1. Purpose:

To effectively determine bacterial count in a bedding sample. This procedure is performed on bedding from Dairy farms, but may be used to determine bacterial counts from similar substances. Interpretation of the results and their relationship with on-farm practices is not conducted by Laboratory for Udder Health technicians. This procedure begins after incubation of culture plates. For culture set-up procedure, refer to LUH.SOP.0071 Bedding and Towel Procedure—Set-up.

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:

Accession Number = Diagnostic Lab Number = D-Lab number
LIMS = Laboratory Information Management System = computer database
LUH = Laboratory for Udder Health

4. Equipment and Material:

Colony counter
Calculator

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 room 340 VDL.

5.4. Specific PPE Required: Employees are required to wear a lab coat and comply with SYS.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.

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. LUH.SOP.0060 General Cleaning Procedure is followed for general cleaning guidelines.

7.2. Refer to LUH.SOP.0071 Bedding and Towel Procedure Set-Up for sample set-up procedures.

7.3. ***Klebisella* Identification:**

7.3.1. Culture plates are examined for growth that is suspect for *Klebsiella sp.* (Large, wet, pink colonies on MacConkey agar)

7.3.2. Suspect colonies are identified on the MALDI.

7.3.2.1. Refer to LUH.SOP.0065 MALDI-TOF Biotyper for the identification procedure.

7.3.2.2. 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.

7.3.3. 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.

7.3.3.1. If the results of the MALDI identification match the results the technician would expect as the plates are re-examined, or no large, wet, pink colonies are observed on the culture plates, the technician may move ahead to procedure step 7.4.

7.3.3.2. If there are colonies that the technician believes may generate a different result, these colonies are run on the MALDI as described in 7.2.2.

7.3.4. **If MALDI identification is performed, the culture is set aside and no results are entered into the LIMS until the identification process is complete.**

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

7.4.1. **Coliforms:** Examine the MacConkey plates for growth. Count only the pick/red colonies growing on this media for this procedure. *Klebsiella spp*. are a sub-set of this group.

7.4.2. **Non-Coliform Gram Negatives:** Examine the MacConkey plate for growth. Count only the tan/clear colonies growing on this media for this procedure. (All colonies not counted as coliforms above.)

7.4.3. **Environmental *Streps*:** Examine the CNA plates for growth and count *Strep* colonies. *Streptococcus* colonies are small, alpha- or non-haemolytic colonies that are catalase negative. They can be grey or white.

7.4.4. ***Staph. spp.*:** Examine the CNA plates for growth and count all *Staph* colonies. Staph colonies are larger, white or yellow colonies which are strongly catalase positive.

7.4.5. ***Bacillus spp.*:** Examine the CNA plates for growth and count all *Bacillus* colonies. Bacillus colonies will be small or tiny, clear, dull, or slightly yellow colonies, which are weakly catalase positive.

7.4.6. The following guidelines will determine which dilution plate the technician will use to make the bacterial count:

7.4.6.1. 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 0 dilution, while the *Bacillus* count may be taken from the 3 dilution plate.

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

7.4.6.3. If the 0 plate has fewer than 25 colonies, count the 0 plate.

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

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

7.4.7. As the counts are made, they are typed into the LIMS system. Count *Klebsiella* colonies (if MALDI was positive) and determine the percent of the total coliforms that were *Klebsiella sp*. This number is entered as a secondary procedure.

7.4.7.1. Enter technician initials on the bedding culture screen for the case.

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

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

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

7.4.7.4.1. U.BBCLS = Bacillus sp.

7.4.7.4.2. U.BCS = Gram negative coliforms

7.4.7.4.3. U.BESS = Environmental Streps

7.4.7.4.4. U.BSS = Staph sp.

7.4.7.4.5. U.GNB = Non-coliform gram negatives

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

7.4.7.6. After entering the results, press Enter. A pop-up screen will ask if a secondary procedure for Coliform ID is required. Type Y on the line to indicate yes and an X next to the procedure. Press Enter.

7.4.7.7. Exit the Bedding Procedure and there will be an additional procedure U.CLID Coliform ID. Enter into this procedure.

7.4.7.8. Type KLE.SP on the Result line. Type initials on the Technician line. Type the percent of the total coliform colonies that were *Klebsiella sp*. on the Value2 line. Type 1 on the line in front of the sample that applies to the results. Press Enter.

7.4.8. Save culture plates until the entire case has been finalized.

7.5. 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 of Results for the LUH.

7.6. Cases not complete after 48 hours:

7.6.1. Pending results shall not be entered into the LIMS until finalized.
7.6.2. 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.)
7.6.3. 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. CNA plates used in this procedure 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 of 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
Standard Methods for the Examination of Dairy Products, 17th Edition, 2004.
Godden, S., R. Bey, K. Lorch, R. Farnsworth, R. Rapnicki. 2008. Ability of Organic and Inorganic Bedding Materials to Promote Growth of Environmental Bacteria. J. Dairy Sci. 91:151-159.

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	2.	Employee stated proper contamination prevention procedures.

Employee Competency

Yes	No	1.	Employee was observed performing this procedure?
Yes	No	2.	Employee's identification matched those of training technician?
Yes	No	3.	Employee has demonstrated competence with MALDI identification procedures?

Comments: