

Udder Health Systems, Inc. Laboratory Operating Standards for Bovine Mastitis Diagnostic Services

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The following outline should be used to evaluate a laboratory's facilities and procedures in the microbiological diagnosis of bovine mastitis. Compliance with these laboratory operating standards insures high quality mastitis microbiology results.

I. Personnel requirements:

A. Academic and training background:

1. Laboratory Technicians: At least 2 years training or bench experience in life sciences with specific training in aseptic techniques and microbiological laboratory methods.
2. Laboratory supervisor: Bachelors in Science, major microbiology
3. Laboratory Director: Doctor of Veterinary Medicine, or Masters Degree in Microbiology

B. Duties

The laboratory is operated by a director, a supervisor and technician. The supervisor is responsible for establishing quality assurance and quality control (QA/QC) policies and ensuring those policies are followed. The supervisor is also the primary laboratory analyst and is responsible for performing analyses on milk, water, bedding sanitation, quality control samples and recording results. In the absence of, or under the direct supervision of the supervisor the technician may also perform analyses on above samples. The supervisor will verify results. The supervisor is also the sample custodian.

The Laboratory Director is responsible for ensuring that the staff is adequately trained and can perform their duties with competence. A staff member with experience in the method of interest, who has been designated by the Laboratory Director, will conduct the training of the appropriate personnel. A specific plan for training technicians will be developed including designation of the trainer, length of training process, and demonstration of proficiency. Proficiency of techniques and methods by trainee will be demonstrated and documented prior to completion of training.

New hire requirements will be determined by the Position Description and /or by the Laboratory Director. Previous education or experience, such as an advanced degree and /or six months of applicable experience, may fulfill the training requirements for the position that he or she is applying. The analyst will be retrained in the analytical methods required of the position if they have not been performed by the analyst for more than one year. Any additional in-house training and retraining needs will be assessed by the Laboratory Director.

C. Training

All laboratory personnel will be trained in the following areas:

- preparation of reagents
- calibration and standardization of instrumentation
- methodology
- health and safety
- quality control

The QA/QC Officer will be designated who conducts training in the overall QA/QC program on an annual basis. Interim training of new personnel is conducted as needed. QA/QC Officer will be trained by an external source on a continuous basis, or at discretion of the Laboratory Director.

II. Facilities Management:

A. Laboratory Safety

The following safety parameters are basic to the laboratory safety program. These parameters ensure a healthy and safe work place. The implementation and effectiveness of the safety program rests on the institution, laboratory director, and laboratory staff themselves.

1. Laboratory personnel are oriented to the Laboratory Safety Program. Each staff member documents date of completion of orientation.
2. Material Safety Data Sheets (MSDSs) will be filed in the Safety Program Manual.
3. The use of Personal Protective Equipment including eyewear, gloves, lab coats, and face shields is described in the Safety Manual.
4. Decontamination of biologically hazardous is disposed of properly (see Safety Manual).
5. Laboratory supplies once contaminated are stored appropriately until decontaminated. All storage containers are securely closed.
6. In the event of a power failure due, an emergency generator is automatically started. Additionally, the Safety Officer will be immediately notified. Duration of power interruption will be noted on all record logs of equipment affected.
7. The location and use of the following Safety Equipment is described in the safety program:
 - a. Fire extinguisher
 - b. Eye washes
 - c. Safety shields
 - d. Safety containers
 - e. Storage facility of hazardous materials
 - f. Safety wall chart.

- 8 The following Laboratory Hazards are identified in the safety program. Preventive measures and post accident actions are described in relation to these hazards.
 - a. Chemical
 - b. Biological
 - c. Physical

B. Policy for Quality Assurance/Quality Control

1. The principal objective for operating the laboratory is to consistently produce complete analytical data, which accurately represent the microbiological content of the samples that are taken.
2. All analytical procedures will be in following with “**Laboratory Handbook on Bovine Mastitis**” Revised Edition 1999, National Mastitis Council, “**Standard Methods For The Examination Of Water and Wastewater**”, 18th Edition 1992 and “**Standard Methods for the Examination of Dairy Products**” 15th Edition , 1985
3. No sample data will be recorded without including results for any analyses of QC samples associated with the data. Data will be entered in indelible ink on printed bench sheets and kept in binders. Data will be kept for at least five years. All data is reviewed and validated prior to release of the data from the laboratory.
4. Initial training for new analyst on analytical methods and QA/QC requirements and procedures will be conducted on a priority basis. Additional training is to be conducted periodically (not less frequently than twice per year) as required to maintain competence in analytical skills. Records of all training are kept in each trainee's personnel folder.
5. Available to all lab personnel are the following manuals: “**Laboratory Handbook on Bovine Mastitis**” Revised Edition 1999 by National Mastitis Council, “**Standard Methods For The Examination Of Water and Wastewater**, 18th Edition 1992” and “**Standard Methods for the Examination of Dairy Products**” 15th Edition 1985 this QA Manual, and, in a readily available binder, safety data sheets for all potentially hazardous chemicals used in the lab.

C. General Laboratory Practices

Good laboratory practices serve as the vehicle that defines the quality control activities in each lab and how they are to be accomplished. The actual procedures and protocols of analyses are to be defined in the Manual of Standard Operations Procedures (SOP).

General guidelines for laboratory practices are listed below.

1. Proper labeling, storage, and/or disposal of all non-essential equipment and supplies.
2. Laboratory:
 - a. bench tops will be disinfected with 70 ppm Chlorine before and after each

- use.
- b. work area adequate for workload and for storage (200 sq. ft/analyst recommended).
 - c. clean, well lighted, ventilated, with adequate temperature control
3. No smoking, drinking, or eating in the laboratory.
 4. The laboratory staff is responsible for minimizing or controlling environmental contamination to the best of their ability.
 5. The lab is kept clean and orderly at all times. Any environmental contamination beyond laboratory control will be reported promptly to the Director or Supervisor.
 6. The laboratory staff is responsible for reporting any equipment breakdowns and /or safety violations observed in the laboratory to the appropriate staff member.
 7. A copy of the Quality Assurance Plan will be located in a known and accessible area for all personnel to obtain.

D. Equipment Maintenance and Monitoring

Proper maintenance and monitoring of all equipment will limit the downtime, maintain calibration, and reduce malfunctions. Monitoring of laboratory apparatus will produce a more efficient laboratory, support the reliability of data, and reduce capital expenses for the organization. Equipment breakdown will be reported to the appropriate personnel as designated on the organizational chart. That person is responsible for taking corrective actions pertaining to the malfunctioning equipment. For equipment, which is deemed critical to the laboratory operation, a back-up piece of equipment will be available.

Small Equipment:

1. Standards Thermometer
 - calibrated by NIST, or is traceable to NIST(certificate available on file), or its equivalent, at the points of use; 0, 35, 44.5, and 121C (maximum registering thermometer)
 - checked annually for accuracy by ice point determination
 - clearly readable gradations
2. Working Thermometers
 - clearly readable gradations
 - calibrated against a NIST standards thermometer annually at points of use
 - labeled with date calibrated last, initials of analyst, and +/- temp. Correction
 - properly immersed as required by manufacturer

3. Balances
 - top loader or equivalent:
 - provides a sensitivity of 0.1 gram at a load of 150 g
 - calibrated quarterly using NIST class S or ASTM Class 1 or 2 weights
 - calibrated annually by a qualified service representative
 - calibration / maintenance recorded
 - analytical balance:
 - provides sensitivity of 1.0 mg at zero and 10.0 g loads
 - calibrated annually by a qualified service representative
 - calibration / maintenance recorded

4. pH meter
 - pH electrodes, consisting of pH half cell and reference half cell or equivalent combination electrode, are free from Ag / AgCl or possess a ion selective barrier preventing passage of Ag / AgCl into the media
 - pH meter with standard accuracy of 0.1 pH unit
 - use at 25 C or use a temperature compensation probe
 - elected accuracy is determined daily or with each use (slope or mV method)
 - if meter used daily, calibrate with 2 buffers in the correct pH range, if meter used infrequently calibrate with each use
 - standard buffer solutions are used once daily and discarded
 - expired buffer solutions are discarded
 - calibration records maintained

5. Colony Counter
 - Quebec colony counter or its equivalent is used to provide the necessary magnification and visibility for plate counts
 - hand tally or other counting device present and records accurately

Large Equipment:

1. Autoclave
 - routine cleaning on weekly basis, drain trap cleaned daily
 - pressure maintained at 15 psi during sterilization
 - temperature maintained between 119 - 123 C for sterilization as determined monthly using a maximum registering thermometer
 - all records of autoclave runs including pressure, ster. Temp., and ster. Time, and total time in and out of autoclave maintained
 - of sufficient size to accommodate workload
 - sterilization efficiency determined quarterly by spore indicators, and heat-sensitive indicator tape used with each batch
 - records of performance checks and servicing maintained

2. Incubators
 - routine cleaning / disinfecting on monthly basis
 - air type, maintain temp. Of 35.0 +/- 0.5 c under any loading capacity
 - thermometers graduated in at least 0.5 C increments
 - thermometers calibrated / tagged appropriately and properly immersed
 - culture dishes limited to stacks of four with spaces of one inch between stacks or incubator walls
 - temperature records maintained twice daily

3. Water baths
 - routine cleaning / disinfecting on weekly basis
 - covered, and adequate water level maintained
 - of sufficient size to accommodate workload
 - level of water covers the level of liquid in the incubating tubes
 - maintain temp. Of 44.5 +/- 0.2 C under any loading capacity
 - thermometer graduated in 0.1 degrees increments
 - thermometers calibrated / tagged appropriately and immersed properly
 - temperature recorded twice daily
 - agar tempering bath maintained at 44 -46 C

4. Refrigerators /freezers
 - temperature maintained at 0 - 4.4 C
 - routine cleaning on monthly basis, all outdated materials properly discarded
 - thermometers properly immersed
 - daily temperature records maintained
 - freezers defrosted as needed (biannually)

5. Laboratory water system for producing Microbiological Suitable Water (MS Water)
 - an on-demand system is recommended
 - maintenance of water system as described by manufacturer
 - an on-line conductivity (or equivalent) monitoring and display device is strongly recommended
 - water produced is free from contaminants and toxic substances, as confirmed annually by the Water Suitability Test (Standard Methods)
 - make-up water is distilled or deionized and exceeds 0.5 megohm cm resistance or is less than 2 micrograms Seimens / cm conductivity at 25 C
 - make-up water is tested monthly for resistance or conductivity
 - make-up water is analyzed monthly for residual chlorine and is at non-detectable levels
 - make-up water is free from trace (< 0.05 mg / L) dissolved metals as determined annually

- make-up water contains <1000 CFU / ml as determined monthly by the heterotrophic plate count method
- distilled water produced by glass, tin-lined or stainless steel still, cleansed routinely as needed
- demineralized water produced by mixed resin cartridges, replaced as required
- reservoir containers for lab water are non-toxic, inert glass or plastic, and are cleansed and sterilized periodically as needed (at least quarterly)
- stored volumes of MS lab water are replaced frequently: weekly suggested
- records maintained for dating of D-I tanks.
- Laboratory MS water is tested by both chemistry and bacteriology laboratories.

Records of the results are kept in the Quality Assurance manual.

<u>Analysis</u>	<u>Frequency</u>	<u>Laboratory</u>
Conductivity	Monthly	In House
Metals	Yearly	Certified Laboratory
Heterotrophic Plate Count	Monthly	In House
Bacterial Quality	Yearly	Certified Laboratory

6. Ovens-hot air
 - provides sterilizing temperature in the range of 160-180 C
 - suitable thermometer used
 - records of temperature and exposure time maintained
 - effectiveness of sterilization determined by spore strips quarterly; records maintained
7. Centrifuges
 - maintenance as described by manufacturer

E. Materials

The use of poor quality materials in any laboratory procedure can adversely affect the results generated. Therefore, good laboratory practices associated with materials are essential to assure the quality of results. All chemicals, reagents, solutions, glassware, and reference materials including standards and prepared bacteriological culture media must meet the specifications which have been determined to be adequate for the methodology or program requirements. Such established specifications are critical quality assurance measures. The following are the minimum QA requirements for materials:

1. Glassware
 - all chipped, etched and broken glassware will be discarded properly
 - all glassware will be rinsed (x3) with tap water immediately after use and before dish washing, sinks will be flushed with hot water when disposing of agar.

- Cleaning and sterilizing of glassware is in accordance with SOP.
2. Pipettes
 - borosilicate glass or non-toxic disposable plastic
 - serological or equivalent
 - error in calibrated delivery volume not exceeding 2.5%
 - deliver accurately and readily, and are appropriately graduated with unbroken tips
 - pipettes larger than 10 ml are not used to deliver 1.0 ml, nor are pipettes larger than 1.0 ml used to deliver 0.1 ml
 3. Petri dishes
 - appropriate size, borosilicate glass or non-toxic disposable plastic sterile dishes used
 - bottoms clearly transparent
 - permanent marker used for labeling
 4. Sample bottles/dilution bottles
 - borosilicate glass or other inert material used
 - of suitable size to contain volume for sample and allow for adequate shaking
 - capable of being properly washed and sterilized
 - closure are water tight to prevent contamination of samples
 - dilution bottles are French square, indelibly marked calibration line at 99 ml, non-toxic plastic screw cap, watertight closures
 5. Flasks/beakers/graduated cylinders
 - borosilicate glass or other inert material used
 - autoclavable
 - graduated cylinders; calibration lines marked, volumes corresponding to calibrations meet ASTM or NIST standards, verified empirically
 6. Chemical/reagents
 - of known and suitable purity and grade for analytical
 - reagents and buffers prepared in sterile, glass or inert no-toxic container properly labeled
 - reagents and buffers stored in sterile, glass or inert no-toxic container proper temperature
 7. Storage of Dehydrated Media
 - all media is stored as specified on the manufacturer's label
 - all media received is labeled with date received and initials of the analyst receiving it

- newly opened media is labeled with the date and initials of analyst opening the media
- dehydrated media is stored alphabetically, new bottles of media are not opened until old bottles are empty or expired
- media which appears discolored, caked, or expired is properly disposed

8. Media Preparation

- culturable media preparation, storage, and expiration are according to the described method or analysis
- General guidelines for storage if not specified by method or manufacturer:
- storage at room temp. For < 7 days (prefer < 48 hrs)
- subsequent storage at 4 C in dark, dry space and total storage time not to exceed one month
- store in a clean dry space where excessive evaporation and possible contamination are minimized
- only distilled or deionized water which exceed 0.5 megohm cm (25 C) resistance with no detectable residual chlorine or trace metals is used in media prep (MS Water)
- tubes or flask is of adequate volume to prevent evaporation baking, and/or accommodate test method
- media is sterilized and dispensed the same day it is prepared
- media is labeled, dated, initialed, and properly stored immediately after preparation
- media stored under refrigeration must be incubated at appropriate temperature for 24 hrs. Prior to use; Durham tubes containing air must be discarded
- Media Controls- Controls on all the media's with positive and negative bacteria and blank. The blank samples are incubated for 72 hours to insure sterility.

9. Miscellaneous

- culture tubes - borosilicate glass, suggested 16 x 150 mm; 20 x 150 mm; 16 x 100 mm
- culture tube closures - fit 16 and 20 mm diameter tubes, stainless steel or non-toxic plastic
- test tube rack - stainless steel or plastic
- fermentation (Durham) tubes - short-form shell borosilicate glass vials; 0.5 or 0.25 dram sizes, flat bottom
- brushes - nylon or equivalent, autoclavable
- knives - stainless steel blades, autoclavable handles, for shucking shellfish
- pipette aides - propipette type or electrical (do not recommend single port bulb type due to potential contamination)
- pipette containers for reusable pipettes - stainless steel or aluminum

F. Data Management

All records are retained at the laboratory for at least five years. After 5 years records are placed in long term storage. Before any result is reported, all raw data and calculations are reviewed for accuracy and signed by supervisor or analyst acting as the quality assurance officer. If data contained on any record is transcribed to facilitate brevity or neatness, the original record is also kept. All data is recorded in ink and corrections are initialed. A list of initials identifying the person to whom they belong is maintained as a permanent lab record.

III. Diagnostic Capability

A. Milk Laboratory Testing

1. Organism capability: The mastitis laboratory should maintain the following important mastitis pathogens as active stock cultures. All laboratory technicians must be capable of identifying colony characteristics of these organisms in individual milk cultures or bulk tank cultures. They must perform the following minimum laboratory routines to confirm a diagnosis as in **“Laboratory Handbook on Bovine Mastitis” Revised Edition 1999** by National Mastitis Council.
2. Test Reagents and Media: The following test reagents and selective media must be available and used to perform the necessary presumptive and confirmatory test for important mastitis pathogens.
 1. Washed Cow Blood Agar
 2. Modified Edwards Agar
 3. MacConkeys Agar
 4. Mycoplasma Agar
 5. Staph Selective Agar
 6. CO2 Incubation
 7. Gram Strain
 8. Catalase Test
 9. Coagulase Test
 10. Esculin Hydrolysis
 11. CAMP Test
 12. Indole and Oxidase Test
 13. API/NFT or substitute
3. Sensitivity of Method
 - a) Individual cow milk culture
 - i) Individual milk samples are plated on a minimum of a quarter of a Blood Agar plate, using a minimum of 0.05 ml of milk, unless an enhancement technique is used (freeze thaw, preincubation, etc.).
 - ii) Usage of selective media is indicated for Mycoplasma detection or very sensitive Strep. ag. detection.

- b) Bulk Tank culture
- i) Bulk tanks are plated onto four medias. .01 ml. is plated onto Blood Agar and Mycoplasma Agar. .1 ml. is plated onto a Staph Selective Agar and Modified Edwards's Agar.

Mastitis Organism Classification Chart

Organism	Presumptive	confirmation
Strep. agalactiae		CAMP (+) Esculin (-)
Staph. aureus	Catalase (+)	Coagulase (+), beta staph.
Mycoplasma	Fried-egg appearance	Fluorescent antibody, PCR
Strep species	Esculin (-) and CAMP (-)	
Strep uberis	Esculin (+) and Inulin (+)	
E- strep	Esculin (+)	
Staph. species	Coagulase (-) and non beta hemolytic pattern	
E. Coli	Indole (+) and Oxidase (-)	API 20 E
Klebsiella pneumoniae	Indole (-) and Oxidase (-) Capsule Production	API 20 E
Klebsiellae species	Indole (-/+) and Oxidase (-)	API 20E
Pseudomonas species	Indole (-) and Oxidase (+)	NFT
Pseudomonas aeruginosa	Indole (-) and Oxidase (+) metallic green sheen and fruity order	NFT
Pasteurella	Oxidase positive No growth MacConkey	NFT
Proteus	Swarming on surface	
Serratia	Red pigment	API 20 E
Bacillus	Large rough edge	
Yeast	Methylene blue stain	Size 5-9 um Budding
Mold	Fluffy colonies with thick hyphae	
Nocardia	Acid-fast filaments	
Prototheca	Methylene blue stain	Size 10-30 um
Acranobacterium pyogenes	Catalase (-) and pin point colony with clear hemolysis at 48 hours	
C. bovis	Catalase (+) dry tiny colony at 48 hours.	

B. Dairy Products Testing

When a laboratory offers testing for traditional dairy product tests such as Standard Plate Count, Direct Microscopic Somatic Cell Count, Laboratory Pasteurized test, or Preincubation test the reference text "**Standard Methods for the Examination of Dairy Products**" should be used for procedural guidelines.

C. Environmental Microbiology

Bedding Culture, Sanitizer, Backflush, etc.

1. Beddings are plated onto three medias: MacConkeys, Modified Edwards and Inulin. Two dilutions are made for each media: a 1 to 10,000 and a 1 to 100,000. An additional dilution is done for the Edwards, a 1 to 1,000,000.
2. All samples are examined for Lactose Fermentors, Nonlactose Fermentors and E Strep. populations. Capability for detection of Klebsiella pneumonia, E. coli, Serratia, and Pseudomonas aeruginosa from environment samples should be verified.
3. Use and verify function of sanitizer neutralizers when they are indicated.

D. Water Culture

When a laboratory offers testing on environmental water samples to look for E. coli, Klebsiella pneumonia, Serratia, or Pseudomonas aeruginosa, the reference test "**Standard Methods For The Examination Of Water and Wastewater**, 18th Edition 1992" should be used for procedural guidelines.

1. A minimum of 100 mls of water needs to be examined.
2. All Samples are examined for lactose fermentors, non-lactose fermentors and for E Strep. populations. Capability for detection of Klebsiella pneumonia, E. coli, Serratia, and Pseudomonas aeruginosa from environment samples should be verified.