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 ±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±2°C for Competitive Assay
      2. 65±2°C for Sequential Assay
      3. 55±2°C for Quantitative Assay
      4. 35±2°C for Cloxacillin Assay
   b. Mixer, Maxi-mixer II or equivalent
   c. Centrifuge, Whisperfuge® or Heraeus® (3400 rpm) or equivalent
   d. Scintillation counter, Charm II or equivalent

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

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

Temperature measuring device for each incubator (App. N item 3)
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 µL 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

      Lab Prep. Date: ________    Lab Exp. Date: ________

   3. Use within 48 hours when stored at 0.0-4.5°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°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)

         Lab Prep. Date: ________    Lab Exp. Date: ________

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

         Lab Prep. Date: ________    Lab Exp. Date: ________

      2. Use within 72 hours when stored at 0.0-4.5°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°C
d. Scintillation fluid expires six (6) months after opening
   
   Date Opened: ________       Lab Exp. Date: ________

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

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<tr>
<th>Penicillin G</th>
<th>Negative Control</th>
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<td>CP _________</td>
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   b. **Sequential Assay Control Point (CP) and Negative Control Average**

   1. Run six 0.008 IU/mL Pen G
   2. Run three Negative Controls

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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)

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

1. Run six 10 ppb Cloxacillin
2. Run three Negative Controls

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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 cannot deviate by more than ±15%
2. For Sequential Assay cannot deviate by more than ±25%
3. For Quantitative Assay cannot deviate by more than ±15%
4. For Cloxacillin Assay cannot deviate by more than ±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 ±20% (±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±2°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±2°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±2°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±2°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±2°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 ¾ 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 µL 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±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