

TETRACYCLINE PILOT PROGRAM

BULK MILK TANKER SCREENING TEST FORM

**IDEXX - SNAP® TETRACYCLINE TEST (DILUTION CONFIRMATION)
(Raw Commingled Cow Milk)**

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

GENERAL REQUIREMENTS

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

SAMPLES

2. See App. N GR item 9 (For Guidance) _____

APPARATUS & REAGENTS

3. **Equipment** _____

- a. Heater block with SNAP insert thermostatically controlled at $45 \pm 5^\circ\text{C}$ _____
 - 1. Check temperature by placing standardized temperature measuring device in a tube containing liquid (bulb submersed); maintain records _____
 - 2. Or, use 6-inch partial immersion thermometer placed directly into small thermometer well in middle of heating unit; maintain records _____
 - 3. Temperature measuring device for each incubator (App. N GR item 3 for guidance) _____
- b. IDEXX Readers for SNAP devices, with printer or data download capability _____
 - 1. SNAPshot® Reader _____
 - a. Check Set, Part Number 87-05856-01 (black skirt) _____
 - 2. SNAPshot® DSR Reader _____
 - a. Check Set, Part Number 87-14761-00 (blue skirt) _____
- c. Pipettor - 450 μL and disposable tips (see App. N GR item 7 for guidance) _____
- d. **FOR SCREENING ONLY** - Single use 450 μL poly-pipet with indicator line to measure amount of sample, supplied by manufacturer _____
- e. Timer _____
- f. Vials for Dilution _____

4. Test Kits

a. SNAP Tetracycline Kit

Lot #: _____ Exp Date: _____

QC Date: _____ By: _____

- 1. Sample tubes containing reagent pellet

5. Sample and control agitation and dilution

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)

b. Determine if sample is to be run diluted or undiluted

- 1. Initial screening of tanker samples and initial testing of producer samples for traceback and re-instatement **MUST** be run Undiluted

- 2. Verification of Initial Positive Tanker Sample, Confirmation of Presumptive Positive Tanker Sample, Confirmation of Producer Traceback on a Confirmed Positive Tanker Sample, and Confirmation of Positive Producer Re-Instatement Sample are all run **Diluted**

- a. Dilute the sample 1/10 with tetracycline negative milk (Item 6.d), one part sample to nine parts tetracycline negative milk

6. Reagent Stability and Preparation

a. Kits must be received within 72 hours if shipped non-refrigerated; over 72 hours must be shipped refrigerated

b. Store kits at 0-7°C, do not use after manufacturer's expiration date

c. Tetracycline Dilution Material

- a. Previously tested tetracycline negative raw milk (fresh or frozen) (Item 6.d)

b. Use within 72 hours when maintained at 0.0-4.5°C

c. 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: _____

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

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

2. Store at 0.0-4.5°C and use within 24 hours. Do not refreeze _____

d. Negative Control - tetracycline negative raw milk (fresh or frozen) _____

1. Previously tested tetracycline negative raw milk _____

2. Milk can be screened (previously tested) by the testing location making and/or using the controls _____

3. Must be undiluted milk _____

4. Negative control must produce less than 0.95 on the IDEXX reader; maintain records _____

Sample ID: _____ Date Tested: _____

Reader value: _____

5. Use within 72 hours when maintained at 0.0-4.5°C _____

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 _____

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

a. Thaw slowly overnight 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 24 hours. Do not refreeze _____

7. Day of use must produce less than 0.95 on the IDEXX reader; maintain records _____

Do Not proceed if out of range _____

e. Positive Control- Manufacturer supplied, do not use after manufacturer's expiration date _____

1. IDEXX ST Positive Control _____

Lot #: _____ Exp Date: _____

- 2. Store according to manufacturer's instructions _____
- 3. Reconstitute as per manufacturer's instructions with fresh or frozen previously screened tetracycline negative raw milk (Item 6.d) _____
- 4. Positive control must produce greater than 1.06 on the IDEXX reader; maintain records _____
 Reader value: _____
- 5. Store reconstituted positive control at 0.0-4.5°C for no more than 24 hours _____
 Lab Prep. Date: _____ Lab Exp. Date: _____
- 6. Day of use must produce greater than 1.06; maintain records _____

Do not proceed if out of range _____

7. Daily Performance and Operation Checks (see App. N GR item 10 for guidance) _____

- 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 _____

TECHNIQUE

8. Initial Test Procedure (First screening sample run UNDILUTED) _____

- 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±5°C, and maintain 45±5°C range for at least 5 min before beginning the test _____
 - 1. Check initial pre-heating with a temperature measuring device (see App. N GR item 3, for guidance); maintain records _____
 - 2. Continuous use block heaters, check temperature daily with temperature measuring device (see App. N GR item 3, for guidance); maintain records _____
- c. Label each device and sample tube _____

- d. Place device(s) on incubator block(s) _____
- e. Verify that pink reagent pellet is in bottom of tube before removing cap. If not in bottom, tap to bring down _____
- f. Remove and discard sample tube cap(s) _____
- g. Mix milk sample(s)/control(s) (See item 5.a) _____
- h. Add 450 uL of mixed sample/control to corresponding labeled tube(s) _____
 - 1. Using Pipettor (item 3.c) with a new tip for each sample/control and holding pipettor vertically draw up 450 μ L avoiding foam and bubbles _____
 - a. Remove tip from liquid _____
 - b. While holding the pipettor vertically, expel test portion to sample tube _____
 - 2. **FOR SCREENING ONLY** - Using a new manufacturer provided single-use 450 μ L poly-pipet (item 3d.) for each sample/control _____
 - a. Draw up 450 uL of sample to indicator line, avoiding foam and bubbles _____
 - b. Remove tip from liquid _____
 - c. While holding poly-pipet vertically, expel test portion to sample tube _____
- i. Agitate sample tube(s) to dissolve reagent pellet _____
- j. Place tube(s) in heater block next to device with the corresponding ID _____
- k. Incubate tube(s) for 5 min (use timer) at $45\pm 5^{\circ}\text{C}$ _____
- l. After incubation, pour contents of each tube into sample well of corresponding device _____
- m. Watch pink 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) _____
- n. Incubate device for 4 min (use timer) at $45\pm 5^{\circ}\text{C}$ _____
- o. At the end of incubation, visually inspect the control and test spots. The test is invalid and the same sample should be retested with a new SNAP device if: _____
 - 1. The control spot fails to develop color _____
 - 2. Blue streaking occurs in the background or the background is the same color as the sample or control spots _____

3. The sample or control spots are not uniform in color or exhibit poor spot Quality _____

p. Insert only valid tests in the reader **IMMEDIATELY (no longer than 30 sec)** after completion of incubation _____

9. Interpretation with IDEXX Reader for SNAP Devices _____

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

10. Verification of Initial (SCREENING UNDILUTED SAMPLE) Positive Tanker Samples done at Same Testing Facility using DILUTION Confirmation Procedure _____

a. Set out four SNAP devices, sample tubes and pipets and label as negative control, positive control, and two devices and tubes with the initial positive sample ID _____

b. Mix milk sample(s) 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting; use within 3 min. (samples must be in appropriate containers to allow the use of vortexing) _____

c. Dilute the sample 1/10 with previously tested tetracycline negative raw milk (Item 6.c), one part sample to nine parts tetracycline negative milk _____

1. 450 uL of sample plus 9 aliquots of 450 uL each of previously tested tetracycline negative raw milk, or _____

2. 1 mL of sample plus 9 mL of previously tested tetracycline negative raw milk _____

d. Mix DILUTED milk sample(s) 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting; use within 3 min. (DILUTED samples must be in appropriate containers to allow the use of vortexing) _____

e. Mix controls 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting; use within 3 min (controls must be in appropriate containers to allow the use of vortexing) _____

f. Add 450 uL of mixed DILUTED sample to corresponding labeled tube(s) _____

1. Using Pipettor (item 3.c) with a new tip for each sample/control and holding pipettor vertically draw up 450 µL avoiding foam and bubbles _____

a. Remove tip from liquid _____

b. While holding the pipettor vertically, expel test portion to sample tube _____

- g. Add 450 uL of mixed control to corresponding labeled tube(s) _____
- 1. Using Pipettor (item 3.c) with a new tip for each sample/control and holding pipettor vertically draw up 450 µL avoiding foam and bubbles _____
 - a. Remove tip from liquid _____
 - b. While holding the pipettor vertically, expel test portion to sample tube _____
- h. Agitate sample tube(s) to dissolve reagent pellet _____
- i. Place tube(s) in heater block next to device with the corresponding ID _____
- j. Incubate tube(s) for 5 min (use timer) at 45±5°C _____
- k. After incubation, pour contents of each tube into sample well of corresponding device _____
- l. Watch pink 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) _____
- m. Incubate device for 4 min (use timer) at 45±5°C _____
- n. At the end of incubation, visually inspect the control and test spots. The test is invalid and the same sample should be retested with a new SNAP device if: _____
 - 1. The control spot fails to develop color _____
 - 2. Blue streaking occurs in the background or the background is the same color as the sample or control spots _____
 - 3. The sample or control spots are not uniform in color or exhibit poor spot quality _____
- o. Insert only valid tests in the reader **IMMEDIATELY (no longer than 30 sec)** after completion of incubation _____

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 the Idexx SNAP Tetracycline test, perform testing technique as outlined in Item 10 (Dilution Confirmation) _____
- b. If performing confirmation on an equivalent test follow confirmation procedure for that test. **(refer to Appendix N Tetracycline Confirmation (current revision))** _____

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 Appendix N Tetracycline Confirmation (current revision) for a listing of test kits to assure equivalence)]

- a. Initial test on producer samples is run on undiluted sample
- b. Any producer sample that is positive must be re-tested
- c. Duplicate testing of producer is performed following the testing technique as outlined in Item 10 (Dilution Confirmation)

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

- a. Initial test on producer samples is run on undiluted sample
- b. Any producer sample that is positive must be re-tested
- c. Duplicate testing of producer is performed following the testing technique as outlined in Item 10 (Dilution Confirmation)

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