APPENDIX N BULK MILK TANKER SCREENING TEST FORM

Neogen BetaStar® Plus (raw commingled cow milk) Beta-Lactam Test

GENERAL REQUIREMENTS

1. See Appendix N General Requirements form items 1-8 & 15 _____

SAMPLES

2. See Appendix N General Requirements form item 9 _____

APPARATUS & REAGENTS

3. Equipment _____

a. Heater Block, 47.5 ± 2.0°C _____

1. Temperature checked daily (day of use), records maintained _____

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

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. Records maintained _____

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

d. Or syringe pipettor with disposable 200 µL tips, 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, syringe pipettor and syringe tips (see item 3d above) _____

   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 5d) _____

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. Reagents and dipsticks stored at 0.0 – 4.4°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, used within 48 hours and maintained at 0.0 – 4.4°C _____

      Lab Prep Date ___/___/___ Lab Exp Date ___/___/___ _____

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

      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. Must be maintained at 0.0 – 4.4°C and used 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. Records maintained _____

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 control(s) for 10 sec at maximum setting, use within 3 min (sample must be in appropriate container to allow 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. **For screening purposes only,** utilize the syringe pipettor provided in the kit _____

   a. Attach a 200 µL pipette tip (provided) to the syringe 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 _____

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 ± 2.0°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 ± 2.0°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-tested. **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 ≤ 1.0) on the reader, sample is an Initial Positive _____

9. Verification of Initial Positive Samples (see App. N GR item 11); Confirmation of Presumptive Positive Samples (see App. N GR item 12); and Producer Traceback (see App. N GR item 13) _____

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

a. Reader tapes or computer print outs maintained _____