APPENDIX N BULK MILK TANKER SCREENING TEST FORM

IDEXX - New SNAP® BETA LACTAM

For Raw Cow Milk

GENERAL REQUIREMENTS

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

SAMPLES

2. See Appendix N General Requirements (GR) form item 9 _____

APPARATUS & REAGENTS

3. Equipment _____

a. Heater block with SNAP inset thermostatically controlled at 45±5C _____

1. Temperature checked by placing standardized thermometer in tube containing liquid (bulb submersed) in heating unit, records maintained _____

2. Or, use 6 inch partial immersion thermometer placed directly into small thermometer well in middle of heating unit, records maintained _____

b. Single use 450 µL ± 50 µL poly pipet with indicator line to measure amount of sample, supplied by manufacturer **(screening only)** _____

c. Fixed volume pipettor to dispense 450 µL ± 50 µL (see App. N GR item 7) _____

d. SNAP Kit _____

Lot # _____ Exp Date _____ _____

e. Sample tubes containing reagent pellet _____

f. Kits received refrigerated _____

g. Store kits at 0-7C _____

h. Timer _____

i. IDEXX Reader for SNAP devices, with printer or data download capability _____

4. Daily Performance and Operation Check (see App. N GR item 10) _____

a. Read Performance Check Set (Device #1 as Negative and Device #2 as Positive)

b. Both devices must read within the limits as indicated on the storage box label of the check set devices _____

Positive Range _____ Negative Range _____ _____

c. If check sets fail, call IDEXX before proceeding _____

5. Controls _____

a. Positive Control, 5.0 ppb ± 0.5 ppb Penicillin G _____

1. Store according to label instructions _____

Mfg. _____ Lot # _____ Exp. Date _____ _____

2. Re-hydrate as per manufacturer's instructions with fresh or frozen previously screened Beta-Lactam negative raw commingled cow milk _____

3. For Positive Control, must produce greater than 1.2 on the IDEXX reader, records maintained _____

Reader value: _____ _____

4. Store reconstituted Positive Control at 0-4.4C for no more than 24 hours _____

b. Negative Control - Beta-Lactam negative raw milk (fresh or frozen) _____

1. For Negative Control purposes, must produce less than 0.95 on the IDEXX reader; records maintained _____

Reader value: _____ _____

2. Store fresh Negative Control milk at 0-4.4C for no more than 72 hours _____

3. Negative Control milk frozen for later use _____

   a. Aliquot within 24 hours and freezing at -15°C or colder in a non frost-free freezer, used within 60 days _____

   b. Thaw frozen milk at 0-4.4C _____

   c. Once thawed mix thoroughly, **Do Not** use if noticeable protein precipitation is present after thawing _____

   d. Thawed negative control milk held at 0-4.4C and used within 24 hours _____

4.Milk controls may not be refrozen _____

TECHNIQUE

6. Test Procedure _____

a. Set out required number of SNAP™ devices, sample tubes and pipets for the samples to be tested _____

   1. Discard unused, un-refrigerated devices at the end of the day _____

b. Pre-warm heater block(s) to 45±5C, and maintain 45±5C range for at least 5 minutes before beginning the test _____

   1. Check initial pre-heating with a reference thermometer, records maintained _____

   2. Continuous use block heaters, check temperature daily with reference thermometer, records maintained _____

c. Label each device and each sample tube _____

d. Place devices on incubator block(s) _____

e. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc, use within 3 minutes _____

f. Look for blue reagent pellet in bottom of tube, if not there tap to bring pellet down _____

g. Remove and discard sample tube caps _____

h. With poly pipets provided, draw up controls or samples (**Screening only)** _____

1. Draw up, avoiding foam and bubbles, expel and draw up again to the indicator lines ± 50µL _____

2. Carefully add all of the control or sample milk to the appropriately labeled tubes. _____

i. Or, using fixed volume pipettor (item 3c), draw up 450 µL ± 50µL of controls and samples _____

1. Draw up, avoiding foam and bubbles, expel and draw up again _____

2. Carefully add to the appropriately labeled tubes _____

j. Use clean poly pipet (or tip) for each control and sample _____

k. Agitate sample tube to dissolve reagent pellet _____

l. Incubate tube(s) in heater block next to device with the corresponding ID _____

m. Incubate tubes for 5 minutes (use timer) at 45±5C _____

n. After incubation, pour contents of tubes into sample well of device _____

o. Watch blue activation circle, as it **begins** to disappear push the Activator firmly until it "snaps" flush with the body of the SNAP™ device (device remains on heater block) _____

p. Incubate device for 4 minutes (use timer) at 45±5C _____

r. Read **IMMEDIATELY (no longer than 30 seconds after final incubation)** with IDEXX Reader for SNAP devices _____

7. Interpretation _____

a. The control spot is on the top and the test spot on the bottom of the Results Window (Correct orientation is with activator button to right and sample well to left) _____

b. Negative result: _____

1. If test spot is darker than or equal to the control spot, sample is **Negative (NF)** _____

c. Positive result: _____

1. If test spot is lighter than control spot, sample is **Initial Positive** _____

d. IDEXX Reader for SNAP devices automatically prints results as **Positive** (initial) or **Negative (NF)** _____

8. Verification of Initial Positive Samples (see App. N GR item 11); Confirmation of Presumptive Positive Samples (see App. N GR item 12); and Producer Trace-Back (see App. N GR item 13) _____

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