incubator lid may be open _____

    c. Charm EZ- reader lid closed _____

    d. Charm EZ-Protect+ not applicable _____

5. Incubator Temperature: _____ _____

6. Timer if not included in incubator _____

b. Charm EZ, EZ-Protect+, or Charm Sciences equivalent with print out or download of data; manual available _____

Serial Number: _____ _____

1. Charm EZ _____

   a. Calibrators – Low and High for use in all assay channels _____

   Solid Color Ranges: Result _____

   Low: _____ _____ _____
   (darker magenta)

   High: _____ _____ _____
   (lighter pink)

   b. Maintain records _____

2. Charm EZ-Protect+ each optics module in base unit _____

   a. Calibrators – Low and High for use in all assay channels. Shall be tested in all optics modules used each day attached to EZ Protect+ base with matching serial number _____

   b. Low and High Calibrators results – IN RANGE=1, OUT OF RANGE=0 _____
   Result(s)

   Low: _____ _____ _____
   (darker magenta)

   High: _____ _____ _____
   (lighter pink)

   c. Maintain records _____

3. Calibrator serial numbers match reader SN or base of EZ Protect+ _____

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 _____

1. 300 µL and disposable tips (see App. N GR item7) _____

d. **FOR SCREENING ONLY -** Single use 300 µL ROSA-pipet with overflow bulb to accurately measure amount of sample, supplied by manufacturer _____

4. Test Strips _____

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

Lot #: _____ Exp. Date: _____ _____

QC Date: _____ By: _____ _____

5. Sample and control agitation _____

a. 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) _____

6. Reagent stability and Preparation _____

a. Charm TRIO reagents must be received within 72 hours if shipped non-refrigerated; over 72 hours must be 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, pink or purple** _____

c. Positive Control _____

1. Manufacturer supplied, do not use after manufacturer's expiration date _____

2. Tablet containing 4 ppb penicillin G, 100 ppb Oxytetracycline, and 10 ppb sulfadimethoxine _____

Lot #: _____ Exp. Date: _____ _____

3. Reconstitute per manufacturers instructions with Negative Control (qualified raw milk) item 6.d.1&2 _____

4. Test +400 or more positive with BLSTE interpretation, used within 48 hours when maintained at 0.0-4.5°C _____

Lab Prep. Date:_____ Lab Exp. Date: _____ _____

5. 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 1 month _____

a. Thaw slowly overnight in refrigerator or more rapidly in cold water. Mix well until sample is homogenous _____

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 _____

6. Day of use, must produce +400 or greater reading and a BLSTE interpretation; maintain records _____

Test Value:_____ Interpretation:_____ _____

Do not proceed if out of range _____

d. Negative Control _____

1. Previously tested negative raw milk _____

2. Milk can be screened (previously tested) by the testing location making and or using the controls _____

3. Negative control must read -600 or more negative _____

Sample ID: _____ Test Value: _____ _____

Date Tested: _____ _____

4. Use within 72 hours when maintained at 0.0-4.5°C _____

5. 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 homogenous _____

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 _____

6. Day of use must produce -600 or more negative; maintain records _____

Do not proceed if out of range _____

e. Optional Use of Negative Control Qualifier (NCQ) – Negative Control Qualifier is used if no previously tested Negative Control (qualified raw milk, item 6.d) is available _____

1. Rehydrate NCQ _____

a. Allow bottle to warm at room temperature for 10 minutes _____

b. Reconstitute with 5.0 mL of 45°C potable water. Shake well _____

c. Allow to stand refrigerated or on ice for 15 minutes _____

d. Shake before use _____

e. Use within 48 hours when maintained at 0.0-4.5°C _____

f. DO NOT use NCQ to rehydrate Positive Control _____

2. Qualification of raw milk as Negative Control _____

a. Test prepared NCQ twice _____

1. Both NCQ readings must be -600 or more negative _____

b. Test raw commingled milk twice _____

1. Both raw commingled milk readings must be -600 or more negative _____

c. Average the two NCQ readings and the two raw commingled milk readings _____

1. The average of the two commingled milk readings must be within ±1300 of the average of the two NCQ readings _____

2. If these conditions are met, the raw commingled milk is qualified as a Negative Control, item 6.d _____

d. Proceed to item 7 to run Performance Monitoring _____

TECHNIQUE

7. Daily Performance and Operation Check _____

a. See App. N GR items 10.b-d. If a reader/optics module is used to test multiple tests, one of those tests shall be performance tested daily _____

b. Use EZ Reader Menu to enter Performance Monitoring mode and “Perf Mon” to enter daily performance check; refer to manual for directions, if using Charm EZ Protect+, use “PM” button and select “Performance Monitoring” to enter daily performance check. Each EZ Protect+ optics module in use must complete Daily Performance and Operation Check, item 7; refer to manual for directions _____

c. Check Calibrators; item 3.b.1.a or 3.b.2.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 _____

f. **Do not proceed if out of range** _____

8. 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). Re-shape dented sample compartments to fit into incubator _____

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 incubator _____

1. EZ reader and EZ Protect+ in incubate and read mode displays appropriate test name and message when in correct temperature range, 55-57°C _____

a. EZ reader displays “add milk to strip and close door”. Follow item 8.i _____

b. EZ Protect+ displays “add milk to strip”. Follow item 8.i within 1 minute of message display _____

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 8.d-g for each sample/control, before starting test of next sample _____

2. Complete all samples within 90 seconds of placing first strip in incubator _____

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

1. Using pipettor (item 3.c.1) 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. DO NOT press on compartment or sponge in compartment _____

h. Charm EZ and EZ Protect+ (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 3 min. Incubate 3 min not to exceed 4 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. Charm EZ and EZ Protect+ automatically sets channel when color/bar coded strip inserted _____

1. Charm EZ – close door; reading and interpretation appear in 5 sec, read strips within 3 min of completion of incubation _____

2. Charm EZ Protect+ - reading and interpretation appear in 5 sec on strip insertion, detection and entering required information _____

i. Charm EZ and EZ Protect+ (incubate and read mode) _____

1. Charm EZ and EZ Protect+ automatically sets channel and incubator temperature when color/bar coded strip inserted. Optionally enter sample ID and/or operator. Wait for “Add milk to strip” message indicating incubator temperature is in range _____

2. Peel strip (8.e) and add milk (8.f) and re-seal strip (8.g) _____

3. Start reading _____

a. Charm EZ- Close door to begin _____

b. EZ-Protect+- Count starts automatically on strip and milk flow detection and times out if milk not detected in specified time _____

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

9. 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 (+)** _____

1. Letters indicating what drug line is causing a positive result follows the positive interpretation:

BL	= Beta-lactam,
TE	= Tetracycline,
S	= Sulfonamide,
BLTE	= Beta-lactam and Tetracycline,
BLS	= Beta-lactam and Sulfonamide,
STE	= Sulfonamide and Tetracycline, and
BLSTE	= Beta-lactam, Sulfonamides, and Tetracyclines

_____

c. From initial positive screen, on Charm EZ press Continue Confirmation or Confirm Later on Charm EZ Protect+ press Verify or Verify Later _____

1. If press Confirm Later, then call the confirmation routine back by pressing Main Menu _____

2. On Charm EZ- Select Perf Mon then from next menu select CNFM-Later button. On Charm EZ Protect+ select PM icon from main menu and select Verify Positive Sample _____

3. Select initial positive sample ID and click OK _____

10. Verification of Beta-lactam and/or Sulfonamide Initial Positive Tanker Samples (BL, BLS, BLTE, STE or BLSTE positive results) _____
[Refer to Item 13 for retesting options of Tetracycline Positive results (TE positive results)] _____

a. Read only mode Required with Charm EZ in confirmation read only option. Use ROSA incubator. All tests must be performed in same reader and same Charm EZ Protect+ optics module as the initial positive _____

1. Set out four TRIO test strips and label as negative control, positive control, and two strips with the initial positive sample ID. Avoid crushing sample compartment(s). Re-shape dented sample compartments to fit into incubator _____

2. 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 votexing) _____

3. Place a labeled strip into incubator _____

    a. Add 300 µL of each sample to the appropriately labeled test strip _____

    b. 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 _____

    c. For these samples, complete steps 10.a.1-4 for each sample/control, before starting test of next test strip _____

    d. Complete pipetting all samples within 90 sec of placing first strip in incubator _____

4. Re-seal plastic firmly around sample pad compartment _____

5. Close lid and latch ROSA incubator to start automatic timer in the incubator. Incubate 3 min not to exceed 4 min _____

6. At end of incubation remove strips from incubator. Visually inspect each strip 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 _____

7. Insert only valid test(s) in reader _____

    a. The Charm EZ in confirmation mode refer to 9.c. Reader automatically sets channel when color/bar coded strip inserted. Charm EZ close door to start count, Charm EZ Protect+ counts automatically _____

        1. Follow Prompts to insert negative and then positive control. Click Continue Confirmation/Verification upon successful completion of positive control _____

        2. Follow prompts to enter the first duplicate sample. Insert the strip and then close door; reading and interpretation appear in 5 sec. Follow prompts to enter the second duplicate sample. Insert the strip and then close door; reading and interpretation appear in 5 sec. Read strips within 3 min of completion of incubation _____

b. Charm EZ Protect+ in incubate and read confirmation option. All tests must be performed in same Charm EZ Protect+ optics module as the initial positive. _____

1. Set out four TRIO test strips and label as negative control, positive control, and two strips with the initial positive sample ID. Avoid crushing sample compartment(s). Re-shape dented sample compartments to fit into incubator _____

2. Perform tests in same optics as the initial positive following the optics prompts for confirmation. Perform Negative Control, then Positive Control then initial positive sample in duplicate _____

3. 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) _____

4. Follow prompts from EZ Protect+ reader and place the appropriately labeled strip into incubator _____

    a. Add 300 µL of the prompted sample to the appropriately labeled test trip _____

    b. While holding strip flat, peel back plastic (to 'peel to here' line) to expose sample pad compartment. Avoid lifting the wick and sponge under the tape _____

5. Re-seal plastic firmly around sample pad compartment _____

6. EZ Protect+ reads results and prompts for next sample in verification sequence. _____

    a. Follow steps 10.b.3-5 _____

    b. Complete all prompted Negative and Positive Controls and sample in duplicate in that order _____

c. The Charm EZ and EZ Protect+ automatically determines Presumptive Positive or Not Found consistent with Items 7.d-f and App. N GR Items 10-12 _____

d. Follow up on Presumptive Positive test for BL, S, BLS, BLTE, STE and BLSTE _____

    1. For BL presumptive positives confirm following Item 11 _____

    2. For S presumptive positives confirm following Item 12 _____

    3. For BLS presumptive positives confirm following Items 11 and 12 _____

    4. For BLTE presumptive positives confirm following Items 11 and 13 _____

    5. For STE presumptive positives confirm following Items 12 and 13 _____

    6. For BLSTE presumptive positives confirm following Items 11, 12 and 13 _____

e. If duplicate testing detects drug(s) other than the drug(s) detected on the initial test, redo Verification of Initial Positive testing using the single drug family test(s) referenced in items 11.a, 12.a or 13.a to test for the new drug(s) detected _____

11. Confirmation of Presumptive Positive Beta-Lactam test result _____

a. For Beta-lactam confirmation, test sample in duplicate along with positive and negative control using the Charm 3 SL3 or use an equivalent test(s) using the approved confirmation procedure for that test(s) _____

1. If Beta-lactam confirmed Not Found with BLS presumptive positive interpretation, additional testing required, refer to Item 12 _____

2. If Beta-lactam confirmed positive with BLS presumptive positive interpretation, additional testing required, refer to Item 12 _____

3. If Beta-lactam confirmed Not Found with BLTE presumptive positive interpretation, additional testing required, refer to Item 13 _____

4. If Beta-lactam confirmed positive with BLTE presumptive positive interpretation, additional testing required, refer to Item 13 _____

5. If Beta-lactam confirmed Not Found with BLSTE presumptive positive interpretation, additional testing required, refer to Items 12 and 13 _____

6. If Beta-lactam confirmed positive with BLSTE presumptive positive interpretation, additional testing required, refer to Items 12 and 13 _____

12. Confirmation of Presumptive Positive Sulfonamide test result _____

a. For Sulfonamide confirmation, test sample in duplicate along with positive and negative control using the Charm ROSA SULF test or use an equivalent test(s) using the approved confirmation procedure for that test(s) _____

1. If Sulfonamide confirmed Not Found with BLS presumptive positive interpretation, additional testing required (if not already performed), refer to Item 11 _____

2. If Sulfonamide confirmed positive with BLS presumptive positive interpretation, additional testing required (if not already performed), refer to Item 11 _____

3. If Sulfonamide confirmed Not Found with STE presumptive positive interpretation, additional testing required, refer to Item 13 _____

4. If Sulfonamide confirmed positive with STE presumptive positive interpretation, additional testing required, refer to Item 13 _____

5. If Sulfonamide confirmed Not Found with BLSTE presumptive positive interpretation, additional testing required, refer to Items 11 (if not already performed) and 13 _____

6. If Sulfonamide confirmed positive with BLSTE presumptive positive interpretation, additional testing required, refer to Items 11 (if not already performed) and 13 _____

13. Retesting Tetracycline Positive Results Additional Testing Required _____

a. For Initial Positive TE results, **DO NOT** retest with TRIO test. Perform Charm ROSA TET-SL (dilution confirmation) or use an equivalent test(s) using the approved confirmation/verification procedure for that test to determine presumptive positive tetracycline _____

1. Follow the procedures as outlined in the PMO, Appendix N Section VI (See M-I-17-2 for Testing Option Flow Charts) _____

b. For presumptive positive BLTE, STE, BLSTE (following testing as outlined in item 10), perform Charm ROSA TET-SL (dilution confirmation) or use an equivalent test(s) using the approved confirmation procedure for that test to determine presumptive positive tetracycline _____

1. Follow the procedures as outlined in the PMO, Appendix N Section VI (See M-I-17-2 for Testing Option Flow Charts) _____

14. Traceback refer to Appendix N – GR Item 13 _____

15. Re-instatement of Producer(s) [Only in an accredited laboratory or by a CIS (refer to M-a-85 current revision for a listing of test kits to assure equivalence)] _____

16. Reporting _____

a. Beta-lactam and Sulfonamide (See App. N GR item 14) _____

b. Tetracycline (Refer to Appendix N Section VI) _____