

APPENDIX N BULK MILK TANKER SCREENING TEST FORM

**Neogen BetaStar® Plus (Raw Commingled Cow Milk) Beta-Lactam Test
IMS# 9-N1**

GENERAL REQUIREMENTS

1. See Appendix N General Requirements (App. N GR) items 1-8 & 15 _____

SAMPLES

2. See App. N GR item 9 _____

APPARATUS & REAGENTS

3. **Equipment** _____

a. Heater Block, 47.5±2.0°C _____

1. Check temperature daily (day of use); maintain records _____

2. Temperature measuring device must be supplied by manufacturer and meet all App. N GR item 3 requirements _____

3. Temperature measuring device for each incubator (App. N GR item 3) _____

b. Accuscan® III Reader (software V 3.551 or higher) with Accuscan III program installed, with printout or download of data. Manual available _____

Serial Number: _____

1. Printer or computer link for hardcopy download _____

2. Maintain records _____

c. Pipettor – 200 µL and disposable tips (see App. N GR item 7) _____

d. Or single use 200 µL Poly-pipet with overflow bulb to accurately measure amount of sample, supplied by manufacturer (**screening only**) _____

e. Timer _____

4. **Reagents** _____

a. BetaStar Plus Test Kit

Lot #: _____

Exp. Date: _____

QC Date: _____ By: _____

1. Kit contains lyophilized receptor vials, dipsticks, and Poly-pipets (item 3.d) _____

a. Verify that first 5 characters of the lot code on the dipstick tube, dipstick and vial matches the first 5 characters of the Test Kit lot code _____

Kit Lot #: _____

2. Verify acceptable status of the moisture indicator card in the dipstick tube prior to use. If all of the dots on the indicator card have changed from blue to pink, do not use the kit _____

b. Positive Control _____

1. Lyophilized 5 ppb Penicillin G/100ppb Desfuroylceftiofur _____

Lot #: _____ Exp. Date: _____

c. Negative control _____

1. Previously tested negative raw milk (item 5.d) _____

5. Reagent Stability (frozen standards are not allowed) _____

a. Test Kit including reagents and dipsticks received refrigerated in insulated chest with cold pack block shipped overnight _____

b. Store reagents and dipsticks at 0.0-4.5°C, maintain no longer than manufacturer's expiration date _____

c. Positive Control – Manufacturer supplied, maintain no longer than manufacturer's expiration date _____

1. Reconstituted with 1 mL of raw commingled cow milk tested at 2.0 or higher ratio with BetaStar Plus Beta Lactam test; use within 48 hours and maintain at 0.0-4.5°C _____

Lab Prep Date: _____ Lab Exp Date: _____

2. Day of use, must produce 0.85 or lower ratio reading; maintain records _____

Test Value: _____

Do not proceed if out of range _____

- d. Negative Control – raw commingled cow milk tested at 2.0 or higher ratio reading with BetaStar Plus Beta-Lactam test. Maintain at 0.0-4.5°C and use within 72 hours

Sample ID: _____ Test Value: _____

Date Tested: _____

Do not proceed if out of range

TECHNIQUE

6. Daily Performance and Operation Check

- a. See App. N GR item 10
- b. Accuscan III Reader Calibration
 - 1. The reader calibration occurs automatically when the Accuscan III Program (V3.551 or higher) is initiated on the reader
 - a. The Accuscan III program must be re-initiated daily prior to use, when the unit is turned off, or when the unit goes off if the battery charge is too low
 - 2. If the reader calibration is unsuccessful, the reader will not operate. A warning message will prompt the user “Calibration unsuccessful. Contact Neogen.”
 - 3. Calibrator cartridges must be read daily. There are two calibrators, one as Positive and one as Negative. Each calibrator has a line for both beta-lactam and Ceftiofur
 - 4. Both lines on each of the calibration cartridges must read within the limits as indicated on the cartridge device

Positive Calibrator Readings:

Beta-lactam: _____

Ceftiofur: _____

Negative Calibrator Reading: _____

Beta-lactam: _____

Ceftiofur: _____

- 5. If Calibrators are out of range, contact Neogen before proceeding
- 6. Maintain records

7. Test Procedure [Precautions – when handling BetaStar Plus test, make sure hands are clean and dry. This will protect against contamination of test reagents]

- a. The test is designed for use under normal ambient environmental conditions (15-30°C). Remove the kit from the refrigerator. Remove the number of test vials required and the dipstick container. Immediately return the balance of the kit to the refrigerator. Allow the dipstick container to equilibrate to room temperature (15-30°C) for 10-15 min prior to opening to prevent condensation
- b. Remove the number of dipsticks required and return the dipstick container to the refrigerator
- c. A maximum of 4 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
- d. Any receptor vials or dipsticks removed from the kit that remain unused at the end of the testing day must be discarded
- e. Dipsticks that have been removed from the dipstick container must be kept clean and dry
- f. Label one vial and one dipstick for each test sample and each control
- g. Gently tap the vial on a hard surface in order to assure all solid material is in bottom of vial
- h. Mix milk sample(s)/control(s) 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting; use within 3 min (sample must be in appropriate containers to allow the use of vortexing)
- i. Carefully remove the cap and rubber stopper from the vial
- j. Pipette 200 µL milk sample into the vial
 - 1. **Certified laboratories must**, 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

2. Using new manufacturer-provided Poly-pipet (item 3.d) for each sample/control [**Screening only**]
 - a. Squeeze top bulb while holding vertically with bulb and overflow reservoir side pointing down, draw up test portion avoiding foam and bubbles. Sample should completely fill pipet shaft and overflow into the bottom half of the overflow reservoir
 - b. Remove tip from liquid
 - c. While holding the Poly-pipet vertically, expel test portion slowly into the vial. Excess portion should remain in reservoir
 - d. Replace the rubber stopper in the vial
- k. Mix the milk and the reagents thoroughly by inverting the vial twice and swirling in a circular motion until all solids are in solution
- l. Remove and discard stopper from vial and place the vial into the heater block and incubate at $47.5 \pm 2.0^{\circ}\text{C}$ for 3 min, not to exceed 3 min and 30 sec
- m. At the completion of the 3 min incubation, place labeled dipstick into the vial in the heater block. The arrows on the dipstick must be oriented downward in the vial
- n. Incubate the dipstick in the vial for 2 min, not to exceed 2 min and 30 sec at $47.5 \pm 2.0^{\circ}\text{C}$
- o. At the completion of the 2 min incubation, remove the dipstick from the vial and visually inspect the control line; an absent, partial or indistinct control line indicates an invalid test and the sample/control must be re-test.
CAUTION: Insert only valid test(s) into the Reader
- p. Place the dipstick arrows first, into the holder, and insert the holder into the Accuscan III reader (software V3.551 or higher)
- q. Read dipsticks within 3 min of completion of the incubation

8. Interpretation with Reader

- a. If there is a negative reading (ratio > 1.0) on the reader, sample is Negative (NF)
- b. If there is a positive reading (ratio \leq 1.0) on the reader, sample is an Initial Positive

9. Verification of Initial Positive Tanker Samples (see App. N GR item 11); Confirmation of Presumptive Positive Tanker Samples (see App. N GR item 12); and Traceback of Producer(s) on a Confirmed Positive Tanker (see App. N GR item 13)

10. Reporting (see App. N GR item 14)

- a. Maintain reader tapes or computer print outs
