

HHS:PHS:FDA:CFSAN:OFS:DPDFS:DEB:MST

5100 Paint Branch Parkway
College Park, MD 20740-3835

M-I-09-2

January 9, 2009

TO: All Regional Food and Drug Directors
Attn: Regional Milk Specialists

FROM: Dairy and Egg Branch (HFS-316)

SUBJECT: Neogen Corporation BetaStar® US Beta-Lactam Test

The Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM) has evaluated data supporting the use of the Neogen Corporation BetaStar® US Beta-Lactam Test for raw, commingled bovine milk.

The FDA evaluation of the data, presented by the Neogen Corporation, indicates that the performance of the test meets the standards established to determine the acceptance of a test for use in raw, commingled bovine milk. The acceptance of the test for raw, commingled bovine milk represents a claim for amoxicillin, ampicillin, cephalixin, cloxacillin and penicillin G. These data have been evaluated in accordance with the standards established for the acceptance of screening tests for monitoring raw, commingled milk in accordance with the provisions of Appendix N-Drug Residue Testing and Farm Surveillance of the *Grade "A" Pasteurized Milk Ordinance*.

The NCIMS Executive Board during a conference call held November 3, 2008 accepted the use of this test when used as labeled. Attached is the letter of acceptance from FDA's CVM.

An electronic version of this memorandum is available for distribution to Regional Milk Specialists, State Milk Regulatory Agencies, State Laboratory Evaluation Officers and State Milk Sanitation Rating Officers in your region. The electronic version should be widely distributed to State Veterinarians, State Veterinary and Pharmacy Boards, Veterinarian Professional Organizations, representatives of the dairy industry and other interested parties and also will be available on the CFSAN Web Site at <http://www.cfsan.fda.gov> at a later date.

CAPT Robert F. Hennes, RS, MPH

Attachment: FDA CVM Memorandum of the acceptance of the test kit
BetaStar® Label Insert



Center for Veterinary Medicine
Office of Research

Food and Drug Administration
8401 Muirkirk Road
Laurel MD 20708

Memorandum

Date January 7, 2009

From Philip J. Kijak, Acting Director
Division of Residue Chemistry

Subject Neogen Corporation BetaStar US Beta-Lactam Test

To Robert F. Hennes, CFSAN, Dairy and Egg Branch/Milk Safety Team

The Neogen Corporation has provided data supporting the use of the Neogen Corporation BetaStar US Beta-Lactam Test for the detection of amoxicillin, ampicillin, cephalosporin, cloxacillin, and penicillin G in raw, commingled bovine milk. These data has been evaluated in accordance with the standards established for the acceptance of screening tests for raw, commingled bovine milk to monitor milk in accordance to the provisions of Appendix N-Drug Residue Testing and Farm Surveillance of the Grade "A" Pasteurized Milk Ordinance (PMO).

The 90/95 percent detection levels and drug concentration responses for the five (5) Bta-lactam drugs are as follows:

90/95 Percent Detection Levels (ppb)

| | |
|--------------|------|
| Amoxicillin | 6.0 |
| Ampicillin | 5.9 |
| Cephapirin | 19.5 |
| Cloxacillin | 9.1 |
| Penicillin G | 4.8 |

Drug Concentration Response (Displayed as Percent Positive)

| Concentration ppb (part per billion) | Amoxicillin | Ampicillin | Cephapirin | Cloxacillin | Penicillin G |
|--|-------------|------------|------------|-------------|--------------|
| 1 | | | | | 0% |
| 2 | 0% | 0% | 0% | 0% | 0% |
| 3 | | | | | 0% |
| 4 | 0% | 0% | 0% | 0% | 27% |
| 5 | | | | | 100% |
| 6 | 93% | 100% | | 0% | |
| 8 | 100% | 100% | 0% | 67% | |
| 10 | 100% | 100% | | 100% | |
| 12 | | | 7% | | |
| 20 | | | 97% | | |

RECOMMENDATION

Our evaluation of the data presented by the Neogen Corporation indicates that the performance of this test meets the standards established for the acceptance of screening tests for monitoring raw, commingled bovine milk in accordance with the provisions of Appendix N of the PMO when used with the Neogen Corporation AccuScan III Reader with AccuScan Program and printer. We recommend that the appropriate announcement be issued to the State Regulatory Agencies and the milk industry advising of the Agency's concurrence with the use of this test as labeled. We also recommend that the next revision of M-a-85 and M-I-96-40 reflect the acceptance of this test.

/ss/

Philip James Kijak, Ph.D.
Acting Director
Division of Residue Chemistry
CVM Office of Research

David G. White , Ph.D., Director, CVM Office of Research:

Concur Do not Concur

Attachment

cc:
TE Graham HFS-450

BetaStar[®] US beta-lactam assay test for Amoxicillin, Ampicillin, Cephapirin, Cloxacillin, and Penicillin G in raw commingled cow milk

PRODUCT INFORMATION

INTRODUCTION

BetaStar US is a receptor assay for rapid detection of the beta-lactam antibiotics penicillin, ampicillin, amoxicillin, cloxacillin, and cephalosporin.

This test is validated for use with raw, commingled cow's milk.

REACTION MECHANISM

The test employs a specific beta-lactam receptor linked to gold particles.

The preliminary incubation of the receptor with milk containing antibiotics will result in interaction of the antibiotics with the receptor. In a second stage, the solution is transferred onto an immunochromatographic medium.

The first line of this medium will capture all the receptors that have not interacted with any antibiotic during the first incubation.

The second line on the immunochromatographic medium serves as a control line.

MATERIALS REQUIRED

The box for 25 tests contains:

- 25 individual vials with lyophilized receptor
- 1 container with 25 dipsticks
- 1 syringe pipettor and 25 disposable tips (for NCIMS screening purposes only)

Do not mix dipsticks and vials from different kits.

Kits must be received with cold packs and must be refrigerated upon receipt.

MATERIALS REQUIRED BUT NOT SUPPLIED

(Available from Neogen)

- Heater block capable of maintaining a temperature of $47.5 \pm 1^\circ\text{C}$
- AccuScan III Reader with AccuScan Program – required for NCIMS testing
- Printer – required for NCIMS testing
- Positive and negative controls (see below)
- For NCIMS certified laboratories, a fixed volume pipettor is required
- Timer

POSITIVE AND NEGATIVE CONTROLS FOR NCIMS TESTING IN THE U.S.

The performance of the test kit must be validated with positive and negative controls each day that the test kit is used and with each new lot of reagents. The controls must be run before milk samples are evaluated.

Negative Control: Raw commingled cow milk tested 2.0 or higher ratio reading with Beta Star US Beta-lactam test. Must be used within 72 hours of testing and temperature maintained at $0 - 4.4^\circ\text{C}$.

Positive Control: Use the Penicillin Positive Control (5.0 ± 0.5 ppb) available from Neogen (item #BT1L13). Follow the insert instructions for preparation of the material. Test for suitability each time prepared, must produce 0.85 or lower ratio reading. Store at $0 - 4.4^\circ\text{C}$.

PERFORMANCE INFORMATION

SENSITIVITY

DOSE RESPONSE INFORMATION

| ppb | Amoxicillin* | Ampicillin* | Cephapirin | Cloxacillin | Penicillin |
|----------------------------|--------------|-------------|------------|-------------|------------|
| 1 | | | | | 0% |
| 2 | 0% | 0% | 0% | 0% | 0% |
| 3 | | | | | 0% |
| 4 | 0% | 0% | 0% | 0% | 27% |
| 5 | | | | | 100% |
| 6 | 93% | 100% | | 0% | |
| 8 | 100% | 100% | 0% | 67% | |
| 10 | 100% | 100% | | 100% | |
| 12 | | | 7% | | |
| 20 | | | 97% | | |
| Tolerance/Safe Level (ppb) | 10 | 10 | 20 | 10 | 5 |
| Calculated 90-95% | 6.0 | 5.9 | 19.5 | 9.1 | 4.8 |

* The drugs indicated with an asterisk (*) have demonstrated a 90-95% sensitivity of the test kit which is at least 25% less than the tolerance or safe level.

Data is presented as percent positive at each concentration. Sensitivity is based on 30 samples per concentration.

SELECTIVITY

60 negative control milk samples were evaluated in an independent laboratory and none of the negative control samples tested positive by BetaStar US.

CROSS-REACTIVITY

DOSE RESPONSE INFORMATION

| ppb | Dicloxacillin | Ticarcillin | Cefadroxyl | Ceftiofur* |
|-----|---------------|-------------|------------|------------|
| 5 | 0% | | | |
| 10 | 100% | 0% | 0% | |
| 20 | 100% | 100% | 0% | |
| 50 | 100% | 100% | 0% | |
| 100 | 100% | 100% | 0% | |
| 500 | | | | 0% |

* As total metabolites.

Data is presented as percent positive at each concentration.

The BetaStar US test does not cross-react with the following drugs at levels of 100 ppb: sulfadiazine, sulfanilamide, sulfathiazole, sulfamethazine, sulfapyridine, sulfadimethoxine, tetracycline, oxytetracycline, chlortetracycline, doxycycline, gentamicin, neomycin, streptomycin, ivermectin, erythromycin, pirlimycin, tilmicosin, novobiocin, furosemide, trichlormethiazide, thiabendazole, chlorothiazide, oxytocin, phenylbutazone, dexamethasone, dipyrrone, flunixin, enrofloxacin, para-aminobenzoic acid (PABA), and fluorphenicol.

TRAINING

The BetaStar US beta-lactam test is recommended for use by personnel who have received training by a Neogen representative. In the United States, under NCIMS recommendations, trained individuals need to maintain proficiency by regular use and/or state sponsored training/certification programs. Individuals needing training should contact Neogen Technical Services at 1-800-234-5333.

DAILY PERFORMANCE AND OPERATION CHECK

1. Run a Positive and Negative control before use on each new lot of kits. The results must be appropriate for the control; if they do not meet specifications, contact Neogen.
2. Run a Positive and Negative control daily to assure appropriate results are achieved. If results are incorrect, re-run the controls. If the problem persists, discontinue testing and contact Neogen for assistance.
3. Reader Calibration occurs automatically when the AccuScan III program is initiated on the reader. If the calibration is unsuccessful, the reader will not operate. A warning message will prompt the user "Calibration unsuccessful. Contact Neogen". Calibration must be performed on each day of use.

TEST PREPARATION

1. A daily temperature check of the heater block is required. Ensure that the heater block has been turned on and preheated and the temperature is maintained at $47.5 \pm 1.0^{\circ}\text{C}$.
2. BetaStar US is designed for use under normal ambient environmental conditions ($15\text{-}30^{\circ}\text{C}$). Remove the kit from the refrigerator and equilibrate the dipstick container to room temperature ($15 - 30^{\circ}\text{C}$) for 10-15 minutes prior to opening to prevent condensation.
3. Remove only enough receptor vials and dipsticks that will be used within the day from kit and immediately return the kit to the refrigerator. Any receptor vials or dipsticks that remain unused at the end of the testing day must be discarded
4. Dipsticks that have been removed from the dipstick container must be kept clean and dry.
5. Label one vial and one dipstick for each test sample and each control.
6. A maximum of 6 tests can be run at one time. If more than one sample is being run, all milk samples should be prepared prior to inserting into the heater block and beginning the incubation period.

TEST PROCEDURE

1. Mix milk sample or control 25 times in seven seconds with a 1 ft movement.
2. Gently tap the vial on a hard surface in order to assure all solid material is in bottom of vial
3. Carefully remove the cap and rubber stopper from the vial
4. Pipette 200 μl milk sample into the vial.
 - i. For NCIMS screening purposes only, utilize the syringe pipettor provided in the kit.
 - a. Attach a 200 μl pipette tip (provided) to the syringe.
 - b. Draw up sample, avoiding foam and bubbles.
 - c. Deliver the sample into the vial by depressing the plunger.
 - d. Replace the rubber stopper in the vial.
 - ii. For NCIMS certified laboratory purposes, utilize a fixed volume pipettor.
 - a. Attach a 200 μl pipette tip to the pipettor.
 - b. Draw up sample, avoiding foam and bubbles.
 - c. Deliver the sample into the vial by depressing the plunger.
 - d. Replace the rubber stopper in the vial.
5. Mix the milk and reagent thoroughly by inverting the vial twice and swirl in a circular motion until all solids are in solution.
6. Remove stopper from vial and place the vial into the heater block and incubate at $47.5 \pm 1.0^{\circ}\text{C}$ for 3 minutes.
7. At the completion of the 3 minute incubation, place labeled dipstick into the vial in the heater block. The arrows on the dipstick must be oriented downward in the vial. Incubate the dipstick in the vial for 2 minutes at $47.5 \pm 1.0^{\circ}\text{C}$.
8. At the completion of the 2 minute incubation, remove the dipstick from the vial, place the dipstick into the holder, and insert the holder into the reader. The dipstick must be read within 3 minutes. (See reader instructions for proper operation.)

TEST INTERPRETATION

If the ratio of test to control line intensities as determined by the reader is > 1.0 , the test is negative. If the ratio is ≤ 1.0 , the test is positive.

If a dipstick with an invalid control line is inserted into the reader, the reader will indicate “Invalid Control Line”. Re-test the control/sample.

The reader printout is formatted to provide test information as follows:

NAME: BetaStar
TEST CATEGORY: Beta-lactam
LOT: Kit lot number
USERNAME: Name of operator
Date of test / Time of test
READER ID: Reader serial number
Sample identification
RATIO: Numerical value with indication of positive/negative

FOR NCIMS TESTING IN THE U.S

- The original test result, if negative, is reported as Not Found.
- The original test result, if positive, is considered an Initial Positive.

RE-TEST OF INITIAL POSITIVE

1. For milk samples yielding an Initial Positive result, promptly re-test the same sample in duplicate, along with Positive and Negative controls.
2. Providing controls are valid, if both duplicate assays are negative, this indicates a Negative Result. Providing both controls are valid, if one or both duplicate samples test positive, the sample is a “Beta-lactam Presumptive Positive Test”.
3. If either control is invalid, repeat the testing. If either control is invalid again, contact Neogen for technical assistance (phone 800/234-5333).
4. In accordance with Appendix N of the PMO, all presumptive positive tests must be reported to the appropriate regulatory agency.

REMARKS

NCIMS TESTING

All NCIMS testing must be performed in accordance with Appendix N of the current PMO. All requirements of the PMO must be followed for all additional testing. Milk testing must be completed within 72 hours of sampling, including the time from the initial test, any necessary retesting of an Initial Positive, confirmation, and/or producer trace-back testing.

STORAGE CONDITIONS

All reagents must be kept refrigerated between 0 and 4.4°C. Before opening the dipstick container, it should be equilibrated to room temperature for at least 10 minutes.

PRECAUTIONS

When handling the BetaStar US test, make sure hands are clean and dry. This will protect against contamination of the test reagents.

If the test sample does not migrate on the dipstick, the test is invalid. This situation will occur when the test is performed on abnormal milk like clotted milk, or if the procedure has not been performed properly.

Do not mix dipsticks and vials from different kits.

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Service can be reached between 8:00 AM and 6:00 PM Eastern time by calling 800/234-5333 or 517/372-9200 and asking for a Neogen sales representative or Technical Service. Assistance is available on a 24-hour basis by calling 800/867-0308. Training on this product, and all Neogen test kits, is available.

MSDS INFORMATION

Material safety data sheets (MSDS) are available for this test kit, and for all of Neogen’s food safety kits, at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

ADDITIONAL INFORMATION

Samples of this test kit were independently evaluated by the AOAC Research Institute and were found to perform to the producer’s specifications as stated in the test kit’s descriptive insert. The producer certifies that this test kit conforms in all respects to the specifications originally evaluated by the AOAC Research Institute as detailed in the PERFORMANCE TESTEDSM certificate number 03080