

**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  $\mu\text{L}$  and disposable tips (see App. N GR item 7 for guidance) \_\_\_\_\_
- d. **FOR SCREENING ONLY** - Single use 450  $\mu\text{L}$  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  $\mu$ 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  $\mu$ 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)]**

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- 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)

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**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)]**

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- 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)

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

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