DETECTION OF INHIBITORY SUBSTANCES IN MILK
APPENDIX N BULK MILK TANKER SCREENING TEST FORM

DELVOTEST® P MINI
(Raw Commingled Cow, Goat and Water Buffalo Milk)
IMS #9-D1

[Unless otherwise stated all tolerances are ±5%]

GENERAL REQUIREMENTS

1. Laboratory Requirements (see Cultural Procedures (CP) items 34 & 35), except: _____

a. For Appendix N testing, see Appendix N General Requirements (App. N GR) items 14 & 15 _____

SAMPLES

2. See CP item 33, except _____

a. For Appendix N testing, see App. N GR form item 9 _____

APPARATUS & REAGENTS

3. See Cultural Procedures, items 1-23, except _____

a. For Appendix N testing, see App. N GR items 1-8 _____

4. Equipment _____

a. Heater block and/or water bath thermostatically controlled at 64±1°C _____

b. Heating block, water bath or other acceptable method to heat to at least 82±2°C, for confirmation _____

c. Pipettor – 100 µL and disposable tips (see App. N GR item 7 or CP item 6) _____

d. Pipets or syringe (supplied by manufacturer) to dispense 100 µL sample test portions (screening only) _____

e. Forceps, Tablet Dispenser, or equivalent _____

f. Test tubes for beta-lactam confirmation _____

g. Timer _____

5. Reagents _____

a. Delvotest P Mini Kit _____

1. Kit: Lot #: _____ Exp. Date: _____ _____

QC Date: _____ By: ____

2. Store kits at 0-15°C _____

3. Bottle of nutrient tablets Lot #: _____ _____

a. Once opened for use, maintain nutrient tablets in original bottle at room temperature with desiccant _____

b. Discard remaining nutrient tablets when last kit ampoule is used. Do not mix with other kits _____

b. Commercial Standard (milk based) 5.0 ppb Penicillin G Positive Control _____

Mfr.: _____ Lot #: _____ Exp. Date: _____ _____

1. Store according to label instructions

2. Rehydrate according to manufacturer's instructions _____

3. Store rehydrated solution according to manufacturer's instructions _____

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

4. Or, aliquot within 24 hours and freeze at –15°C or colder in non frost-free freezer or in an insulated foam container in a frost-free freezer; use within 2 months. (Once thawed, maintain control according to manufacturer's instructions and use within 24 hours) _____

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

c. Negative Control _____

1. Inhibitor Free Raw Milk _____

a. Sample ID: _____ Date Tested: _____ _____

b. Store solution at 0.0-4.5°C for no more than 72 hours _____

c. Or, aliquot within 24 hours and freeze at –15°C or colder in non frost-free freezer or in an insulated foam container in a frost-free freezer; use within 2 months (Once thawed, store control at 0.0-4.5°C and use within 24 hours) _____

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

2. Commercially Available Negative Control _____

Mfr.: _____ Lot #: _____ Exp. Date: _____ _____

a. Store according to label instructions _____

b. Rehydrate according to manufacturer's instructions _____

c. Store rehydrated solution according to manufacturer's instructions _____

d. Or, aliquot within 24 hours and freeze at –15°C or colder in non frost-free freezer or in an insulated foam container in a frost-free freezer; use within 2 months (Once thawed, maintain according to manufacturer's instructions) _____

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

d. Beta-lactamase (not required if beta-lactamase is not used for confirmation) _____

Mfr.: _____ Lot #: _____ Exp. Date: _____ _____

1. Store according to manufacturer's instructions _____

2. Do not use beyond expiration date _____

TECHNIQUE

6. Performance Checks (see App. N GR item 10.a) _____

a. Positive and negative controls give appropriate color reactions prior to any sample analysis (refers to new lot numbers) _____

b. Take corrective action for inappropriate color reaction(s) _____

c. Maintain records _____

7. Test Procedure _____

a. Remove one ampoule for each sample/control to be tested and identify _____

b. Use one positive and one negative control with each set of samples tested (item 5) _____

c. Punch hole through top foil _____

1. Using forceps or tablet dispenser (or equivalent), add one nutrient tablet to each ampoule _____

d. Mix milk sample(s)/control(s) by shaking 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) _____

e. Add 100 µL of mixed sample/control to appropriate ampoule _____

1. Using pipettor (item 4.c) with new tip for each sample/control, draw up 100 µL avoiding foam and bubbles _____

a. Remove tip from liquid _____

b. Expel test portion into appropriate ampoule _____

1. If pipettor has two (2) stops, depress plunger to second stop _____

2. Using manufacturer-provided syringe (Screening Only) with new tip for each control/sample _____

a. Depress plunger completely, draw up test portion avoiding foam and bubbles _____

b. Remove tip from liquid _____

c. Expel test portion into appropriate ampoule _____

f. Incubate at 64±1°C for the time period specified by the manufacturer. Time is approximate and test is complete when controls give proper color reactions _____

g. Remove ampoules from heater block or water bath, visually read against a white background and compare to color chart _____

8. Results _____

a. A yellow or yellow/purple color of the agar indicates the absence of inhibitory substances. Result is negative _____

b. A purple color of the agar indicates the presence of inhibitory substances. Result is an initial or presumptive positive. Confirm as in 9 below _____

c. Maintain records _____

9. Confirmation of PMO Section 6 Samples or Verification of Appendix N Initial Positive Tanker Samples (see App. N GR item 11); Confirmation of Presumptive Positive Tanker Samples (see App. N GR item 12); and if applicable, Producer Traceback on a Confirmed Positive Tanker (see App. N GR item 13). PROMPTLY retest the SAME sample in DUPLICATE along with a positive and negative control as described below (9.a.1-10) _____

a. Inhibitor confirmation/verification and optional beta-lactamase confirmation _____

1. Confirmation (without beta-lactamase) _____

a. Prepare a tube for each suspect sample _____

b. Prepare a tube of positive control milk (item 5.b) _____

c. Prepare a tube of negative control (item 5.c) _____

d. Heat all tubes to 82±2°C for 2 min (TC required) _____

e. Remove and cool rapidly in an ice bath to room temperature or below _____

2. Confirmation using beta-lactamase **(Optional by State Regulatory Agency)** _____

a. Prepare two tubes of each suspect sample and two tubes for the positive and negative controls _____

b. Heat all tubes to 82±2°C for 2 min (TC required) _____

c. Remove and cool rapidly in an ice bath to room temperature or below _____

d. Add beta-lactamase to one tube of each sample and control _____

3. Remove one ampoule for each tube _____

4. Punch hole through top foil and add one nutrient tablet to each ampoule _____

5. Mix tubes, as in 7.d, and add 100 µL of mixed sample to corresponding ampoule as in 7.e _____

6. Change pipettor/syringe tips or use new pipet for each sample/control _____

7. Incubate at 64±1°C for the time period specified by the manufacturer. Time is approximate and test is complete when controls give proper color reactions _____

8. Remove ampoules from heater block or water bath, visually read against a white background and compare to color chart _____

9. Record the color reactions of all samples and controls _____

10. Controls give appropriate color reactions, if not, repeat testing of all samples and controls _____

    a. If control(s) fail again, contact State regulatory and send sample, along with temperature control, to an accredited laboratory for confirmation **(must comply with M-a-85 (latest revision) and App. N of the PMO)** _____

    b. Seek technical assistance _____

b. Results of Presumptive Positive and Confirmation Tests _____

1. A yellow or yellow/purple color of the agar in both duplicates indicates the absence of inhibitory substances. Result is negative _____

2. A purple color of the agar in either or both duplicates indicates the presence of an inhibitory substances. Result is confirmed positive _____

3. Maintain records

c. Interpretation of optional beta-lactamase test: _____

1. If the agar of the untreated milk sample is yellow or yellow/purple and the corresponding agar of the beta-lactamase treated milk sample is yellow or yellow/purple, inhibitor not detected _____

2. If the agar of the untreated milk sample is purple and the corresponding beta-lactamase treated milk sample is yellow or yellow/purple sample is positive for beta-lactam _____

3. If the agar of the untreated milk sample is purple and the corresponding agar of the beta-lactamase treated milk sample is also purple, sample is positive for inhibitor (non-beta-lactam) _____

4. If the agar of the untreated milk sample is yellow or yellow/purple and the corresponding agar of the beta-lactamase treated milk sample is purple, test is invalid; repeat test _____

5. Maintain records _____

d. **Confirmation of Appendix N samples**, see Appendix N GR form items 12-13, perform confirmation as in items 9.a.1-10 above (**use of beta-lactamase required**) and interpret as in item 9.b-c above _____

e. **Verification of Initial Positive Tanker (see App. N GR item 11**). Samples using beta-lactam specific test kit; conduct test as in respective FORM FDA/NCIMS 2400 for the test kit; **if beta-lactam not detected in either sample duplicate, verify sample using the Delvotest P test kit as described in item 9.a.1-10 above** _____

10. Recording and Reporting (for Appendix N also see App. N GR item 14) _____

a. Record results of samples and controls performed _____

b. Report presence of inhibitor only for heated milk samples _____

c. If inhibitor not detected, report as Not Found (NF) _____

d. Report presence of inhibitor as **Positive** (+) or **Positive for beta-lactam** (if confirmed with beta-lactamase as in item 9.a.2 & 9.c); **report to State Regulatory Agency** _____

e. If inhibitor is present, bacteria counts cannot be reported _____