

APPENDIX N BULK MILK TANKER SCREENING TEST FORM

**CHARM SL (raw commingled cow, sheep, water buffalo and goat milk),
SL-6 (raw commingled cow milk) AND
SL-3 (raw commingled cow milk)
BETA-LACTAM TESTS**

GENERAL REQUIREMENTS

1. See Appendix N General Requirements form items 1-8 & 15 _____

SAMPLES

2. See Appendix N General Requirements (GR) form item 9 _____

APPARATUS & REAGENTS

3. Equipment _____

- a. Charm Sciences Strip Incubator:
56±1C 8 minute timer- SL Beta-Lactam test;
45±2C 8 minute timer- SL-6 Beta-Lactam Test;
56±1C 3 minute with internal timer- SL-3 Beta-Lactam test _____

1. Clean and level. Temperature checked daily (day of use),
records maintained _____

2. Thermometer, for each incubator (App N GR #3) _____

3. Lid closed (slightly sprung so that timer not active), when
not running tests _____

4. Incubator Temperature: _____

5. Timer if not included in incubator
Incubation Time of internal timer: _____

- 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. SL Beta-Lactam test- ROSA Reader V1.03 or higher (or if ROSA Pearl Reader see 3.b.3) _____
 - a. Calibrators- 2 line for SL Beta-Lactam _____

Two Line Range(s): Read

Low: _____

High _____

 2. SL-6 Beta-Lactam test- ROSA Reader V1.07 or higher (or if ROSA Pearl Reader see 3.b.3) _____
 - a. Calibrators- 3 lines for SL-6 Beta-Lactam _____

Three Line Range(s): Read

Low: _____

High _____

 3. SL-3 Beta-Lactam test- ROSA Pearl Reader V3.00 or higher _____
 - a. Calibrators- Low and High for use in all assay channels _____

Range(s)
Solid color Ranges: Read

Low calibrator: _____
(darker magenta)

High Calibrator: _____
(lighter pink)

 4. Calibrator serial numbers match ROSA reader SN _____
 5. **Do not proceed if out of range.** Manufacturer should be contacted for corrective actions _____
 6. Printer or computer link for hardcopy download _____
 7. Records maintained _____
- c. Pipettor - 300 μ L 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 SL-6 or SL-3 Beta-lactam Test) - mini or equivalent (1200-2000 x g) for frozen controls _____

4. Reagents _____

- a. Test Strips _____

Lot #: _____ Exp. Date: _____

QC Date: ___/___/___ by _____

- b. Positive Control _____

- 1. Lyophilized or tablet 5 ppb Penicillin G for SL and SL-3 Beta-lactam Tests _____

Lot #: _____ Exp Date: _____

- 2. Lyophilized or tablet 5ppb Penicillin G/10ppb Cloxacillin for SL-6 Beta-lactam Test _____

Lot # _____ Exp. Date: _____

- c. Negative Control _____

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

5. Reagent stability _____

- a. SL-6 and SL-3 reagents received refrigerated _____

- b. Reagents stored at 0-4.4C, desiccant blue, maintain no longer than manufacturer's expiration Date _____

- 1. **Do not use if desiccant indicator is white or pink** _____

- c. Negative Control - raw milk tested –600 or more negative with SL and SL-3 Beta-lactam Tests or –400 or more negative with SL-6 Beta-lactam (SL Test Negative Control can be any of the approved species milk) _____

Sample ID: _____ Test Value: _____

Date tested: ___/___/___

1. Used within 72 hours when maintained at 0-4.4C _____
2. Or, frozen immediately (within 6 hours) and stored in a non-frost-free freezer, or in a styrofoam container in a frost free freezer, for no more than 2 months at -15C or below _____

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 visible protein precipitation** _____

- b. Cool and use within 24 hours, do not refreeze _____

- c. For SLBL **ONLY**, centrifuge 3 minutes and cool _____

1. Test portion below fat layer without mixing, step 7b _____

3. Day of use must produce -600 or more negative with SL and SL-3 Beta-Lactam Tests or -400 or more negative with SL-6 Beta-Lactam test, record maintained _____

Do not proceed if out of range. _____

- d. Positive Control- Manufacturer supplied, maintain no longer than manufacturer's expiration date _____

1. Reconstituted with Negative Control (raw milk), tested +400 or more positive, used within 48 hours when maintained at 0-4.4C _____

Lab Date prep: ___/___/___ Lab Exp. Date: ___/___/___ _____

2. Or, frozen immediately (within 6 hours) and stored in a non-frost-free freezer, or in a styrofoam container in a frost-free freezer, for no more than 2 months (3 weeks for SL-3 Test) at -15C or below _____

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 visible protein precipitation** _____

- b. Cool and use within 24 hours, do not refreeze _____

c. For SLBL **ONLY**, centrifuge 3 minutes and cool _____

1. Test portion below fat layer without mixing,
step 7b. _____

3. Day of use, must produce +400 or greater reading,
records maintained _____

Test Value: _____

Do not proceed if out of range _____

TECHNIQUE

6. Daily Performance and Operation Check _____

a. See App. N GR (item 10) _____

b. If using reader Versions 1.05 and higher, or ROSA-Pearl, use
ESC 5 reader function to enter performance monitor mode of
reader, refer to manual for directions _____

c. Check Calibrators, records maintained _____

d. Positive and negative controls must give appropriate readings
prior to any sample analysis _____

1. Controls in-range when in performance monitoring mode,
reader version 1.05 and higher _____

2. If out of range, manufacturer should be contacted for
corrective action, 800-343-2170. _____

e. **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 _____

1. Discard unused test strips at the end of the day. _____

b. Mix milk sample(s) or control 25 times in 7 seconds with a 1 ft
movement or vortex control for 10 seconds at maximum
setting, use within 3 minutes _____

c. Label test strips, one for each test sample and each control.
Avoid crushing sample compartment(s) _____

- d. Place strip into appropriate incubator _____
 - 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 minutes (1 minute 15 seconds for SL-3 Test) of placing first strip in incubator _____
- e. While holding strip flat, peel (to 'peel to here' line) back plastic to expose sample pad compartment. Avoid lifting the wick and sponge under tape _____
- f. Using pipettor (item 3c), draw up 300 uL of controls and samples _____
 - 1. Draw up, avoiding foam and bubbles _____
- g. Using ROSA-pipet (**screening only**) _____
 - 1. While holding ROSA-pipet almost vertically with bulb and overflow reservoir side pointing down, depress top bulb and insert into sample (avoiding foam and bubbles) _____
 - 2. Release bulb and sample should completely fill pipet shaft and overflow into bottom ½ of overflow reservoir _____
 - 3. Excess sample from overflow should remain in reservoir when pipetting. Do not re-use ROSA-pipet _____
- h. Position pipet or ROSA-pipet upright and vertically above strip with tip in either side well of sample pad compartment (if applicable, use etched line on incubator as location guide). Slowly pipette 300 µL sample test portion to one side of the sample pad compartment _____
- i. Re-seal plastic firmly around sample pad compartment _____
- j. Repeat Step 7 d-g for each sample and/or control test strip in incubator. Complete all samples within 2 minutes (1 minute 15 seconds for SL-3 Test) of placing first test strip in incubator _____
- k. Close and latch incubator cover to start automatic timer in the incubator. If no automatic timer in incubator, set timer for 8 minutes. For SL and SL-6 test incubate 8 minutes not to Exceed 9 minutes. For SL-3 test incubate 3 minutes not to exceed 3 minutes and 30 seconds. _____

l. 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

m. Insert only valid test(s) in reader (set to appropriate channel)

1. SLBL slow blink for SL Beta-lactam Test

2. SLBL rapid blink for SL-6 Beta-lactam Test

3. SLBL solid (no blink) for SL-3 Beta-lactam Test

n. Press ENTER, reading and interpretation appear in 5 seconds, read strips within 5 minutes (3 minutes with SL-3) of completion of incubation

8. Interpretation with ROSA Reader

a. If there is a negative or zero reading on the reader, sample is **Negative (NF)**

b. If there is a positive reading on the reader, sample is **Initial Positive**

9. Verification of Initial Positive Samples (see App. N GR item 11); Confirmation of Presumptive Positive Samples (see App. N GR item 12); and Producer Traceback (see App. N GR item 13)

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

APPENDIX N BULK MILK TANKER SCREENING TEST FORM

IDEXX - NEW SNAP® BETA-LACTAM TEST

(raw commingled cow milk)

GENERAL REQUIREMENTS

1. See Appendix N General Requirements form items 1-8 & 15 _____

SAMPLES

2. See Appendix N General Requirements (GR) form item 9 _____

APPARATUS & REAGENTS

3. **Equipment** _____

- a. Heater block with SNAP inset thermostatically controlled at 45±5C _____

1. Temperature checked by placing standardized thermometer in tube containing liquid (bulb submersed in heating unit, records maintained) _____

2. Or, use 6 inch partial immersion thermometer placed directly into small thermometer well in middle of heating unit, records maintained _____

- b. Single use 450 µL ± 50 µL poly-pipet with indicator line to measure amount of sample, supplied by manufacturer **(screening only)** _____

- c. Pipettor to dispense 450 µL ± 50 µL (see App. N GR item 7) _____

- d. SNAP Kit _____

Lot # _____ Exp Date _____

QC Date ___/___/___ by _____

- e. Sample tubes containing reagent pellet _____

- f. Kits received refrigerated _____

- g. Store kits at 0-7C _____

- h. Timer _____

- i. IDEXX Readers for SNAP devices, with printer or data download capability _____

 - 1. SNAPshot Reader _____

 - a. Check Set, Part Number 87-05856-01 (black skirt) _____

 - 2. SNAPshot DSR Reader _____

 - b. Check Set, Part Number 87-14761-00 (blue skirt) _____

4. Daily Performance and Operation Check (see App. N GR item 10) _____

- a. Read Performance Check Set (Device #1 as Negative and Device #2 as Positive) _____
- b. Both devices must read within the limits as indicated on the storage box label of the check set devices _____

Positive Range _____ Negative Range _____

- c. If check sets fail, call IDEXX before proceeding _____

5. Controls _____

- a. Positive Control, 5.0 ppb ± 0.5 ppb Penicillin G _____

 - 1. Store according to label instructions _____

Mfg. _____ Lot # _____ Exp Date __/__/__ _____

 - 2. Re-hydrate as per manufacturer's instructions with fresh or frozen previously screened Beta-Lactam negative raw cow milk _____
 - 3. For Positive Control, must produce greater than 1.2 on the IDEXX reader, records maintained _____

Reader value: _____

 - 4. Store reconstituted Positive Control at 0-4.4C for no more than 24 hours _____

- b. Negative Control - Beta-Lactam negative raw milk (fresh or frozen) _____
- 1. For Negative Control purposes, must produce less than 0.95 on the IDEXX reader; records maintained _____
 Sample ID _____ Date Tested __/__/__ _____
 Reader value: _____ _____
- 2. Store fresh Negative Control milk at 0–4.4C for no more than 72 hours _____
- 3. Negative Control milk frozen for later use _____
 - a. Aliquot within 24 hours and freezing at -15C or colder colder in a non frost-free freezer or in a styrofoam container in a frost free freezer, used within 60 days _____
 - b. Thaw frozen milk at 0-4.4C _____
 - c. Once thawed mix thoroughly, **Do Not** use if noticeable protein precipitation is present after thawing _____
 - d. Thawed negative control milk held at 0-4.4C and used within 24 hours _____
- 4. Milk controls may not be refrozen _____

TECHNIQUE

6. Test Procedure _____

- a. Set out required number of SNAP devices, sample tubes and pipets for the samples to be tested _____
 - 1. Discard unused, un-refrigerated devices at the end of the day _____
- b. Pre-warm heater block(s) to 45±5C, and maintain 45±5C range for at least 5 minutes before beginning the test _____
 - 1. Check initial pre-heating with a reference thermometer, records maintained _____
 - 2. Continuous use block heaters, check temperature daily with reference thermometer, records maintained _____
- c. Label each device and each sample tube _____

- d. Place devices on incubator block(s) _____
- e. Mix milk sample(s) or control(s) 25 times in 7 seconds with a 1 ft movement or vortex for 10 seconds at maximum setting, use within 3 minutes _____
- f. Look for blue reagent pellet in bottom of tube, if not there tap to bring pellet down _____
- g. Remove and discard sample tube caps _____
- h. With poly-pipets provided, draw up controls or samples **(screening only)** _____
 - 1. Draw up, avoiding foam and bubbles, to the indicator lines $\pm 50\mu\text{L}$ _____
 - 2. Carefully add all of the control or sample milk to the appropriately labeled tubes. _____
- i. Or, using pipettor (item 3c), draw up $450\ \mu\text{L} \pm 50\mu\text{L}$ of controls and samples _____
 - 1. Draw up, avoiding foam and bubbles _____
 - 2. Carefully add to the appropriately labeled tubes _____
- j. Use clean poly-pipet (or tip) for each control and sample _____
- k. Agitate sample tube to dissolve reagent pellet _____
- l. Incubate tube(s) in heater block next to device with the corresponding ID _____
- m. Incubate tubes for 5 minutes (use timer) at $45\pm 5\text{C}$ _____
- n. After incubation, pour contents of tubes into sample well of device _____
- o. Watch blue activation circle, as it begins to disappear push the Activator firmly until it "snaps" flush with the body of the SNAP device (device remains on heater block) _____
- p. Incubate device for 4 minutes (use timer) at $45\pm 5\text{C}$ _____
- q. Read **IMMEDIATELY (no longer than 30 seconds after final incubation)** with IDEXX Reader for SNAP devices _____

7. Interpretation

a. The control spot is on the top and the test spot on the bottom of the Results Window (Correct orientation is with activator button to right and sample well to left)

b. Negative result:

1. If test spot is darker than or equal to the control spot, sample is **Negative (NF)**

c. Positive result:

1. If test spot is lighter than control spot, sample is **Initial Positive**

d. IDEXX Reader for SNAP devices automatically prints results as **Positive** (initial) or **Negative (NF)**

8. Verification of Initial Positive Samples (see App. N GR item 11); Confirmation of Presumptive Positive Samples (see App. N GR item 12); and Producer Trace-Back (see App. N GR item 13)

9. Reporting (see App. N GR item 14)

APPENDIX N BULK MILK TANKER SCREENING TEST FORM

CHARM II BETA-LACTAM ASSAYS

GENERAL REQUIREMENTS

1. See Appendix N General Requirements form items 1-8 & 15 _____

SAMPLES

2. See Appendix N General Requirements (GR) form item 9 _____

APPARATUS & REAGENTS

3. **Equipment** _____

- a. Analyzer heater for 13 x 100 mm tubes _____

1. 85±2C for Competitive Assay _____

2. 65±2C for Sequential Assay _____

3. 55±2C for Quantitative Assay _____

4. 35±2C for Cloxacillin Assay _____

5. Temperature checked by electronic display, or by placing standardized thermometer in tube containing liquid (bulb submersed) in heating unit, records maintained _____

6. Or, use 6 inch partial immersion thermometer placed directly into small thermometer well in middle of heating unit, records maintained _____

- b. Mixer, Maxi-mixer II or equivalent _____

- c. Centrifuge, Whisperfuge or Heraeus (3400 rpm) or equivalent _____

- d. Scintillation counter, Charm II or equivalent _____

- e. Scintillation fluid dispenser, set to dispense 3 mL _____

1. Checked quarterly with Class A graduate cylinder and record _____

- f. Cotton swabs _____

- g. Borosilicate test tubes, 13 x 100 mm _____

- h. Plastic stoppers for tubes _____

- i. Pipettors- Fixed Volume (see App. N GR item 7) _____
- 1. 300 μ L and appropriate tips _____
- 2. 5.0 mL and appropriate tips _____
- j. Timer _____

4. Reagents _____

- a. Scintillation fluid, Optifluor or equivalent supplied by manufacturer of Beta-Lactam Assays _____
- b. Competitive, Sequential or Quantitative Assay _____
 - 1. Reagent blister packages: microbial binder (green) tablet, tracer reagent (yellow) tablet _____
 - Lot # _____ Exp. Date _____
 - 2. 0.008 IU/mL Penicillin G standard _____
 - Lot # _____ Exp. Date _____
 - 3. Zero control standard _____
 - Lot # _____ Exp. Date _____
- c. Cloxacillin Assay _____
 - 1. Reagent blister packages: microbial/antibody binder (white) tablet, tracer reagent (blue) tablet _____
 - Lot # _____ Exp. Date _____
 - 2. 10 ppb Cloxacillin standard _____
 - Lot # _____ Exp. Date _____
 - 3. Zero control standard _____
 - Lot # _____ Exp. Date _____

5. Reagent stability _____

- a. All tablet reagents stored at -15C or below _____

b. Positive Control – Lyophilized 0.008 IU/mL penicillin G or 10 ppb Cloxacillin standard for Cloxacillin assay, 1 year and reconstituted for 48 hours at 0-4.4C

1. Reconstitute with 100 mL (measured) Zero Control (allow to sit 15 minutes prior to use or aliquotting)

Date prep. ___/___/___ Lab Exp. Date ___/___/___

2. For Quantitative Only: Dilute reconstituted 0.008 IU/mL penicillin G standard 1:4 with Zero Control and use within 48 hours

3. Or, freeze immediately and store in a non frost-free freezer, or in a styrofoam container in a frost-free freezer, for no more than 2 months at -15C or below

Date prep. ___/___/___ Lab Exp. Date ___/___/___

a. Thaw and use within 24 hours

c. Negative Control – Lyophilized Zero Control Standard (ZCS), expiration date and reconstituted for 72 hours at 0-4.4C. Alternatively, raw milk qualified to test average (N=3) within $\pm 10\%$ of zero control standard

Date prep. ___/___/___ Lab Exp. Date ___/___/___

1. Or freeze immediately and store in a non frost-free freezer, or in a styrofoam container in a frost free freezer, for no more than 2 months at -15C or below

Date prep. ___/___/___ Lab Exp. Date ___/___/___

a. Thaw and use within 24 hours

d. Scintillation fluid expires 6 months after opening

Date opened ___/___/___ Lab Exp. Date ___/___/___

TECHNIQUE

6. Control point and Negative Control average to be determined for each new lot of reagents. Steps 6, 7, and 8 are for the various Charm beta-Lactam screening methods and it is operator choice which method is followed

a. Competitive Assay control point (CP) and Negative Control average

1. Run six 0.008 IU/mL pen G

2. Run three negative controls

Penicillin G

Negative Control

1. _____

1. _____

2. _____

2. _____

3. _____

3. _____

4. _____

Av. _____

5. _____

6. _____

Av. _____

+15% _____

CP _____

b. Sequential Assay control point (CP) and Negative Control average

1. Run six 0.008 IU/mL pen G

2. Run three negative controls

Penicillin G

Negative Control

1. _____

1. _____

2. _____

2. _____

3. _____

3. _____

4. _____

Av. _____

5. _____

6. _____

Av. _____

+25% _____

CP _____

c. Quantitative Assay control point (CP) and negative Control average

1. Run six Negative Controls

2. Run three 0.002 IU/mL pen G (1 part 0.008 IU/mL and 3 parts Negative Control)

Negative Control

Penicillin G

1. _____

1. _____

2. _____

2. _____

3. _____

3. _____

4. _____

Av. _____

5. _____

6. _____

Av. _____

-15% _____

CP _____

d. Cloxacillin Assay control point (CP) and Zero Control average

1. Run six 10 ppb Cloxacillin

b. Run three Negative Controls

Cloxacillin

Negative Control

1. _____

1. _____

2. _____

2. _____

3. _____

3. _____

4. _____

Av. _____

5. _____

6. _____

Av. _____

+15% _____

CP _____

7. Acceptability of control point determinations

a. If any of the 6 control point determinations deviate from the average, redo that determination

1. For Competitive Assay can not deviate by more than $\pm 15\%$

2. For Sequential Assay can not deviate by more than $\pm 25\%$

3. For Quantitative Assay can not deviate by more than $\pm 15\%$

4. For Cloxacillin Assay can not deviate by more than $\pm 15\%$

b. If the re-determined value is within the allowed deviation recalculate the average and proceed with testing

- c. If the value is not within allowed deviation then another set of 6 standards must be run

8. Daily Performance and Operation Check (also see App. N GR item 10)

- a. The negative control tests $\pm 20\%$ ($\pm 15\%$ for Quantitative Assay) established for each new kit lot
- b. The positive control tests less than or equal to the control point
- c. If these conditions are not met re-determine control point(s)
 - 1. Conditions met, proceed with testing
 - 2. Conditions not met, discontinue testing and seek technical assistance

9. Beta-Lactam (all except Cloxacillin) Test Procedures

- a. Label test tubes, one for each test sample
- b. Add 1 green tablet to each tube
- c. Add 300 μL water to each tube
- d. Breakup tablets in tubes by mixing tubes 10 times on mixer in a rise and fall motion in 10 seconds, if necessary continue mixing, green tablets must be completely suspended before proceeding
- e. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc, use within 3 minutes
- f. Add 5.0 mL milk sample (draw up, avoiding foam and bubbles, expel and draw up again) to the appropriately labeled tubes
- g. Competitive Assay
 - 1. The following steps must be completed within 40 seconds (all sample tubes being assayed)
 - a. Add yellow tablet to each tube
 - b. Mix tubes 10 times on mixer in a rise and fall motion in 10 seconds (yellow tablets do not breakup)
 - 2. Incubate tubes for 3 minutes at $85 \pm 2\text{C}$
 - 3. Remove tubes and centrifuge for 3 minutes, optionally for 5 minutes (use same time used to determine control point)
 - 4. Skip to item 11

h. Sequential Assay

1. Mix tubes 10 times on mixer in a rise and fall motion in 10 seconds
2. Incubate tubes for 2 minutes at $65\pm 2C$
3. The following steps must be completed within 40 seconds (all sample tubes being assayed)
 - a. Add yellow tablet to each tube
 - b. Mix tubes as in item 1 above
4. Incubate tubes for 2 minutes at $65\pm 2C$
5. Remove tubes and centrifuge for 3 minutes, optionally for 5 minutes (use same time used to determine control point)
6. Skip to item 11

i. Quantitative Assay

1. Mix tubes 10 times on mixer in a rise and fall motion in 10 seconds
2. Incubate tubes for 7 minutes at $55\pm 2C$
3. The following steps must be completed within 40 seconds (all sample tubes being assayed)
 - a. Add yellow tablet to each tube
 - b. Mix tubes as in item 1 above
4. Incubate tubes for 2 minutes at $55\pm 2C$
5. Remove tubes and centrifuge for 3 minutes, optionally for 5 minutes (use same time used to determine control point)
6. Skip to item 11

10. Cloxacillin Test Procedure

a. Competitive Assay

1. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc, use within 3 minutes
2. Fill labeled test tubes $\frac{3}{4}$ full with milk samples and centrifuge for 5 minutes

3. Cool tubes to 0-4.4C _____
4. Label empty test tubes, one for each test sample _____
5. Add 1 white tablet to each new empty tube _____
6. Add 300 μ L water to each tube _____
7. Breakup tablets in tubes by mixing tubes 10 times on mixer in a rise and fall motion in 10 seconds, if necessary continue mixing, white tablets must be completely suspended before proceeding _____
8. Draw up 5.0 mL milk sample from below the fat layer, use new tip for each sample and add to the appropriately labeled tubes with white tablets (do not expel as in item 12f) _____
9. The following steps must be completed within 40 seconds (all sample tubes being assayed) _____
 - a. Add blue tablet to each tube _____
 - b. Mix tubes 10 times on mixer in a rise and fall motion in 10 seconds (blue tablets do not breakup) _____
10. Incubate tubes for 3 minutes at 35 \pm 2C _____
11. Remove tubes and centrifuge for 5 minutes _____

11. After centrifugation step in Beta-Lactam (9g3, 9h4, and 9i4) and Cloxacillin (10a11) test procedures _____

- a. Immediately pour off milk _____
- b. While still draining tubes, remove fat ring with 2 or more cotton swabs, continue until dry, do not touch pellet (do not go much below the fat ring) _____
- c. Add 300 μ L of water to tubes and break up pellets using vortex mixer _____
- d. Pellets must be completely suspended before proceeding to next step _____
- e. Add 3 mL of scintillation fluid to each tube, cap and vortex until uniformly mixed _____
- f. Count tubes on scintillation counter for 1 minute using [14C] channel _____
- g. Record counts as counts per minute (CPM) _____

12. Interpretation

- a. If the beta-Lactam assay (not applicable to Cloxacillin Assay) result in the analyzer is at least 50 points greater than the control point, then the sample is Negative (NF)
- b. If Cloxacillin assay result is greater than the control then the sample is Negative (NF)
- c. If the beta-Lactam assay result in the analyzer is less than or equal to the control point then the sample is Presumptive Positive
- d. If the beta-Lactam assay (not applicable to Cloxacillin Assay) result in the analyzer is less than 50 points greater than the control point, then the sample must be re-counted
 - 1. If on re-count the result is greater than the control point, then the sample is Negative (NF)
 - 2. If on re-count the result is equal to or less than the control point then the sample is Presumptive Positive

13. Verification of Initial Positive Samples (see App. N GR item 11); Confirmation of Presumptive Positive Samples (see App. N GR item 12); and Producer Traceback (see App. N GR item 13). For Quantitative Assay: PROMPTLY retest the SAME sample using the Sequential Assay or Competitive Assay, and when these beta-Lactam assays give Not Found [NF] the Cloxacillin Assay is required

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

15. Handling of exempt quantities of radioactive materials

- a. No mouth pipetting
- b. No smoking, eating or use of cosmetics while reagents are being handled
- c. NRC licensed facilities must meet license requirements as they relate to the use of gloves, other protective measures, and handling of waste
- d. Wash hands thoroughly after handling reagents
- e. Wipe up spills immediately and thoroughly
- f. Properly dispose of all contaminated waste

APPENDIX N BULK MILK TANKER SCREENING TEST FORM

CHARM II COMPETITIVE ASSAYS

FOR SULFONAMIDES, CHLORAMPHENICOL AND TETRACYCLINES

GENERAL REQUIREMENTS

1. See Appendix N General Requirements form items 1-8 & 15 _____

SAMPLES

2. See Appendix N General Requirements (GR) form item 9 _____

APPARATUS & REAGENTS

3. **Equipment** _____

- a. Analyzer heater for 13 x 100 mm tubes _____

1. 85±2C for Sulfonamide Assay _____

2. 35±2C for Tetracycline Assay _____

3. Temperature checked by electronic display, or by placing standardized thermometer in tube containing liquid (bulb submersed) in heating unit, records maintained _____

4. Or, use 6 inch partial immersion thermometer placed directly into small thermometer well in middle of heating unit, records maintained _____

- b. Ice-water bath, 0-4.4C for Chloramphenicol and Other Amphenicol Assay _____

- c. Mixer, Maxi-mixer II or equivalent _____

- d. Centrifuge, whisperfuge or Heraeus (3400 rpm) or equivalent _____

- e. Scintillation counter, Charm II or equivalent _____

- f. Scintillation fluid dispenser, set to dispense 3 mL _____

1. Checked quarterly with Class A graduate cylinder and record _____

- g. Cotton swabs (not applicable for Amphenicol Assay) _____

- h. Borosilicate test tubes, 13 x 100 mm _____

- i. Plastic stoppers for tubes _____

- j. Pipettors- Fixed Volume (see App. N GR item 7) _____
 - 1. 300 μ L and appropriate tips _____
 - 2. 5.0 mL and appropriate tips _____
 - 3. 1.0 mL and appropriate tips (not applicable Sulfa Drug Assay) _____
- k. Timer _____

4. Reagents _____

- a. Scintillation fluid, Optifluor or equivalent supplied by manufacturer _____
- b. Sulfonamide Assay _____
 - 1. Reagent blister packages: microbial/antibody binder (white) tablet, tracer reagent (pink) tablet _____
 - Lot # _____ Exp. Date _____ _____
 - 2. 10 ppb Sulfamethazine standard or multi-standard _____
 - Lot # _____ Exp. Date _____ _____
 - 3. Zero control standard _____
 - Lot # _____ Exp. Date _____ _____
- c. Chloramphenicol or other Amphenicol Assay _____
 - 1. Reagent blister packages: reagent (white tablet), Tracer reagent (green tablet) and Charcoal (black tablet) _____
 - Lot # _____ Exp. Date _____ _____
 - 2. 1 ppb Chloramphenicol standard or multi-standard _____
 - Lot # _____ Exp. Date _____ _____
 - 3. Zero control standard _____
 - Lot # _____ Exp. Date _____ _____
- d. Tetracycline Assay _____
 - 1. Reagent blister packages: microbial/antibody binder (white) tablet, tracer reagent (orange) tablet _____
 - Lot # _____ Exp. Date _____ _____

2. 30 ppb Oxytetracycline standard or multi-standard _____

Lot # _____ Exp. Date _____

3. Zero control standard _____

Lot # _____ Exp. Date _____

5. Reagent stability _____

a. All tablet reagents stored at -15C or below _____

b. Positive Control – Lyophilized 10 ppb Sulfamethazine,
30 ppb Oxytetracycline and 1 ppb Chloramphenicol standards
stable for 1 year, and reconstituted for 48 hours at 0-4.4C _____

1. Reconstitute with 100 mL (measured) Zero Control (allow to
sit 15 minutes prior to use or aliquotting) _____

Date prep. ___/___/___ Lab Exp. Date ___/___/___

2. Or, freeze immediately and store in a non frost-free freezer,
or in a styrofoam container in a frost-free freezer, for no more
than 2 months at -15C or below _____

Date prep. ___/___/___ Lab Exp. Date ___/___/___

a. Thaw and use within 24 hours _____

c. Negative Control – Lyophilized zero control standard, stable for
1 year and reconstituted for 72 hours at 0-4.4C. Alternatively, raw
milk qualified to test average (N=3) within $\pm 10\%$ of zero control
standard _____

Date prep. ___/___/___ Lab Exp. Date ___/___/___

1. Or, freeze immediately and store in a non frost-free freezer,
or in a styrofoam container in a frost free freezer, for no more
than 2 months at -15C or below _____

Date prep. ___/___/___ Lab Exp. Date ___/___/___

a. Thaw and use within 24 hours _____

d. Optifluor expires 6 months after opening _____

Date opened ___/___/___ Lab Exp. Date ___/___/___

TECHNIQUE

6. Control point and Zero Control average to be determined for each new lot of reagents

a. Sulfonamide Assay control point (CP) and Negative Control average

1. Run six 10 ppb sulfamethazine

2. Run three negative controls

Sulfamethazine

Negative Control

1. _____

1. _____

2. _____

2. _____

3. _____

3. _____

4. _____

Av. _____

5. _____

6. _____

Av. _____

+24% _____

CP. _____

b. Chloramphenicol or other Amphenicol Assay control point (CP) and Negative Control average

1. Run six 1 ppb chloramphenicol

2. Run three Negative controls

Chloramphenicol

Negative Control

1. _____

1. _____

2. _____

2. _____

3. _____

3. _____

4. _____

Av. _____

5. _____

6. _____

Av. _____

+25% _____

CP. _____

c. Tetracycline Assay control point (CP) and Negative Control average _____

1. Run six 30 ppb oxytetracycline

2. Run three Negative controls

Oxytetracycline

Negative Control

1. _____

1. _____

2. _____

2. _____

3. _____

3. _____

4. _____

Av. _____

5. _____

6. _____

Av. _____

+23% _____

CP. _____

7. Acceptability of control point determinations _____

a. If any of the 6 control point determinations deviate from the average, redo that determination _____

1. For Sulfonamide Assay can not deviate by more than $\pm 24\%$ _____

2. For Tetracycline Assay can not deviate by more than $\pm 23\%$ _____

3. For Chloramphenicol Assay can not deviate by more than $\pm 25\%$ _____

b. If the re-determined value is within the allowed deviation recalculate the average and proceed with testing _____

c. If the value is not within allowed deviation then another set of 6 standards must be run _____

8. Daily Performance and Operation Check (also see App. N GR item 10) _____

a. The Negative control tests $\pm 20\%$ established for each new kit lot _____

b. The positive control tests less than or equal to the control point _____

c. If these conditions are not met re-determine control point(s) _____

1. Conditions met, proceed with testing _____

2. Conditions not met, discontinue testing and seek technical assistance _____

9. Test Procedures

a. Sulfonamide Assay

1. Label test tubes, one for each test sample
2. Add 1 white tablet to each tube
3. Add 300 μ L water to each tube
4. Breakup tablets in tubes by mixing tubes 10 times on mixer in a rise and fall motion in 10 seconds, white tablets must be completely suspended before proceeding
5. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc, use within 3 minutes
6. Add 5.0 mL milk sample (draw up, avoiding foam and bubbles, expel and draw up again) to the appropriately labeled tubes
7. The following steps must be completed within 40 seconds (all sample tubes being assayed)
 - a. Add pink tablet to each tube
 - b. Mix tubes 10 times on mixer in a rise and fall motion in 10 seconds (pink tablets do not breakup)
8. Incubate tubes for 3 minutes at $85\pm 2^{\circ}\text{C}$
9. Remove tubes and centrifuge for 3 minutes, optionally for 5 minutes (use same time used to determine control point)
10. After centrifugation, immediately pour off milk
11. While still draining tubes, remove fat ring with 2 or more cotton swabs, continue until dry, do not touch pellet (do not go much below the fat ring)
12. Add 300 μ L of water to tubes and break up pellets using vortex mixer
13. Pellets must be completely suspended before proceeding to next step
14. Add 3 mL of scintillation fluid to each tube, cap and vortex until uniformly mixed
15. Count tubes on scintillation counter for 1 minute using [3H] channel

16. Record counts as counts per minute (CPM) _____

- b. Chloramphenicol or Other Amphenicol Assay _____
 1. Label test tubes, one for each test sample _____
 2. Add 1 white tablet to each tube _____
 3. Add 300 μ L water to each tube _____
 4. Breakup tablets in tubes by mixing tubes 10 times on mixer in a rise and fall motion in 10 seconds, white tablets must be completely suspended before proceeding _____
 5. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc or vortex, use within 3 minutes _____
 6. Add 1.0 mL milk sample (draw up, avoiding foam and bubbles, expel and draw up again) to each tube using new tip for each sample _____
 7. The following steps must be completed within 40 seconds (all assay tubes being assayed) _____
 - a. Add 1 green tablet to each tube _____
 - b. Mix tubes as in 4 above _____
 - c. Add black tablet to each tube _____
 - d. Mix tubes as in 4 above _____
 8. Incubate tubes in an ice bath (50% ice, 50% water) at 0-4.4C for 3 minutes _____
 9. Remove tubes and centrifuge for 5 minutes _____
 10. Using 300 μ L pipettor immediately add 300 μ L of centrifuged sample (removed avoiding fat and with-out disturbing pellet) to a new labeled tube _____
 11. Use fresh tip for each sample _____
 12. Add 3 mL of scintillation fluid to each tube, cap and vortex until uniformly mixed _____
 13. Count tubes on scintillation counter for 1 minute using [3H] channel _____
 14. Record counts as counts per minute (CPM) _____

c. Tetracycline Assay

1. Label test tubes, one for each test sample
2. Mix samples/controls by shaking 25 times in 7 seconds through 1 ft arc, use within 3 minutes. Dilute 1 ml of sample with 9 ml of Zero Control, repeat mixing. **Controls are not diluted before testing**
3. Add 1 white tablet to each empty tube
4. Add 300 μ L water to each tube
5. Breakup tablets in tubes by mixing tubes 10 times on mixer in a rise and fall motion in 10 seconds, white tablets must be completely suspended before proceeding
6. Add 5.0 mL diluted milk sample or undiluted controls (draw up avoiding foam and bubbles, expel and draw up again) to the appropriately labeled tubes
7. The following steps must be completed within 40 seconds (all sample tubes being assayed)
 - a. Add orange tablet to each tube
 - b. Mix tubes 15 times on mixer in a rise and fall motion in 20 seconds (orange tablets do not breakup)
8. Incubate tubes for 3 minutes at $35\pm 2C$
9. Remove tubes and centrifuge for 5 minutes
10. After centrifugation immediately pour off milk
11. While still draining tubes, remove fat ring with 2 or more cotton swabs, continue until dry, do not touch pellet (do not go much below the fat ring)
12. Add 300 μ L of water to tubes and break up pellets using vortex mixer
13. Pellets must be completely suspended before proceeding to next step
14. Add 3 mL of scintillation fluid to a tube, cap and vortex until uniformly mixed. Count tubes on scintillation counter for 1 minute using [3H] channel
15. Repeat step 14 with each tube to be analyzed.

16. Record counts as counts per minute (CPM)

10. Interpretation

- a. If the number of the measured activity in the analyzer is greater than the control point, then the sample is Negative (NF)
- b. If the number of the measured activity in the analyzer is less than or equal to the control point then the sample is Presumptive Positive

11. Verification of Initial Positive Samples (see App. N GR item 11); Confirmation of Presumptive Positive Samples (see App. N GR item 12);and Producer Traceback (see App. N GR item 13)

12. Reporting (see App. N GR item 12)

13. Handling of exempt quantities of radioactive materials

- a. No mouth pipetting
- b. No smoking, eating or use of cosmetics while reagents are being handled
- c. NRC licensed facilities must meet license requirements as they relate to the use of gloves, other protective measures, and handling of waste
- d. Wash hands thoroughly after handling reagents
- e. Wipe up spills immediately and thoroughly
- f. Properly dispose of all contaminated waste

**PHOSPHATASE TEST - CHARM FAST ALKALINE PHOSPHATASE TEST
USING CHARM NOVALUM**

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

SAMPLES

- 1. Laboratory Requirements (see CP, items 33 & 34)** _____

APPARATUS

- 2. CP, items 1 - 32 (as necessary)** _____

- a. Unless otherwise stated, "shake vigorously" refers to standard microbiological mixing, i.e., 25 times in a one foot arc in seven seconds _____

- 3. Pipettors and Pipets** _____

- a. Fixed volume or electronic, 100 μ L _____
- b. Calibration checked as specified in CP item 6e, records maintained _____
- c. Disposable, 10 mL (ASTM) pipet with 0.1 mL graduations _____

- 4. Microtube adapter for NovaLUM** _____

- 5. NovaLUM Analyzer** _____

- a. Operating instructions available _____
1. Channels configured for FAP assay _____
- a. FAP MILK – 45 second time _____
- b. FAP CREAM – 90 Seconds time _____
- c. FAP CHOC – 90 Seconds time _____
2. Thermoprobe connected with NovaLUM positioned upright in Stand _____
- a. Probe measuring ambient room temperature, DO NOT IMMERSSE IN WATER (Ambient room temperature must be between 18-24C to run the test) _____
- b. Definitions: _____
1. FAP MILK- Fluid white milks - including skim through whole fat milk _____

- 2. FAP CREAM- Unflavored liquid dairy products - including half and half, buttermilk, creams (light, medium and whipping), etc _____
- 3. FAP CHOC- Flavored liquid dairy products - Liquid products that can be accurately pipetted, containing flavor additives and/or thickening agents including flavored milks, and etc _____

6. **Water Bath, circulating, 34±1C and 63±1C (or 66±1C if fat > 10%), or 13 x 100 test tube dry well heater blocks acceptable (Confirmation procedure)** _____

7. **Centrifuge - Charm II Heraeus (3,400 RPM), minifuge, or equivalent (1,200 - 2,000 g)** _____

8. **Handling and storage** _____

a. Kit contains Reagent FAP Vials and Calibrator Tablets _____

Kit: Lot # _____ Rcd. Date: ___/___/___ Exp Date: ___/___/___ _____

Calibrator Lot # _____ Exp Date: ___/___/___ _____

b. Reagents stored at 0-4.4C until expiration date _____

c. FAP vials must be at 18-24C at time of use, may be stored at room temperature, expiration date marked 3 weeks from room temperature storage _____

CONTROLS

9. **Negative Calibrator/Control** _____

a. Product type. Prepare at least 20 mL of negative sample for use as a negative calibrator/control and to rehydrate 350mU/L positive calibrator/ control _____

1. Fluid white milk - heat a sample of product (highest fat content) to 95±1C for 1 minute with stirring _____

2. Flavored liquid dairy products - heat a chocolate sample (highest fat content) to 95±1C for 1 minute with stirring _____

a. Cool rapidly in an ice bath and hold at 0-4.4C _____

b. Centrifuge for 3 minutes and decant supernatant _____

3. Unflavored liquid dairy products - heat pasteurized light cream to 95±1C for 1 minute with stirring _____

4. Note: if product precipitates during negative sample preparation, e.g. sheep milk, heating sample to 63C for 45 minutes is acceptable. If using 13 x 100 test tube dry well heater block at 95C, it takes 10 minutes to heat product to 95C for one minute, use temperature control _____
- b. Cool rapidly in an ice bath and hold at 0-4.4C _____
- c. Kept at 0-4.4C, the Negative Control/Sample may be used for up to 48 hours _____
- d. If desired, distribute 1 mL quantities into small tubes (see 5.a.2.b for product definitions), seal and freeze in a non-frost-free freezer or in a styrofoam container placed in the center of a frost-free freezer for no more than 2 months at -15C or below, vials labeled with preparation and expiration dates _____

10. Positive 350 mU/L Calibrator/Control _____

- a. Prepare Positive Calibrator/Control _____
 1. Rehydrate a calibrator tablet with 100 uL water, mix to disperse tablet, wait 1 minute and mix again _____
 2. Add 2.5 mL of Negative Calibrator/Control to dissolve calibrator tablet _____
 3. Shake vigorously and let settle 10 minutes at 0-4.4C for re-suspension _____
 4. Shake vigorously again and use for test _____
- b. Positive calibrator/control held at 0-4.4C may be used for 48 hours _____

CALIBRATION

11. With each new kit lot # calibrate analyzer _____

- a. Prepare Negative Calibrator/Control and Positive Calibrator/Control, sections 9 and 10 _____
- b. Calibrate NovaLUM by entering 'Calibration Menu' from Main Menu _____
 1. Press 8 in Main Menu or scroll down to menu item 8 _____
 2. Press Enter _____

3. Select FAP assay, menu item 3

Note: Calibration menu of previously calibrated instruments may also be entered from 'Programmed Plans', selecting channel, and then selecting 'calibrate' from the menu list

- c. Select appropriate channel for calibration and follow prompts.
Note: Previously calibrated channels will list a selection menu, select 'calibrate', follow prompts

1. Test a negative calibrator/control, section 13c
2. Test a positive calibrator/control, section 13c
3. Instrument will make internal adjustments
4. Test another negative calibrator/control, section 13c
5. Test another positive calibrator/control, section 13c
6. If performance of negative (<15) and positive is in range (320-400), instrument will prompt calibration successful. If performance out of range, instrument will recalculate settings and prompt to perform another positive and negative calibrator/control
7. Repeat steps 4-6. If out of range NovaLUM will prompt a re-calibration, step 1

DAILY PERFORMANCE CHECKS

12. Daily test a Negative Control/sample (item 9) and Positive Control (item 10), for at least one product

- a. Verify FAP vial stored at room temperature. Select NovaLUM 'programmed plans', select appropriate FAP channel and select menu 3 'Control Check'. Follow Prompts
 1. Test positive calibrator/control, section 13c. Positive Control valid, 247-453 mU/L
 2. Test negative calibrator/control, section 13c. Negative Control valid or less than or equal to 15 mU/L

TEST PROCEDURE

13. Procedure

- a. Prepare sample
 - 1. Invert filled retail container 25 times, each inversion a full cycle down and up. Negative control – shake vigorously or vortex at least 10 seconds at maximum setting
 - 2. For flavored dairy products (not including controls, item 9 & 10)
 - a. Add 1 mL of sample into an appropriate tube or vial (NOT FAP vial)
 - b. Centrifuge for 3 minutes
 - c. Use liquid extract in item 15c
- b. Select NovaLUM programmed plans, select appropriate
 - 1. NovaLUM FAP channel and select menu item 1, “RUN”
 - 2. Select appropriate programmed plan
 - 3. NovaLUM should be in ‘RUN SAMPLE’ Screen
- c. Verify FAP vial stored at room temperature.
 - 1. Pierce foil top with clean pipet tip.
- d. Dispense 100 μ L of the prepared sample (item 13a) or mixed controls (items 9 & 10) into the FAP vial liquid and then immediately press enter on NovaLUM
 - 1. Follow prompt and vortex FAP vial with sample for 5 seconds at maximum setting
 - 2. Follow prompt and attach microtube adapter to threaded side of vial. Then fully insert vial into NovaLUM chamber. This step must be completed while screen is flashing (30 seconds)
- e. At the end of pre-programmed time, the screen will stop flashing and count the sample. The mU/L phosphatase level will be displayed on screen. Press OK to print and prepare for next sample

- f. Samples with ≥ 350 mU/L of ALP activity are suspect positive and must be tested for microbial, and reactivated phosphatase (items 14 & 15)

CONFIRMATION

14. Microbial Phosphatase

- a. Heat 1.0 mL of suspect sample at $63 \pm 1^\circ\text{C}$ for 30 minutes, stirring or mixing every 10 minutes
 - 1. If fat content is $>10\%$, heat at $66 \pm 1^\circ\text{C}$ for 30 minutes
- b. Cool sample rapidly to $0-4.4^\circ\text{C}$ in an ice bath
- c. Test positive and negative controls following item 13
- d. Test heated sample and unheated sample (original sample) following item 13
- e. Interpretation
 - 1. Controls test as specified in item 12
 - 2. If heated and unheated sample have equal activity ($\pm 30\%$, mU/L or RLU) the sample is regarded Not Found for residual phosphatase, the activity originally measured is microbial
 - 3. If the heated sample is more than 30% below unheated sample (mU/L or RLU), the sample contains milk phosphatase activity, either residual or reactivated

15. Reactivated Phosphatase

- a. Magnesium acetate solution commercially available
- b. Or, prepared in laboratory
 - 1. Dissolve 35.4 g of magnesium acetate tetra-hydrate, $\text{Mg}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$ in 25 mL water, warming slightly to aid dissolution
 - 2. Pour solution into 100 mL volumetric flask, rinse original container several times and add rinses to flask
 - 3. After cooling to room temperature, make up to 100 mL (stable for 1 year at $0-4.4^\circ\text{C}$)
- c. Procedure
 - 1. Label separate test tubes as "Blank" and "Test"

2. Add a 5.0 mL aliquot of sample (unheated, original sample not prepared as in 13a) to each test tube _____
3. Add 0.1 mL MS water to the sample labeled "Blank", and 0.1 mL magnesium acetate solution to the sample labeled "Test" _____
4. Cap tubes and heat both aliquots for 1 hr at 34±1C _____
5. Remove samples from water bath and cool rapidly to 0-4.4C in an ice bath _____
6. Dilute 1 mL of sample containing magnesium acetate (Test) with 5 mL (1:6 dilution) of negative control product (item 9), label tube as "Diluted Test" _____
7. Test undiluted sample containing no magnesium acetate (Blank) and diluted sample containing magnesium acetate (Diluted Test) for phosphatase activity following item 13 _____

d. Interpretation _____

1. If the diluted aliquot containing magnesium acetate (Diluted Test) has equal (±30%) or greater phosphatase activity than the undiluted aliquot containing no magnesium (Blank), the sample is regarded as Not Found for residual phosphatase, and the phosphatase originally measured is of reactivated origin _____

$$\text{Dil. w/Mg (Test)} \geq \text{Undil. (Blank)} = \text{Reactivated}$$

2. If the diluted aliquot (Diluted Test) contains less (30% below or less) activity than the undiluted aliquot (Blank) the sample is considered Positive for residual phosphatase _____

$$\text{Dil. w/Mg (Test)} < \text{Undil. (Blank)} = \text{Residual}$$

3. A false-positive for residual phosphatase may also be obtained if a reactivatable sample has been allowed to stand at elevated temperatures (20C) for periods of 1 hr or more before testing (SPC < 20,000/mL) _____

REPORT

16. Report as: _____

- a. Residual phosphatase Not Found (NF) _____

1. Record as <88 mU/L _____

2. Report as Not Found (NF) _____

- b. Residual phosphatase Positive _____
 - 1. Microbial and reactivatable phosphatase are not demonstrated _____
 - 2. Suspect positives greater than or equal to 350 mU/L, must be tested for microbial and reactivated phosphatase (items 14 and 15) _____
 - 3. Report mU/L values when equal to or greater than 88 mU/L _____
- c. Report as Not Found (NF) for residual phosphatase if: _____
 - 1. If microbial phosphatase present _____
 - 2. If reactivated phosphatase present _____
 - c. If there is documentation to show that the product was treated such that reactivated phosphatase may be present _____