

DETECTION OF INHIBITORY SUBSTANCES IN MILK

**DELVOTEST® P 5 PACK/Visual & DelvoScan® Reader
(Raw Commingled Cow Milk, Raw Commingled Goat Milk and
NCIMS Accepted Pasteurized Cow and Goat Milk Products)
IMS #9D3**

[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 item 9 _____

APPARATUS & REAGENTS

- 3. **See CP items 1-23, except** _____
 - a. For Appendix N testing, see App. N GR items 1-8 _____
- 4. **Equipment** _____
 - a. Dry incubator and/or water bath thermostatically controlled at 64±2°C _____
 - b. Heating block, water bath or other acceptable method to heat to at least 82±2°C, for confirmation _____
 - c. Pipettor - 100µL and disposable tips (see App. N GR Item 7 or CP item 6) _____
 - d. Forceps, Tablet Dispenser, or equivalent _____
 - e. Test tubes for beta-lactam confirmation _____
 - f. Timer _____
 - g. DelvoScan Reader (optional) (approved for white milk only) _____
 - 1. Software version: _____

- 2. Scanner: (_____)

 - a. Instrument calibrated once every 30 days
 - b. Latest calibration date: _____

- 3. Computer with Windows operating system
- 4. Printer: _____
- 5. Kodak Q-60 5x7" color reference photo card
- 6. Black/Dark cloth for scanner background

5. Reagents

- a. Delvotest P 5 Pack 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 wells are 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 $0.0-4.5^{\circ}\text{C}$ for no more than 72 hrs

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 $0.0-4.5^{\circ}\text{C}$ 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 Check (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. Depending on the number of samples to be tested, take out a sufficient number of whole multi-plates and/or cut off the number of wells needed
- b. Identify samples/controls
- c. Use one positive and one negative control with each set of samples tested, ≤ 94 samples (item 5)
- d. Remove aluminum top foil
- e. Using forceps or tablet dispenser (or equivalent), add one nutrient tablet to each test well
- f. Sample agitation
 - 1. Mix raw milk sample(s)/control(s) (approx $\frac{3}{4}$ full), subsample(s) of retail milk containers or 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/controls must be in appropriate container to allow the use of vortexing)
 - 2. Mix retail milk samples by inverting containers top to bottom then bottom to top (a complete half circle or 180 degrees) without pausing, 25 times; use within 3 min
- g. Add 100 μ L of mixed sample/control to appropriate test well
 - 1. Using pipettor (item 4.c) with new tip for each sample/control, draw up 100 μ L avoiding foam and bubbles
 - 2. Remove tip from liquid
 - 3. Expel test portion into appropriate test well
 - a. If pipettor has two (2) stops, depress plunger to second stop

- h. Seal wells with adhesive strips provided with test kits _____
- i. Incubate at $64\pm 2^{\circ}\text{C}$ for the time period specified by the manufacturer. Time is approximate and test is complete when controls give proper color reactions _____
- j. Remove from dry incubator or water bath and visually read test result from the bottom _____

8. Results _____

- a. Visual reading of multi-plates _____
 - 1. A yellow or yellow/purple color of the agar indicates the absence of inhibitory substances. Result is negative _____
 - 2. A purple color of the agar indicates the presence of inhibitory substances. Result is an initial or presumptive positive. Confirm as in item 9 below _____
- b. Optionally read multi-plates with DelvoScan Reader (Multi-plates from water bath must be dried off prior to further handling) _____
 - 1. Start DelvoScan software by double click on icon _____
 - a. Select test (Delvo P 5 Pack) and press next _____
 - b. Input operator, sample and test kit data _____
 - 1. Enter number of test on scan-bed _____
 - 2. Enter test kit lot number _____
 - 3. Enter sample data _____
 - c. Press 'SCAN' and results will be displayed _____
 - d. Press 'PRINT' to obtain hard copy result data _____
 - e. Samples that read DelvoScan negative (<NEG>), inhibitor not detected _____
 - f. Samples that read DelvoScan positive (<POS>), must be confirmed (see item 9) _____
 - 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, Traceback of Producer(s) 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.1-12)** _____

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

1. Confirmation (without beta-lactamase) _____

a. Prepare a tube of 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. Cut off enough wells for all sample and control tubes _____

a. Or, alternatively Delvotest P/SP Mini ampoules may be used for raw samples (analyst(s) must be certified for this procedure) _____

4. Remove top foil and add one (1) nutrient tablet to each test well _____

5. Mix tubes, as in 7.f.1, and add 100 μL of mixed sample/control to corresponding test well as in 7.g _____

6. Change pipettor tips for each sample/control _____

7. Seal wells with adhesive strips provided with test kit _____

8. Place sealed blocks in pre-warmed dry incubator or water bath and incubate at 64±2°C for the time period specified by the manufacturer. Time is approximate and test is complete when controls give proper color reactions _____
 9. Remove from dry incubator or water bath and visually read test result from the bottom side _____
 10. Optionally read multi-plates with DelvoScan Reader (Multi-plates from water bath must be dried off prior to further handling). See item 8.b for instructions _____
 11. Record the color reactions (item 8.a) of all samples and controls _____
 12. Controls give appropriate reactions/colors, 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. Yellow or yellow/purple color of the agar in both duplicates OR, DelvoScan negative (<NEG>), indicates the absence of inhibitory substances. Result is negative _____
 2. Purple color of the agar OR a DelvoScan positive (<POS>), indicates the presence of inhibitory substances. Result is confirmed positive _____
 3. Maintain records _____
- c. Results of optional beta-lactamase test: _____
1. If the agar of the untreated milk sample is yellow or yellow/purple, or DelvoScan negative (<NEG>) **and** the corresponding agar of the beta-lactamase treated milk sample is yellow or yellow/purple, or DelvoScan negative (<NEG>), inhibitor not detected _____
 2. If the agar of the untreated milk sample is purple or DelvoScan positive (<POS>) **and** the corresponding agar of the beta-lactamase treated milk sample is yellow or yellow/purple, or DelvoScan negative (<NEG>), sample is **positive for beta-lactam** _____

- 3. If the agar of the untreated milk sample is purple or DelvoScan Positive (<POS>) **and** the corresponding agar of the beta-lactamase treated milk sample is also purple or DelvoScan positive (<POS>), sample is **positive for inhibitor (non-beta-lactam)** _____
- 4. If the agar of the untreated milk sample is yellow or yellow/purple or DelvoScan negative (<NEG>) **and** the corresponding agar of the beta-lactamase treated milk sample is purple or DelvoScan positive (<POS>), test is invalid, repeat test _____
- 5. Maintain records _____

d. **Confirmation of Appendix N samples**, see App. N GR form items 12-13, perform confirmation as in items 9.a.1-12 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) or Producer (see App. N GR item 13.c-g)**. Duplicate samples tested using beta-lactam specific test kit; conduct test as in respective FORM FDA 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.1-12 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 is 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 _____