

**APPENDIX N BULK MILK TANKER SCREENING TEST FORM**

**PENZYME<sup>®</sup> MILK/ PENZYME<sup>®</sup> III TESTS**

**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. Heating unit thermostatically controlled at 47±1C, checked daily \_\_\_\_\_

1. Temperature checked by placing standardized thermometer in vial containing ½ inch of liquid (bulb submersed) in heating block well, records maintained \_\_\_\_\_

2. Or, use 6 inch partial immersion thermometer placed directly into small well in middle of heating block, records maintained \_\_\_\_\_

b. Timer \_\_\_\_\_

**Penzyme Milk Test**

c. Syringe and tips to dispense 200 µL of sample, supplied by manufacturer \_\_\_\_\_

d. Optionally, use fixed volume 200 µL pipettor (see App.N GR item 7) \_\_\_\_\_

e. Penzyme Milk Test Kit \_\_\_\_\_

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_

1. Kit contains enzyme vials, reagent tablets, syringe, tips and forceps \_\_\_\_\_

2. Store kits at 0.5-4.4C \_\_\_\_\_

**Penzyme III Test**

f. Pipets to dispense 1.5 mL to enzyme vial \_\_\_\_\_

g. Fixed volume pipettors and tips (specified by manufacturer) to dispense 10 µL of enzyme solution and 50 µL of milk sample (see App. N GR item 7) \_\_\_\_\_

h. Penzyme III Test Kit \_\_\_\_\_

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_

1. Kit contains enzyme vial, reagent tablets and plastic forceps \_\_\_\_\_

2. Store kits at 0.5-4.4C \_\_\_\_\_

i. Enzyme vial (vial #1) \_\_\_\_\_

1. Reconstitute with 1.5 mL of cold (0.5-4.4C) deionized water \_\_\_\_\_

2. Shake gently and allow 10 minutes for contents to dissolve properly \_\_\_\_\_

3. Once reconstituted enzyme must be used within 30 days \_\_\_\_\_

Date prep. \_\_\_\_\_

4. Store reconstituted enzyme at 0.5-4.4C when not in use \_\_\_\_\_

j. Disposable, clear test tubes - size compatible with heating unit \_\_\_\_\_

4. Commercial Standard, 5.0 ppb Penicillin G \_\_\_\_\_

a. Store according to label instructions \_\_\_\_\_

Mfg. \_\_\_\_\_ Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

b. Re-hydrate as per manufacturer's instructions \_\_\_\_\_

c. Test for suitability each time prepared, must produce appropriate reaction, records maintained \_\_\_\_\_

d. Store solution at 0-4.4C for no more than 2 days \_\_\_\_\_

- e. Or, distribute sufficient amount in small containers, seal and freeze at -15C or below in non-frost-free freezer (or in a small styrofoam box, placed in center of frost-free freezer) for no more than 2 months

Date prep. \_\_\_\_\_ Lab Exp. Date \_\_\_\_\_

1. Thaw and use within 24 hours

5. Phosphate Buffered Saline

- a. Dissolve 2 grams Potassium dibasic phosphate and 8.0 grams of mono-basic potassium phosphate and make up to 1 liter. pH  $6.0 \pm 0.05$

6. Na or K Penicillin G Standard (USP or Human injectable)

- a. Store according to label instructions

Mfg. \_\_\_\_\_ Lot # \_\_\_\_\_ Exp. \_\_\_\_\_

- b. Use a 4 or 5 place analytical balance to weigh out the penicillin G

- c. Calculate the equivalent penicillin G base by using the appropriate correction factor, potency in IU/mg  $\div$  potency of Pen G<sup>-</sup> (1782 IU/mg) (ex. K PenG potency = 1596 IU/mg, purity equal to  $1596 \div 1782 = 0.895$  mg PenG<sup>-</sup>/mgKPenG)

- d. Make a 1 mg/mL stock solution by adding drug (100 mg PenG<sup>-</sup>  $\div$  item 18c) (ex.  $100 \div 0.895 = 111.7$  mg KpenG) to a 100 mL volumetric flask and making up with buffer (item 4)

- e. Make 1:100 serial dilution of the stock solution, using 100 mL volumetric flask (10  $\mu$ g/mL stock)

- f. Make the final dilution in inhibitor free milk (item 19 or 20) to yield the 5.0 ppb standard (ex. 0.5 mL of item 18.e. + 999.5 mL milk = 1000 mL of 5 ppb PenG<sup>-</sup>)

Date prep. \_\_\_\_\_

- g. Test for suitability each time prepared, must produce appropriate reaction, records maintained

h. Store 5.0 ppb standard at 0-4.4C for no more than 2 days \_\_\_\_\_

i. Or, distribute sufficient amount in small containers, seal and freeze at -15C or below in non-frost-free freezer (or in a small styrofoam box, placed in center of frost-free freezer) for no more than 2 months \_\_\_\_\_

Date prep. \_\_\_\_\_ Lab Exp. Date \_\_\_\_\_

1. Thaw and use within 24 hours \_\_\_\_\_

7. Inhibitor Free Milk (fluid milk product with milkfat 0.00 to 3.5%, total solids < 13%) \_\_\_\_\_

a. Test for suitability each time prepared, must produce appropriate reaction; records maintained \_\_\_\_\_

#### TECHIQUE

8. Daily Performance and Operation Check (see App. N GR item 10) \_\_\_\_\_

9. Test Procedures \_\_\_\_\_

a. **Penzyme Milk Test** \_\_\_\_\_

1. Have milk samples collected ready to use \_\_\_\_\_

2. Tap vial(s) gently on base to dislodge any enzyme from cap. Carefully remove tear-off seal and rubber cap from enzyme vial  
(**CAUTION:** tear-off seal has sharp edges) \_\_\_\_\_

3. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc, use within 3 minutes \_\_\_\_\_

4. With syringe (disposable tip in place) add 200 µL of sample(s) (draw up, avoiding foam and bubbles, expel and draw up again) to the corresponding labeled vial and replace stopper \_\_\_\_\_

a. Optionally use 200 µL pipettor to add samples to vials \_\_\_\_\_

5. Roll and shake vial(s) to get all of the enzyme into solution \_\_\_\_\_

6. Place capped vial(s) into pre-warmed 47±1C heater block and incubate for 5 minutes \_\_\_\_\_
7. Remove vial(s) from incubator \_\_\_\_\_
8. Remove stopper and add one (1) reagent tablet to each vial \_\_\_\_\_
9. Replace stoppers and shake sample vial(s) for 5 seconds to start tablet disintegration \_\_\_\_\_
10. Place vial(s) back in heater block and incubate for an additional 15 minutes \_\_\_\_\_
11. About 5 minutes into second incubation remove vial(s), shake contents for about 5 seconds and return to heating block \_\_\_\_\_
12. Remove vial(s) from heating block and gently swirl contents \_\_\_\_\_
13. Read results immediately by comparing color to chart enclosed with kit \_\_\_\_\_

b. **Penzyme III Test** \_\_\_\_\_

1. Have sample(s) ready to use \_\_\_\_\_
2. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc, use within 3 minutes \_\_\_\_\_
3. With pipettor (tip in place) added 10 µL of enzyme solution to disposable test tube(s) \_\_\_\_\_
4. With pipettor (tip in place) added 50 µL of each sample (draw up, avoiding foam and bubbles, expel and draw up again) to the appropriately labeled disposable tube \_\_\_\_\_
5. Use separate tips for each sample and discard after use \_\_\_\_\_
6. Thoroughly mix enzyme and sample(s) in test tubes \_\_\_\_\_
7. Place tubes in 47±1C heating unit and incubate for 5 minutes \_\_\_\_\_
8. Remove tubes from heating unit and add one (1) reagent tablet to each tube \_\_\_\_\_

9. Shake tubes for 5 seconds to start tablet disintegration

\_\_\_\_\_

10. Place tubes back in heating unit and incubate for an additional 10 minutes

\_\_\_\_\_

11. After about 5 minutes into the second incubation, remove tubes and shake contents for about 5 seconds and return tubes to heating unit

\_\_\_\_\_

12. Remove tubes from the heating unit and gently swirl the contents

\_\_\_\_\_

13. Read results immediately by comparing color to the color chart enclosed with the test kit

\_\_\_\_\_

10. Interpretation

\_\_\_\_\_

a. If result of daily negative control and sample correspond to the color chart for a negative result, sample is **Negative (NF)**

\_\_\_\_\_

b. If result is any color other than that for a negative result sample is **Presumptive Positive**

\_\_\_\_\_

11. Confirmation of Presumptive Positive Samples (see App. N GR item 11)

\_\_\_\_\_

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

\_\_\_\_\_