DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

MILK LABORATORY EVALUATION FORM

LABORATORY	
LOCATION	LAB #
DATE	X = DEVIATION U = UNDETERMINED O = NOT USED NA = NOT APPLICABLE

DETECTION OF INHIBITORY SUBSTANCES IN MILK
Delvotest 5 Pack
For Raw and Finished Cow and Goat Milk
[Unless otherwise stated all tolerances are ±5%]

SAMPLES

1. Laboratory Requirements (see CP, items 33 and 34), except
a. For Appendix N testing, see Appendix N General Requirements form, item 9 ..... _____

APPARATUS

2. See Cultural Procedures, items 1-23, except ..... _____
a. For Appendix N testing, see Appendix N General Requirements form, items 1 - 8 ..... _____
3. Dry incubator and/or water bath 64±2C (see CP item 15) ..... _____
4. Heating block and/or water bath thermostatically controlled at 82±2C, for confirmation ..... _____
5. Fixed Volume 100 µL pipettor (_____) ..... _____
6. Forceps, Tablet Dispenser, or equivalent ..... _____
7. Test tubes, 10 mL or greater for beta-lactam confirmation (optional) ..... _____
8. Timer ..... _____

MATERIALS

9. See Cultural Procedures, items 24-32 ..... _____
10. Delvotest P 5 Pack Kit ..... _____
a. Kit: Lot # _____ Exp. Date _____ ..... _____
b. Bottle of nutrient tablets Lot # _____ ..... _____
c. Store kits at 0-15C ..... _____
d. Opened bottles of nutrient tablets at room temperature
Date Opened: _____ ..... _____
e. Run a positive control (item 12 or 14) and negative control (item 15) with each new lot of kits, give appropriate reactions; records maintained ..... _____
11. Beta-lactamase, 10,000,000 IU/mL (not required if beta-lactamase is not used for confirmation) ..... _____
a. Stored as per manufacturer's instructions ..... _____
b. Do not use beyond expiration date ..... _____
Mfg. _____ Lot # _____ Exp. Date _____ ..... _____
c. Test each lot for suitability, add beta-lactamase to 5.0 ppb positive control (item 12 or 14) and add to one (1) well, beta-lactamase neutralizes positive control; records maintained ..... _____
12. Commercial Standard, 5.0 ppb Penicillin G ..... _____
a. Store according to label instructions ..... _____
Mfg. _____ Lot # _____ Exp. Date _____ ..... _____
b. Rehydrate as per manufacturer's instructions ..... _____
c. Test for suitability *each* time prepared, add to one (1) well, must produce appropriate reaction (purple); 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 _____ ..... _____
13. Phosphate Buffer ..... _____
a. Dissolve 2 grams Potassium dibasic phosphate and 8.0 grams of monobasic potassium phosphate and make up to 1 liter. pH 6.0 ± 0.05) ..... _____
14. Na or K Penicillin G Reference Standard (USP or Human injectable) ..... _____
a. Store according to label instructions ..... _____
Mfg. _____ Lot # _____ Exp. Date _____ ..... _____
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 ÷ potency of Pen G^- (1782 IU/mg) (ex. K PenG potency = 1596 IU/mg, purity equal to 1596 ÷ 1782 = 0.895 mg $PenG^-$ /mgKPenG) ..... _____
d. Make a 1 mg/mL stock solution by adding drug (100 mg $PenG^-$ ÷ item 18c) (ex. 100 ÷ 0.895 = 111.7 mg KpenG) to a 100 mL volumetric flask and making up with buffer (item 13) ..... _____
e. Make 1:100 serial dilution of the stock solution, using 100 mL volumetric flask (10 µg/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 $PenG^-$) ..... _____
Date prep. _____ ..... _____
g. Test for suitability each time prepared, add to one (1) well, must produce appropriate reaction (purple); 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 _____ ..... _____
15. Inhibitor Free Milk (fluid milk product with milkfat 0.00 to 3.5%, total solids < 13%) and tests negative with test kit ..... _____
a. Test for suitability, add to one (1) well, must produce appropriate reaction (yellow); records maintained ..... _____

TECHNIQUE

16. Test Procedure ..... _____
a. Identify samples ..... _____

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b. Use one negative and one positive control with each set of samples run (≤ 94 samples) .....
c. Depending on the number of samples to be tested, take out a sufficient number of whole multiplates and/or cut off the number of wells needed .....
d. Remove aluminum top foil and using forceps or tablet dispenser add one nutrient tablet to each test well .....
e. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc or vortex for 10 seconds, use within 3 minutes .....
f. Pipetting procedure .....
1. With tip securely fastened to the end of the pipettor and the pipettor in a vertical position, depress the plunger to the first stop .....
2. With the plunger still depressed, insert tip 1 cm below surface of the sample (avoid foam) .....
3. Release plunger **slowly** allowing tip to fill (quickly releasing the plunger will cause inaccurate filling and may foul pipettor) .....
4. Remove tip from sample and depress plunger to empty tip back to sample .....
5. If blow out type pipettor used, press the plunger to the second stop to completely empty the tip .....
6. Press plunger to first stop and repeat 2 and 3 above .....
7. Touch off to a dry spot on the inside of the sample container .....
g. Add 100 µL of mixed sample/control to a test well, record position on template, repeat for all samples and controls ...
h. Change pipettor tips for each sample and control .....
i. Close used wells carefully with sealing sheets, provided with test kit .....
j. Place sealed multiplates and/or blocks in prewarmed dry incubator or water bath and incubate at 64±2C for the time period specified by the manufacturer. Time is approximate and test is complete when controls give proper color reactions .....
k. Remove from dry incubator or water bath and read test result from the bottom side .....
l. Samples with purple color on all **or part** of solid medium must be promptly confirmed .....
m. Wells that are yellow after incubation, inhibitor not detected **(Not Found)** .....

17. Laboratory Procedure, Confirmation .....

a. Inhibitor confirmation .....
1. Prepare and label tubes with 5 mL of each suspect sample .....
2. Prepare and label a tube with 5 mL of inhibitor free milk (item 15) .....
3. Prepare and label a tube with 5 mL of positive control milk .....
4. Heat all tubes to 82±2C for 2 minutes (TC required) .....
5. Remove and cool rapidly in an ice bath to room temperature .....
6. Optional use of beta-lactamase (**optional by State Regulatory Agency**) .....
a. Prepare and label two tubes with 5 mL for each suspect sample and two tubes for the positive and negative controls .....
b. Heat all tubes to 82±2C for 2 minutes (TC required) .....
c. Remove and cool rapidly in an ice bath to room temperature .....
d. Add 200 µL (2 x 100 µL) of beta-lactamase to one tube of each sample and control .....
e. Agitate, shake or vortex, to thoroughly mix tubes and let stand 15 minutes at room temperature .....
7. Cut off enough wells for all sample and control tubes .....
a. Or, alternatively Delvotest P ampules may be used (must be certified for this procudure) .....
8. Remove top foil and add one (1) nutrient tablet to each test well .....
9. Vortex tubes and add 100 µL of mixed sample/control to a test well (as per 16f 1-7 above, identify samples, repeat for all samples and controls .....
10. Change pipettor tips for each sample and control .....
11. Close wells carefully with sealing strips, enclosed in test kit .....
12. Place sealed blocks in prewarmed dry incubator or water bath and incubate at 64±2C for the time period specified by the manufacturer. Time is approximate and test is complete when controls give proper color reactions .....
13. Remove from dry incubator or water bath and read test result from the bottom side .....
14. Record the color reactions of all samples and controls .....
15. Controls give appropriate reactions/colors, if not repeat test .....

b. Interpretation of Confirmation Tests .....
1. Wells that are yellow or yellow/purple after incubation, inhibitor not detected **(Not Found)** .....
2. Wells that are purple after incubation, inhibitor present **(Positive)** .....
3. Interpretation of optional beta-lactamase test: .....
a. If the untreated milk sample is yellow or yellow/purple **and** the corresponding beta-lactamase treated milk sample is yellow or yellow/purple, inhibitor not detected **(Not Found)** .....
b. If 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** .....
c. If the untreated milk sample is purple **and** the corresponding beta-lactamase treated milk sample is also purple, sample is **Positive for inhibitor** (other than beta-lactam), **report to State Regulatory Agency** .....

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d. If the untreated milk sample is yellow or yellow/purple **and** the corresponding beta-lactamase treated milk sample is purple, test is invalid, repeat test ..... _____

c. Test for Beta-lactam (optional) ..... _____

1. Use approved beta-lactam screen test, if positive report as in 18d. If Not Found then must confirm for inhibitor as in item 17a ..... _____

d. **Confirmation of Appendix N samples**, see Appendix N General Requirements form item 11, perform confirmation as in items 17a 1-15 above (**use of beta-lactamase required**) and interpret as in item 17b 3 above ..... _____

18. Recording and Reporting (for Appendix N also see Appendix N General Requirements form) ..... _____

a. Record test performed, interpretation of unknowns (samples) and controls ..... _____

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

c. If inhibitor is not detected report as **Not Found** ..... _____

d. Report presence of inhibitor as **Positive** (+) or **Positive for beta-lactam** (if confirmed with beta-lactamase or item 17c) ..... _____

e. Report as positive for inhibitor (other than beta-lactam) where demonstrated, **report to State Regulatory Agency** ..... _____

f. If inhibitor is present, plate counts cannot be reported ..... _____