

**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)

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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

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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

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Date prep. \_\_\_/\_\_\_/\_\_\_      Lab Exp. Date \_\_\_/\_\_\_/\_\_\_

a. Thaw and use within 24 hours

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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

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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

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d. Scintillation fluid expires 6 months after opening

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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

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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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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

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

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- 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**

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- 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 (avoiding foam and bubbles) to the appropriately identified 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±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±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±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±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 identified test tubes ¾ 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 identified 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  $\mu$ 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