

APPENDIX N BULK MILK TANKER SCREENING TEST FORM

**Neogen BetaStar Advanced Test for Tetracyclines
(Raw Commingled Cow Milk)
IMS #9-N2**

[Unless otherwise stated all tolerances are $\pm 5\%$]

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. Neogen Corporation, Raptor[®] Integrated Analysis System (Manual available).
Thermostatically controlled at $65\pm 5^{\circ}\text{C}$ _____

Serial Number: _____

1. Temperature checked daily on the screen and printout (day of use),
records maintained (Printout acceptable for daily temperature check) _____

a. Incubator Temperature: _____

b. Annual temperature verification performed; records maintained _____

1. Date of last verification: _____

b. Reader calibrators _____

1. Positive: _____

2. Negative: _____

c. Pipettor - 400 μL and disposable tips (see App. N GR item 7) _____

d. Or disposable 400 μL single-use poly-pipettes (**screening only**) _____

4. Test Kits _____

a. BetaStar Advanced Test for Tetracyclines Kit _____

Lot #: _____ Exp. Date: _____

QC Date: _____ By: _____

5. Sample and control agitation

- a. 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 (samples/controls must be in appropriate containers to allow the use of vortexing)

6. Reagent Stability and Preparation

- a. Test Kit including strips are received under ambient temperature
- b. Strips stored at 18 - 30°C (64 - 86°F), maintain no longer than manufacturer's expiration date
- c. Negative Control
 - 1. Previously negative tested raw milk
 - 2. Milk can be screened (previously tested) by the testing location making and/or using the controls
 - 3. Negative control must result in a ratio of ≥ 1.15 ; maintain records

Sample ID: _____ Date Tested: _____

Ratio: _____

- 4. Use within 72 hours when maintained at 0.0-4.5°C
- 5. Or, aliquot within 24 hours and freeze at -15°C or colder in a non frost-free freezer or in an insulated foam container in a frost-free freezer; use within 2 months

Lab Prep. Date: _____ Lab Exp. Date: _____

- a. Thaw slowly in refrigerator or more rapidly in cold water. Mix well until sample is homogeneous

1. Do Not use if there is visible protein precipitation

- b. Store at 0.0-4.5°C and use within 48 hours. Do not refreeze

- 6. Day of use must result in a ratio of ≥ 1.15 ; maintain records

Do Not proceed if out of range

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

1. BetaStar Advanced for Tetracyclines Positive Control _____

a. Lyophilized 300.0 ±30.0 ppb Oxytetracycline _____

Lot #: _____ Exp. Date: _____

2. Store according to label instructions _____

3. Reconstitute with 1.0 mL of fresh or previously frozen previously screened tetracycline negative raw commingled cow milk _____

4. Positive control must produce a ratio of ≤ 0.85 ; maintain records _____

Ratio: _____

5. Store reconstituted positive control at 0.0-4.5°C for no more than 48 hours _____

Lab Prep. Date: _____ Lab Exp. Date: _____

6. Or, aliquot within 24 hours and freeze at -15°C or colder in a non frost-free freezer or in an insulated foam container in a frost-free freezer; use within 2 months. **Do Not** freeze positive control if it was made with previously frozen negative control _____

Lab Prep. Date: _____ Lab Exp. Date: _____

a Thaw slowly in refrigerator or more rapidly in cold water. Mix well until sample is homogeneous _____

1. **Do Not use if there is visible protein precipitation** _____

b. Store at 0.0-4.5°C and use within 48 hours; do not refreeze _____

7. Day of use must produce a ratio of ≤ 0.85 ; maintain records _____

TECHNIQUE

7. Daily Performance and Operation Check _____

a. See App. N GR items 10.b-d _____

b. Raptor® Integrated Analysis System _____

1. At Raptor® start-up, calibration of camera and LED occurs automatically when instrument is turned on _____

2. If the calibration is unsuccessful, the reader will not operate. A warning message will prompt the user, "Calibration unsuccessful. Contact Neogen." _____
3. Annual calibration defines x and y offsets for the Raptor system _____
 - a. User performed annual calibration is required every 365 days. Verify annual calibration was performed within last 365 days. Please see user manual for more details _____

Date of last calibration: _____
4. Daily reader check calibration _____
 - a. The reader check calibration must be performed daily in each of three ports in the Raptor System _____
 - b. There are three calibration test strips within each cartridge, all positive or all negative _____
 - c. Both positive and negative calibration cartridges must read within the limits specified ≤ 0.85 for positive and ≥ 1.15 for negative; maintain records _____
 - d. Positive Calibrator Ratios: (Specification ≤ 0.85) _____

Port 1: _____ Port 2: _____ Port 3: _____
 - e. Negative Calibrator Ratios: (Specification ≥ 1.15) _____

Port 1: _____ Port 2: _____ Port 3: _____
5. If reader check calibrations are out of range, contact Neogen before proceeding _____

8. Test Procedure _____

- a. Make sure hands are clean and dry before handling test kits _____
- b. Set out required number of cartridges and place them in a dry labeled container at room temperature, or take out cartridges as needed _____
 1. Cartridges that have been removed from the protective storage container must be kept clean and dry _____
 2. Any cartridges removed from the kit that remain unused at the end of the testing day must be discarded _____
- c. Cartridges are pre-loaded with one test strip. Up to two more test strips may be loaded into the cartridge. One cartridge, loaded with up to three test strips, can be used to test one milk sample _____

- d. Place cartridge with test strip(s) into any of the three ports. When cartridge is inserted into the port, the port will automatically begin to adjust to the proper temperature _____
- e. The bar code on the test device will be read. If the QR (quick response) code for the lot of strips has not been entered into the system, the bar code reader in the front of the reader will turn on automatically. Scan the QR code found on the container storing the test strips _____
- f. Instrument will prompt user for the milk sample ID. Scan or enter the sample ID at this time _____
- g. Mix milk sample(s)/control(s) (See item 5.a) _____
- h. The user will be prompted to add the milk sample when the port reaches $65.0 \pm 5.0^{\circ}\text{C}$. **Do Not** add milk sample until prompted to do so _____
- i. Add 400 uL of mixed sample/control into the back of the cartridge _____
 - 1. Using Pipettor (item 3.c) with a new tip for each sample/control and holding pipettor vertically draw up 400 μL avoiding foam and bubbles _____
 - a. Remove tip from liquid _____
 - b. While holding the pipettor vertically, expel test portion into cartridge _____
 - c. After sample is delivered into cartridge, eject pipette tip into the back of the cartridge to prevent double loading of the same sample or loading a second sample into the same cartridge _____
 - 2. **FOR SCREENING ONLY** - Using a new manufacturer provided single-use 400 μL poly-pipet (item 3d.) for each sample/control _____
 - a. Squeeze top bulb while holding single-use pipet vertically and draw up test portion avoiding foam and bubbles. Insure that pipet shaft is completely full and sample overflows into the bottom half of the overflow reservoir _____
 - b. Remove tip from liquid _____
 - c. While holding the single-use pipet vertically, expel test portion slowly into the back of the cartridge. Excess portion should remain in reservoir _____
 - d. After loading milk sample into the cartridge, leave the used pipette in the back of the cartridge. This will prevent double loading the same sample or loading a second sample into the same cartridge _____
- j. Press "Next" after sample has been added. The unit will begin the 5 minute incubation after the system identifies the fluid front of the sample wicking up the device _____

- k. After 5 minutes the result will be displayed on the screen, an audible tone will sound, and the test result will automatically print _____
- l. Remove cartridge containing test strip(s) from the reader and discard the entire cartridge _____

9. Interpretation with Reader _____

- a. If there is a ratio of ≥ 1.00 on the reader, sample is a Negative (NF) _____
- b. If there is a ratio of < 1.00 on the reader, sample is an Initial Positive _____

10. Verification of Initial Positive Tanker Samples (see App. N GR item 11) _____

11. Confirmation of Presumptive Positive Tanker Samples (see App. N GR item 12) [Only in an accredited laboratory or by a CIS] _____

- a. If performing confirmation on an equivalent test follow confirmation procedure for that test (refer to M-a-85 current revision for a listing of test kits to assure equivalence) _____

12. Traceback of Producer(s) on a Confirmed Positive Tanker (see App. N GR item 13) [Only in an accredited laboratory or by a CIS (refer to M-a-85 current revision for a listing of test kits to assure equivalence)] _____

13. Re-instatement of Producer(s) [Only in an accredited laboratory or by a CIS (refer to M-a-85 current revision for a listing of test kits to assure equivalence)] _____

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