

<p style="text-align: center;">UNIVERSITY OF MINNESOTA VETERINARY DIAGNOSTIC LABORATORY Standard Operating Procedure (SOP)</p>	Doc. No.: LUH.SOP.0006
	Revision: 11
Category: Laboratory for Udder Health Section, Test Method	Active Date: 06Apr2018
Title: Bulk Tank Procedure—Reading Cultures	Page 1 of 5

1. Purpose:

To effectively identify and count the bacteria growing on a bovine Bulk Tank culture and enter this information into the LIMS. Refer to LUH.SOP.0069 Bulk Tank Set-Up Procedure for culture set-up instructions.

2. Responsibility:

It is the responsibility of the VDL Section Head to ensure training for staff that will perform this SOP. It is the responsibility of laboratory personnel using this procedure to read, understand, receive training for, and agree to follow the procedure described in this SOP

3. Definitions:

BT = Bulk Tank Sample

Accession Number = Diagnostic Lab Number = D-Lab Number

LIMS = Laboratory Information Management System = computer database

LUH = Laboratory for Udder Health

4. Equipment and Material:

Biohazard Trash Can

Colony Counter

Calculator

Incubated BT Culture Plates

5. Safety:

5.1. Training for this procedure includes review of hazards and accident prevention, personal protective equipment (PPE) and other safety requirements based on potential risks associated with this procedure. Specific requirements may be found in the body of this document. University of Minnesota safety information and safety policies are available from University Health and Safety (UHS) on their website www.dehs.umn.edu. All biological, chemical and radioactive waste is disposed of according to state, federal and U of M requirements as found at www.dehs.umn.edu "Hazardous Waste."

5.2. Biosafety Level = 2

5.3. Safety Data Sheets (SDS) and / or Material Safety Data Sheets (MSDS) are available in binders on the north wall of 340 VDL.

5.4. Specific PPE Required: Employees are expected to wear a lab coat and comply with SYSY.REF.30 Dress Code Policy for VDL

5.5. Hazards: Biohazard Level 2 Organisms

5.6. Occupational Health Recommendations: N/A

5.7. Accident / Exposure Response

5.7.1. Consult SAFETY.REF.001, VDL Emergency Information, for appropriate response to Serious Incidents

5.7.2. Copies of Serious incident reports should also be sent to the VDL Director and DSO.

6. Training:

Laboratory personnel will receive training and will follow appropriate document review schedule. Training status is maintained within the sections or retained in Q-Pulse.

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7. Procedure:

7.1. The following precautions shall be followed to avoid contamination:

- 7.1.1. SYS.SOP.5.4.002 Contamination Prevention at the VDL shall be followed as general contamination prevention procedures.
- 7.1.2. Plates remain with lid down to ensure that condensation does not drip onto plates.
- 7.1.3. LUH.SOP.0060 General Cleaning Procedure is followed for general cleaning guidelines.

7.2. Bulk tank cultures—24 hour incubation

- 7.2.1. Culture plates are examined for growth that is suspect for *Staph. aureus* and *Strep. agalactiae*. (Beta-haemolytic colonies on Factor® and FOCUS™ plates, respectively)
- 7.2.2. Suspect colonies are identified on the MALDI.
 - 7.2.2.1. Refer to LUH.SOP.0065 MALDI-TOF Biotyper for the identification procedure.
 - 7.2.2.2. Maldi results must meet the Acceptance Criteria as stated in section 8 of LUH.SOP.0065 MALDI-TOF Biotyper before they are accepted as final results.
- 7.2.3. The MALDI result, case number, and sample ID are transferred from the MALDI results report to a post-it note which is placed on the culture plates.
- 7.2.4. The culture plates are incubated for an additional 24 hours.

7.3. Bulk tank cultures—48 hours incubation

- 7.3.1. Culture plates are examined for growth that is suspect for *Staph. aureus* and *Strep. agalactiae*. (Beta-haemolytic colonies on Factor® and FOCUS™ plates, respectively.) The results of the MALDI identification performed at 24 hours are compared to the growth on the Factor® and FOCUS™ plates.
 - 7.3.1.1. If the results of the MALDI identification match the results the technician would expect as the plates are examined, or no beta-haemolytic colonies are observed on the culture plates, the technician may move ahead to procedure step 7.3.3.
 - 7.3.1.2. If there are colonies that the technician reading the 48 hour plates believes are possible *S. aureus* or *S. agalactiae*, these colonies are identified on the MALDI.

Note: Possible reasons identification would be performed at 48 hours include newly beta-haemolytic colonies not noted the previous day or the technician believes a different colony may generate a different result.


 - 7.3.1.2.1. Refer to LUH.SOP.0065 MALDI-TOF Biotyper for the identification procedure.
 - 7.3.1.2.2. Maldi results must meet the Acceptance Criteria as stated in section 8 of LUH.SOP.0065 MALDI-TOF Biotyper before they are accepted as final results.
- 7.3.2. **If MALDI identification is performed at 48 hours, the culture is set aside and no results are entered into the LIMS until the identification process is complete.**
- 7.3.3. Use a colony counter, as needed, to count the number of colonies for each classification of bacteria. For this procedure, counts will be taken for only specific

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categories of bacteria. There may be other colonies of growth that will not belong to these groups and, therefore, will not be counted.

7.3.3.1. **Coliforms:** Examine the MacConkey plates for growth. Count only the pick/red colonies growing on this media for this procedure.

7.3.3.2. ***Strep. agalactiae*:** Examine the FOCUS™ plates for growth. Count beta-haemolytic colonies only if the MALDI result was positive for *S. agalactiae*.

 7.3.3.3. **Non-ag Steps (Environmental *Strep. spp.*):** Examine the FOCUS™ plates for growth. Count all non-haemolytic colonies. In addition, count beta-haemolytic colonies only if the MALDI result was negative for *S. agalactiae*.

7.3.3.4. ***Staph. aureus*:** Examine the Factor® plates for growth and count all *S. aureus* colonies. *S. aureus* colonies are large, white or yellow, beta-haemolytic, catalase positive colonies. Count only if the MALDI result was positive for *S. aureus*.


7.3.3.5. ***Staph. spp.*:** Examine the Factor® plates for growth and count all *Staph* colonies that were not counted as *S. aureus* above. *Staph* colonies are larger, white or yellow colonies which are strongly catalase positive. In addition, count beta-haemolytic colonies if the MALDI result was negative for *S. aureus*.

7.3.4. The following guidelines will determine which dilution plate (5 or 50) the technician will use to make the bacterial count:

7.3.4.1. Count the number of colonies for each of the classifications of bacteria (see above) from the dilution plate that has between 25 and 250 colonies for each classification. For example, the *Staph. sp.* count may be taken off of the 50 dilution, while the *S. aureus* count may be taken from the 5 dilution plate.

7.3.4.2. If no plate falls within this range, count the closest.

7.3.4.3. If both plates have fewer than 25 colonies, count the 5 plate.

7.3.4.4.  If the 50 dilution has over 250 colonies, 250 colonies on the 50 dilution will be the maximum number recorded for that classification.

7.3.4.5. If the count is zero, this information is recorded as 0 colonies on the 5 dilution

7.3.5. As the counts are made, they are typed into the LIMS system.

7.3.5.1. Enter technician initials on the bulk tank culture screen for the case.

7.3.5.2. Press 7 (Components) in front of the line for the sample being examined. Press Enter.

7.3.5.3. A list with the following codes will appear on the new screen. Press F11 (Toggle Dilution)

7.3.5.4. The following codes will appear on the left side of the screen (page down for the last set of codes)

7.3.5.4.1. U.BTC.SC = coliforms

7.3.5.4.2. U.NAS.SC = non-Ag Streps

7.3.5.4.3. U.SAGA.SC = Strep ag

7.3.5.4.4. U.SAUR.SC = Staph aureus

7.3.5.4.5. U.STA.SC = Staph sp.

7.3.5.5. The colony count will be entered on Plt1: _____ and the dilution of the plate will be entered on Dilu: _____ for each type of bacteria.

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7.3.5.6. After entering the results, press Enter. A pop-up screen will ask if a secondary procedure for Coliform ID is required. Press F12 to exit from this screen.

7.4. Cases are ready for results to be finalized when all growth falling into the specific categories for this procedure have been identified and counted. Refer to LUH.SOP.0054 Finalizing Test Results for the LUH.

7.5. Cases not complete after 48 hours:

7.5.1. Pending results shall not be entered into the LIMS until finalized.

7.5.2. Place a note on the culture plates indicating that the plates need to be saved. (This will ensure plates are available for double checking if questions arise when finalizing results.)

7.5.3. Place a note in the file indicating that the case is not complete, which tests are pending, and a date that the case is expected to be finished.

8. Acceptance Criteria:

8.1. Plates used in this procedure that were produced in the LUH media lab must pass the standards set in LUH.SOP.0035 Media Batch Quality Assurance

8.2. Each day the procedure is followed the following quality control SOPs will be followed:

8.2.1. LUH.SOP.0031 Daily Media QA Procedure

8.2.2. LUH.SOP.0032 Air Quality Control

8.3. Technicians performing this procedure have passed the annual proficiency test. Refer to LUH.SOP.0026 Annual Proficiency Testing for additional information.

9. Interpretation of Results:

9.1. Refer to LUH.SOP.0065 MALDI-TOF Biotyper for interpretation of results.

10. References:

SYS.SOP.5.4.002 Contamination Prevention at the VDL

LUH.SOP.0060 General Cleaning Procedure

LUH.SOP.0065 MALDI-TOF Biotyper

LUH.SOP.0054 Finalizing Results for the LUH

LUH.SOP.0035 Media Batch Quality Assurance

LUH.SOP.0031 Daily Media QA Procedure

LUH.SOP.0032 Air Quality Control

LUH.SOP.0026 Annual Proficiency Testing

LUH.FORM.0069 Bulk Tank Set-Up Procedure

Laboratory Handbook on Bovine Mastitis by the National Mastitis Council Inc., revised edition 1999.

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Employee Training and Competency Record

Employee Name (print)

Trainer Name (print)

Employee Read SOP		Employee Understands Safety Instructions		Employee Observed Procedure		Employee Performed Procedure		Employee Demonstrated Competency	
Employee Initials	Trainer Initials	Employee Initials	Trainer Initials	Employee Initials	Trainer Initials	Employee Initials	Trainer Initials	Employee Initials	Trainer Initials
Date	Date	Date	Date	Date	Date	Date	Date	Date	Date

The Employee initials this document to indicate he/she has read and understands the SOP and/or manual.
 The Trainer initials this document to confirm she/he completed a review of the procedure, review of safety instructions, procedure training and/or competency testing with the Employee as indicated.

Procedure Training

Correctly Answered (Circle one)

Yes	No	1.	Employee stated proper safety precautions?
Yes	No	3.	Employee stated proper contamination prevention procedures?

Employee Competency

Yes	No	1.	Employee was observed performing this procedure?
Yes	No	2.	Employee's results matched those of training technician?
Yes	No	3.	Employee has demonstrated competence with all identification charts and necessary biochemical tests associated with this procedure?

Comments:
