

**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  $\pm 5\%$ ]

**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  $64 \pm 1^\circ\text{C}$
- b. Heating block, water bath or other acceptable method to heat to at least  $82 \pm 2^\circ\text{C}$ , for confirmation
- c. Pipettor – 100  $\mu\text{L}$  and disposable tips (see App. N GR item 7 or CP item 6)
- d. Pipets or syringe (supplied by manufacturer) to dispense 100  $\mu\text{L}$  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  $-15^{\circ}\text{C}$  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  $64\pm 1^{\circ}\text{C}$  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

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- 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  $82\pm 2^{\circ}\text{C}$  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  $82\pm 2^{\circ}\text{C}$  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  $\mu\text{L}$  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  $64\pm 1^{\circ}\text{C}$  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**

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

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- 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

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