1. **Purpose:** To effectively determine bacterial count in a bedding sample or on a towel 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 SOP details the set-up procedures for these tests. For additional information, refer to the full SOPs.

   LUH.SOP.0004 Bedding Procedure
   LUH.SOP.0021 Towel Procedure
   LUH.SOP.0042 Prototheca Reading Procedure

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:**
   LUH.EQ.142 -20 Freezer
   Permanent ink sharpie marker
   Biohazard trash can
   Bench-top biohazard bucket lined with biohazard waste bag
   Clean 50 ml beakers
   Secondary containment bucket
   Sterile water
   Sterile Whirl-Pak bags
   Sterile 14 ml round bottom tubes
   One of the following timers:
      LUH.EQ.119
      LUH.EQ.144
      LUH.EQ.153
      Sterile 2 ml dilution tubes
   One of the following 1000 µl pipettes:
      LUH.EQ.57-60
      LUH.EQ.79
      LUH.EQ.156-160
      LUH.EQ.165-174
   Sterile, bendable, inoculating loops
   Milk racks
   Sharpie markers
   Ruler
   Sterile, filtered pipette tips

Absorbent workspace cover
Cart
Ziploc Bags

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 shall wear a lab coat and nitrile gloves when preforming this procedure. Employees shall comply with SYS.REF.30 Dress Code Policy for VDL.

5.5. Hazards: N/A

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. A workspace cover may be placed on the work surface to contain spills.

7.1.3. New, sterile, Whirl-Pak bags and round bottom tubes are used for each bedding sample.

7.1.4. Care is taken to ensure the surfaces of the petri plates and the inside surface of the Whirl-Pak do not come into contact with hands or other non-sterile objects. If a plate becomes contaminated, it is replaced with a new, sterile plate.

7.1.5. Plates remain closed until they are inoculated, and only exposed to laboratory air for a limited period of time while being inoculated.

7.1.6. Plates remain with lid down to ensure that condensation does not drip onto plates.

7.1.7. If, in the process of inoculating the plate, the pipette tip comes into contact with a non-sterile surface, the contaminated tip is discarded and a new tip is used.

7.1.8. If, in the process of spreading the bedding water across a plate with a bent inoculating loop, the loop comes in contact with a non-sterile surface, the loop is discarded and a new, sterile loop is used.

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

7.2. Refer to LUH.SOP.0002 Receiving of Mastitis Samples for Pre-Test tracking and labeling procedures.

7.3. **Bedding Samples**

7.3.1. **Sample Storage—**To be performed on the day samples arrive in the lab.

7.3.1.1. Label a Whirl-Pak bag with Diagnostic Lab number and LIMS Animal ID number.

7.3.1.2. Measure the bedding sample into a clean 50 ml beaker - pack sample lightly until it is level at 50 ml mark.

7.3.1.3. Place measured bedding sample in the Whirl-Pak bag.

7.3.1.4. Place bag with measured sample in the plastic container in the -20 freezer LUH.EQ.07.

7.3.1.5. Samples are stored in LUH.EQ.07 until they are tested. Samples must be frozen prior to testing. To ensure this is the case, samples received on Mondays are tested the following week.

7.3.1.6. The remainder of the sample is placed in the daily sample storage bag according to LUH.SOP.0038 Sample Storage Procedure.

7.3.2. **Making a Water Slurry**

7.3.2.1. Add 250 ml sterile water per 50 ml sample. This is a 1:5 dilution.

7.3.2.2. Mix bedding sample by shaking the bag containing the bedding.

7.3.2.3. Allow to soak for at least 10 minutes

7.3.2.4. Shake sample again and pour about 10 ml into a labeled round bottom tube.

7.3.3. **Labeling Media and Dilution Tubes**

7.3.3.1. Label three 2ml water tubes (dilution tubes) with 1, 2, and 3 respectively. Alternatively, the tubes may be arranged in a rack in such a manner as to make it clear to the technician which tube corresponds with each dilution number. (Left to right, lowest to highest, for example.)

7.3.3.2. Label 4 each of the MacConkey and CNA plates with the Diagnostic Lab number, the LIMS Animal ID Number, and the dilution number.

7.3.3.2.1. The Diagnostic Lab number is written horizontally across the middle of the plate.

7.3.3.2.2. The LIMS Animal ID number is written at the top of the plates.

7.3.3.2.3. The dilution number (0, 1, 2, or 3) is written at the bottom of the plate.

7.3.4. **Inoculation of Dilution Tubes**

7.3.4.1. Invert the round bottom tube to mix the sample.

7.3.4.2. Using a 1000µL pipette and a sterile tip, transfer 200µL of the sample in the round bottom tube into dilution tube 1. Eject tip into a bench-top biohazard bucket.

7.3.4.3. Using a new, sterile pipette tip—aspirate the liquid in dilution the 1 in and out of the pipette tip several times to mix the sample. Use this pipette tip to

transfer 200µL from tube 1 into tube 2. Eject tip into a bench-top biohazard bucket.

7.3.4.4. Using a new, sterile pipette tip—aspirate the liquid in dilution tube 2 in and out of the pipette tip several times to mix the sample. Use this pipette tip to transfer 200µL from tube 2 into tube 3. Eject tip into a bench-top biohazard bucket.

7.3.4.5. Using a new, sterile pipette tip—aspirate the liquid in dilution tube 3 in and out of the pipette tip several times to mix the sample.

7.3.5. **Inoculation of Media**

7.3.5.1. Use a 1000µL pipette and a new, sterile tip, to transfer 200µL to transfer liquid between the tubes and the plates. The same pipette tip may be used between transfers as long as the tip does not become contaminated and the technician is working consecutively from more dilute samples toward the most concentrated.

7.3.5.2. Transfer 200µL from dilution tube 3 to the MacConkey plate labeled 3. Transfer 200µL from dilution tube 3 to the CNA plate labeled 3.

7.3.5.3. Use the same pipette tip to transfer 200µL from dilution tube 2 to the plates labeled 2.

7.3.5.4. Work in the same manner to transfer from the dilution tube 1 to the plates labeled 1 and from the round bottom tube to the plates labeled 0. Eject the used pipette tip into the bench-top biohazard bucket.

7.3.5.5. Sterilely remove a disposable loop from its container and bend it into an “L” shape by pressing the tip against the inside of a sterile plate lid.

7.3.5.6. Starting with the plates labeled 3, use to loop to spread the liquid across the entire surface of the agar. The same loop may be used for all the plates as long as the loop does not become contaminated and the technician is working consecutively from more dilute samples toward the most concentrated.

7.4. **Towel Samples**

7.4.1. **Sample Storage**—To be performed on the day the sample arrives in the lab.

7.4.1.1. Label a Whirl-Pak bag and round bottom tube with the Diagnostic Lab number and sample ID.

7.4.1.2. With gloved hands, measure the towel in INCHES with the ruler and determine the area of the towel (length x width) in square inches.

7.4.1.3. Place the towel in the Whirl-Pack bag.

7.4.1.4. Record the size of the towel on the outside of the bag.

7.4.1.5. Place Whirl-Pack in the plastic tray in the -20 freezer LUH.EQ.07.

7.4.1.6. Samples are stored in LUH.EQ.07 until they are tested. Samples must be frozen prior to testing. To ensure this is the case, samples received on Mondays are tested the following week.

7.4.2. **Making a Water Slurry**

7.4.2.1. Add an amount of sterile water in ml equal to the area of the towel in square inches. (i.e. area=144in^2 then add 144ml of sterile water)

7.4.2.2. Close the Whirl-Pak bag and mix the water around the towel by kneading the bag containing the towel and water.

7.4.2.3. Allow the towel to soak for at least 10 minutes. (Set a timer or refer to the wall clock)

7.4.3. **Inoculation of Media**

7.4.3.1. Knead sample again and pour about 10ml of the water into the round bottom tube.

7.4.3.2. Label two CNA and two MacConkey plates with the Diagnostic Lab Number centered vertically on the plate and the LIMS ID number(s) at the top or bottom of the plates.

7.4.3.3. Label the bottom of one CNA and one MacConkey plate with 5 and the other set of plates with 50. This is the dilution factor.

7.4.3.4. Use a 1000µl pipette and sterile, filtered tips to transfer 200µl of the sample into a dilution tube.

7.4.3.5. Mix the sample in the dilution tube by pipetting the liquid in the tube up and down in the tip several times.

7.4.3.6. Using a new pipette tip, transfer 200µl of the liquid from the dilution tube to the CNA plate labeled 50 and transfer 200µl of the liquid from the dilution tube to the MacConkey plate labeled 50. Finally, transfer 200µl of the original sample from the round bottom tube to the CNA plate labeled 5 and 200µl of the original sample in the round bottom tube to the MacConkey plate labeled 5. If performed in this order, the pipette tip need not be changed between tubes.

7.4.3.7. Use the inside lid of the Petri dish to bend a sterile blue loop into an “L” shape.

7.4.3.8. Use this bent loop to evenly spread the liquid across the surface of each plate, starting with the plates labeled 50, and then those labeled 5.

7.5. **Stacking plates**

7.5.1. Plates are stacked in sets with the CNA plates on top of the MacConkey plates and the two sets stacked on top of each other on the top level of a cart.

7.6. **Special Note: Prototheca Only Requests**

7.6.1. Prototheca only testing may be set up from both bedding and towel samples when requested. If Prototheca testing is requested in addition to general testing of Bedding or Towel, Prototheca can be read off of the plates set up in the standard procedure for Bedding/Towel.

7.6.2. A sample of the water slurry is swabbed onto Factor and MacConkey media and incubated for 48 hours at 37°C. Refer to LUH.SOP.0042 Prototheca Reading Procedure for additional information.

7.7. **Clean Up**

7.7.1. Upon completion of sample set-up, the workspace cover is discarded, and LUH.SOP.0060 Cleaning Procedures for the LUH is followed for the clean-up process.

7.7.2. All 15ml Tubes and bags containing towels are stored for 2 weeks in LUH.EQ.04.

7.7.3. All other items used in this procedure may be discarded in the biohazard trash can.

8. Acceptance Criteria:

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

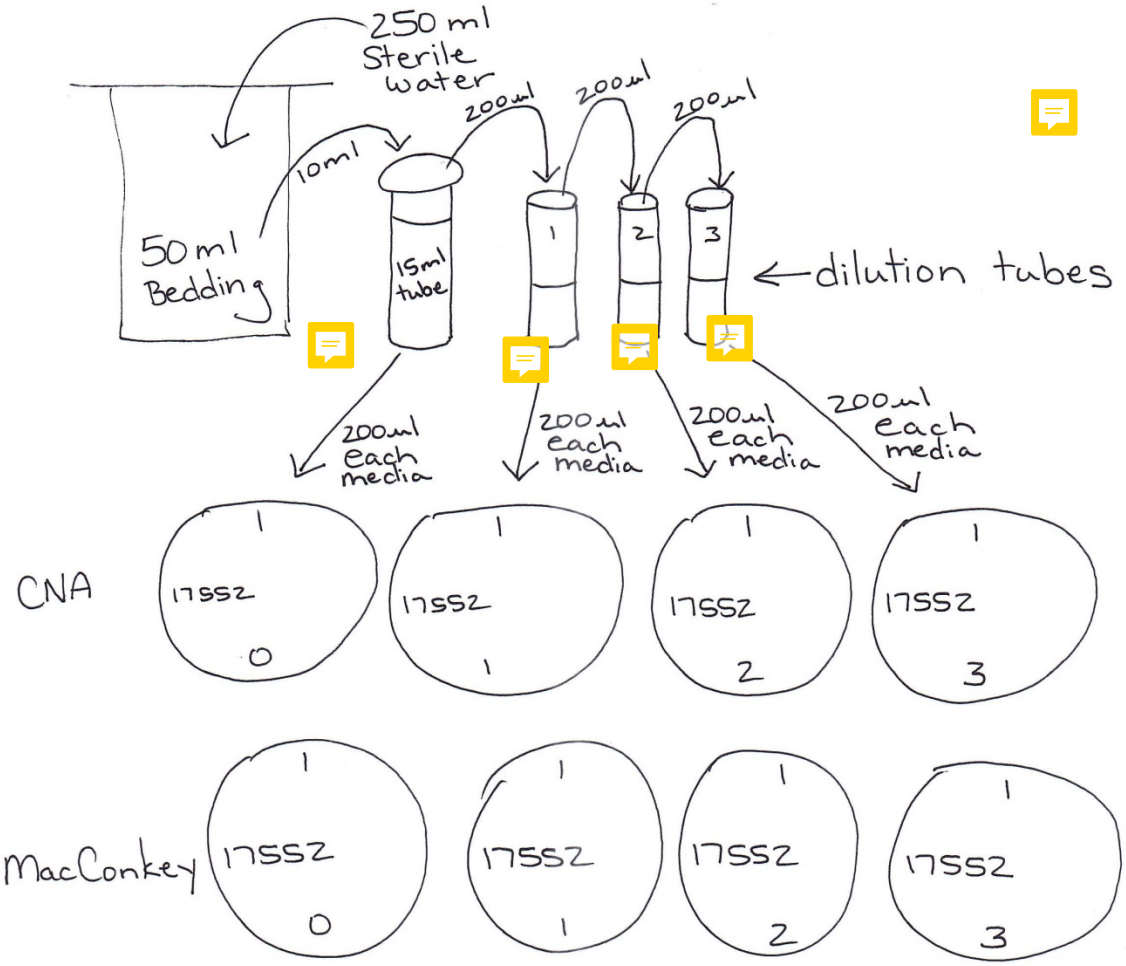
8.2. Each day the procedure is followed the following quality control SOPs will be followed:
  8.2.1. LUH.SOP.0031 Daily Media QA Procedure
  8.2.2. LUH.SOP.0032 Air Quality Control
8.3. Technicians performing this procedure have passed the annual proficiency test. Refer to LUH.SOP.0026 Annual Proficiency Testing for additional information.

9. Interpretation of Results:

9.1. Refer to interpretation of results for individual tests.

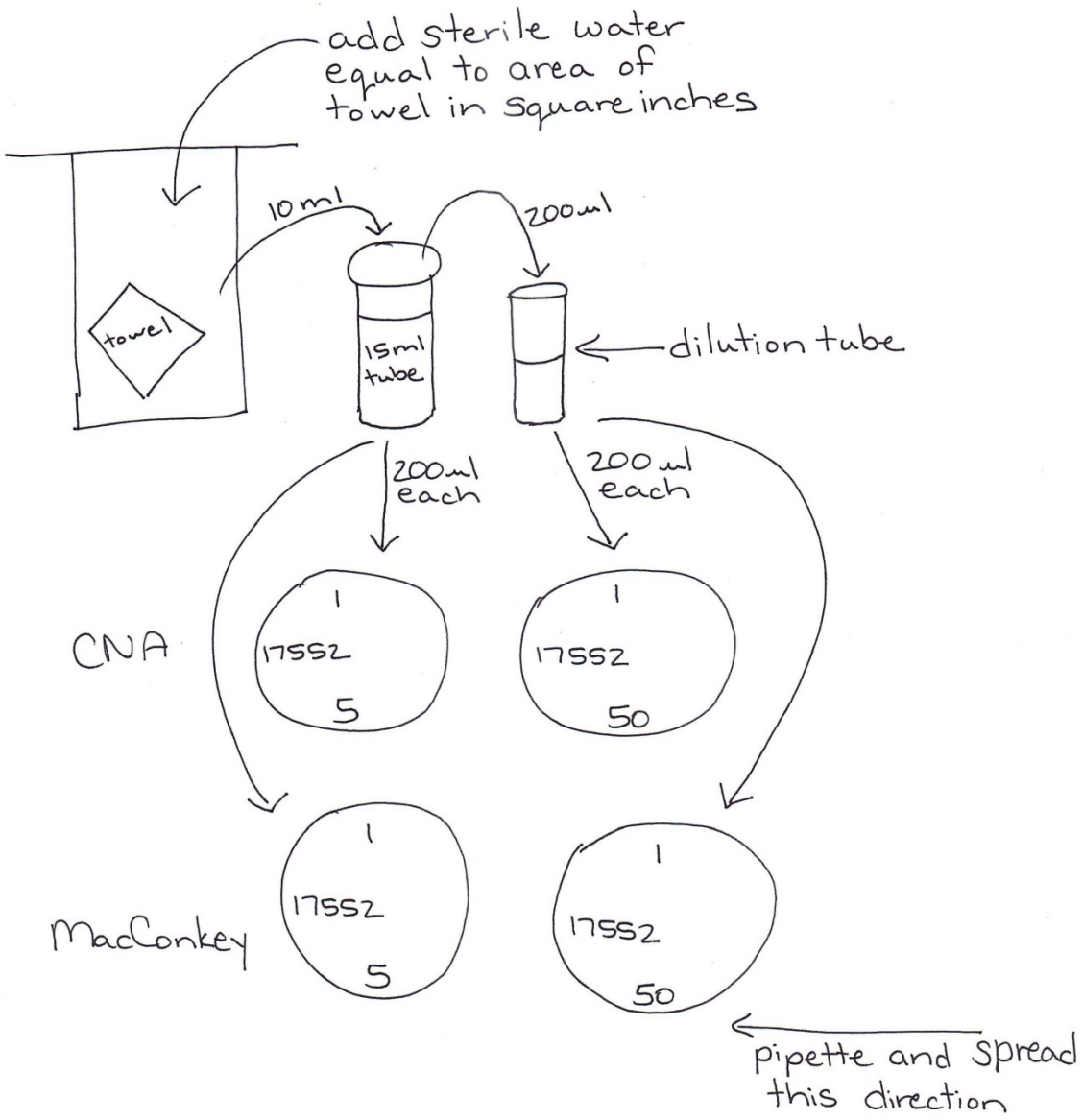
10. References:

SYS.SOP.5.4.002 Contamination Prevention at the VDL
LUH.SOP.0060 General Cleaning Procedure
LUH.SOP.0002 Receiving of Mastitis Samples
LUH.SOP.0038 Sample Storage Procedure
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.SOP.0004 Bedding Procedure
LUH.SOP.0021 Towel Procedure
LUH.SOP.0042 Prototheca Reading Procedure
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.



pipette and use Spreader in this direction

Bedding Set-Up



Towel Culture Set-Up

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 demonstrated proper sample set-up procedure?
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 identification matched those of training technician?

Comments: