

**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  $\mu$ 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  $\mu$ 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  $\geq 350$  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 \_\_\_\_\_

**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  $\mu$ 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**

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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**

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**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).**

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**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)**

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**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  $\mu$ 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 \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

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\_\_\_\_\_

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\_\_\_\_\_

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 \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

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**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

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- 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

\_\_\_\_\_

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\_\_\_\_\_

**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  $\mu\text{L}$  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

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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  $\mu$ 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  $35\pm 2$ C \_\_\_\_\_
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  $\mu$ 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  $\mu$ 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. \_\_\_\_\_
- 2. \_\_\_\_\_
- 3. \_\_\_\_\_
- 4. \_\_\_\_\_
- 5. \_\_\_\_\_
- 6. \_\_\_\_\_
- Av. \_\_\_\_\_
- +24% \_\_\_\_\_
- CP. \_\_\_\_\_

- 1. \_\_\_\_\_
- 2. \_\_\_\_\_
- 3. \_\_\_\_\_
- Av. \_\_\_\_\_

\_\_\_\_\_

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. \_\_\_\_\_
- 2. \_\_\_\_\_
- 3. \_\_\_\_\_
- 4. \_\_\_\_\_
- 5. \_\_\_\_\_
- 6. \_\_\_\_\_
- Av. \_\_\_\_\_
- +25% \_\_\_\_\_
- CP. \_\_\_\_\_

- 1. \_\_\_\_\_
- 2. \_\_\_\_\_
- 3. \_\_\_\_\_
- Av. \_\_\_\_\_

\_\_\_\_\_

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  $\mu$ 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  $85\pm 2C$
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  $\mu$ 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  $\mu$ 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  $\mu$ L pipettor immediately add 300  $\mu$ 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  $\mu$ 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  $\mu$ 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)

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**10. Interpretation**

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- 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

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**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)**

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

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**13. Handling of exempt quantities of radioactive materials**

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- 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

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