BEST PRACTICE GUIDELINES FOR A BACTERIOLOGY/MYCOLOGY QUALITY SYSTEM FOR AAVLD-ACCREDITED VETERINARY DIAGNOSTIC LABS

PURPOSE: The intent of this document is not to describe in detail a comprehensive quality program for any particular laboratory, but rather to provide guidance for a program framework and outline the core elements that should be included in a Quality System for diagnostic bacteriology and mycology laboratories in order for them to comply with the AAVLD Requirements for an Accredited Veterinary Medical Diagnostic Laboratory.

DEFINITIONS: Previous documents on Quality Assurance (QA) and Quality Control (QC) made a distinction between QA and QC, where QA was the set of policies for monitoring and tracking quality, while QC was the set of specific technical procedures used to monitor test quality, performance of equipment, and the media and reagents used in the testing. These distinctions have been removed and updated to what is now referred to as the Quality System (QS). A Quality System is defined in the AAVLD Requirements for an Accredited Veterinary Medical Diagnostic Laboratory as “A set of interrelated or interacting elements that organizations use to implement and direct quality planning, quality control, quality assurance, and quality improvement”.

1. Overall Goal of the Quality System. The QS of the laboratory should include policies and procedures to ensure that accurate, precise, reproducible, traceable results are provided to the client, and to provide procedures to reduce to a minimum the extent of test variation within and between laboratories. Typical and suggested general areas to address include:

   A. Proper collection and transport of specimens where this will directly impact the quality of test results

   B. Specimen tracking through records of specimen accession, unique identification and specimen handling in the laboratory

   C. Policies and procedures for specifying performance parameters and setting limits for acceptable performance

   D. Policies and procedures to ensure that proper media and isolation techniques are used for the recovery of bacterial and fungal pathogens

   E. Policies and procedures for ongoing monitoring and evaluation of test results

   F. Policies and procedures to document and correct non-conformances through Corrective Actions (CA) and Preventive Actions (PA).

   G. Policies and procedures that specify turnaround times for all testing

   H. Policies and procedures for training and ongoing competency
I. Policies and procedures to control and update laboratory documents and procedures

J. Laboratory safety

K. Policies and procedures to ensure use of calibrated equipment for all testing.

2. General Implementation and specific components of the Quality System as it relates to Bacteriology and Mycology laboratories.

A. The laboratory should assist the client with proper specimen collection and transport to the laboratory. Examples include resources such as user guides, direct consultation, newsletters, and provision of transport media/systems. The laboratory should also have a policy and procedure for assessing specimen quality where it will directly impact the quality of test results, and have a policy and procedure to deal with inappropriate specimens. In general, when appropriate, the client needs to be contacted and advised that the specimen quality may adversely affect test results.

B. The laboratory should have policies and procedures to keep track of specimens submitted to the laboratory, from accession to disposal.

1) All specimens should be uniquely identified

2) Specimens should be traceable while being handled in the laboratory

3) There should be a procedure on how and where specimens are stored

4) There should be a procedure that specifies when and how submitted specimens are disposed of

C. The laboratory should maintain a written procedure manual (often referred to as Standard Operating Procedures or SOPs) that addresses the following:

1) Explicit directions for all methods and test procedures, including limits for acceptable performance, used in the laboratory

NOTE: Bacteriology testing is a bit unique in that a typical specimen may contain unknown and/or multiple organisms in combination and interpretation of significance of these can be specimen-dependent. An SOP is not required to identify each and every organism – the SOP for this type of testing should indicate that standard bacteriologic methods and tests as outlined in reference texts, journal articles, etc. will be used. Agent-specific SOPs should be used for specialized cultures to detect and/or identify specific agents when the laboratory has defined criteria for such identification, when mandated procedures are specified by regulatory agencies,
performance of specific key tests used in isolations and identification (e.g. toxin identification, CAMP test, or similar), and for equipment.

2) References and/or documentation for the validation of each procedure or test used in the laboratory are required. However, validation can include the use of standard microbiologic reference texts, journal articles, and other published information.

3) A system to review and update the procedure manual on a regular basis.

4) A policy to ensure the availability of the procedure manual with current and controlled procedures in the work area.

5) Policies and procedures to document and correct non-conformances in testing through Corrective Actions (CA) and Preventive Actions (PA).

D. Proficiency testing and ongoing monitoring of test results

1) The goal of proficiency testing is to assess the technical capabilities of personnel and the reliability of test procedures.

2) Acceptable proficiency testing might include enrollment in a proficiency testing program that provides bacterial specimens via surveys or check tests, provide internal "blind" unknowns to technicians for their evaluation, inter-laboratory exchange programs, and quality assurance surveys such as the AAVLD quality assurance survey.

3) Verification of organism identification by alternate means such as identification by a reference laboratory, or rRNA gene sequencing.

4) Ongoing monitoring and evaluation of test results can also occur as testing is performed using, for example, test controls, replicate testing using the same or different methods, or correlation of results for different characteristics of a bacterial specimen.

E. Record-keeping. Many of the SOPs that follow may already be included in more general laboratory policies and procedures

1) Maintain or have access to documentation for the performance of personnel, media, reagents, stains, equipment, susceptibility disks and tests, test kits, antisera and any other materials used for isolation and identification of bacteria and fungi.

2) Keep records of traceability for reagents used in testing so that the test could be repeated in conditions as close to the original test as possible. This includes lot numbers of reagents and controls, and times/temperatures used during testing.
3) Retain records for the length of time required by the laboratory’s quality manual, which will vary with federal, state and institutional policies

F. Reporting

1) Documentation is required for the performance of personnel, media, reagents, stains, equipment, susceptibility disks and tests, test kits, antisera and any other materials used for isolation and identification of bacteria and fungi.

2) The laboratory should have a procedure that specifies the method(s) of reporting results, who has reporting authority within the laboratory, and a policy for providing and documenting preliminary reports given to clients on cases that are not completed.

3) The laboratory should have a procedure to monitor data entry and imported data to assure consistency with manual worksheets and instrument output.

G. Personnel

1) Personnel will be hired, evaluated, paid and otherwise managed administratively as directed by institutional policies and procedures

2) Appendix I of the AAVLD Requirements has minimum and preferred qualifications for technical staff and sections heads, repeated here for Bacteriology/Mycology personnel:

<table>
<thead>
<tr>
<th>POSITION</th>
<th>MINIMAL QUALIFICATIONS</th>
<th>PREFERRED QUALIFICATIONS</th>
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<tbody>
<tr>
<td>Section Head</td>
<td>MS Microbiology + 2 yrs experience, or BS certified Med Tech + 5 yrs experience in veterinary diagnostic microbiology</td>
<td>DVM/MS, DVM/PhD, or PhD. Diplomate ACVM + 5 yrs experience in veterinary diagnostic microbiology</td>
</tr>
<tr>
<td>Technical Staff</td>
<td>High school + 2 yrs experience; or comply with existing state or university policy</td>
<td>BS/MT/VT/HT/HTL as appropriate and 2 yrs experience</td>
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3) Have a policy to provide initial training and documentation of that training for all relevant testing methods, as well as all policies and procedures outlined in the laboratory’s Quality System

4) Have a policy to provide documented initial and ongoing competency through programs such as proficiency tests, quality assurance tests, continuing education,
and affiliation and certification by appropriate professional organizations.

H. Laboratory safety

1) Safety procedures in the laboratory are generally governed by the institution and their applicable state and/or federal guidelines.

2) In general, at a minimum, laboratories will be expected to anticipate required levels of biosafety, and to provide a manual with documented safety precautions including compilations of material safety data sheets (MSDS) on all chemicals used in the lab and maintain in a file or electronically, ensure that these are readily accessible to all staff, and require and document employees to review the safety manual and MSDS documents periodically.

3. Specific and detailed suggestions for key technical areas unique for bacteriology and mycology laboratories. The following information is not intended to be inclusive, or detail actual requirements, but as general guidelines as used by AAVLD accredited laboratories. Other laboratories, as outlined and justified in their Quality Systems, may use different parameters. The parameters can often be derived from resources such as the American Society for Microbiology (ASM) and the Clinical and Laboratory Standards Institute (CLSI).

A. Equipment

1) Document performance, maintenance, calibrations, certifications, servicing and repair of all equipment and maintain records for the life of the instrument as required by the laboratory’s quality system.

2) Perform routine maintenance as specified by the manufacturer or as established by user to guarantee accuracy of results.

3) Monitor temperature-dependent activities each working day using calibrated temperature measuring devices.

**Suggested** temperature tolerances for key equipment

- incubators +/- 2°C
- water baths and heating blocks +/- 1°C
- refrigerators 4-8°C
- standard freezers +/- 5°C (-20°C, but must be below zero)
- ultra-low freezers +/- 10°C (-80°C, but must be below -60°C)

4) Suggested monitoring schedule for instruments typically found in diagnostic bacteriology laboratories.
i. AEROBIC/CO2 INCUBATORS

- each working day: record temperatures; monitor CO2 concentrations in capneic incubators (may be done periodically with Fyrite system); check gas cylinder contents
- weekly: check humidity, if applicable
- annually: clean inside and outside; check tubing for leaks; check that unit is level

ii. ANAEROBIC CHAMBERS

- each working day: check anaerobic indicators (methylene blue indicator solution/strips, reazurin strips); biological indicators (failure of an obligate anaerobe such as Cladobium novyi to grow, or growth of a strict aerobe such as Pseudomonas aeruginosa); check gas pressure
- biweekly: check catalyst and desiccant
- monthly: clean interior and check humidity
- semi-annually: check for leaks; check filters
- annually or as necessary: follow manufacturer's maintenance guidelines

iii. ANAEROBIC JARS/POUCHES FOR INCUBATION

- each working day or with each use: check atmosphere indicators; change catalyst; check jar and lid for cracks
- weekly: clean and disinfect jars
- monthly: check seal rings and lubricate, if needed

iv. AUTOCLAVE

- each working day: clean and drain, record temperatures and pressures for each cycle if the unit has automatic devices; for older units that lack such devices, each cycle should be monitored with heat sensitive tape
- weekly: check safety valve
- monthly: perform sterility tests using spore solutions or strips; check seals and clean or routine maintenance as recommended by in a service contract

v. AUTOMATED ANTIMICROBIAL SUSCEPTIBILITY AND IDENTIFICATION INSTRUMENT: follow manufacturer's guidelines

vi. BIOLOGICAL SAFETY CABINET:

- daily or with each use: follow manufacturer’s instructions for proper use, as well as those specified by the BMBL, to maintain effective barrier functions; wipe all surfaces with disinfectant; check airflow
- annually: perform inspection and certification
vii. MICROSCOPE

- each working day: clean oil from lenses; cover to protect from dust
- annually: professional inspection and cleaning; calibrate ocular micrometer

viii. CENTRIFUGE

- daily or with each use: balance loads; record temperatures, if applicable; wipe interior with disinfectant, if applicable; maintain a user logbook
- monthly: check electrical connections
- semiannually: verify temperature, if applicable; check brushes
- annually: calibrate speed and inspect rotors

ix. HEATING BLOCK

- daily or with each use: record temperature
- monthly: clean out all holes

x. HOT AIR OVEN

- daily or with each use: record temperature with calibrated thermometer
- quarterly: verify temperature with calibrated thermometer

xi. pH METER

- with each use: calibrate with standards and rinse electrodes
- each working day: check electrode solutions
- quarterly: replace or recondition electrodes, if needed

xii. PIPETTORS/CALIBRATED LOOPS

- daily or with each use: inspect for damage, calibrate disposable loops with each new lot
- annually: calibrate pipettors

xiii. REFRIGERATORS/FREEZERS

- each working day: record temperatures
- semiannually: defrost; clean interior and exterior; check gaskets
- annually: check that unit is level; check alarm function, if applicable

xiv. THERMOMETERS
• with each use: check for damage
• quarterly: verify temperature, if subdivisions are < 0.1 C

gxv. WATER BATH

• with each use: check temperature; check for leaks; check water level; check for contamination or deposits
• monthly or as needed: drain, clean and remove mineral deposits

gxv. TIMERS

• timers need to be appropriately calibrated

B. Media

1) Commercial or in-house

i. Shall be sterile
ii. Shall be able to support or inhibit the growth of certain microorganisms, dependent on the type of medium employed
iii. Shall give the appropriate biochemical response, if applicable

2) Batch refers to all the tubes, plates or containers of medium prepared at the same time or those received at the same time that are of the same lot number; lot numbers of all received media and of all reagents used to prepare media shall be tracked

3) Commercial media

i. Inspect each shipment for damage and quality
ii. Log in upon receipt with name of vendor and date
iii. Retain current hard copy version or be able to access online the manufacturer's documentation indicating compliance with CLSI protocols
iv. Document any deficiencies and corrective actions
v. Store according to manufacturer's directions
vi. Track lot number use
vii. Do not use past the expiration dates
viii. Note that some commercial media, such as chocolate and campylobacter agars, are also recommended by CLSI to have in-house quality control performed

4) In-house prepared media

i. Evaluate sterility
Example of procedure: remove 1 unit from each small batch or 3 units from each
batch >100, incubate at 35C for 24 hr. For media containing blood, incubate an
additional 24 hr at room temperature to check for contamination with
psychrophiles. Discard media that has been used for sterility checks

ii. Evaluate growth

Example of procedure: should support growth of low numbers of test organisms.
Make a 1:10 dilution in sterile saline of a 0.5 McFarland turbidity standard of test
organism and use a 1 ul loop to inoculate a plate of test medium (may use
instead a 1:100 dilution and a 10 ul loop). If this does not yield isolated colonies
on the medium being tested, a 10-fold lighter inoculum may be used

iii. Evaluate selective characteristics: check ability of media to support growth of
low numbers of selected organisms, as in section ii above

iv. Evaluate inhibitory characteristics: check ability to inhibit growth of undesirable
organisms by using a heavier inoculum

Example of procedure: Make a 1:10 dilution in sterile saline of a 0.5 McFarland
turbidity standard of test organism and use a 10 ul loop to inoculate a plate of
test medium. NOTE: a ten-fold inoculum may be required to avoid overwhelming
some types of media

iv. Evaluate for biochemical response

Example of procedure: test with a minimum of 2 control organisms to evaluate
both positive and negative results.

v. Tubed media

Example of procedure: inoculate each tube with 10 ul of a 0.5 McFarland
suspension of test organism in sterile saline. With slow growing organisms, it
may be necessary to conduct QC simultaneously with actual cultures e.g.
Mycobacterium paratuberculosis

vii. Place media into routine use when batches of media pass performance checks

viii. Discard any medium that fails to meet performance criteria

ix. Refer to references and CLSI documents for suggested organisms to use in
performance testing

x. Label containers of dehydrated media with the date of receipt, date of first use,
and do not use past the expiration dates

xi. Develop a method of labeling and recording batches of media

xii. Develop an expiration date system

Example: in general, 20 weeks for heavy-filled plates (e.g. Sab-Dex), 12 weeks for media with aseptically-added ingredients, 4 weeks for phenylethyl alcohol agar plates, and 3 weeks for motility test media and O-F media

xiii. Practice proper storage of all media and additives

Examples:
- Store plates at 4-8°C in plastic bags or in universal containers to prevent desiccation
- Close screw capped tube media or place tubed media with other closure types in plastic bags to prevent desiccation and store at 4-8°C
- Store hygroscopic media additives in a desiccator jar at the temperature recommended by the manufacturer
- Store antimicrobial powders desiccated at refrigerator temperatures
- Dispense antimicrobial stock solutions in small amounts at the time of their preparation, store at or below -14°C and once thawed, discard any unused portion

xiv. Maintain a written media log and include dates of preparation and performance checks, results of performance checks, etc.

5. Mycology media

i. Evaluate each lot to determine whether it supports or inhibits growth of known organisms
ii. Inoculate directly from working stocks
iii. Do not need to standardize inocula except when dealing with yeast, which should be standardized dependent on whether testing for growth or for inhibition
iv. Incubate either at room temperature or at 30°C

C. Stains, reagents, antisera and kit systems

1) Label with: contents, concentration, date placed in use, expiration date and storage requirements

2) Do not use outdated products or those that fail performance tests
3) Evaluate bacterial metabolites with control organisms known to give both positive and negative results

4) Maintain written records of performance tests

5) Suggested frequency of quality control

i. Check each new vial/batch and every day test is performed:
   - catalase
   - oxidase
   - direct antigen detection reagents (DNA probes, latex agglutination)

ii. Check each new vial/batch and/or once per week:
   - ONPG disks
   - XV factor/d-ALA disks
   - optochin disks
   - beta-lactamase tests
   - Gram stains, Kinyoun and Ziehl-Neelsen stains, other stains
   - bacitracin disks
   - anaerobic differentiation disks

iii. Check each new vial/batch and/or once per month:
   - Antisera for aerobic culture identification
   - Fluorescent antibody antisera

iv. Check each new vial/batch only:
   - coagulase plasma with following provisions: storage is in accordance with manufacturer’s guidelines and is not used past the expiration date

v. Check all other products not listed above or those products that are listed above but used infrequently with each new vial/batch and every day the test is performed

D. Biochemical test systems

1) Includes miniaturized commercial biochemical methods and automated methods for biochemical identification

2) Store according to manufacturer's directions

3) Do not interchange kit components between different lot numbers of kits

4) Do not use outdated kits
E. **Antimicrobial susceptibility testing**

1) Follow guidelines set forth in the most current version of the CLSI M31 document: *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*

2) For automated testing, use manufacturer guidelines or as established by user to guarantee accuracy of results

3) Maintain written records

F. **Maintenance of stock cultures:** Stock cultures of reference microorganisms must be maintained appropriately, and their source or origin recorded. Maintenance of cultures in storage and on the bench must be documented. Typical procedures might be as follows, and CLSI also has guidelines for the maintenance of stock cultures:

1) Handle pathogenic organisms with appropriate precautions and maintain so as to minimize alterations in growth characteristics and contamination

2) Sources - obtain directly from the American Type Culture Collection (ATCC) or other similar reputable source

3) Specific methods for storage, maintenance of bacterial stock cultures

   i. **Lyophilization**
      - Method of choice
      - Maintains preservation for many years
      - Preserve in small, single-use aliquots

   ii. **Frozen cultures**
      - An acceptable method
      - Make a dense suspension of bacterial growth from a solid medium in a cryoprotective medium (e.g. sterile defibrinated blood, soybean casein digest broth with 10-15% glycerol, sterile skim milk, on beads)
      - Dispense in small volumes into sterile cryovials
      - Prepare enough stock for 1 year
      - If quickly frozen at or below -45C, organisms will survive for an extended period of time
      - Subcultures used to make new batches of frozen stocks are acceptable, however, organisms need to be re-characterized

   iii. **Working control cultures**
      - Working controls must be identified as traceable to the original reference standard or material
- Up to 2 serial subcultures from primary working culture may be prepared to provide tertiary working
- May be kept on agar slants or plates of an appropriate medium and stored at 2-8°C for up to 4 weeks (dependent upon viability of the organism)
- Replenish primary working cultures monthly from frozen, lyophilized or commercial cultures to minimize any genotypic or phenotypic activity
- When needed, a single vial of stock organism is either thawed or rehydrated and transferred to an appropriate growth medium
- Isolated colonies are used to initiate performance tests or to serve as primary working cultures

iv. Anaerobic stock cultures
- Maintain working cultures in chopped meat or other suitable broth medium
- Following overnight incubation, may store at room temperature
- Perform monthly transfers and viability checks before returning to stock culture status

v. Methods of storage and maintenance of working fungal stock cultures
- Regular cultures are time-consuming and usually result in fungi becoming atypical and non-viable
- Maintain the most frequently used organisms on slants of fungal media with monthly transfers
- Maintenance of less frequently used organisms
  - May store cultures on slants in screw-capped tubes at 4°C for up to 6 months
  - Subculture the more fastidious fungi more frequently than every 6 months e.g., the zygomycetes

vi. Maintenance of permanent fungal stock cultures
a. Water cultures
  - Growth from an actively-sporulating culture on potato dextrose agar is placed into 5 ml of sterile water contained in a screw-capped tube
  - Tubes are stored at room temperature and remain viable for years
  - Additional sterile water is added as it evaporates
  - To reactivate stocks, shake tube and inoculate a small amount of suspension onto appropriate fungal medium

b. Sterile mineral oil overlay
  - Sterile mineral oil is added to a screw-capped tube containing an actively-sporulating fungal culture on potato dextrose agar
  - Cap of tube is tightened and culture is stored in refrigerator until needed
  - Cultures remain viable for up to 1 year
  - To reactivate, flame the open mouth of the tube, remove a visible portion of the fungal growth using a sterile inoculating needle, allow excess oil to drip away and transfer the fungal growth to a plate of
appropriate fungal medium

c. Freezing
  - A slant culture of actively-sporulating organism growing on potato dextrose agar is placed at -70 C
  - To reactivate, quickly remove from the freezer, aseptically tease away some growth and inoculate it onto a plate of appropriate fungal medium
  - Stock culture slant shall be returned to the freezer before thawing occurs
  - If stock culture thaws, a new slant shall be prepared

Suggested Readings and Resources


Clinical Laboratory Standards Institute publication M31A3. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard - Third Edition

Clinical Laboratory Standards Institute publication M37A3. Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline -Third Edition

Clinical Laboratory Standards Institute publication M42A. Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated From Aquatic Animals; Approved Guideline

Clinical Laboratory Standards Institute publication M49A. Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated From Aquatic Animals; Approved Guideline

Difco and BBL manual (web version 2009)
http://www.bd.com/ds/technicalCenter/misc/difcobblmanual_2nded_lowres.pdf


Requirements for an Accredited Veterinary Medical Diagnostic Laboratory, American Association of Veterinary Laboratory Diagnosticians, Inc., Version 5.0


Revised Document

This document was revised October, 2010 by the AAVLD Bacteriology Subcommittee. The working committee that revised this document included (alphabetically): Doreene Hyatt, Pat Lukens, Carol Maddox, J. Lindsay Oaks, Karen Post, Debra Royal, Deepanker Tewari, Amy Swinford, and Ching Ching Wu. The document was presented to, discussed by, and approved by the full Subcommittee at the November 2010 AAVLD meeting in Minneapolis, MN.