

# CHARM® II BETA-LACTAM ASSAYS

## APPENDIX N BULK MILK TANKER SCREENING TEST FORM

Competitive (Raw Commingled Cow Milk and Pasteurized White Milks) IMS #9-C2  
Sequential (Raw Commingled Cow and Goat Milk) IMS #9-C3  
Quantitative (Raw Commingled Cow Milk) IMS #9-C4  
Cloxacillin (Raw Commingled Cow Milk) IMS #9-C9

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

### GENERAL REQUIREMENTS

1. See Appendix N General Requirements (App. N GR) items 1-8 & 15 \_\_\_\_\_

### SAMPLES

2. See App. N GR item 9 \_\_\_\_\_

### APPARATUS & REAGENTS

3. **Equipment** \_\_\_\_\_

- a. Analyzer heater for 13 x 100 mm tubes \_\_\_\_\_

1.  $85\pm 2^\circ\text{C}$  for Competitive Assay \_\_\_\_\_

2.  $65\pm 2^\circ\text{C}$  for Sequential Assay \_\_\_\_\_

3.  $55\pm 2^\circ\text{C}$  for Quantitative Assay \_\_\_\_\_

4.  $35\pm 2^\circ\text{C}$  for Cloxacillin Assay \_\_\_\_\_

5. Temperature checked by electronic display, or by placing accuracy checked temperature measuring device in tube containing liquid (bulb submersed) in heating unit; maintain records \_\_\_\_\_

6. Or, use 6 inch partial immersion thermometer placed directly into small thermometer well in middle of heating unit; maintain records \_\_\_\_\_

7. Temperature measuring device for each incubator (App. N item 3) \_\_\_\_\_

- 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. Check every six (6) months with Class A graduated cylinder and record; maintain records \_\_\_\_\_
- f. Cotton swabs \_\_\_\_\_
- g. Borosilicate test tubes, 13 x 100 mm \_\_\_\_\_
- h. Plastic stoppers for tubes \_\_\_\_\_
- i. Pipettors - Fixed Volume or Electronic (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 test kits \_\_\_\_\_
- 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  $-15^{\circ}\text{C}$  or below \_\_\_\_\_

b. Positive Control – Lyophilized 0.008 IU/mL penicillin G or 10 ppb Cloxacillin standard for Cloxacillin assay \_\_\_\_\_

1. Reconstitute with 100 mL (measured) Negative Control (allow to sit 15 min prior to use or aliquotting) \_\_\_\_\_

Lab Prep. Date: \_\_\_\_\_ Lab Exp. Date: \_\_\_\_\_

2. For Quantitative Only: Dilute reconstituted 0.008 IU/mL Penicillin G standard 1:4 with Zero Control Standard \_\_\_\_\_

3. Use within 48 hours when stored at  $0.0-4.5^{\circ}\text{C}$  \_\_\_\_\_

4. Or, aliquot within 24 hours and freeze at  $-15^{\circ}\text{C}$  or colder in a non frost-free freezer or in an insulated foam container in a frost-free freezer; use within 2 months \_\_\_\_\_

Lab Prep. Date: \_\_\_\_\_ Lab Exp. Date: \_\_\_\_\_

a. Thaw and use within 24 hours. Store at  $0.0-4.5^{\circ}\text{C}$  \_\_\_\_\_

c. Negative Control – Lyophilized Zero Control Standard (ZCS) or alternatively, raw milk qualified to test similar to ZCS \_\_\_\_\_

Lab Prep. Date: \_\_\_\_\_ Lab Exp. Date: \_\_\_\_\_

1. Reconstitute ZCS according to manufacture instructions. (Allow to sit 15 min prior to use or aliquotting) \_\_\_\_\_

a. To qualify raw milk, test sample 3 times and average results. Average must be within  $\pm 10\%$  of ZCS \_\_\_\_\_

Lab Prep. Date: \_\_\_\_\_ Lab Exp. Date: \_\_\_\_\_

2. Use within 72 hours when stored at  $0.0-4.5^{\circ}\text{C}$  \_\_\_\_\_

3. Or, aliquot within 24 hours and freeze at  $-15^{\circ}\text{C}$  or colder in a non frost-free freezer or in an insulated foam container in a frost-free freezer; use within 2 months \_\_\_\_\_

Lab Prep. Date: \_\_\_\_\_ Lab Exp. Date: \_\_\_\_\_

a. Thaw and use within 24 hours. Store at  $0.0-4.5^{\circ}\text{C}$  \_\_\_\_\_

d. Scintillation fluid expires six (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. \_\_\_\_\_
- 2. \_\_\_\_\_
- 3. \_\_\_\_\_
- 4. \_\_\_\_\_
- 5. \_\_\_\_\_
- 6. \_\_\_\_\_
- Av. \_\_\_\_\_
- 15% \_\_\_\_\_
- CP \_\_\_\_\_

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

d. Cloxacillin Assay Control Point (CP) and Zero Control Average \_\_\_\_\_

- 1. Run six 10 ppb Cloxacillin \_\_\_\_\_
- 2. Run three Negative Controls \_\_\_\_\_

Cloxacillin

Negative Control

- 1. \_\_\_\_\_
- 2. \_\_\_\_\_
- 3. \_\_\_\_\_
- 4. \_\_\_\_\_
- 5. \_\_\_\_\_
- 6. \_\_\_\_\_
- Av. \_\_\_\_\_
- +15% \_\_\_\_\_
- CP \_\_\_\_\_

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

**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 cannot deviate by more than  $\pm 15\%$  \_\_\_\_\_
- 2. For Sequential Assay cannot deviate by more than  $\pm 25\%$  \_\_\_\_\_
- 3. For Quantitative Assay cannot deviate by more than  $\pm 15\%$  \_\_\_\_\_
- 4. For Cloxacillin Assay cannot 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, run another set of six (6) standards \_\_\_\_\_
- d. A common control point for multiple analysts may be used \_\_\_\_\_
  - 1. Control point determination performed by one analyst only \_\_\_\_\_
  - 2. Control point determination rotated and inclusive of all certified/approved analysts \_\_\_\_\_
  - 3. If daily performance check fails and is not resolved by using fresh controls, technique should be reviewed for consistency and corrective action taken as necessary \_\_\_\_\_

**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  $\mu\text{L}$  water to each tube \_\_\_\_\_
- d. Breakup tablets in tubes by mixing tubes 10 times on mixer in a rise and fall motion in 10 sec, if necessary continue mixing, green tablets must be completely suspended before proceeding \_\_\_\_\_
- e. Mix milk sample(s)/control(s) 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting, use within 3 min (samples must be in appropriate container to allow the use of vortexing) \_\_\_\_\_
- f. Add 5.0 mL of mixed sample/control to corresponding tube \_\_\_\_\_
  - 1. Using pipettor (item 3.i.2) with new tip for each sample/control, draw up 5 mL avoiding foam or bubbles \_\_\_\_\_
  - 2. Remove tip from liquid \_\_\_\_\_
  - 3. Expel test portion into appropriate tube \_\_\_\_\_

g. Competitive Assay

1. The following steps must be completed within 40 sec (all sample tubes being assayed)
  - a. Add yellow tablet to each tube
  - b. Vortex tubes 10 times in a rise and fall motion in 10 sec (yellow tablets do not breakup)
2. Incubate tubes for 3 min at  $85\pm 2^{\circ}\text{C}$
3. Remove tubes and centrifuge for 3 min; optionally for 5 min (same time used to determine control point)
4. Skip to item 11

h. Sequential Assay

1. Vortex tubes 10 times in a rise and fall motion in 10 sec
2. Incubate tubes for 2 min at  $65\pm 2^{\circ}\text{C}$
3. The following steps must be completed within 40 sec (all sample tubes being assayed)
  - a. Add yellow tablet to each tube
  - b. Vortex tubes as in item 9.h.1 above
4. Incubate tubes for 2 min at  $65\pm 2^{\circ}\text{C}$
5. Remove tubes and centrifuge for 3 min; optionally for 5 min (same time used to determine control point)
6. Skip to item 11

i. Quantitative Assay

1. Vortex tubes 10 times in a rise and fall motion in 10 sec
2. Incubate tubes for 7 min at  $55\pm 2^{\circ}\text{C}$
3. The following steps must be completed within 40 sec (all sample tubes being assayed)
  - a. Add yellow tablet to each tube
  - b. Vortex tubes as in item 1 above
4. Incubate tubes for 2 min at  $55\pm 2^{\circ}\text{C}$

5. Remove tubes and centrifuge for 3 min; optionally for 5 min (same time used to determine control point) \_\_\_\_\_
6. Skip to item 11 \_\_\_\_\_

**10. Cloxacillin Test Procedure** \_\_\_\_\_

a. Competitive Assay \_\_\_\_\_

1. Mix milk sample(s)/control(s) 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting, use within 3 min (samples must be in appropriate containers to allow the use of vortexing) \_\_\_\_\_
2. Fill identified test tubes  $\frac{3}{4}$  full with milk samples, avoiding foam and bubbles, and centrifuge for 5 min \_\_\_\_\_
3. Cool tubes to 0.0-4.5°C \_\_\_\_\_
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 vortexing tubes 10 times on mixer in a rise and fall motion in 10 sec, if necessary continue vortexing, white tablets must be completely suspended before proceeding \_\_\_\_\_
8. Draw up 5.0 mL of centrifuged sample/control from below the fat layer \_\_\_\_\_
  - a. Use new tip for each sample/control \_\_\_\_\_
  - b. Remove tip from liquid \_\_\_\_\_
  - c. Expel test portion into appropriate tube \_\_\_\_\_
9. The following steps must be completed within 40 sec (all sample tubes being assayed) \_\_\_\_\_
  - a. Add blue tablet to each tube \_\_\_\_\_
  - b. Vortex tubes 10 times in a rise and fall motion in 10 sec (blue tablets do not breakup) \_\_\_\_\_
10. Incubate tubes for 3 min at 35 $\pm$ 2°C \_\_\_\_\_
11. Remove tubes and centrifuge for 5 min \_\_\_\_\_

**11. After Centrifugation Step in Beta-Lactam (9.g.3, 9.h.5, and 9.i.5) and Cloxacillin (10.a.11) 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 µL 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 or shake until uniformly mixed
- f. Count tubes on scintillation counter for 1 min 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 result 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. Nuclear Regulatory Commission (NRC) licensed facilities must meet requirements as they relate to the use of gloves, other protective measures, and handling of wastes
- 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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