1. **Purpose:** To set up cultures in such a way as to effectively determine the bacterial count in a bovine Bulk Tank sample. Refer to the SOPs for reading the cultures for further information on this procedure:

   LUH.SOP.0006 Bulk Tank Procedure-Reading Cultures
   LUH.SOP.0011 Mycoplasma Reading Procedure
   LUH.SOP.0042 Prototheca Reading Culture

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:**
   Factor agar plates
   MacConkey agar plates
   MTKT agar plates
   Permanent ink Sharpie marker
   One of the following 1000ul pipettes
      LUH.EQ.56
      LUH.EQ.57
      LUH.EQ.58
      LUH.EQ.59
      LUH.EQ.60
      LUH.EQ.70
      LUH.EQ.78
      LUH.EQ.79
      LUH.EQ.86
      LUH.EQ.156
      LUH.EQ.157
      LUH.EQ.158
      LUH.EQ.159
      LUH.EQ.160
   Sterile pipette tips
   Bench-top biohazard bucket lined with biohazard waste bag
   Sterile 2oz vials
   Cart
   Workspace Cover
   Milk Tube Rack

Biohazard Trash Can
Sterile, bendable, inoculating loops
Sterile 2ml water tubes

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 340VDL.

5.4. Specific PPE Required: Lab Coat is required when working in the laboratory. Protective Nitrile gloves are required when using chemicals or handling biological samples. Gloves are not required during the reading/data-entry process.

5.5. Hazards: Biosafety Level 2 Microorganisms

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

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

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

7.1.6. If the pipette tip comes into contact with a non-sterile surface, the contaminated tip is discarded and a new tip is used.

7.1.7. If a loop comes in contact with a non-sterile surface, the loop is discarded and a new sterile loop is used.

7.1.8. 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. Labeling Media

7.3.1. Label 2 each of MacConkey, Factor, and MTKT plates with the Diagnostic Lab number, the LIMS Animal ID Number, and/or BT, and the dilution number.

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

7.3.1.2. The LIMS Animal ID number/BT is written at the top of the plates.

7.3.1.3. The dilution (5 or 50) is written at the bottom of the plate.

7.3.1.4. If Prototheca is also requested, also write “PRO” at the tops of the plates.

7.4. Pooling samples—this step shall be skipped when individual testing has been requested.

7.4.1. Transfer 2000µL of each sample into a sterile 2oz milk vial with a 1000µL pipette and a sterile tip (1000µL twice). Eject tip into a bench-top biohazard bucket between each new sample.

7.5. Inoculation of Dilution Tube

7.5.1. Obtain one 2ml sterile dilution tube.

7.5.2. Invert the pooled milk sample tube to mix the sample.

7.5.3. Using a 1000µL pipette and a sterile tip, transfer 200µL of the pooled sample into the dilution tube. Eject tip into a bench-top biohazard bucket.

7.5.4. Using a new, sterile pipette tip—aspirate the liquid in and out of the pipette tip several times to mix the sample. Eject tip into a bench-top biohazard bucket.

7.6. Inoculation of Media

7.6.1. Use a 1000µL pipette and a new, sterile tip, to transfer 200µL of 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. The order of the types of media does not matter, but the order of the concentration DOES matter.

7.6.1.1. Transfer 200µL from the dilution tube to the MacConkey plate labeled 50.
Transfer 200µL from the dilution tube to the Factor plate labeled 50.
Transfer 200µL from the dilution tube to the MTKT plate labeled 50.

7.6.1.2. Use the same pipette tip to transfer 200µL from the pooled sample to each of the plates labeled 5.

7.6.1.3. Eject the used pipette tip into the bench-top biohazard bucket.

7.6.2. 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.

7.6.3. Starting with the plates labeled 50, use the 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 spreads the liquid on the plates labeled 50 prior to the plates labeled 5. Discard the bent loop in a bench-top biohazard bucket.

7.7. Mycoplasma culture on bulk tank sample

7.7.1. Mycoplasma culture is included in a bulk tank culture unless the client specifically indicates that Mycoplasma is not required.

7.7.2. Mycoplasma culture is performed on the pooled sample.
7.7.2.1. Label a Mycoplasma plate with the Diagnostic Lab Number written horizontally across the middle of the plate. At the top of the plate, label “BT” and the LIMS Animal ID number.
7.7.2.2. Mix the pooled sample by gently inverting the sample several times.
7.7.2.3. Open the milk vial.
7.7.2.4. Dip a sterile swab into the sample.
7.7.2.5. Swab the entire surface of the Mycoplasma plate.

7.8. Stacking plates
7.8.1. Plates for each bulk tank are stacked in the following order:
7.8.1.1. The plates are grouped by media-type with the 5 dilution on top of the 50 dilution plates for each media-type.
7.8.1.2. Factor plates on top, MTKT plates in the middle, and MacConkey plates on the bottom.
7.8.1.3. Each bulk tank stack is placed on the top of a cart for processing at the end of the day.
7.8.2. The Mycoplasma plate is not placed on the cart, but rather the Mycoplasma plates are grouped together at the end of the counter top for sorting later in the day.

7.9. Sample Storage
7.9.1. When inoculation of media is complete, samples are bagged and placed on the bottom of a cart in preparation of processing at the end of the day.
7.9.2. The sample storage procedure is detailed in LUH.SOP.0038 Sample Storage Procedure.

8. Acceptance Criteria:

8.1. Factor and MTKT 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.

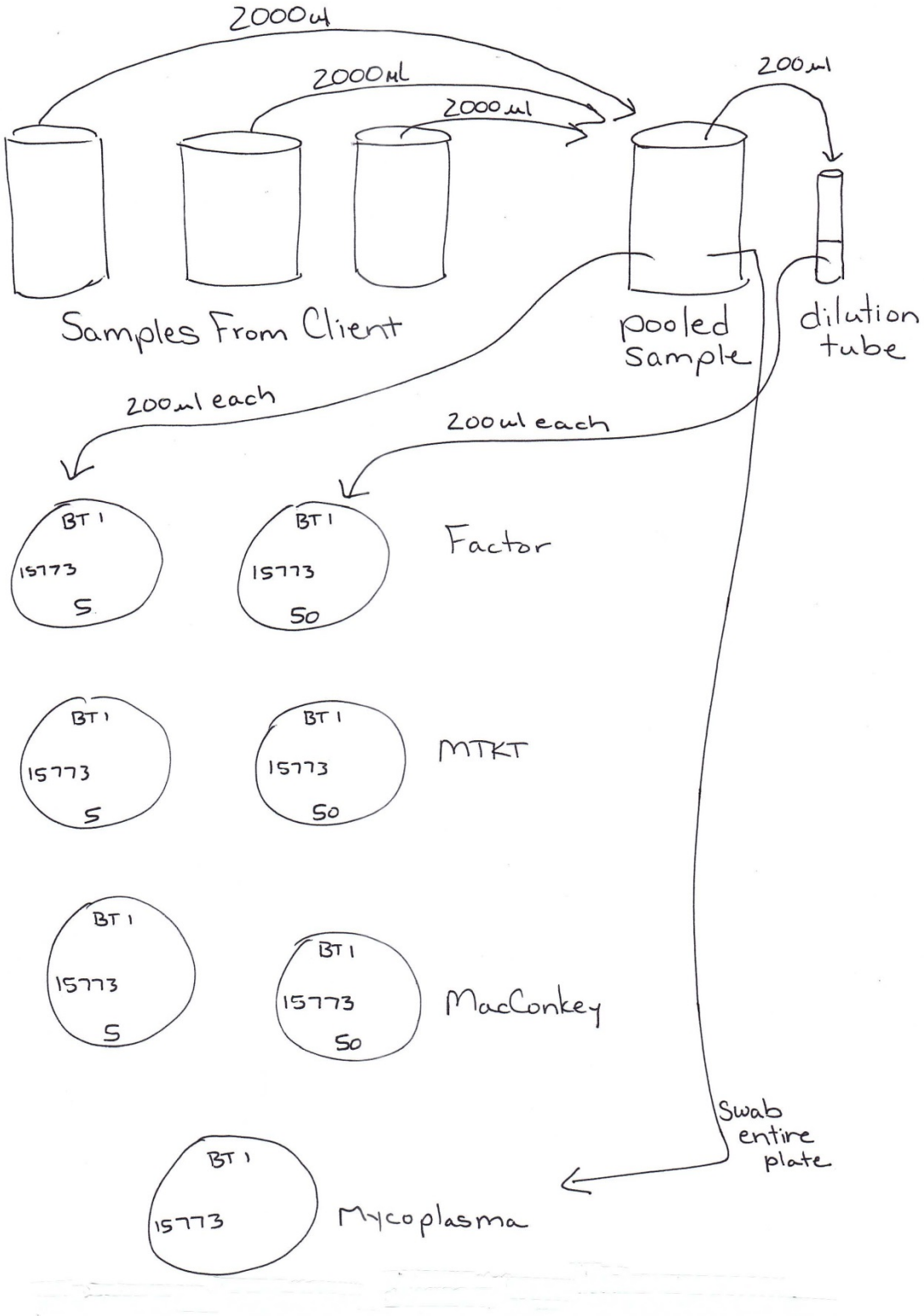
9. Interpretation of Results:

Refer to LUH.SOP.0006 Bulk Tank Procedure—Reading Cultures for interpretation of results.

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.0006 Bulk Tank Procedure—Reading Cultures
LUH.SOP.0011 Mycoplasma Reading Procedure
LUH.SOP.0042 Prototheca Reading Culture
Laboratory Handbook on Bovine Mastitis by the National Mastitis Council Inc., revised edition 1999.


2000µl
2000ML
2000µl
200µl
Samples From Client
pooled Sample
dilution tube
200µl each
200µl each
BT 1
15773
S
BT 1
15773
So
Factor
BT 1
15773
S
BT 1
15773
So
MTKT
BT 1
15773
S
BT 1
15773
So
MacConkey
Swab entire plate
BT 1
15773
Mycoplasma

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.	Results matched those of training technician?

Comments:

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