



Appendix N Training

Antibiotic Screening for beta lactam Drugs as Required by the State of VT, FDA and the National Conference of Interstate Milk Shipments(NCIMS)

The Pasteurized Milk Ordinance (PMO)

If you have a copy of the PMO you should review the antibiotic testing program details in Appendix N. Paste the following link into your web browser to access the document online:

- <http://www.fda.gov/downloads/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Milk/UCM513508.pdf>
- It will be helpful to have a copy of the Appendix N General Requirements form and the form for the specific test kit that you will be using (Delvotest P Mini, Charm SL or Idexx SNAP) in front of you while you're reviewing this Power Point. Both can be printed from the VT Agency of Agriculture website under the laboratory forms section.





Topics of Discussion

- Appendix N Program
- Sample Handling
- Equipment Requirements
- Test Procedure
- Record Keeping
- What to Do When You Get a Positive
- Achieving Approved Status
 - Split Samples and the On-Site Evaluation



Appendix N Program

The state of Vermont is charged with making sure that all Grade A milk is tested for beta lactam antibiotics prior to being processed for human consumption.

To assure consistency across the state and around the country there are specific rules regarding sample handling, record keeping and testing procedures.



Sample Handling

- The sample should be homogenous and representative of the whole that will be used in making the product.
- Take two samples, preferably in clean 2 oz. vials. One will serve as a temperature control (TC) and the other is the sample that will be tested. Keep both refrigerated until testing is complete. In the event of a positive the original sample will travel with the TC to a Certified Lab for confirmation testing.
- If you are testing immediately after sampling (within 5 minutes), then you can use the temperature of the bulk tank as the temperature of the sample at the time of testing. This temperature will be recorded on the Drug Residue Test Record (see next slide).

Equipment Requirements

- Refrigerator with temperature range of 0.0-4.5°C
- Freezer that is at least -15.0°C
 - If freezer is frost free then controls must be stored in a Styrofoam container to avoid repeated freezing and thawing cycles
- Incubator block specific to the test kit you choose
- Reader and printer for Idexx SNAP and Charm SL
- Small centrifuge for spinning Charm SL frozen controls
- Timer if not built into the incubator



Equipment Requirements - Thermometers

- Thermometers for use in the program are in °C and should be checked for accuracy before first use and then annually. All temperatures should be recorded to the nearest half degree using a decimal point.

A minimum of 2 and as many as 4 are required as described below:

- The test kits and samples need to be stored at 0.0-4.5 °C. Therefore, you will need to record the temperature of the refrigerator daily or at a minimum on days of testing.
- The frozen controls need to be stored in a freezer that is at least -15.0 °C. Therefore, you will need to record the temperature of the freezer daily or at a minimum on days of testing.
- A thermometer is necessary for ascertaining the temperature of the sample at the time of testing unless the sample is being tested within 5 minutes of being taken from the bulk tank. In that case the temperature of the bulk tank in °F may be used as the temperature of the sample at the time of testing on the Drug Residue Test Record. Otherwise the temperature of the sample should be taken in °C with a previously checked thermometer.
- If the temperature of the incubator can not be read digitally, then a thermometer will be necessary for taking the temperature of the incubator.
- Thermometers can be ordered from and checked by the State Central Dairy Laboratory. You may also order thermometers from your own supplier and send them to the lab for initial and annual accuracy check for a small fee.



Test Procedure

You will need to decide which test best suits your needs. The three most commonly used tests are:

- Delvotest P Mini-approved for cow, goat and water buffalo milk (2.5-3 hour test)
- Charm SL-approved for cow, goat, sheep and water buffalo milk (8 min test)
- Idexx SNAP-approved for cow milk (9 min test)



Test Procedure-Lot Testing

Each time you get a new test kit or group of kits you will keep a separate record called a Lot Test Record.

On this record you will record the results you get from the positive and negative controls the first time you use the kit along with the kit lot number and expiration date. If you get several kits in a single batch with the same lot number one lot test is sufficient for the batch. See the next slide for an example of a Lot Test Record

Test Procedure-Controls

Each test has a specific temperature and incubation time. Reviewed here are themes common to all of the tests.

- Positive and negative controls must be run along with the sample each day the test is performed.
- Follow the manufacturer's instructions for preparing the positive control. For the Charm SL and Delvotest P Mini, controls can be frozen for up to two months. The Idexx SNAP requires a fresh positive control.
- The negative control is your own previously screened known negative raw milk.
- A record must be kept each time that a new positive control is reconstituted and each time that a new negative control is chosen. See the next slide for an example of the Antibiotic Controls Record.
- Once reconstituted, distribute positive control into small vials and freeze. Vials can be thawed quickly in a small amount of cool water just prior to testing or left in the refrigerator to thaw overnight.
- Negative control can also be frozen. Fresh negative milk is good for use as a control for up to 72 hours. Often it is easier and requires less record keeping to freeze negative control at the same time that a new positive control is frozen.
- To avoid using controls beyond their expiration date record the expiration date on the outside of the container used to store the controls in the freezer.





Test Procedure

- Thaw the positive and negative controls, for the Charm SL centrifuge thawed controls for 3 minutes
- Retrieve the sample and temperature control (TC) from the bulk tank
- Record on the Drug Residue Test Record:(see slide 6)
 - the temperature of the sample at the time of testing in °C from the TC
 - the complete date (mm/dd/yy) and time of testing
 - the temperature of the incubator
 - What is being tested i.e. positive/negative controls and whatever you are calling your sample (producer #, xyz farm, etc.)

Test Procedure

- Identify the test devices with what you are testing, i.e. positive control, negative control and sample(s).
- Shake the sample(s) and controls 25 times in seven seconds through a one foot arc (do not shake centrifuged controls for the Charm SL).
- Distribute the sample/controls to their test devices within 3 minutes of shaking.
- Follow manufacturer's instructions for the amount of sample to deliver for each test kit.
- Incubate the sample for the specified time period, use a timer if one is not built into the incubator.





Test Procedure-Reading and Interpretation

- The Charm SL and Idexx SNAP tests require readers which include calibrator devices that must be read prior to reading the controls and sample(s). Follow the manufacturer's instructions for how to use the readers.
- After the Calibrator Strips/Devices have been read as valid the negative and positive controls are read next.
- Once the controls are read and give the expected results you are ready to read the sample.
- For the Delvotest P Mini a purple result is positive and a yellow result is negative.
- To report the results on the Drug Residue Test Record, record a value or color and interpretation for each of the items tested, i.e. Pos Control: purple/pos, Negative Control: yellow/negative, Sample: yellow/Not Found (NF). **Take note that negative samples are reported as Not Found (NF) on the Drug Residue Test Record.** For a test that has a value from a printer, record the value and interpretation, for example: Pos Control: +2678/Pos, Neg Control: -1876/Neg, sample: -2545/NF.



Record Keeping

- Lets review the records that we've discussed thus far:
 - Drug Residue Test Record –where antibiotic testing is recorded. The Charm SL and Idexx SNAP printouts should be stapled to their corresponding Drug Residue Test Record.
 - Antibiotic Controls Record-where controls are recorded when you make a new positive and select a new negative.
 - Lot Test Record-record results you get for pos/neg controls the first time a new lot of test kits is run.
 - Refrigerator Record-temperature recorded daily.
 - Freezer Record-temperature recorded daily.

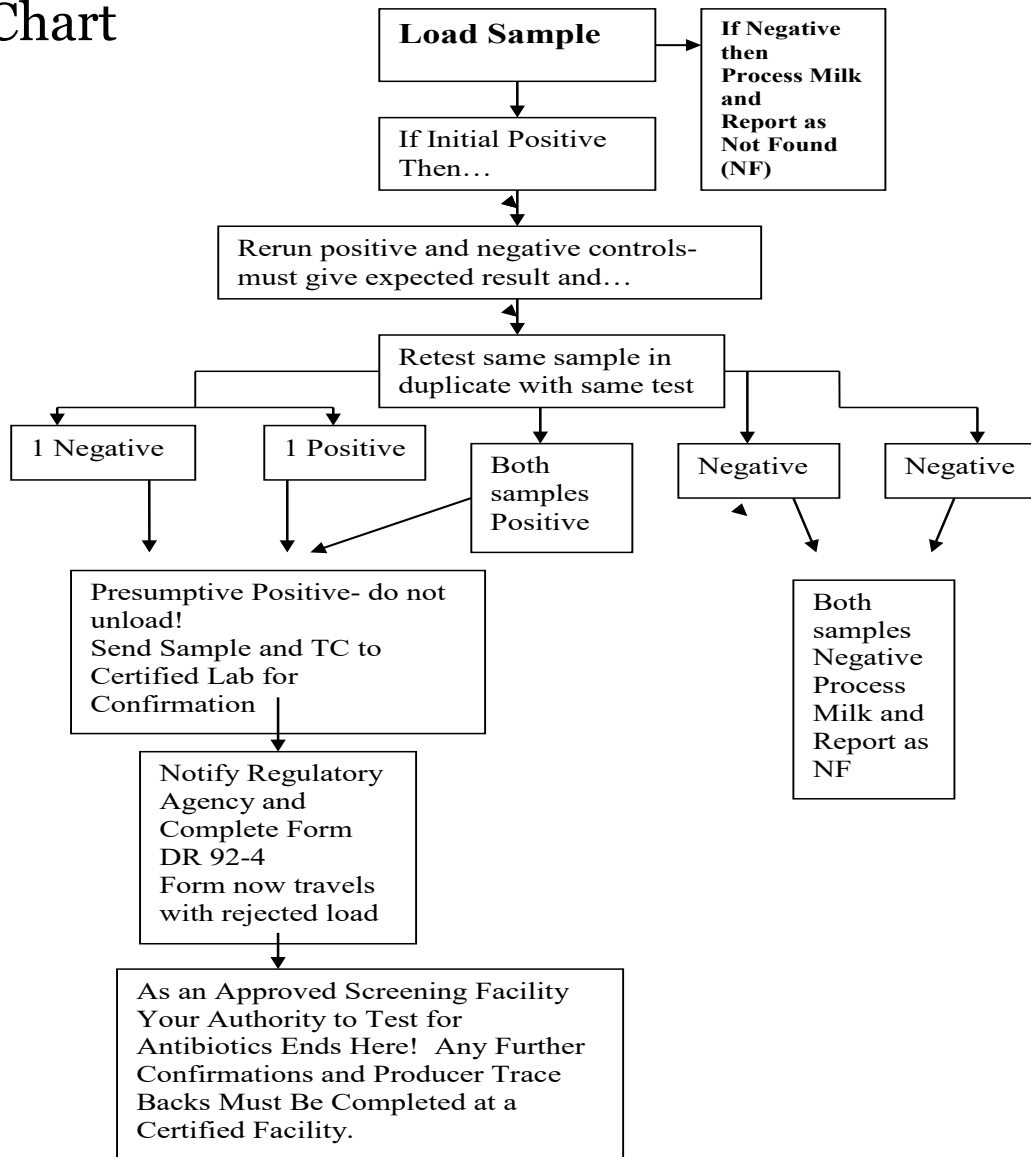


What do I do if I find a positive?

- Rerun the same sample in duplicate with the positive and negative controls.
- If the controls give the expected results and both of the duplicate samples come out negative, then milk may be processed.
- If the controls give the expected results and one or both of the duplicate samples is positive, then it is considered a Presumptive Positive.
- As an Approved Screen Only facility your authority to test for antibiotics ends here. Final Confirmation, producer trace-back and clearing must be performed in a Certified laboratory.
- See Flow Chart on next slide

What do I do if I find a positive?

Flow Chart





What do I do if I find a positive?

Charm SL and Idexx SNAP

- For those tests that require a reader, the positive and negative controls that are run with the duplicate samples must be read by the reader under the same function as the daily controls.
- For the Charm SL Press ESC 5 to get to the Perf Monitoring channel, but bypass the calibration devices by pressing next twice, then run the controls at the usual prompts.
- For the Idexx SNAP, run the controls under the usual numbers that have been chosen for them (i.e. 01-Neg, 02-Pos).
- Once the controls have been read and determined to be acceptable, then the duplicate samples are read.
- All print-outs are saved and stapled to the corresponding Drug Residue Test Record.

What do I do if I find a positive?



- Original Sample and TC should be transported on ice and water to a Certified Lab for a Final Confirmation by the same or an equivalent test.
- If you own the milk you may decide to discard it in the appropriate manner without confirmation testing, but the Dairy Regulatory Section must be notified.
- In either of the above scenarios Form DR92-4 must be completed and either transported with the sample to a certified facility or if the milk is discarded w/o confirmation DR92-4 must be completed and mailed/faxed to Dairy Regulatory.

See next slide for a copy of Form DR92-4, the areas that are your responsibility for completing have been highlighted.

What do I do if I find a positive? Form DR92-4

FORM DR 92-4



REPORT OF POSITIVE DRUG RESIDUE TEST RESULT & MILK DISPOSITION FOR BULK MILK PICKUP TRUCKS/TANKERS*

RECEIVING PLANT NAME: _____ DATE: _____
PLANT ADDRESS: _____
SOURCE OF MILK FIPS NO.: _____ LBS. OF MILK REJECTED: _____
TRUCK #: _____ HAULER: _____

Screening Test Analyst: _____ Type of Drug Found _____
Test Method: _____ Test Result: _____ +/- Control Results _____ / _____

Presumptive Positive Test Analyst _____
Test Method: _____ (Sample Run in Duplicate with +/- Controls)
Test Result 1: _____ 2: _____ +/- Control Results _____ / _____

Final Confirmation Analyst: _____ Certified Facility _____
Test Method: _____ (Sample Run in Duplicate with +/- Controls)
Test Result 1: _____ 2: _____ +/- Control Results _____ / _____

Final Confirmation of Positive Producer Analyst _____
Test Method: _____ (Sample Run in Duplicate with +/- Controls)
Test Result 1: _____ 2: _____ +/- Control Results _____ / _____

DISPOSITION OF MILK: _____

DISPOSITION CERTIFIED BY: _____
(signature) (date)
NAME/ADDRESS/ID# OF POSITIVE PRODUCER(S) _____

REPORT TO AGENCY OF AGRICULTURE, FOOD & MARKETS
CALL THE DAIRY DIVISION (802) 828-2433 FAX (802) 828-5983
CALLER: _____

PERSON NOTIFIED: _____

TIME/DATE OF NOTIFICATION: _____

FAX COMPLETED REPORT FORM TO THE DAIRY DIVISION. MAINTAIN A
COPY OF COMPLETED REPORT FORM FOR YOUR RECORDS.

*A copy of this form shall travel with rejected load to its final disposition destination.

What do I do if I find a positive?

List of Certified Laboratories Where a Final Confirmation Can Be Done



Contact List-Certified Laboratories

VT Agency of Agriculture Lab

UVM Hills Building Rm 236

105 Carrigan Drive

Burlington, VT 05405

Phone: (802) 585-4441

Contact: Kristen Needham: 585-4439 or Wendy Blackman: 585-4438

Tests Certified for: Delvotest, Charm SL, SNAP, Charm II Sulfa and Tetracycline

HP Hood LLC/Booth Bros. Dairy Lab

219 Allen St.

Barre, VT 05641

Phone: (802) 476-6605

Fax: (802) 476-7497

Contact: Scott Ferris

Tests Certified for: Charm SL

Cabot Creamery Cooperative Lab

Cabot, VT

Phone: (802) 563-3924

Fax: (802) 563-2263

Contact: Ed Verasko

Test Certified for: Charm SL

Thomas Dairy

2096 US Route 7 North

Rutland, VT 05701

Phone: (802) 773-6788

Fax: (802) 747-7121

Contact: Richard Thomas Jr.

Test Certified for: Charm SL

St. Albans Cooperative Lab

140 Federal St.

St. Albans, VT 05478

Phone: (802) 524-6581

Fax: (802) 527-1769

Contact: Spencer LaBarge

Tests Certified for: Charm SL, Delvotest

AgriMark/Cabot Lab

869 Exchange St.

Middlebury, VT 05753

Phone: (802) 388-6731

Contact: Alexandra Keimer

Test Certified for: Charm SL



Achieving Approved Status

Split Samples and the On-Site Evaluation

- In order to achieve Approved Status in the Appendix N program you must:
 - Participate in the Annual Split Sample Proficiency Program
 - Undergo an Initial and then Biennial On-Site Evaluations

Achieving Approved Status

Split Sample Proficiency Program



- Split Samples are performed annually in October
- Typically you will be notified 3-4 weeks in advance of the date to expect the samples
- There are 8 blind samples that need to be tested within 72 hours of receipt
- Each person who tests at a facility is expected to run the 8 samples along with positive and negative controls
- Results are mailed or emailed to the LEOs and should include information about the kit lot #, controls used, date/time of testing, temp of samples and results (see next slide for an example of the report)
- The LEOs compile all results and provide a written report including an update of each facility's status

Achieving Approved Status

Split Sample Proficiency Program



Test Kit/Controls	Manufacturer	Lot #	Expiration Date
Test Kit			
Positive Control			
Negative Control			

Date Analyzed:	Time:	Temp at receipt: Temp at analysis:	Incubation Temp:
Sample #			
Result*		Interpretation	
1			
2			
3			
4			
5			
6			
7			
8			
+ Control			
- Control			
Analyst Name: (please print)			Date:
Comments:			

*Record color reaction or numerical value from a reader.

Interpretation:
 NF= Not Found
 IP= Initial Positive



Achieving Approved Status

On-Site Evaluation

- Every two years an LEO will visit your facility for an on-site evaluation
- During the evaluation he/she will:
 - Observe all analysts setting up the antibiotic test
 - Look at all equipment relevant to the program (refrigerator, freezer, incubator, thermometers)
 - Review all records for the previous 2 years

Achieving Approved Status

On-Site Evaluation



- At the end of the evaluation there will be a discussion of the findings and you will be told the current status of your facility
- The LEO will write a report based on the findings
- You may be required to respond to the report within a given time frame
- In order to maintain Approved Status in the program you must complete the process by following the requests made in the final report
- The report is sent to FDA and the Regional Milk Specialist

Contact Information For LEOs:

Please contact us anytime with questions!



- Wendy Blackman
 - Phone: (802) 585-4438
 - Email: wendy.blackman@Vermont.gov
- John Jaworski
 - Phone: (802) 585-4441
 - Email: john.jaworski@Vermont.gov

Mailing address:

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UVM Hills Building Rm. 202
105 Carrigan Drive
Burlington, VT 05405

This is the end of the Appendix N Training Power Point. Thanks for your interest!

