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±2ºC for Competitive Assay ____ _____
2. 65±2ºC for Sequential Assay ____ _____
3. 55±2ºC for Quantitative Assay ____ _____
4. 35±2ºC 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 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 –15ºC 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 0.0-4.5ºC

4. Or, aliquot within 24 hours and freeze at –15ºC 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 0.0-4.5ºC _____

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 ± 10% of ZCS _____

Lab Prep. Date: _____ Lab Exp. Date: _____ _____

2. Use within 72 hours when stored at 0.0-4.5ºC _____

3. Or, aliquot within 24 hours and freeze at –15ºC 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 0.0-4.5ºC _____

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.	_____	1.	_____
2.	_____	2.	_____
3.	_____	3.	_____
4.	_____	Av.	_____
5.	_____		
6.	_____		
Av.	_____		
–15%	_____		
CP	_____		

_____

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.	_____	1.	_____
2.	_____	2.	_____
3.	_____	3.	_____
4.	_____	Av.	_____
5.	_____		
6.	_____		
Av.	_____		
+15%	_____		
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 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±2ºC _____

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±2ºC _____

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±2ºC _____

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±2ºC _____

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±2ºC _____

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 0.0-4.5ºC _____

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±2ºC _____

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 _____