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 #9-D3

[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°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°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 ¾ 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 µL of mixed sample/control to appropriate test well _____

1. Using pipettor (item 4.c) with new tip for each sample/control, draw up 100 µL 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±2°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±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. 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 _____