

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 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 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°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°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°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. _____
- 2. _____
- 3. _____
- 4. _____
- 5. _____
- 6. _____
- Av. _____
- +15% _____
- CP _____

- 1. _____
- 2. _____
- 3. _____
- Av. _____

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

- 1. _____
- 2. _____
- 3. _____
- Av. _____

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 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 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 μ 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
