

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 & 13 _____

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 _____

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h. Plastic stoppers for tubes _____

i. Pipettors (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 _____

Beta Lactam Assays

b. Reagent blister packages: microbial binder (green) tablet, tracer reagent (yellow) tablet _____

Lot # _____ Exp. date _____

c. 0.008 IU/mL Penicillin G standard _____

Lot # _____ Exp. date _____

d. Zero control standard _____

Lot # _____ Exp. date _____

Cloxacillin Assay

e. Reagent blister packages: microbial/antibody binder (white) tablet, tracer reagent (blue) tablet _____

Lot # _____ Exp. date _____

f. 10 ppb cloxacillin standard _____

Lot # _____ Exp. date _____

g. Zero control standard _____

Lot # _____ Exp. date _____

5. Reagent stability _____

a. All tablet reagents stored at -15C or below _____

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b. Lyophilized 0.008 IU/mL penicillin G and 10 ppb

cloxacillin standards, 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) _____

2. **For Quantitative Only:** Dilute reconstituted 0.008 IU/mL penicillin G standard 1:4 with Zero Control and use within 48 hours _____

3. Test for suitability each time prepared, must produce appropriate reaction, records maintained _____

4. 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 _____

c. Lyophilized zero control standard, 1 year and reconstituted for 72 hours at 0-4.4C _____

1. Test for suitability each time prepared, must produce appropriate reaction, records maintained _____

2. 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 _____

d. Optifluor expires 6 months after opening _____

Date opened _____ Lab Exp. Date _____

6. **Competitive Assay** control point (CP) and Zero Control average

a. Run six 0.008
IU/mL pen G

b. Run three zero
controls

Penicillin G

Zero Control

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

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

7. **Sequential Assay** control point (CP) and Zero Control average

a. Run six 0.008
IU/mL pen G

b. Run three zero
controls

Penicillin G

Zero Control

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

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

8. **Quantitative Assay** control point (CP) and Zero Control

average

a. Run six Zero Controls

b. Run three Pen G controls

Zero Control

Pen G Control

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

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

9. **Cloxacillin Assay** control point (CP) and Zero Control average

a. Run six 10 ppb cloxacillin

b. Run three zero controls

Cloxacillin

Zero Control

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

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

10. 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 $\pm 15\%$

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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
- c. If the value is not within allowed deviation then another set of 6 standards must be run

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

- a. The zero 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

12. Test Procedures

Beta Lactam Assays

- 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 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 (draw up, avoiding foam and bubbles, expel and draw up again) to the appropriately labeled tubes

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- 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 $85 \pm 2C$ _____

3. Remove tubes and centrifuge for 3 minutes, optionally for 5 minutes (use same time used to determine control point) _____

4. Skip to item 12 1 _____

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 $65 \pm 2C$ _____

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 $65 \pm 2C$ _____

5. Remove tubes and centrifuge for 3 minutes, optionally for 5 minutes (use same time used to determine control point) _____

6. Skip to item 12 1 _____

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 ± 2 _____

3. The following steps must be completed within 40 seconds (all sample tubes being assayed) _____

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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 121 _____

Cloxacillin Assay

k. **Cloxacillin Assay** _____

- 1. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc, use within 3 minutes _____
- 2. Fill labeled test tubes $\frac{3}{4}$ 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 µL 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 labeled 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±2C _____

11. Remove tubes and centrifuge for 5 minutes _____

1. After centrifugation (all assays) _____

1. Immediately pour off milk _____

2. 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)** _____

3. Add 300 µL of water to tubes and break up pellets using vortex mixer _____

4. **Pellets must be completely suspended before proceeding to next step** _____

5. Add 3 mL of scintillation fluid to each tube, cap and vortex until uniformly mixed _____

6. Count tubes on scintillation counter for 1 minute using [¹⁴C] channel _____

7. Record counts as counts per minute (CPM) _____

13. Interpretation _____

a. If the number of the measured activity in the analyzer is at least 50 points greater than the control point, then the sample is **Negative (NF)** _____

b. If the number of the measured activity in the analyzer is less than or equal to the control point then the sample is **Presumptive Positive** _____

c. If the number of the measured activity 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** _____

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14. Confirmation of Presumptive Positive Samples (also see

App. N GR item 11)

- a. **Quantitative Assay: PROMPTLY** retest the **SAME** sample using the **Sequential Assay, and if necessary (Sequential Assay gives Not Found [NF]) the Cloxacillin Assay (Required)**

15. Reporting (see App. N GR item 12)

16. 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