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 ±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±5°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: ___

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 sample MUST be run undiluted
      2. Verification of Initial Positive Tanker Sample, Confirmation of Presumptive Positive Tanker Sample, Traceback of Producer(s) on a Confirmed Positive Tanker Samples and Producer Re-Instatement Samples 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: ________

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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

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 blue 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 µL 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 µL poly-pipet (item 3d.) for each sample/control

   a. Draw up 450 µL 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±5°C

l. After incubation, pour contents of each tube into sample well of corresponding device

m. 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)

n. Incubate device for 4 min (use timer) at 45±5°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 µL of sample plus 9 aliquots of 450 µL 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 µL 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 µL 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 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) ________

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. Reporting (Refer to the 2015 NCIMS Proposal 211 Pilot Program Accepted Tetracycline Test Kit Using Both Undiluted and Diluted Steps and Appendix N Pilot Program Q&A - Current Revisions) ________

12. Producer Reinstatement and Reporting (Refer to the 2015 NCIMS Proposal 211 Pilot Program Accepted Tetracycline Test Kit Using Both Undiluted and Diluted Steps and Appendix N Pilot Program Q&A - Current Revisions) ________