APPENDIX N BULK MILK TANKER SCREENING TEST FORM

IDEXX - SNAP® TETRACYLINE TEST (DILUTION CONFIRMATION) (Raw Commingled Cow Milk) IMS #9-12 FDA/NCIMS Revision 4/20

[Unless otherwise stated all tolerances are ±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. 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 #:		xp Date:			
		QC I	Date: By	r:			
		1.	Sample tubes containir	ng reagent pellet			
5.	Sam	ple a	and control agitation a	nd 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 tank for traceback and re-ins	ter samples and initial testing of producer samplesstatement MUST be run Undiluted			
		2.	Verification of Initial Po Presumptive Positive T Traceback on a Confirr Positive Producer Re-I	ositive Tanker Samples, Confirmation of Fanker Samples, Confirmation of Producer med Positive Tanker Samples, and Confirmation of nstatement Samples are all run Diluted			
			a. Dilute the sample one part sample to	1/10 with tetracycline negative milk (Item 6.d), o nine parts tetracycline negative milk			
6.	Rea	gent	Stability and Preparat	ion			
	a.	Kits 72 h	must be received within ours must be shipped re	72 hours if shipped non-refrigerated; over efrigerated			
	b.	Store kits at 0-7°C, do not use after manufacturer's expiration date					
	C.	Tetracycline Dilution Material		al			
		1.	Previously tested tetrac (Item 6.d)	cycline negative raw milk (fresh or frozen)			
		2.	Use within 72 hours wh	nen maintained at 0.0-4.5°C			
		3.	Or, aliquot within 24 ho free freezer or in an ins within 2 months	ours and freeze at -15°C or colder in a non-frost- sulated foam container in a frost-free freezer; use			
			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					
d.	Neg	gative 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 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					
	6.						
		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.	Pos exp	sitive Control- Manufacturer supplied, do not use after manufacturer's iration date					
	1.	IDEXX ST Positive Control					
		Lot #: Exp Date:					
	2.	Store according to manufacturer's instructions					

	 Reconstitute as per manufacturer's instructions with fresh or frozen previously screened tetracycline negative raw milk (Item 6.d) 					
	 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				
Dai gui	ly Pe danc	erformance and Operation Checks (see App. N GR item 10 for e)				
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					
	Positive Range: Negative Range:					
C.	If check sets fail, call IDEXX before proceeding					
		TECHNIQUE				
Init	ial Te	est 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.	 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					

7.

8.

- 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 µL 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 singleuse 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
- I. 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±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 Qua	sample or control spots are not uniform in color or exhibit poor spot ality			
	p.	Insert only valid tests in the reader IMMEDIATELY (no longer than 30 sec) after completion of incubation					
9.	Inter	erpretation with IDEXX Reader for SNAP Devices					
	a.	IDE> Nega	XX Ro ative	eader for SNAP devices automatically prints results as Positive or (NF)			
10.	Verification of Initial (SCREENING UNDILUTED SAMPLE) Positive Tanker Samples done at Same Testing Facility using DILUTION Confirmation Procedure						
	a.	Set o cont sam	our SNAP devices, sample tubes and pipets and label as negative positive control, and two devices and tubes with the initial positive D				
	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.d), 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 milk	L of sample plus 9 mL of previously tested tetracycline negative raw			
	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 maximum setting; use within 3 min (c to allow the use of vortexing)				rols 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at n setting; use within 3 min (controls must be in appropriate containers he use of vortexing)			
f. Add 450 μL of mixed DILUTED san		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 μ L avoiding foam and bubbles					
			a.	Remove tip from liquid			
			b.	While holding the pipettor vertically, expel test portion to sample tube			

Add 450 µL of mixed control to corresponding labeled tube(s) g.

		1. Using pipett	g Pipettor (item 3.c) with a new tip for each sample/control and holding tor 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 s	sample tube(s) to dissolve reagent pellet			
	i.	 Place tube(s) in heater block next to device with the corresponding ID Incubate tube(s) for 5 min (use timer) at 45±5°C After incubation, pour contents of each tube into sample well of corresponding device 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) 				
	j.					
	k.					
	I.					
	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 colo	e streaking occurs in the background or the background is the same or as the sample or control spots			
		3. The qua	e sample or control spots are not uniform in color or exhibit poor spot ality			
	0.	Insert on after com	nly valid tests in the reader IMMEDIATELY (no longer than 30 sec) mpletion 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					

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)]

- a. If performing traceback on the IDEXX SNAP Tetracycline test, perform testing technique as outlined below:
 - 1. Initial test on producer samples is run on undiluted sample
 - 2. Any producer sample that is positive must be re-tested
 - 3. Duplicate testing of producer is performed following the testing technique as outlined in Item 10 (Dilution Confirmation)
- b. If performing traceback 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)
- 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)] ______
 - a. If performing re-instatement on the IDEXX SNAP Tetracycline test, perform testing technique as outlined below:
 - 1. Initial test on producer samples is run on undiluted sample
 - 2. Any producer sample that is positive must be re-tested
 - 3. Duplicate testing of producer is performed following the testing technique as outlined in Item 10 (Dilution Confirmation)
 - b. If performing traceback 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)
- 14. Reporting (see App. N GR item 14)