SUGGESTED BACTERIOLOGY/MYCOLOGY QUALITY ASSURANCE AND QUALITY CONTROL GUIDELINES FOR AAVLD-ACCREDITED VETERINARY DIAGNOSTIC LABS

I. Quality Assurance
    A. Definition
        1. Program to evaluate that lab procedures yield relevant and timely data to the user
    B. Involves quality assessment and improvement
        1. Quality is assessed by specifying performance parameters and setting limits for acceptable performance
        2. Quality improvement is achieved by anticipating potential problems and correcting them before they occur or by correcting problems as they occur and preventing their reocurrence
    C. Suggested general areas to address
        1. Proper specimen collection and transport
        2. Proper media and isolation techniques for the recovery of bacterial and fungal pathogens
        3. Error tracking
        4. Turnaround time
        5. Monitor and evaluate tests
        6. Conduct periodic surveys of users to assess how the lab is perceived

II. Quality Control
    A. Definition and purpose
        1. Program that ensures that the lab information is accurate, reliable and reproducible
        2. Goal is to eliminate or reduce to a minimum the extent of test variation within and between laboratories
    B. Elements of a quality control program
        1. Written procedure manual
            a. Explicit directions for all methods and test procedures used in the lab
            b. References and/or documentation for the validation of each procedure used in the lab is desirable
            c. Section on proper specimen collection and transport
            d. Review and update on a regular basis
            e. Copy in the work area for technicians
            f. Refer to reference 6 for format
        2. Record-keeping
            a. Documentation for the performance of personnel, media, reagents, stains, equipment, susceptibility disks and tests, test kits, and antisera
            b. Retain records for at least 2 years
        3. Personnel
            a. Provide written position description and performance standards
            b. Annual evaluation
            c. Provide continuing education and retain

documentation
   d. Encourage affiliation with and certification by professional organizations
   e. Require all employees to become familiar with procedure manual
   f. Provide orientation period for all new employees
   g. Maintain "library" of current reference texts and pertinent journals
   h. Tactfully mandate reporting the occurrence of any abnormal situation to the lab supervisor
4. Proficiency testing
   a. Measurement of technical capabilities of personnel and reliability of procedures
   b. Enrollment in a proficiency testing program that provides bacterial specimens via surveys is to be encouraged
   c. May provide internal "blind" unknowns to technicians for their evaluation
5. Laboratory safety
   a. Provide manual with documented safety precautions
   b. Compile material safety data sheets (MSDS) on all chemicals used in the lab and maintain in a file
   c. Require employees to review safety manual and MSDS documents periodically
   d. Store volatile and flammable materials properly
   e. Presence of fire extinguishers, fire blankets and eye wash stations in appropriate locations and require employees to be familiar with proper use
6. Equipment
   a. Document performance and maintenance of all equipment and maintain records for the life of the instrument
   b. Perform routine maintenance as specified by the manufacturer
   c. Monitor temperature-dependent activities on a daily basis using calibrated thermometers
      i. Suggested temperature tolerances
         -incubators +/- 2 C
         -waterbaths/heating blocks +/- 1 C
         -refrigerators 4-8 C
         -standard freezers +/- 5 C
         -ultra-low freezers +/- 10 C (as long as is below -60 C)
   d. Suggested QC schedule for instruments
      i. Anaerobic chamber
         each working day:check anaerobic indicators (methylene blue indicator solution/strips, reazurin strips); biological indicators (failure of an obligate anaerobe such as Clostridium 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
semiannually:check for leaks; check filters
annually or as necessary:follow manufacturer's guidelines regarding maintenance

ii. Anaerobe jar/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

iii. Autoclave
each working day: clean 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

iv. Automated antimicrobial susceptibility and identification instrument
-follow manufacturer's guidelines

v. Biological safety cabinet
daily or with each use: wipe all surfaces with disinfectant; check airflow
annually: perform inspection and certification

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

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

viii. Incubators
each working day: record temperatures; monitor CO_2 concentrations in capneic incubators; check gas cyclinder contents
weekly: check humidity, if applicable
annually: clean inside and outside; check tubing for leaks; check that unit is level

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

quarterly: verify temperature

xi. pH meter

each working day: check electrode solutions

with each use: calibrate with standards; rinse electrodes

quarterly: replace or recondition electrodes, if needed

xii. Pipetters/calibrated loops

daily or with each use: inspect for damage; calibrate disposable loops with each new lot

annually: calibrate pipetters

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

xv. Water bath

with each use: check temperature; check for leaks; check water level; check for contamination or deposits

monthly: drain and clean

semiannually: remove mineral deposits

7. Media

a. 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

b. 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

c. Commercial

i. Inspect each shipment for damage and quality

ii. Log in upon receipt with name of vendor and date

iii. Retain manufacturer's documentation indicating compliance with NCCLS QA protocols

iv. Document any deficiencies and corrective actions

v. Store according to manufacturer's directions

vi. Do not use past the expiration date

d. In-house

i. Evaluate sterility
- -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
- -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 ml loop)
- -if this method does not yield isolated colonies on the medium being tested, a 10-fold lighter inoculum may be used

iii. Evaluate selective characteristics
- -check ability to support growth of low numbers of elected organisms, as in ii above

iv. Evaluate inhibitory characteristics
- -check ability to inhibit growth of undesirable organisms by using a heavier inoculum
- -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 innoculum may be required to avoid overwhelming some types of media

v. Evaluate for biochemical response
- -test with a minimum of 2 control organisms to evaluate both positive and negative results
- -with slow growing organisms, it may be necessary to conduct QC simultaneously with actual cultures e.g. Mycobacterium paratuberculosis

vi. Tubed media
- -inoculate each tube with 10 ul of a 0.5 McFarland suspension of test organism in sterile saline

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

viii. Discard any medium that fails to meet performance criteria

ix. Refer to references 4, 5, 9 and 11 for suggested organisms to use in performance

testing
Label containers of dehydrated media with the date of first use and do not use past the expiration date

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

xii. Develop an expiration date system
-in general:20 wks. for heavy-filled plates (Sab-Dex), 12 wks. for media with aseptically-added ingredients, 4 weeks for phenylethyl alcohol agar plates, 3 weeks for motility test media and O-F media

xiii. Practice proper storage of media and additives -Store plates at 4-8C in plastic bags or in universal containers to prevent desiccation
-Screw cap tubed media or place in plastic bags to prevent desiccation and store at 4-8C
-Store hygroscopic media additives in a desiccator jar at the temperature recommended by the manufacturer
-Store antimicrobic powders desiccated at refrigerator temperatures
-Dispense antimicrobic stock solutions in small amounts at the time of their preparation, store at or below -14 C and once thawed, discard any unused portion

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

e. 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 testing for growth vs. inhibition by methods in headings II7dii or II7div, respectively.
iv. Incubate either at room temp. or 30C

8. Stains, reagents, antisera and kit systems
a. Label with:contents, concentration, date placed in use, expiration date and storage requirements
b. Do not use outdated products or those that fail performance tests
c. Evaluate bacterial metabolites with control organisms known to give both positive and negative results
d. Maintain written records of performance tests
e. Frequency of suggested quality control
i. Check each new vial/batch and every day test is performed :

-catalase
-oxidase
-direct antigen detection reagents (DNA probes,latex coagglutination)

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

iii. Check each new vial/batch and once/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 the reagent 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

9. Biochemical test systems
   a. Includes miniaturized commercial biochemical methods and automated methods for biochemical identification
   b. Store according to manufacturer's directions
   c. Do not interchange kit components between different lot numbers of kits
   d. Do not use outdated kits
10. Antimicrobial susceptibility testing
   a. Refer to reference 8 for recommendations
   b. Other recommendations will be forth-coming from from the AAVLD Subcommittee on Antimicrobial Susceptibilty Testing
   c. Maintain written records

Appendix I

Maintenance of Stock Cultures of Microorganisms

I. Handle pathogenic organisms with appropriate precautions and maintain as to minimize alterations in growth characteristics and contamination

II. Sources
   A. Obtain directly from the American Type Culture Collection (ATCC) or other reputable source

III. Methods for storage and maintenance of bacterial stock cultures

A. Lyophilization
    1. Method of choice
    2. Maintains preservation for many years
    3. Preserve in small, single use aliquots
B. Frozen cultures
    1. Acceptable method
        Make a dense suspension of bacterial growth from a solid medium in a cryoprotective medium
        a. sterile defibrinated blood
        b. soybean casein digest broth with 10-15% glycerol
        c. sterile skim milk
        d. on beads
    3. Dispense in small volumes into sterile cryovials
    4. Prepare enough stock for 1 year
    5. If quickly frozen at or below -45 C, organisms will survive for an extended period of time
    6. Subcultures used to make new batches of frozen stocks are acceptable, however, organisms need to be re-characterized
C. Working control cultures
    1. Up to 2 serial subcultures from primary working culture may be prepared to provide tertiary working controls
    2. May be kept on agar slants or plates of an appropriate medium and stored at 2-8C for up to 4 weeks (dependent upon viability of the organism)
    3. Replenish primary working cultures monthly from frozen, lyophilized or commercial cultures to minimize any genotypic or phenotypic activity
        a. When needed, a single vial of stock organism is either thawed or rehydrated and transferred to an appropriate growth medium
        b. Isolated colonies are used to initiate performance tests or to serve as primary working cultures
D. Anaerobic stock cultures
    1. Maintain working control cultures in chopped meat or other suitable broth medium
    2. Following overnight incubation, may store at room temperature
    3. Perform monthly transfers and viability checks before returning to stock culture status

IV. Methods of storage and maintenance of fungal stock cultures
A. Regular cultures are time-consuming and usually result in fungi becoming atypical and non-viable
B. Maintain the most frequently used organisms on slants of fungal media with monthly transfers
C. Maintenance of less frequently used organisms
    1. May store cultures on slants in screw-capped tubes at 4C for up to 6 months
    2. Subculture the more fastidious fungi more frequently than every 6 months e.g.the zygomycetes
D. Maintenance of permanent stock cultures
    1. Water cultures
        a. Growth from an actively-sporulating culture on

potato dextrose agar is placed into 5 ml of sterile water contained in a screw-capped tube
   b. Tubes are stored at room temperature and remain viable for years
   c. Additional sterile water is added as it evaporates
   d. To reactivate stocks, shake tube and inoculate a small amount of suspension onto appropriate fungal medium
2. Sterile mineral oil overlay
   a. Sterile mineral oil is added to a screw-capped tube containing an actively-sporulating fungal culture on potato dextrose agar
   b. Cap of tube is tightened and culture is stored in refrigerator until needed
   c. Cultures remain viable for up to 1 year
   d. 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
3. Freezing
   a. A slant culture of actively-sporulating organism growing on potato dextrose agar is placed at -70 C
   b. To reactivate, quickly remove from the freezer, aseptically tease away some growth and inoculate it onto a plate of appropriate fungal medium
   c. Stock culture slant shall be returned to the freezer before thawing occurs
   d. If stock culture thaws, a new slant shall be prepared

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