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Microbiology Categories

Former Standard and Guidance	*Proposed Standard and Guidance*
The following Microbiology standards are applicable to the subspecialty testing categories as follows: Bacteriology (MB S1-S11); Mycobacteriology (MB S1-S9); Mycology (MB S1-S11); Parasitology (MB S6, MB S8-S9); Virology (MB S1, MB S6, MB S8-S11).	**Deleted**
The following specialty sustaining standards of practice and applicable general system standards shall be incorporated into the laboratory's quality management system, where applicable to the scope of services provided. Effective May 1, 2011.	**Deleted**
Mycobacteriology Sustaining Standard of Practice 1 (TB S1): Biological Safety Cabinet (BSC) A class II or higher biological safety cabinet (BSC) shall be used when: a) processing specimens submitted for mycobacteriological testing, including slide preparation; b) handling or processing unsealed mycobacteriology cultures; c) performing any other procedures that have the potential to create aerosols. **Guidance –** Operational guidelines for biological safety cabinets can be found in the Safety Standards of the General Systems Standards Part 1.	**Microbiology Standard of Practice 1 (MB S1): Biological Safety Cabinet** A class II or higher biological safety cabinet (BSC) must be used when: a) processing specimens submitted for mycobacteriological testing, including slide preparation or handling unsealed mycobacteriology cultures; b) processing patient specimens submitted for isolation of pathogenic fungi or handling cultures of pathogenic fungi; c) inoculating cell cultures with clinical specimens and for all procedures involving the maintenance and processing of inoculated cell cultures and culture-

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Mycology Sustaining Standard of Practice 1 (MY S1): Biological Safety Cabinet (BSC) A class II or higher biological safety cabinet (BSC) shall be used whenever: a) processing patient specimens submitted for isolation of pathogenic fungi; b) handling or processing cultures of pathogenic fungi. **Guidance –** Additional required use of the BSC should be established by the laboratory director based on an infectious agent risk assessment (refer to Safety Standards).	amplified materials; or d) performing any other procedures that have the potential to create infectious aerosols. **Guidance –** Additional required use of the BSC should be established by the laboratory director based on an infectious agent risk assessment (refer to Laboratory Safety Standard of Practice 7 (LS S7): Biohazard Risk Assessment.
Mycobacteriology Sustaining Standard of Practice 2 (TB S2): Centrifugation Safety For all mycobacteriology procedures that use centrifuges: a) aerosol-free centrifuge cups shall be used; b) centrifuge cups shall be opened in a class II or higher BSC. **Mycology Sustaining Standard of Practice 2 (MY S2): Centrifugation** For all mycology procedures that use centrifuges: a) aerosol-free centrifuge cups shall be used; b) centrifuge cups shall be opened in a class II or higher BSC.	**Microbiology Standard of Practice 2 (MB S2): Centrifugation Safety** For all mycobacteriology, mycology and virology procedures involving centrifugation of potentially infectious materials: a) aerosol-free centrifuge cups must be used; and b) centrifuge cups must be opened in a class II or higher biological safety cabinet (BSC). **Guidance –** Proper safety practices are important for centrifugation of shell vial cultures.

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<tr><th>Former Standard and Guidance</th><th>Proposed Standard and Guidance</th></tr>
<tr><td>Microbiology Sustaining Standard of Practice 1 (MB S1): Quality Control Stock Cultures

The laboratory shall maintain stock cultures for all quality control procedures.

Guidance – Maintenance of stock cultures should be standardized in a manner that minimizes the opportunity for contamination or alteration of relevant characteristics. Stock cultures should consist of low-passage material rather than laboratory-adapted high passage material.

Validated patient isolates, proficiency testing specimens, or commercially prepared controls may be used unless otherwise required by manufacturer. American Type Culture Collection (ATCC) controls are not required, except for use in susceptibility testing.</td><td>Microbiology Standard of Practice 3 (MB S3): Quality Control Stock Cultures

The laboratory must have quality control procedures for all stocks cultures to minimize contamination or alteration of relevant characteristics.

Guidance –

Maintenance of stock cultures should be standardized in a manner that minimizes the opportunity for contamination or alteration of relevant characteristics. Stock cultures should consist of low-passage material rather than laboratory-adapted high passage material.

Validated patient isolates, proficiency testing specimens, or commercially prepared controls may be used unless otherwise required by manufacturer.</td></tr>
<tr><td>Microbiology Sustaining Standard of Practice 2 (MB S2): Commercial Medium

Each lot or shipment of commercially prepared media shall be tested:

a) on-site for growth, selectivity, and/or inhibition and biochemical responses; or,

b) by the manufacturer in accordance with established criteria. Quality control checks for sterility, growth, selectivity and/or inhibition and biochemical responses need not be retested by the user provided that:

i. for each shipment or lot of media, the laboratory has</td><td>Microbiology Standard of Practice 4 (MB S4): Microbial Growth Medium

Each lot or shipment of commercially prepared or in-house prepared media must be tested:

a) on-site for growth, selectivity, and/or inhibition and biochemical responses; or

b) by criteria established by the manufacturer or the laboratory in absence of manufacturer instructions. Quality control (QC) checks for sterility, growth, selectivity and/or inhibition and biochemical responses need not be retested by the laboratory provided that:</td></tr>
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documentation on the media label, package insert, technical manual, or other document, that the manufacturer's quality control practices conform to specifications; and, ii. the laboratory documents receipt and condition of each shipment or lot of media, and notifies the media manufacturer of: - cracked Petri dishes; - unequal filling of plates; - cracked media in plates; - hemolysis; - freezing; - excessive number of bubbles; or - contamination. **Guidance –** Media may be tested concurrent with initial use provided results are reviewed prior to release of patient results.	i. for each shipment or lot of media, the laboratory has documentation on the media label, package insert, technical manual, or other document, that the manufacturer's or in-house QC practices conform to specifications; and ii. the laboratory documents receipt and condition of each shipment or lot of media, and notifies the media manufacturer or in-house preparer of: - cracked Petri dishes; - unequal filling of plates; - cracked media in plates; - hemolysis; - freezing; - excessive number of bubbles; or - contamination. **Guidance –** Media may be tested concurrent with initial use provided QC results are reviewed prior to release of patient results.

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Microbiology Sustaining Standard of Practice 4 (MB S4): Expiration Date Prepared In-House The expiration date for each batch of in-house prepared microbiological media shall not exceed 8 weeks from the preparation date for plated and non-screw cap tubed media and six months from the preparation date for screw cap tubed media provided the laboratory: a) has taken into account the inclusion of labile components such as antibiotics; b) stores the media under required conditions (e.g. temperature, shielded from light, proper humidity); and, c) has previously tested at least one batch of each medium type and shown it to perform as expected at the end of the designated shelf life. **Guidance –** This testing should be repeated when changing vendors.	**Microbiology Standard of Practice 5 (MB S5): Expiration Date Prepared In-House** The expiration date for each batch of in-house prepared microbiological media must not exceed eight (8) weeks from the preparation date for plated and non-screw cap tubed media and six (6) months from the preparation date for screw cap tubed media
Microbiology Sustaining Standard of Practice 9 (MB S9): Reports Reports shall include: a) the test methodology; b) an interpretation, when necessary, to explain the significance of the test result; c) a qualifier identifying an assay limitation, if appropriate; and,	**Microbiology Standard of Practice 6 (MB S6): Reports** In addition to the requirements in Reporting Standard of Practice 2, test reports must include: a) the test method; b) qualifiers for viral cultures that are incomplete or uninterpretable or when isolate identification is considered presumptive, i.e., an isolate is not confirmed by a specific viral identification system; and

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<tr><td>d) a recommendation for follow-up testing, if appropriate.

Guidance – These requirements are in addition to those required by Reporting Sustaining Standard 1 (Reporting S1).

a) Examples of assay methodology include culture, EIA, PCR, etc. Specific test systems are not required to be listed on a test report.
b) Report qualifiers are used to convey information that would affect the significance and/or clinical interpretation of the test result.
c,d) Rapid antigen tests for influenza virus are particularly vulnerable to assay sensitivity issues because of antigenic variations among circulating influenza sub-types/strains and/or the emergence of novel sub-types/strains. The laboratory should be alert to these performance issues and include qualifiers and recommendations for follow-up testing as appropriate.</td><td>c) a recommendation for follow-up testing, if appropriate.

Guidance –

a) Examples of assay methodology include culture, EIA, PCR, etc. Specific test systems are not required to be listed on a test report.</td></tr>
<tr><td>Microbiology Sustaining Standard of Practice 10 (MB S10): Laboratory Response Network (LRN)

The laboratory shall have a section in the SOPM describing policies and practices related to their activities as a Laboratory Response Network (LRN) sentinel (formerly level A) laboratory, if applicable, including:

a) maintaining updated LRN guidelines and protocols related to the testing, identification and reporting of select and emergent agents including information regarding special handling and safety practices to be employed;</td><td>Microbiology Standard of Practice 7 (MB S7): Laboratory Response Network

In addition to the requirements for Test Procedure Content Standard of Practice 1, the laboratory must have a section in the standard operating procedure describing policies and practices related to their activities as a Laboratory Response Network (LRN) sentinel laboratory, if applicable, including:

a) maintaining updated guidelines and protocols related to the testing, identification and reporting of select and emerging infectious agents including information</td></tr>
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b) providing staff with information regarding the biosafety level(s) (BSL) recommended for the microbiological testing being performed and identifying the highest BSL available for each category of microbiological testing;
c) identifying the LRN reference laboratory for their facility and contact information for individual(s) to be contacted if a select agent is suspected; and,
d) distribution of information to health care providers regarding specimen collection and submission instructions that should be followed when infection with a select agent or other infectious agent requiring special handling is suspected.

Guidance – Laboratories holding a clinical laboratory permit in either Bacteriology –Comprehensive or Virology are currently considered LRN sentinel (formerly Level A) laboratories, unless designated as a LRN reference laboratory.

Information regarding laboratory testing for critical and emergent agents is available to all laboratories on the CDC website. LRN sentinel laboratories holding NYS clinical laboratory permits are advised to regularly access the NYSDOH Health Commerce System (HCS) for updated information related to testing, identification and reporting of these agents. Information regarding NYS HCS accounts can be obtained at 1-(866) 529-1890, option 1. Laboratories serving NYC should also access the NYC Department of Health and Mental Hygiene’s Health Alert Network (HAN); for information, contact 1-888-NYCMED-9 or nycmed@health.nyc.gov.

The Wadsworth Center may define the levels of testing (e.g.
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regarding special handling and safety practices to be employed;
b) providing staff with information regarding the biosafety level(s) (BSL) recommended for the microbiological testing being performed and identifying the highest BSL available for each category of microbiological testing;
c) identifying the LRN reference laboratory for their facility and contact information for individual(s) to be contacted if a select agent is suspected; and
d) distribution of information to health care providers regarding specimen collection and submission instructions that should be followed when infection with a select agent or other infectious agent requiring special handling is suspected.

Guidance –

Laboratories holding a New York State clinical laboratory permit in either Bacteriology or Virology are currently considered LRN sentinel laboratories, unless designated as an LRN reference laboratory.

Information regarding laboratory testing for select and emerging infectious agents is available to all laboratories on the American Society of Microbiology website.

The Wadsworth Center may define the levels of testing (e.g., rule out only) and identification (e.g., presumptive only) and the reporting pathway for a particular agent. The Wadsworth Center’s LRN distributes this information as needed to sentinel laboratories by e-mail or e-fax to the laboratory director and
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rule out only) and identification (e.g. presumptive only) and the reporting pathway for a particular agent. The Wadsworth Center's LRN distributes this information as needed to sentinel laboratories by fax and/or electronic copy to the laboratory director and posts these announcements on the HCS. NYS and NYC LRN reference laboratory contacts and other LRN information is available on the NYSDOH LRN website which is accessed through the HCS. The Wadsworth Center LRN program staff can be contacted at 26TLRNexec@health.state.ny.us26T. Biosafety levels and associated recommendations and practices are described in the CDC publication "*Biosafety in Microbiological and Biomedical Laboratories*" (BMBL) and on the CDC website at 26Twww.cdc.gov26T. Laboratories must comply with infectious disease reporting requirements as outlined in the Public Health Sustaining Standard of Practice 1 (Public Health S1): Reporting. Laboratories must comply with pertinent items of USA Patriot Act and the Select Agent Rule (e.g. disposal/transfer of select rule—see Microbiology Sustaining Standard 11 (MB S11)).	posts these announcements on the HCS. New York State and New York City LRN reference laboratory contacts and other LRN information is available on the Wadsworth Center LRN website. The Wadsworth Center LRN program staff can be contacted at: LRNexec@health.state.ny.us. Biosafety levels and associated recommendations and practices are described in the CDC publication "*Biosafety in Microbiological and Biomedical Laboratories*" (BMBL) and on the CDC website at: https://www.cdc.gov/labs/BMBL.html. Laboratories must comply with infectious disease reporting requirements as outlined in the Public Health Reporting Standards of Practice 1 and 2.
Microbiology Sustaining Standard of Practice 11 (MB S11): Inventory and Track of Select Agents The laboratory shall establish and implement an inventory and tracking system that ensures that all samples and their derivatives suspected or confirmed to contain select agents are accounted for until laboratory findings establish the absence of a select agent. If a select agent is confirmed then	**Microbiology Standard of Practice 8 (MB S8): Select Agent Inventory** The laboratory must ensure that all samples and their derivatives suspected or confirmed to contain select agents are accounted for until laboratory findings establish the absence of a select agent. If a select agent is confirmed, documentation of

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<tr><td>documentation of its transfer including record of appropriate packing and shipping or destruction within seven days must be completed.

Guidance – A list of select agents (Biological Diseases/Agents List) can be found at the federal Centers for Disease Control and Prevention website at http://www.selectagents.gov.

Inventory and tracking documentation shall include the identity of all individuals accessing such materials, as well as completion of APHIS/CDC forms 3 (Report of Theft, Loss or Release of Select Agents or Toxins) and 4 (Report of Identification of a Select Agent or Toxin) for organisms and toxins isolated from clinical specimens. This tracking system includes select agents used as control material and for those specimens that are suspected to be positive for a select agent. Tracking will begin at the time it is suspected that a specimen contains a select agent.</td><td>its transfer including record of appropriate packing and shipping or destruction within seven days must be completed.

Guidance –

A list of select agents (Biological Diseases/Agents List) can be found at the federal Centers for Disease Control and Prevention website at: http://www.selectagents.gov.

Laboratories must comply with pertinent items of the Select Agent Rule (e.g., disposal/transfer of select agents)

Inventory and tracking documentation should include the identity of all individuals accessing such materials, as well as completion of APHIS/CDC forms 3 (Report of Theft, Loss or Release of Select Agents or Toxins) and 4 (Report of Identification of a Select Agent or Toxin) for organisms and toxins isolated from clinical specimen. Additional information is available at: http://www.selectagents.gov.</td></tr>
<tr><td>Microbiology Sustaining Standard of Practice 3 (MB S3): Media Prepared In-House

A sample of each batch of microbiological media prepared in-house shall be tested, prior to or concurrent with initial use, for sterility, ability to support growth, selectivity and/or inhibition, and biochemical responses.

Guidance – Media may be tested concurrent with initial use provided results are reviewed prior to release of patient results.</td><td>Standard deleted

Required under Reagents and Media Standard of Practice 2 (RGM S2): Verification of Reagents and Media – Control Procedures</td></tr>
<tr><td>Microbiology Sustaining Standard of Practice 5 (MB S5): Media for Satellite Locations</td><td>Standard deleted</td></tr>
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Laboratories that supply media to satellite locations are responsible for either sending or maintaining quality control documentation and for notification of each satellite location of a recall. **Guidance –** In compliance with Reagents Sustaining Standard 4 (REAG S4), satellite laboratories are responsible for maintaining inventory control documentation which includes the name of the central laboratory provider.	
Microbiology Sustaining Standard of Practice 6 (MB S6): Specimen Criteria The SOPM shall define specimen types acceptable for each assay and shall include collection, storage and transport criteria, and rejection criteria.	**Standard deleted** **Required under Test Procedure Content Standard of Practice 1 (TPC S1): Test Procedure Content**
Microbiology Sustaining Standard of Practice 7 (MB S7): Automated Identification Systems The laboratory shall check each new lot number or shipment received of reagents with positive and negative reactivity quality control organisms for automated identification systems (QC). A streamlined quality control may be instituted when using microbial automated identification systems (MIS) provided that they follow a written protocol that ensures that they: a) performed a verification study; b) maintain documentation that the manufacturer has performed adequate QC to ensure that the system	**Standard deleted** **Required under Reagents and Media Standard of Practice 2 (RGM S2): Verification of Reagents and Media – Control Procedures**

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performs appropriately; c) maintain documentation that states that the distributor has followed all the manufacturer's requirements for shipping and storage; d) store and maintain the system according to the manufacturer's requirements; e) perform streamlined QC as directed by the manufacturer that integrates the manufacturer's risk mitigation information ; f) maintain records of all QC performed; g) remediate all QC failures and repeat QC in triplicate before resuming patient testing; and h) notify the manufacturer and distributor of the unresolved QC failure. **Guidance –** The streamlined quality control protocol is subject to the requirements of Quality Control Sustaining Standard of Practice (QC Design S1): Design of Individualized Quality Control Plan. Automated systems used exclusively to screen for bacterial contamination of blood components must also follow this standard. Non-automated screening tests for bacterial contamination of blood components, such as pH or glucose, are covered under the permit category of Blood Services – Transfusion or Blood Services – Collection. Identification of the organism requires a Bacteriology –Comprehensive permit.	

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a) A review of historical data may be used for the verification study. Refer to Reagents Sustaining Standard of Practice 2 (REAG S2): Verification- General Requirement and Reagents Sustaining Standard of Practice 3 (REAG S3): Verification of Reagents and Media for quality control requirements for all other reagents.	
Microbiology Sustaining Standard of Practice 8 (MB S8): Laboratory Worksheets Laboratory records shall include worksheets and/or electronic records that include all tests and test results that led to the identification of microorganisms. **Guidance –** Worksheets and/or electronic records should include identification of the media or host systems used and the corresponding observations for each medium as well as biochemical test reactions where appropriate.	**Standard deleted** **Required under Document and Specimen Retention Standard of Practice 8 (DSR S8): Analytic System Records Retention**

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Unless otherwise stated these standards apply to FDA-approved assays, modified FDA-approved assays and laboratory-developed nucleic acid amplification assays and sequencing assays used for the detection/identification/characterization of infectious agents. Laboratories may not report results obtained using laboratory-developed assays or modified FDA-approved assays without prior approval of the assay protocol by the Clinical Laboratory Evaluation Program as per Validation Sustaining Standard of Practice 5 (Validation S5). Application and submission guidelines are available at http://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval. These standards apply to all microbial agents including HIV and supplement the general systems standards that pertain to molecular test methods.	**Deleted**
The following specialty sustaining standards of practices shall be incorporated into the laboratory's quality management system, where applicable to the scope of services provided. Effective May 1, 2011.	**Deleted**
Microbiology Nucleic Acid Amplification Assays Sustaining Standard of Practice 2 (MNA S2): Prevention and Remediation of Nucleic Acid Contamination The SOPM shall include a description of practices and procedures intended to prevent nucleic acid contamination including: a) a workflow pattern that utilizes separate areas and moves	**Standard move to General Systems Standards** **Required under Test Procedure Content Standard of Practice 2 (TPC S2): Test Procedures for Unidirectional Workflow**

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unidirectionally from pre- to post-amplification processes; b) dedicated pre-amplification equipment, reagents, supplies, and PPE that have been neither stored nor used in post-amplification areas or other areas that may result in exposure to amplicon, plasmids, and culture-amplified materials; c) the handling, processing and storing of clinical specimens and pre-amplification reagents and supplies (e.g. extraction reagents, mastermix, probes) in a manner that prevents exposure to amplicon; d) a decontamination and remediation plan to be implemented in the event that amplicon contamination is identified. **Guidance –** Item a of this standard does not apply to FDA approved Closed System Amplification Tests (CSATs). Pre-amplification activities include the storage, processing and extraction of clinical specimens and preparation of assay reagents. Post-amplification activities include those processes that occur after molecular amplification has been performed and result in an exponential increase in the amount of nucleic acid product (amplicon). a) The unidirectional workflow pattern is intended to ensure that pre-amplification procedures are performed in a work area that excludes amplification products (amplicon). The high level of concern is based on the significant risk of generating false-positive test results due to amplicon contamination of patient specimens and/or pre-amplification	

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supplies and reagents. Failure to adhere to the established unidirectional workflow pattern requires implementation and documentation of additional measures for monitoring and preventing amplicon contamination. These measures may include the use of UNG in PCR assays, use of amplicon contamination monitoring programs such as swipe testing of molecular areas, and the use of decontamination products designed to eliminate nucleic acid contaminants. a) The practices and space designation policies should be tailored to the laboratory's test menu and design. Ideally, a laboratory should have 3 separate rooms for performing nucleic acid amplification assays: a pre-amplification reagent preparation room; a room used for specimen preparation/nucleic acid extraction and for template addition; and, a room dedicated to post-amplification processes. An alternative arrangement may be developed within a room where reagent preparation (e.g. mastermix set-up and template addition) are performed in distinct areas provided that **strictly dedicated and delineated areas, PCR workstations, supplies, reagents, etc.** are utilized for separating the two pre-amplification phases of work. However, it remains a high priority that post-amplification procedures be performed in a separate room. a) It is suggested that negative controls in addition to those required when performing FDA approved assays be included when "open amplicon" systems are utilized in a laboratory that does not have at least two separate <u>rooms</u> for pre- and post-amplification activities.	

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a) Individuals performing CSATs may return to pre-amplification areas since the closed systems do not release amplicon into the environment provided that assay and discard procedures are followed. b) This refers to all equipment, furniture, instruments, supplies, reagents and PPE, including, but not limited to, pipets, pipettors, bulbs, tips, pens, discard containers, and clerical and cleaning supplies. PPE includes all laboratory coats/smocks, booties, hair bonnets, gloves, safety glasses and other individually-worn barriers. Worksheets and manuals that have been in post-amplification areas must not be brought into pre-amplification areas. b) Plugged (aerosol barrier) tips or positive displacement pipets are recommended for pre-amplification procedures. c) Ideally, a room under positive pressure relative to the post-amplification room should be used for preparation of mastermix and other "clean" reagents. d) The remediation plan should: define the decontamination procedure(s) to be employed; include root cause investigation, corrective action, competency assessment with retraining if necessary, and evidence supporting the adequacy of the remediation/decontamination procedures (e.g. environmental monitoring, increasing the number of negative controls per run).	
Microbiology Nucleic Acid Amplification Assays Sustaining Standard of Practice 3 (MNA S3): Instrumentation	**Standard move to General Systems Standards** **Required under Laboratory Equipment and Instruments Standard of Practice 9 (LEI S9): Thermal Cyclers and**

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<tr><td>The laboratory shall:
a) operate instruments and run internal performance checks according to the manufacturer’s instructions and/or the laboratory’s validated procedures; and,
b) verify the uniformity of temperature across all sample chambers at inception, annually, and after servicing.
Guidance –
a) Instruments include all instruments used for nucleic acid testing such as thermal cyclers, real time PCR instruments, optical instruments, heat blocks, automated extraction systems, and sequencing instrumentation.
b) Documentation of manufacturer verification is acceptable. Verification should include monitoring of temperature ramping rates where applicable. This may be met by using a verified low positive control in every well or an electronic check for temperature homogeneity.
b) Cross platform verification can be performed by monitoring positive controls utilized in each instrument run.</td><td>Polymerase Chain Reaction</td></tr>
<tr><td>Microbiology Nucleic Acid Amplification Sustaining Standard of Practice 4 (MNA S4): Reagent Storage
Probes, primers and other labile reagents used in nucleic acid amplification assays shall be stored and maintained in accordance with manufacturer’s instructions. In the absence of these, the laboratory’s own validation data shall be used to establish acceptable storage and maintenance parameters.</td><td>Standard move to General Systems Standards
Required under Reagents and Media Standard of Practice 5 (RGM S5): Reagent and Media Storage</td></tr>
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Former Standard and Guidance	***Proposed Standard and Guidance***
Guidance – Probes, primers and mastermix should be stored in small aliquots to minimize the number of freeze-thaw cycles. An acceptable number of freeze-thaw cycles may be stated by the manufacturer or established by monitoring control results that are appropriate (e.g. low level analyte) for identifying reagent deterioration. Bulk mastermix storage and preparation criteria should be validated by the laboratory to ensure the integrity of the reagent over the designated shelf life interval. Expiration dates for these reagents shall be based on laboratory validation studies where appropriate.	
Microbiology Nucleic Acid Amplification Assay Sustaining Standard of Practice 5 (MNA S5): Quality Control Samples for Laboratory Developed and Modified FDA-approved MNAAs Each assay protocol for all laboratory developed MNAA assays and modified FDA approved assays shall define the acceptable detection range for all controls and each run shall include at least: a) one control capable of detecting amplification inhibition by patient specimens unless the CLRS-approved application/method exempts the requirement; b) for qualitative assays, a negative control and a low range positive control that assess the entire assay, including specimen preparation/extraction (except for sequence based assays); c) for quantitative assays, a negative control and at least 2	**Microbiology Nucleic Acid Amplification Assay Standard of Practice 1 (MNA S1): Quality Control Samples for Laboratory Developed Tests** Each assay protocol for all laboratory developed tests (LDTs) for MNAA assays and modified FDA approved assays must meet the requirements in Quality Control Standard of Practice 2, 3 and 4 to define the acceptable detection range for all controls and each run must include at least: a) one (1) control capable of detecting amplification inhibition by patient specimens unless the LDT approved by the Department exempts the requirement; b) for qualitative single target assays, a negative control and a low range positive control that assess the entire assay, including specimen preparation/extraction (except for sequence based assays); c) for qualitative multi-target assays at least one (1)

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positive controls that assess a reasonable portion of the linear range of the assay including specimen preparation/extraction; and, d) additional negative controls in laboratories that manipulate amplicon but do not use separate rooms for pre- and post-amplification processes. **Guidance –** This standard applies to controls to be used with laboratory developed assays and FDA-approved assays that have been modified by the laboratory. Controls for unmodified FDA-approved assays should minimally include those recommended by the manufacturer and those that meet the requirements of other applicable standards. Negative controls including template-free mastermix controls not only serve to identify technical and/or reagent issues but also help identify amplicon contamination. The negative controls may include a reagent processing control that serves as both a template-free mastermix reagent control as well as a processing/extraction negative control. For laboratories preparing mastermix to be used on multiple instruments, the template-free mastermix control should be utilized for each run of each instrument. a) Inhibition controls may be excluded if there are sufficient data showing that the inhibition rate is less than 1% for a specimen type for the assay. It is possible to extend inhibition data to other analytes when applying the same extraction procedure and specimen matrix and utilizing the same amplification methodology.	specific target positive control; d) for quantitative assays, a negative control and at least two (2) positive controls that assess the linear range of the assay including one (1) control within two (2) logs of the lower limit of quantitation (LLOQ) and one (1) control in the upper half of the linear range including specimen preparation/extraction; and e) additional negative controls in laboratories that manipulate amplicon but do not use separate rooms for pre- and post-amplification processes. **Guidance –** This standard applies to controls to be used with laboratory developed tests (LDTs). Information on Departmental approval of LDTs is available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval. Negative controls including template-free mastermix controls not only serve to identify technical and/or reagent issues but also help identify amplicon contamination. The negative controls may include a reagent processing control that serves as both a template-free mastermix reagent control as well as a processing/extraction negative control. For laboratories preparing mastermix to be used on multiple instruments, the template-free mastermix control should be utilized for each run of each instrument. b) Inhibition controls may be excluded if there are sufficient

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Microbiology Nucleic Acid (MNA) Amplification Assay

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a) Inhibition controls are not required if the run includes isolates only and not patient specimens. b,c) A low-range positive is defined as having a value of not more than 10-fold above the assay detection limit. b,c) For multiplex assays, a low range control is required for each target. These may be run on a rotating basis and may include pools of 3-4 targets. d) Processes that involve manipulation of amplicon include conventional PCR and nucleic acid sequencing. d) It is recommended that the number of negative controls equal at least 2% of the patient specimen test number and that these are interspersed randomly throughout patient specimens (e.g. 2 per 96-well plate). Note: Refer to the Application for Approval of Infectious Agent Nucleic Acid Amplification Tests (http://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval/submission-checklists) for additional guidance related to assay control ranges or exemptions from use of inhibition controls.	data showing that the inhibition rate is less than one (1) percent for a specimen type for the assay. It is possible to extend inhibition data to other analytes when applying the same extraction procedure and specimen matrix and utilizing the same amplification methodology. b) Inhibition controls are not required if the run includes isolates only and not patient specimens. b, c) A low-range positive is defined as having a value of not more than ten (10) fold above the assay detection limit. b, c) For multiplex assays, a low range control is required for each target. These may be run on a rotating basis and may include pools of three (3) to four (4) targets. d) Processes that involve manipulation of amplicon include conventional PCR and nucleic acid sequencing. d) It is recommended that the number of negative controls equal at least two (2) percent of the patient specimen test number and that these are interspersed randomly throughout patient specimens (e.g. two (2) per 96-well plate).

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Microbiology Nucleic Acid (MNA) Amplification Assay

Former Standard and Guidance	*Proposed Standard and Guidance*
Microbiology Nucleic Acid Amplification Assay Sustaining Standard of Practice 6 (MNA S6): Quality Control Samples for Sequencing Assays Each sequencing assay shall include a a) negative amplification control; b) negative sequencing control; c) positive sequencing control; and d) positive amplification and inhibition controls when testing primary specimens using laboratory developed assays or modified FDA approved assays. **Guidance –** Laboratories using a core facility do not need to provide negative and positive sequencing amplification controls if the assay performed by the core facility includes negative and positive sequencing controls. a,b) The negative amplification control may also be used as the negative sequencing control. a) Purified plasmid that is supplied with a commercially available kit may be used as a positive sequencing control. Previously tested and well characterized PCR product of the target from clinical samples can also be used as sequencing controls. d) Positive amplification controls and inhibition controls are not necessary when performing sequencing on clinical isolates. d) Positive amplification and inhibition controls may be omitted	**Microbiology Nucleic Acid Amplification Assay Standard of Practice 2 (MNA S2): Quality Control Samples for Sequencing Assays** Each sequencing assay must include a: a) negative amplification control; b) negative sequencing control; c) positive sequencing control; and d) positive amplification and inhibition controls when testing primary specimens for the detection or identification of an infectious agent, unless an individualized quality control plan (IQCP), performed according to Quality Control Standards of Practice 2, 3 and 4 is approved by the Department as a laboratory developed test (LDT). **Guidance –** Information on Departmental approval of LDTs is available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval. Laboratories using a core facility do not need to provide negative and positive sequencing amplification controls if the assay performed by the core facility includes negative and positive sequencing controls.

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<tr><th colspan="2">Microbiology</th></tr>
<tr><th colspan="2">Microbiology Nucleic Acid (MNA) Amplification Assay</th></tr>
<tr><th>Former Standard and Guidance</th><th>Proposed Standard and Guidance</th></tr>
<tr><td>when testing primary specimens for genotyping assays for prognostic purposes. If there is no amplification product or genotype resulting from a primary specimen, the report must document that the result may be due to the following: virus was below the limit of detection in the sample, mutations were present in the virus genome, or inhibitors were present in the sample that prevented amplification.

Note: Refer to the Application for Approval of Infectious Agent Nucleic Acid Amplification Tests (http://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval/submission-checklists) for further information.</td><td>a, b)
The negative amplification control may also be used as the negative sequencing control.

b) Purified plasmid that is supplied with a commercially available kit may be used as a positive sequencing control. Previously tested and well characterized PCR product of the target from clinical samples can also be used as sequencing controls.

d) Positive amplification controls and inhibition controls are not necessary when performing sequencing on clinical isolates.

d) Positive amplification and inhibition controls may be omitted when testing primary specimens for genotyping assays for prognostic purposes.</td></tr>
<tr><td>Microbiology Nucleic Acid Amplification Assay Sustaining Standard of Practice 7 (MNA S7): Reports for Laboratory Developed Sequence-based Assays

Reports shall describe the relationship between the observed result and the predicted phenotype.

Guidance – This standard does not apply to sequence-based assays for identification.

For unmodified FDA -approved assays reporting should be consistent with the manufacturer’s instructions.

Reports containing test results generated using sequencing, probe-based, and other genotype assays should include information stating the relationship between the observed</td><td>Microbiology Nucleic Acid Amplification Assay Standard of Practice 3 (MNA S3): Reports for Laboratory Developed Sequence-based Assays

In addition to the requirements in Reporting Standard of Practice 2, reports must include disclaimers on all viral based sequencing tests from primary specimens that have no amplification or genotype results.

Guidance –

The disclaimer on the report is needed to document that the result may be due to the following: virus was below the limit of detection in the sample, mutations were present in the virus</td></tr>
</table>

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Microbiology Nucleic Acid (MNA) Amplification Assay

Former Standard and Guidance	*Proposed Standard and Guidance*
result(s) and the related characteristic such as prediction of drug resistance or virulence. Mutations should not be reported as indicative of drug resistance or virulence unless there is well-supported documentation in peer-reviewed literature. Refer to the Application for Approval of Infectious Agent Nucleic Acid Amplification Tests (http://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval/submission-checklists) for additional requirements.	genome, or inhibitors were present in the sample that prevented amplification.
Microbiology Nucleic Acid Amplification Assay Sustaining Standard of Practice 8 (MNA S8): Task Separation for FDA-Approved Closed System Amplification Test (CSATS) The laboratory shall: a) Handle, process, and store clinical specimens, reagents and supplies in a manner that prevents exposure to amplicon, plasmids, and culture-amplified materials; and b) Locate the instrument in an area free of open amplicon systems. **Guidance –** A CSAT refers to an assay in which all steps, including post-amplification steps, are performed and contained within a closed system. A closed system is defined as an instrument in which the patient specimen is directly added to the test unit, device, or cartridge, sealed, and then the testing	**Microbiology Nucleic Acid Amplification Assay Standard of Practice 4 (MNA S4): Task Separation for FDA-Approved Closed System Amplification Test** For a closed system amplification test (CSAT), the laboratory must: a) handle, process, and store clinical specimens, reagents and supplies in a manner that prevents exposure to amplicon, plasmids, and culture-amplified materials; and b) locate the instrument in an area free of open amplicon systems. **Guidance –** A CSAT refers to an assay in which all steps, including post-amplification steps, are performed and contained within a closed system. A closed system is defined as an instrument in which the patient specimen is directly added to the test unit,

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<tr><th colspan="2">Microbiology</th></tr>
<tr><th colspan="2">Microbiology Nucleic Acid (MNA) Amplification Assay</th></tr>
<tr><th>Former Standard and Guidance</th><th>Proposed Standard and Guidance</th></tr>
<tr><td>process is initiated with no additional external manipulation or addition of reagents (either manually or robotically).

CSAT instrumentation should be segregated from areas in which specimens are routinely processed in order to avoid cross-contamination.

An individual performing CSAT may return to pre-amplification areas since the closed systems do not release amplicon into the environment provided that assay and discard procedures are followed.</td><td>device, or cartridge, and then the testing process is initiated with no additional external manipulation or addition of reagents unless approval is received by the Department.

CSAT instrumentation should be segregated from areas in which specimens are routinely processed in order to avoid cross-contamination.

An individual performing CSAT may return to pre-amplification areas since the closed systems do not release amplicon into the environment provided that assay and discard procedures are followed.</td></tr>
<tr><td>Microbiology Nucleic Acid Amplification Assays Sustaining Standard of Practice 1 (MNA S1): Employee Training and Competency

The laboratory’s training and competency program shall:

a) include practices and procedures that must be implemented in order to reduce the likelihood of cross contamination and other technical errors;

b) include direct observation adequate to confirm technical competence in all aspects of each molecular technique;

c) require successful testing of a blinded assessment panel that includes samples representative of those expected in the laboratory;

d) include documentation that items b and c have been satisfied prior to approving an individual to perform independent testing; and</td><td>Standard deleted

Required under Human Resources Sustaining Standard of Practice 6 (HR S6): Training for Testing and Non-technical Personnel and

Human Resources Standard of Practice 8 (HR S8): Competency Assessment – Testing Personnel</td></tr>
</table>

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Microbiology Nucleic Acid (MNA) Amplification Assay	
Former Standard and Guidance	***Proposed Standard and Guidance***
e) include documentation of annual competency assessments for each individual performing molecular assays. **Guidance –** This standard includes requirements in addition to those stated in Human Resources Sustaining Standard 6 (HR S6) and Human Resources Sustaining Standard 8 (HR S8). b,e) A single representative assay may be used to assess competency when using the same methodology, including specimen preparation/extraction, for more than one agent. d,e) Documentation of training should include a direct observation of operator adherence to molecular workflow. Assessment panels may include proficiency test samples or other characterized materials. Sample ranges should be representative of the entire assay range where appropriate however it certain instances it is impractical to obtain high concentrations. A panel should include representative samples with at least one sample at or near the limit of detection. See also Proficiency Test Sustaining Standards and Quality Assessment Sustaining Standard 3 (QA S3).	
Microbiology Nucleic Acid Amplification Assay Sustaining Standard of Practice 9 (MNA S9): Controls for FDA-Approved Closed System Amplification Tests (CSATs) Positive target controls and negative controls that assess the entire assay shall be run on each new lot number or shipment prior to reporting patient results and on each day of use for FDA-Cleared Closed System Amplification tests (CSATs)	**Standard deleted** **Required under Quality Control Standard of Practice 1 (QC S1): Minimum Quality Control Requirements**

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unless the laboratory has validated a reduced quality control schedule that minimally includes a: a) positive target control run at least monthly; b) negative control run at least weekly. **Guidance –** The validation studies should demonstrate acceptable quality control results at the interval corresponding to the laboratory's quality control schedule. The laboratory is not required to run quality control every day in the time period to verify reagent stability.	

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Former Standard and Guidance	***Proposed Standard and Guidance***
The following specialty sustaining standards of practices shall be incorporated into the laboratory's quality management system, where applicable to the scope of services provided. The laboratory must also follow the Microbiology and Molecular Microbiology Standards. Effective May 1, 2011; BT S9 and BT S11 revised and effective July 1, 2014.	Laboratories that perform testing for *Chlamydia* must follow applicable Virology Culture standards.
Bacteriology Standard of Practice 1 (BT S1): Reagent QC The laboratory shall check positive and negative reactivity with control organisms as follows: a) each day of use for beta-lactamase and all stains other than Gram stain; b) each week of use for Gram stain; c) every 6 months for antisera. **Guidance –** c) Polyvalent antisera should be tested with at least one organism from each polyvalent group.	**Bacteriology Standard of Practice 1 (BT S1): Reagent Quality Control** Unless an Individualized Quality Control Plan (IQCP) is established according to Quality Control Standards of Practice S2, S3 and S4, the laboratory must check positive and negative reactivity with control organisms as follows: a) each day of use for beta-lactamase and all stains other than Gram stain; b) each week of use for Gram stain; c) every six (6) months for antisera **Guidance –** c) Polyvalent antisera should be tested with at least one (1) organism from each polyvalent group.
Bacteriology Standard of Practice 2 (BT S2): Urine Loops Non-disposable urine loops shall be calibrated monthly. **Guidance –** Calibration may be performed using a blue-dye	**Bacteriology Standard of Practice 2 (BT S2): Urine Culture Inoculating Loops** Verification of calibration of non-disposable urine culture

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methodology or by using a calibrated drill bit.	inoculating loops must be performed monthly or done as specified in manufacturer instructions. **Guidance –** Verification of calibration may be performed using a blue-dye methodology or by using a calibrated drill bit. Verification of non-disposable loops used in automated instruments can be performed during manufacturer provided preventative maintenance.
Bacteriology Standard of Practice 3 (BT S3): Anaerobic Containers The environmental conditions of anaerobic bags, jars, and glove boxes shall be monitored and documented each day of use. **Guidance –** An oxygen sensitive indicator such as methylene blue, resazurine, or a control culture of *Clostridium novyi B* should be placed in anaerobic jars or chambers to ensure anaerobic conditions are met.	**Bacteriology Standard of Practice 3 (BT S3): Anaerobic Containers** The environmental conditions of anaerobic bags, jars, and glove boxes must be monitored and documented each day of use. **Guidance –** An oxygen sensitive indicator such as methylene blue, resazurine, or a control culture of *Clostridium novyi B* should be placed in anaerobic jars or chambers to ensure anaerobic conditions are met.
Bacteriology Standard of Practice 4 (BT S4): Aerobic Blood Cultures Macroscopically negative aerobic blood cultures shall be subcultured at some point before discarding. **Guidance –** Subcultures need not be done on blood cultures performed by radiometric methods or automated non-	**Bacteriology Standard of Practice 4 (BT S4): Aerobic Blood Cultures** Macroscopically negative aerobic blood cultures must be subcultured before discarding. **Guidance –** Subcultures need not be done on blood cultures if the bottles

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<tr><th colspan="2">Microbiology</th></tr>
<tr><td colspan="2">Bacteriology</td></tr>
<tr><td>Former Standard and Guidance</td><td>Proposed Standard and Guidance</td></tr>
<tr><td>radiometric methods if the bottles are monitored for five days.</td><td>are monitored for five (5) days.</td></tr>
<tr><td colspan="2">Antimicrobial susceptibility Testing: Disk diffusion and minimal inhibitory concentration</td></tr>
<tr><td>Bacteriology Standard 5 (BT S5): Defining Antibiotic Panels

Antibiotic panels appropriate to the specimen source and organism isolated shall be defined.

Guidance – Guidelines should be established for the number and type of antibiotics tested and/or reported for organisms isolated from different sources. It is recommended that, in a hospital setting, the laboratory periodically reviews the most current formulary established by the pharmacy and/or the Infection Control Committee.</td><td>Bacteriology Standard 5 (BT S5): Defining Antibiotic Panels

In addition to the requirements for Test Procedure Content Standard of Practice 1, the standard operating procedure must define antibiotic panels appropriate to the specimen source and organism isolated.

Guidance –

Guidelines should be established for the number and type of antibiotics tested and/or reported for organisms isolated from different sources. It is recommended that, in a hospital setting, the laboratory periodically reviews the most current formulary established by the pharmacy and/or the Infection Control Committee.</td></tr>
<tr><td>Disk diffusion methods (Standards 6-9)</td><td>Disk diffusion methods (Standards 6-9)</td></tr>
<tr><td>Bacteriology Standard of Practice 6 (BT S6): Media QC for Disk Diffusion Methods

Each batch of media used for antimicrobial susceptibility testing shall be checked with the appropriate control strains before, or concurrent with, initial use utilizing approved reference organisms.</td><td>Bacteriology Standard of Practice 6 (BT S6): Media Quality Control for Disk Diffusion Methods

Each batch of media used for antimicrobial susceptibility testing must be verified with the appropriate reference organisms strains before, or concurrent with, initial use.

Guidance –</td></tr>
</table>

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Former Standard and Guidance	***Proposed Standard and Guidance***
Guidance – If performed concurrently with patient testing, QC results should be reviewed prior to release of patient results.	If performed concurrently with patient testing, quality control (QC) results should be reviewed prior to release of patient results.
Bacteriology Standard of Practice 7 (BT S7): Antibiotic QC for Disk Diffusion Methods Using known reference organisms, the laboratory shall check each new lot of antimicrobial disks before, or concurrent with, initial use. **Guidance –** If performed concurrently with patient testing, QC results should be reviewed prior to release of patient results.	**Bacteriology Standard of Practice 7 (BT S7): Antibiotic Quality Control for Disk Diffusion Methods** Each new lot of antimicrobial disks must be verified with the appropriate reference organisms before, or concurrent with, initial use. **Guidance –** If performed concurrently with patient testing, quality control (QC) results should be reviewed prior to release of patient results.
Bacteriology Standard of Practice 8 (BT S8): Antibiotic Disk Distribution Antibiotic disks shall be evenly distributed over the culture plate not less than 15 mm from the outer edge of the plate and no closer than 24 mm from center to center except when specifically directed otherwise by the manufacturer's directions or generally accepted standards of practice. **Guidance –** Generally, no more than 12 disks should be distributed on a 150 mm petri plate and no more than 5 disks on a 100 mm plate. For *Haemophilus* species, *Neisseria gonorrhoeae*, and *Streptococcus* species (including *Streptococcus pneumoniae*), no more than 9 disks per 150 mm plate and no more than 4 disks per 100 mm plate should be	**Bacteriology Standard of Practice 8 (BT S8): Antibiotic Disk Distribution** Antibiotic disks must be evenly distributed over the culture plate not less than fifteen (15) mm from the outer edge of the plate and no closer than twenty-four (24) mm from center to center except when specifically directed otherwise by the manufacturer's directions. **Guidance –** Generally, no more than twelve (12) disks should be distributed on a one hundred and fifty (150) mm petri plate and no more than five (5) disks on a one hundred (100) mm plate. For *Haemophilus* species, *Neisseria gonorrhoeae*, and *Streptococcus* species (including *Streptococcus pneumoniae*),

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<tr><th>Former Standard and Guidance</th><th>Proposed Standard and Guidance</th></tr>
<tr><td>used.</td><td>no more than nine (9) disks per one hundred and fifty (150) mm plate and no more than four (4) disks per one hundred (100) mm plate should be used.</td></tr>
<tr><td>Bacteriology Standard of Practice 9 (BT S9): Disk Diffusion QC Frequency, Assessment and Recording

For antimicrobial susceptibility disk diffusion testing, the laboratory shall:

a) use the appropriate control organism(s) to check the procedure each day of testing; or

b) test each appropriate control strain a minimum of once each week during which patients are tested, provided the laboratory has demonstrated satisfactory performance of quality control testing by testing control strains each day of use for at least 20 days;

c) record zone sizes for each antimicrobial quality control test; and

d) verify quality control results are within established zone diameter ranges.

Guidance –

b) This alternative quality control practice is subject to the requirements of Quality Control Sustaining Standard of Practice (QC Design S1): Design of Individualized Quality Control Plan.

c) Zone sizes may be measured using a ruler, sliding calipers, templates, or other appropriate measurement devices</td><td>Bacteriology Standard of Practice 9 (BT S9): Disk Diffusion Quality Control Frequency, Assessment and Recording

For antimicrobial susceptibility disk diffusion testing, the laboratory must:

a) use the appropriate control organism(s) each day of testing; and

b) record zone sizes for each antimicrobial quality control test; or

c) meet the requirements of Quality Control Standard of Practice 2, 3 and 4; and

d) document that quality control results are within established zone diameter ranges.

Guidance –

b) Zone sizes may be measured using a ruler, sliding calipers, templates, or other appropriate measurement devices.

d) The laboratory may establish zone diameter ranges using relevant references.</td></tr>
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Former Standard and Guidance	***Proposed Standard and Guidance***
prepared for this purpose. d) The laboratory may establish zone diameter ranges using relevant references.	

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Mycobacteriology

Former Standard and Guidance	*Proposed Standard and Guidance*
The following specialty sustaining standards of practices shall be incorporated into the laboratory's quality management system, where applicable to the scope of services provided. The laboratory must also follow the designated Microbiology Standards. Effective July 15, 2018.	**Deleted**
Mycobacteriology Sustaining Standard of Practice 3 (TB S3): Specimen Centrifugation Specimens shall be centrifuged for a minimum of 15 minutes at greater than or equal to 3,000 x g.	**Mycobacteriology Standard of Practice 1 (TB S1): Specimen Centrifugation** Specimens must be centrifuged for a minimum of fifteen (15) minutes at greater than or equal to 3,000 x g.
Mycobacteriology Sustaining Standard of Practice 4 (TB S4): Cross-Contamination The laboratory shall design procedures that minimize the possibility of cross-contamination including but not limited to: a) only one patient specimen shall be opened and manipulated at a time in a BSC; b) positive control organisms shall not be present in the BSC when patient specimens are being processed. **Guidance –** Batch staining with jars or dishes is not good laboratory practice. This does not apply to automated staining systems. b) False positive results have been reported due to contamination with a control that contained a high concentration of organisms i.e. TB or NTM culture.	**Mycobacteriology Standard of Practice 2 (TB S2): Cross-Contamination** The laboratory must design procedures that minimize the possibility of cross-contamination including, but not limited to: a) opening and manipulating only one (1) patient specimen at a time in a biological safety cabinet (BSC); and b) excluding positive control organisms from the BSC while patient specimens are being processed. **Guidance –** Batch staining with jars or dishes should not be utilized. This does not apply to automated staining systems.

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Former Standard and Guidance	***Proposed Standard and Guidance***
Mycobacteriology Sustaining Standard of Practice 5 (TB S5): Staining Quality Control For mycobacteriological staining, a positive and negative control shall be run with each new shipment or lot of stain and each time of use.	**Mycobacteriology Standard of Practice 3 (TB S3): Staining Quality Control** For mycobacteriological staining, a positive and negative control must be run with each new shipment or lot of stain and each time of use or meet the requirements of Quality Control Standards of Practice 2, 3 and 4.
Mycobacteriology Sustaining Standard of Practice 6 (TB S6): Fluorochrome Stains The laboratory shall have documentation that positive fluorochrome stains in newly diagnosed patients are: a) confirmed by carbol fuchsin stain; or, b) independently evaluated by a second person. **Guidance –** Carbol fuchsin stain is the preferred method for confirmation in newly diagnosed patients.	**Mycobacteriology Standard of Practice 4 (TB S4): Fluorochrome Stains** Laboratories using fluorochrome staining must confirm positive results in newly diagnosed patients by: a) carbol fuchsin stain; or b) independent evaluation by a second person. **Guidance –** Carbol fuchsin is the preferred stain for confirmation in newly diagnosed patients.
Mycobacteriology Sustaining Standard of Practice 7 (TB S7): Reporting Smear Results Reports of all positive and negative smear stain results shall: a) be communicated to the ordering physician or other authorized person within 30 hours of the receipt of the specimen; and b) indicate that culture is being performed. **Guidance –**	**Mycobacteriology Standard of Practice 5 (TB S5): Reporting Smear Results** In addition to report requirements in Reporting Standard of Practice 2, reports of all smear stain results must: a) be communicated to the ordering physician or other authorized person within thirty (30) hours of the receipt of the specimen; and b) indicate that culture is being performed.

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<tr><th colspan="2">Mycobacteriology</th></tr>
<tr><th>Former Standard and Guidance</th><th>Proposed Standard and Guidance</th></tr>
<tr><td>a) Reporting time should be periodically monitored to ensure compliance.
b) Culture is necessary due to the limited sensitivity of microscopy.</td><td>Guidance –
a) Reporting time should be periodically monitored to ensure compliance.</td></tr>
<tr><td>Mycobacteriology Sustaining Standard of Practice 8 (TB S8): Smears Only Permit Category - Specimen Submission and Result Notification
Laboratories testing under the Smears Only permit category shall:
a) submit specimens for culture to a laboratory holding a New York State permit in the appropriate Mycobacteriology category; and
b) notify the reference laboratory if the specimen being sent is the first smear positive specimen from the patient.
Guidance – Part (a) of the standard is to be followed regardless of the smear result.
b) This notification is essential so that the reference laboratory can comply with TBS 15.
b) The patient smear history can be reviewed in the LIMS system of the referring laboratory.</td><td>Mycobacteriology Standard of Practice 6 (TB S6): Laboratories testing only Smears - Specimen Submission and Result Notification
Laboratories testing only smears must:
a) refer all specimens for culture to a laboratory holding a valid New York State clinical laboratory permit in the category of Mycobacteriology; and
b) notify the reference laboratory if the specimen being sent is the first smear positive specimen from the patient.
Guidance –
b) This notification is essential so that the reference laboratory can comply with Mycobacteriology Sustaining Standard of Practice 13 (TB S13).
b) The patient smear history can be reviewed in the laboratory information system of the referring laboratory.</td></tr>
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<tr><th>Former Standard and Guidance</th><th>Proposed Standard and Guidance</th></tr>
<tr><td>Mycobacteriology Sustaining Standard of Practice 9 (TB S9): Retention of Stained Slides

Stained slides of direct smears from primary specimens shall be retained until the final culture report has been issued.

Guidance –

Fluorochrome slides will fade with time, so they should be retained in the dark. The slides may be restained with a carbol fuchsin method if necessary.</td><td>Mycobacteriology Standard of Practice 7 (TB S7): Retention of Stained Slides

Stained slides of direct smears from primary specimens must be retained until the final culture report has been issued according to Document and Specimen Retention Standard of Practice 10.

Guidance –

Fluorochrome slides will fade with time, so they should be retained in the dark. The slides may be restained with a carbol fuchsin method if necessary.</td></tr>
<tr><td>Mycobacteriology Sustaining Standard of Practice 10 (TB S10): Nucleic Acid Amplification

Nucleic acid amplification for M. tuberculosis complex shall be performed on all primary respiratory specimens that test smear positive and are from patients who have not been previously diagnosed with tuberculosis.

Guidance – If the laboratory does not have the capability to perform nucleic acid amplification testing, an additional respiratory specimen shall be immediately requested and sent to a New York State permitted laboratory that performs nucleic acid amplification.

Non-amplified nucleic acid assays do not satisfy this standard.

Specimens from patients with a known history of non-tuberculous Mycobacteria (NTM) infection and without clinical suspicion of tuberculosis (e.g., cystic fibrosis patients) do not</td><td>Mycobacteriology Standard of Practice 8 (TB S8): Nucleic Acid Amplification

Nucleic acid amplification testing for M. tuberculosis complex must be performed on all primary respiratory specimens that test smear positive and are from patients who have not been previously diagnosed with tuberculosis.

If the laboratory does not have the capability to perform nucleic acid amplification testing, an additional respiratory specimen must be immediately sent to a laboratory holding a valid New York State clinical laboratory permit in the category of Mycobacteriology to perform nucleic acid amplification.

Guidance –

Non-amplified nucleic acid assays do not satisfy this standard.

Specimens from patients with a known history of non-tuberculous Mycobacteria (NTM) infection and without clinical</td></tr>
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Mycobacteriology

Former Standard and Guidance	***Proposed Standard and Guidance***
need nucleic acid amplification testing performed.	suspicion of tuberculosis (e.g., cystic fibrosis patients) do not need nucleic acid amplification testing performed.
Mycobacteriology Sustaining Standard of Practice 11 (TB S11): Media For all specimens other than blood, at least one solid and one liquid medium shall be inoculated for culturing acid fast bacilli (AFB).	**Mycobacteriology Standard of Practice 9 (TB S9): Media** For all specimens other than blood, at least one (1) solid and one (1) liquid medium must be inoculated for culturing acid fast bacilli (AFB).
Mycobacteriology Sustaining Standard of Practice 12 (TBS 12): Culture Purity The purity of a positive liquid culture shall be verified by: a) acid fast staining microscopy; and b) plating on enriched, non-selective culture media (eg. Chocolate agar) to ensure that cultures are not contaminated with non-acid fast bacilli.	**Mycobacteriology Standard of Practice 10 (TBS 10): Culture Purity** To ensure that positive liquid cultures are not contaminated, the laboratory must perform: a) acid fast staining microscopy; and b) plating on enriched, non-selective culture media (e.g., Chocolate agar).
Mycobacteriology Sustaining Standard of Practice 13 (TBS 13): Retention of Isolates Laboratories shall save the original and subsequent *M. tuberculosis* complex isolates from all patients for 12 months. **Guidance –** Multiple isolates may be requested from the same patient for public health investigation. Isolates may be retained on appropriate media and stored at 4-8 degrees C or may be frozen at -70 degrees C to -80 degrees C.	**Mycobacteriology Standard of Practice 11 (TBS 11): Retention of Isolates** Laboratories must save all original and subsequent *M. tuberculosis* complex isolates for twelve (12) months according to Document and Specimen Retention Standard of Practice 10. **Guidance –** Multiple isolates may be requested from the same patient for public health investigation. Isolates may be retained on appropriate media and stored at

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Former Standard and Guidance	**_Proposed Standard and Guidance_**
	-70 to -80 degrees Celsius.
Mycobacteriology Sustaining Standard of Practice 14 (TB S14): Identifying _M. avium_ complex and _M. gordonae_ Laboratories shall use only AFB morphology and NYS or FDA approved methods to identify *M. avium* complex and *M. gordonae*. **Guidance –** Identification of *M. avium* complex or *M. gordonae* by biochemical methods is not satisfactory. However, new technologies may be acceptable as long as they are appropriately validated and approved by NYS Clinical Laboratory Reference System or cleared by the FDA.	**Mycobacteriology Standard of Practice 12 (TB S12): Identifying _M. avium_ complex and _M. gordonae_** Detection of *M. avium* complex and *M. gordonae* by biochemical methods must be confirmed by another method.
Mycobacteriology Sustaining Standard of Practice 15 (TB S15): Submission of Isolates to a Public Health Laboratory Laboratories shall submit to either the Wadsworth Center or the NYC Public Health Laboratories: a) all initial isolates of *Mycobacterium tuberculosis* complex from newly diagnosed patients within the next business day of a positive identification of *M. tuberculosis* complex; b) all *M. tuberculosis* complex isolates presenting a change in susceptibility pattern. The initial isolate and the subsequent isolate demonstrating an altered susceptibility pattern shall both be submitted. **Guidance –** Isolates recovered from patients residing in New York City should be submitted to the NYC Public Health Laboratories; isolates from patients residing outside of NYC	**Mycobacteriology Standard of Practice 13 (TB S13): Submission of Isolates to a Public Health Laboratory** Laboratories must submit to either the Wadsworth Center or the New York City (NYC) Public Health Laboratory: a) all initial isolates of *Mycobacterium tuberculosis* complex from newly diagnosed patients by the next business day of a positive identification of *M. tuberculosis* complex; and b) all *M. tuberculosis* complex isolates presenting a change in susceptibility pattern. The initial isolate and the subsequent isolate demonstrating an altered susceptibility pattern must both be submitted. **Guidance –** Isolates recovered from patients residing in New York City

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(upstate and out-of-state) should be submitted to the Wadsworth Center in Albany, NY. Refer to the latest version of the Laboratory Reporting and Specimen Submission Requirements for Communicable Diseases available at http://www.wadsworth.org/regulatory/clep/laws. For all laboratories, *M. tuberculosis* complex isolated by the New York State Fast-Track Program do not need to be resubmitted to either public health laboratory by the original submitter. a) To expedite genotype testing, an aliquot of at least 1 ml of the primary broth medium should be sent rather than waiting for a mature subculture on a slant. b) A change in drug susceptibility may be identified by the health care provider or through the patient's history. b) If an initial isolate has already been submitted, there is no need to re-submit that isolate with a subsequent isolate demonstrating an altered susceptibility pattern.	(NYC) should be submitted to the NYC Public Health Laboratory; isolates from patients residing outside of NYC should be submitted to the Wadsworth Center. Refer to the latest version of the Laboratory Reporting and Specimen Submission Requirements for Communicable Diseases available at http://www.wadsworth.org/regulatory/clep/laws. a) To expedite genotype testing, an aliquot of at least one (1) ml of the primary broth medium should be sent rather than waiting for a mature subculture on a slant. b) A change in drug susceptibility may be identified by the health care provider or through the patient's history.
Mycobacteriology Sustaining Standard of Practice 16 (TB S16): Referral of Positive Isolates for Susceptibility Testing If susceptibility is not performed in-house, the initial positive culture on a newly diagnosed patient shall be submitted to a New York State permitted laboratory by the next business day of identification as *Mycobacterium tuberculosis* complex. **Guidance –** Whenever possible, the initial positive culture (i.e., equal to or greater than 1 ml broth aliquot or slant) should be submitted and a subculture should be retained in the originating	**Mycobacteriology Standard of Practice 14 (TB S14): Referral of Positive Isolates for Susceptibility Testing** If susceptibility is not performed in-house, the initial positive *M. tuberculosis* complex culture on a newly diagnosed patient must be referred by the next business day for susceptibility testing to a laboratory holding a valid New York State clinical laboratory permit in the category of Mycobacteriology. **Guidance –** Whenever possible, the initial positive culture (i.e., equal to or

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laboratory The submitting laboratory should provide the date of identification to the reference laboratory. For laboratories not performing susceptibility testing in-house, the submitting laboratory should periodically monitor whether the interval between the initial identification and the receipt of culture by the reference laboratory is acceptable.	greater than one (1) ml broth aliquot or slant) should be submitted and a subculture should be retained in the originating laboratory The submitting laboratory should provide the date of identification to the reference laboratory. For laboratories not performing susceptibility testing in-house, the submitting laboratory should periodically monitor whether the interval between the initial identification and the receipt of culture by the reference laboratory is acceptable.
Mycobacteriology Sustaining Standard of Practice 17 (TB S17): Susceptibility Testing Susceptibility testing shall be performed using the indirect testing method. **Guidance –** Indirect susceptibility testing utilizes a pure culture as inoculum. Using a specimen as inoculum (direct susceptibility method) is not acceptable.	**Mycobacteriology Standard of Practice 15 (TB S15): Culture-Based Susceptibility Testing** Susceptibility testing must be performed using the indirect testing method. **Guidance –** Indirect susceptibility testing utilizes a pure isolate as inoculum. Using a specimen as inoculum (direct susceptibility method) is not acceptable.
Mycobacteriology Sustaining Standard of Practice 18 (TB S18): First-Line Tuberculosis Drugs All initial isolates of *M. tuberculosis* complex shall, at a minimum, be tested for susceptibility to the following first-line tuberculosis drugs: Rifampin, Isoniazid, Pyrazinamide, Ethambutol using culture or nucleic acid based methods. All isolates predicted to be resistant by nucleic acid based methods shall be confirmed by culture-based susceptibility	**Mycobacteriology Standard of Practice 16 (TB S16): First-Line Tuberculosis Drugs** All initial isolates of *M. tuberculosis* complex must at a minimum be tested against the following first-line tuberculosis drug using culture or nucleic acid based methods: Rifampin, Isoniazid, Pyrazinamide, and Ethambutol. If the laboratory does not perform pyrazinamide susceptibility testing, the isolate must be submitted within one (1) business

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testing. Isolates predicted to be susceptible by nucleic acid methods other than whole genome sequencing shall be confirmed by culture-based susceptibility testing. **Guidance –** For all isolates identified as *M. tuberculosis* complex: If the laboratory does not perform pyrazinamide susceptibility testing, the isolate should be submitted within 24 hours to a New York State permitted laboratory for pyrazinamide testing.	day for pyrazinamide testing to a laboratory holding a valid New York State clinical laboratory permit in the category of Mycobacteriology. All isolates predicted to be resistant by nucleic acid-based methods must be confirmed by culture-based susceptibility testing. All isolates predicted to be sensitive by nucleic acid-based methods other than whole genome sequencing must be confirmed by culture-based susceptibility testing.
Mycobacteriology Sustaining Standard of Practice 19 (TB S19): Verification of Reagents for Susceptibility Testing For anti-mycobacterial susceptibility tests, the laboratory must shall check each batch of media and each lot number and shipment of anti-mycobacterial agent(s) before or concurrent with initial use, using an appropriate control organism(s) and a) establish limits for acceptable control results; b) use the appropriate control organism(s) to check the procedure each week tests are performed; c) use a control strain of *M. tuberculosis* that is fully susceptible to first line drugs for susceptibility tests performed on *M. tuberculosis* complex isolates; d) verify that the results for the control organism(s) are within established limits before reporting patient results; and e) document the results of all control procedures performed.	**Mycobacteriology Standard of Practice 17 (TB S17): Verification of Reagents for Culture-Based Susceptibility Testing** For anti-mycobacterial culture-based susceptibility tests, the laboratory must check each batch of media and each lot number and shipment of anti-mycobacterial agent(s) before or concurrent with initial use, using an appropriate control organism(s) and: a) establish limits for acceptable control results; b) use the appropriate control organism(s) to check the procedure each week tests are performed; c) use a control strain of *M. tuberculosis* that is fully susceptible to first line drugs for susceptibility tests performed on *M. tuberculosis* complex isolates; d) verify that the results for the control organism(s) are within established performance specifications before

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	reporting patient results; and e) document the results of all control procedures performed.
Mycobacteriology Sustaining Standard of Practice 21 (TB S21): Second-Line Drugs Additional culture or nucleic acid based susceptibility testing shall be performed for second-line drugs for all initial positive cultures of *M. tuberculosis* complex from newly diagnosed patients if culture-based resistance is detected for one or more first-line drugs, with the exception of mono-resistance to pyrazinamide. If second-line drug susceptibility cannot be performed in-house, the isolate shall be referred within 24 hours to a New York State permitted laboratory for testing. All isolates predicted to be resistant by nucleic acid based methods shall be confirmed by culture-based susceptibility testing. Isolates predicted to be susceptible by nucleic acid based methods other than whole genome sequencing shall be confirmed by culture based susceptibility testing. **Guidance –** Whenever possible, the initial positive culture (i.e., 3 ml broth aliquot or slant) should be immediately referred and a subculture should be retained in the originating laboratory.	**Mycobacteriology Standard of Practice 18 (TB S18): Second-Line Drugs** Additional culture or nucleic acid-based susceptibility testing must be performed for second-line drugs for all initial positive cultures of *M. tuberculosis* complex from newly diagnosed patients if culture-based resistance is detected for one (1) or more first-line drugs, with the exception of mono-PZA resistance. If second-line drug susceptibility cannot be performed in-house, the isolate must be referred within one (1) business day to a laboratory holding a valid New York State clinical laboratory permit in the category of Mycobacteriology. All isolates predicted to be <u>resistant</u> by nucleic acid-based methods must be confirmed by culture-based susceptibility testing. Isolates predicted to be <u>sensitive</u> by nucleic acid-based methods, other than whole genome sequencing, must be confirmed by culture-based susceptibility testing.
Mycobacteriology Sustaining Standard of Practice 22 (TB S22): Reporting First-Line Drugs Susceptibility test results for first-line drugs shall be reported	**Mycobacteriology Standard of Practice 19 (TB S19): Reporting Susceptibility Test Results** All susceptibility test results must be reported within one (1)

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within 24 hours of findings. If applicable, the report shall specify that second line drug susceptibility testing is being performed. **Guidance –** Test results for susceptibility to first line drugs should not be held pending the results of the additional testing.	business day of findings. **Guidance –** Test results for susceptibility to first line drugs should not be held pending the results of the additional testing.
Mycobacteriology Sustaining Standard of Practice 23 (TB S23): Turn Around Time for Susceptibility Testing For initial diagnostic specimens, the average time from identification of *M. tuberculosis* complex from culture to reporting of susceptibility results for first line drugs shall not exceed 17 days for 70% of such specimens. **Guidance –** The laboratory receiving the primary specimen is responsible for ensuring that the turn-around-time requirement is met. This standard applies to laboratories performing smears only and laboratories performing susceptibility testing.	**Mycobacteriology Standard of Practice 20 (TB S20): Turnaround Time for Susceptibility Testing** For initial diagnostic specimens, the average time from identification of *M. tuberculosis* complex from culture to reporting of susceptibility results for first line drugs must not exceed seventeen (17) days for seventy (70) percent of specimens. The laboratory receiving the primary specimen is responsible for ensuring that the turn-around time requirement is met. This standard applies to laboratories performing smears and laboratories performing susceptibility testing.

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The following specialty sustaining standards of practices shall be incorporated into the laboratory's quality management system, where applicable to the scope of services provided. Effective July 14, 2014	**Deleted**
Mycology Sustaining Standard of Practice 3 (MY S3): **Microscopy** Identification of molds and yeast shall include direct microscopic examination of the clinical specimen or the isolate using an appropriate mounting medium or stain unless a New York State- or FDA-approved nucleic acid or mass spectroscopic identification method is used. **Guidance –** Based upon clinical history and nature of the clinical specimen, a direct examination may be performed with one of the following reagents or stains: potassium hydroxide; India ink; Cellufluor; Gram stain; Giemsa stain, ethenamine silver stain, or other appropriate method(s). (Note: The listed examples are not all-inclusive). Mold and yeast isolates may be examined with Cellufluor, India ink, Giemsa stain, Gram stain, or other appropriate method(s). (Note: The listed examples are not all-inclusive).	**Mycology Standard of Practice 1 (MY S1): Microscopy of Primary Specimens** Identification of molds and yeast must utilize direct microscopic examination of the primary specimen using an appropriate mounting medium or stain. **Guidance –** Based upon clinical history and nature of the clinical specimen, a direct examination may be performed with one of the following reagents or stains: potassium hydroxide; India ink; Cellufluor; Gram stain; Giemsa stain, ethenamine silver stain, or other appropriate method(s). (Note: The listed examples are not all-inclusive).
Mycology Sustaining Standard of Practice 4 (MYS4): Culture Incubation The mycology laboratory shall: a) incubate cultures at 30 ± 2° C; and	**Mycology Standard of Practice 2 (MYS2): Culture Incubation** The mycology laboratory must: a) incubate cultures at 30 ± 2 degrees Celsius unless

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b) provide sufficient humidity to prevent drying of inoculated plates. **Guidance –** b) Insufficient humidity is evident when agar is cracked before the end of the incubation period.	otherwise instructed by the manufacturer; and b) provide sufficient humidity to prevent drying of inoculated plate. **Guidance –** b) Insufficient humidity is evident when agar is cracked before the end of the incubation period.
Mycology Sustaining Standard of Practice 5 (MYS5): Culture Media for Isolation of Molds and Yeasts Laboratories shall utilize a combination of culture media for isolation of molds and yeasts as appropriate for clinical specimens being tested. **Guidance –** Examples of suitable media are as follows (Note: The listed examples are not all-inclusive): a) general purpose media such as: Sabouraud dextrose agar--no antibacterial or antifungal agents,; b) general purpose media with cycloheximide such as: Sabouraud dextrose agar - with antibacterial agents and cycloheximide,; c) enriched media such as: BHI agar, SABHI agar d) specialized media such as: chromogenic agars and formulations that might aid in isolation and presumptive identification of fastidious and dimorphic pathogenic fungi and *Malassezia* species.	**Mycology Standard of Practice 3 (MYS3): Culture Media for Isolation of Molds and Yeasts** Laboratories that perform culture must utilize a combination of culture media for isolation of molds and yeasts as appropriate for clinical specimens being tested. **Guidance –** Examples of suitable media are as follows (Note: The listed examples are not all-inclusive): • general purpose media such as: Sabouraud dextrose agar--no antibacterial or antifungal agents; • general purpose media with cycloheximide such as: Sabouraud dextrose agar - with antibacterial agents and cycloheximide; • enriched media such as: BHI agar, SABHI agar; and • specialized media such as: chromogenic agars and formulations that might aid in isolation and presumptive identification of fastidious and dimorphic pathogenic fungi

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<tr><td></td><td>and Malassezia species.</td></tr>
<tr><td>Mycology Sustaining Standard of Practice 6 (MY S6): Identification of Pathogenic Molds
Methods for identification of pathogenic molds shall include:
a) a medium to stimulate production of characteristic spores and biochemical tests to differentiate fungi; or
b) an FDA-approved or NYS-approved diagnostic system(s).</td><td>Mycology Standard of Practice 4 (MY S4): Identification of Pathogenic Molds
Methods for identification of pathogenic molds must include:
a) a medium to stimulate production of characteristic spores and biochemical tests to differentiate fungi; or
b) a nucleic acid method; or
c) a MALDI-TOF mass spectrometry method.</td></tr>
<tr><td>Mycology Sustaining Standard of Practice 7 (MY S7): Identification of Pathogenic Yeasts
Methods for identification of pathogenic yeasts shall include:
a) media for phenotypic tests; or
b) FDA-approved or NYS-approved diagnostic systems.
Guidance –
a) Examples of appropriate media for the identification of yeast include Cornmeal or cream of rice medium with Tween 80, rapid assimilation of trehalose (RAT), and urease.</td><td>Mycology Standard of Practice 5 (MY S5): Identification of Pathogenic Yeasts
Methods for identification of pathogenic yeasts must include:
a) media for phenotypic tests; or
b) a nucleic acid method; or
c) a MALDI-TOF mass spectrometry method.
Guidance –
a) Examples of appropriate media for the identification of yeast include Cornmeal or cream of rice medium with Tween 80, rapid assimilation of trehalose (RAT), and urease.</td></tr>
<tr><td>Mycology Sustaining Standard of Practice 8 (MY S8): Reference Material</td><td>Mycology Standard of Practice 6 (MY S6): Reference Material</td></tr>
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<tr><th>Former Standard and Guidance</th><th>Proposed Standard and Guidance</th></tr>
<tr><td>A reference collection of photographs or reference materials to identify microscopic fungal elements shall be readily available in the laboratory for comparison with diagnostic specimens.</td><td>A reference collection of photographs or reference materials to identify microscopic fungal elements must be readily available in the laboratory for comparison with diagnostic specimens.</td></tr>
<tr><td>Mycology Sustaining Standard of Practice 9 (MY S9): Quality Control of Probes and Stains

The laboratory shall minimally check the reactivity of each batch (prepared in-house), lot number (commercially prepared), and shipment when prepared or opened as follows:

a) a positive control as required by CLIA for lactophenol cotton blue;

b) positive and negative control organisms for all stains and probes.

Guidance – The laboratory should establish frequency of QC checks based upon published guidelines from consensus organizations.</td><td>Mycology Standard of Practice 7 (MY S7): Quality Control of Stains

The laboratory must check the reactivity of each batch (prepared in-house), lot number (commercially prepared), and shipment when prepared or opened by using:

a) a positive control for lactophenol cotton blue;

b) positive and negative control organisms for all stains.</td></tr>
<tr><td>Mycology Sustaining Standard of Practice 10 (MY S10): Antifungal Susceptibility Testing Quality Control

For antifungal susceptibility tests, the laboratory shall:

a) verify manufacturer’s limits or establish limits for acceptable control results;

b) check each batch of media and each lot number and shipment of antifungal agent(s) using appropriate control organism(s) before or concurrent with initial use; and</td><td>Mycology Standard of Practice 8 (MY S8): Antifungal Susceptibility Testing Quality Control

In addition to the requirements in Test Procedure Content Standard of Practice 1, the laboratory must have procedures for antifungal susceptibility tests, that include requirements to:

a) utilize antibiotic panels appropriate to the specimen source and organism isolated;

b) verify manufacturer’s limits or establish limits for acceptable control results;</td></tr>
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c) verify that the results for the control organism(s) are within established limits before reporting patient results. **Guidance –** ***Clinical and Laboratory Standards Institute11T35T*** (11T35T***CLSI11T35T***) 35Tor European Committee on Antimicrobial Susceptibility Testing (EUCAST) approved methods include guidelines for appropriate quality control strains.	c) verify each batch of media used for antimicrobial susceptibility testing with the appropriate reference organism strains before, or concurrent with, initial use; and d) document that quality control (QC) results are within established limits before reporting patient results.
Mycology Sustaining Standard of Practice 11 (MY S11): Susceptibility Testing Reports Susceptibility testing reports shall include: a) an interpretation, to explain the significance of the test result; and/or b) a qualifier identifying an assay limitation, if appropriate. **Guidance –** a) Interpretative guidelines based upon the CLSI or EUCAST documents or FDA approved commercial products may be used.	**Standard deleted** **Required under Reporting Standard of Practice 2 (REP S2): Test Report Content**

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The following specialty sustaining standards of practices shall be incorporated into the laboratory's quality management system, where applicable to the scope of services provided. Effective July 25, 2012	**Deleted**
Parasitology Sustaining Standard of Practice 1 (PS1): Stool Specimen Preservation for Morphological Examination Stool specimens to be used for parasitological identification based on morphology shall be a) examined immediately; b) preserved immediately upon collection using the fixative appropriate for the test being ordered; or c) refrigerated for no more than three hours from time of collection to either examination or preservation. **Guidance –** The laboratory should choose the fixative that is most appropriate for its testing purposes, eg. PVA for Trichrome, 10% formalin or SAF for acid-fast stain, ethanol for DNA extraction. When it is anticipated that the time of collection will not be recorded or transport time will be prolonged, laboratories are encouraged to provide stool transport kits with preservatives to clients. Specimens must be refrigerated and preserved with 3-hours of collection when not examined immediately. Specimens to be used for antigen testing or DNA extraction may be stored in ethanol, potassium dichromate, frozen,	**Parasitology Standard of Practice 1 (PS S1): Stool Specimen Preservation for Morphological Examination** Stool specimens to be used for parasitological identification based on morphology must be: a) examined within one (1) hour of collection; or b) preserved within one (1) hour of collection using the fixative appropriate for the test being ordered; or c) refrigerated for no more than three (3) hours to preservation. **Guidance –** The laboratory should choose the fixative that is most appropriate for its testing purposes, e.g., PVA for Trichrome, ten (10) percent formalin or SAF for acid-fast staining. When it is anticipated that the time of collection will not be recorded or transport time will be prolonged, laboratories are encouraged to provide stool transport kits containing preservatives. It is recommended that ova and parasite examinations include a concentration step whenever compatible with subsequent testing, as the concentration step significantly increases recovery of parasites.

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refrigerated or in Cary-Blair transport medium depending on the assay utilized. Consult the package insert or CLRS-approved laboratory-developed method for instructions. It is recommended that ova and parasite examinations include a concentration step whenever compatible with subsequent testing, as the concentration step is designed to facilitate recovery of parasites.	
Parasitology Sustaining Standard of Practice 2 (PS2): Quality Controls for Staining Permanent stains shall be checked using a positive and negative control, at a minimum, with each new shipment or lot, and once per month of use. **Guidance –** Controls may be obtained commercially, previously tested patients or proficiency-testing samples.	**Parasitology Standard of Practice 2 (PS S2): Quality Controls for Staining** Permanent stains for fecal specimens must be checked using a positive and negative control, at a minimum, with each new shipment or lot, and once per month of use. Permanent stains for other specimen types must be checked with a positive and negative control each day of use, unless an individualized quality control plan (IQCP) is developed according to Quality Control Standard of Practice 2, 3 and 4 to allow quality control to be performed on each new shipment or lot and once per month of use. **Guidance –** Controls may be obtained commercially, previously tested patients or proficiency-testing samples as long as the sample used demonstrates the characteristics of the stain.
Parasitology Sustaining Standard of Practice 3 (PS3): Antigen Detection Assay Controls Excluding single use devices, each antigen detection assay shall be verified for performance with at least one positive	**Standard deleted**

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control and one negative control each time the test is performed. **Guidance –** Positive and negative controls supplied with commercially available tests are acceptable. Quality control plans for single use devices must follow either Quality Control Sustaining Standard or Practice 1 (QC Design S1): Design of Individualized Quality Control Plan or Quality Design Sustaining Standard of Practice 2a (QC Design S2a): Minimum Requirements.	
Parasitology Sustaining Standard of Practice 4 (PS S4): Ocular Micrometer Calibration Laboratories shall calibrate ocular micrometers: a) annually for each objective; and b) with any change in objective or eyepiece. **Guidance –** Calibration figures or conversion factors for determining size using each objective should be readily available at the microscope. a) The SOP should contain instructions for calibration and examples to show how each objective is calibrated.	**Parasitology Standard of Practice 3 (PS S3): Ocular Micrometer Calibration** Laboratories must calibrate ocular micrometers: a) annually for each objective; and b) with any change in objective or eyepiece. **Guidance –** Calibration figures or conversion factors for determining size using each objective should be readily available at the microscope. a) The standard operating procedure should contain instructions for calibration and examples to show how each objective is calibrated.
Parasitology Sustaining Standard of Practice 5 (PS S5): Ova and Extracellular Parasite Measurement	**Parasitology Standard of Practice 4 (PS S4): Ova and Extracellular Parasite Measurement**

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Using a calibrated ocular micrometer, laboratories shall a) measure and document the size of all ova and extracellular protists; b) demonstrate annually that each analyst can accurately measure organisms or objects. **Guidance –** Size is an important criterion for clinical parasitology and may be a critical factor to distinguish morphologically similar organisms (e.g. eggs of *Paragonimus westermani* vs. *Fasciola*). a) Documentation may be on worksheets or electronic records. b) Proficiency may be established by calibration of the ocular micrometer for each analyst or measurement of an organism or object as part of annual competency assessment.	Using a calibrated ocular micrometer, laboratories must: a) measure and document the size of all ova and extracellular protists; and b) demonstrate annually that each analyst can accurately measure organisms or objects. **Guidance –** Size is an important criterion for clinical parasitology and may be a critical factor to distinguish morphologically similar organisms (e.g. *Cryptosporidium* and *Cyclospora*). a) Documentation may be on worksheets or electronic records. b) Proficiency may be established by calibration of the ocular micrometer for each analyst or measurement of an organism or object as part of annual competency assessment.
Parasitology Sustaining Standard of Practice 6(PS S6): Examination of Blood Smears The SOP for identification of blood borne parasites include: a) a description of the number of fields that need to be examined for both positive and negative results; and b) examination at 10X for the detection of filarids, if such testing is indicated. **Guidance –** Giemsa stain is recommended; however, Wright's	**Parasitology Standard of Practice 5 (PS S5): Examination of Blood Smears** In addition to the requirements in Test Procedure Content Standard of Practice 1, the laboratory must have a standard operating procedure for identification of blood borne parasites that includes: a) a description of the number of fields that need to be examined for both positive and negative results; and b) examination at 10X for the detection of filarids, if such

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stain or a Wright-Giemsa combination stain may also be used. a) an adequate number of fields [e.g., 300 oil immersion fields (10x100)] should be examined under oil immersion before calling a specimen negative.	testing is indicated. **Guidance –** Giemsa stain is recommended; however, Wright's stain or a Wright-Giemsa combination stain may also be used. a) an adequate number of fields [e.g., 300 oil immersion fields (10x100)] should be examined under oil immersion before calling a specimen negative.
Parasitology Sustaining Standard of Practice 7 (PS S7): Reference Material A reference collection of slides, photographs, or gross specimens of identified parasites shall be readily available in the laboratory for comparison with diagnostic specimens. **Guidance –** Textbooks with photographs, bench aids, tables including drawings and sizes, previously stained slide preparations, preserved specimens, or slides from proficiency testing programs are examples of acceptable reference material.	**Parasitology Standard of Practice 6 (PS S6): Reference Material** A reference collection of slides, photographs, or gross specimens of identified parasites must be readily available in the laboratory for comparison with diagnostic specimens. **Guidance –** Digital images, textbooks with photographs, bench aids, tables including drawings and sizes, previously stained slide preparations, preserved specimens, or slides from proficiency testing programs are examples of acceptable reference material.
Parasitology Sustaining Standard of Practice 8 (PS8): Report Content Reports for ova and parasite examination shall indicate if the examination did not include tests to detect *Cryptosporidium spp., Giardia intestinalis* or *Entamoeba histolytica/dispar*.	**Parasitology Standard of Practice 7 (PS S7): Report Content** In addition to the requirements in Reporting Standard of Practice 2, reports for ova and parasite examination must indicate if the examination did not include tests to detect *Cryptosporidium spp., Cyclospora, Giardia duodenalis* or

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Guidance – The tests included in an ova and parasite exam may vary considerably. This standard is intended to inform clinicians if any of the three most common parasites in New York State are not tested for when an Ova and Parasite test is performed. If a test for a specific organism is ordered, (e.g. Giardia antigen detection) this standard does not apply.	*Entamoeba histolytica/dispar.* **Guidance –** The tests included in an ova and parasite exam may vary considerably. This standard is intended to inform clinicians if any of the most common parasites in New York State are not tested for when an Ova and Parasite test is performed. If a test for a specific organism is ordered, (e.g. Giardia antigen detection) this standard does not apply.
Parasitology Sustaining Standard of Practice 9 (PS S9): Single-Use Antigen Assays Reports based solely on an immunochromatographic card test (lateral flow, rapid tests) shall include statements recommending that results from these tests be confirmed by another method. **Guidance –** It is recommended that all specimens be confirmed by another method.	**Parasitology Standard of Practice 8 (PS S8): Single-Use Antigen Assays** In addition to the requirements in Reporting Standard of Practice 2, reports based solely on an immunochromatographic card test (lateral flow, rapid tests) must include statements recommending that results from these tests be confirmed by another method. **Guidance –** It is recommended that all specimens be confirmed by another method.
Parasitology Sustaining Standard of Practice 9 (PS S9): Reporting Negative Results If a single specimen is submitted for testing and the results are negative, reports shall indicate that one negative specimen does not rule out the possibility of a parasitic infection **Guidance –** Many parasites can be easily missed if a single	**Parasitology Standard of Practice 9 (PS9): Reporting Negative Results** In addition to the requirements in Reporting Standard of Practice 2, if a single stool specimen is submitted for testing and the results are negative, reports must indicate that one (1) negative specimen does not rule out the possibility of a

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blood or stool specimen is tested. To improve detection, it is recommended that three specimens be collected on separate days over a period of not more than seven days.	parasitic infection. **Guidance –** Many parasites can be easily missed if a single stool specimen is tested. To improve detection, it is recommended that three (3) specimens be collected on separate days over a period of seven (7) days.
Parasitology Sustaining Standard of Practice 11 (PS11) Specimen Retention For positive stool or blood specimens, the laboratory shall retain, for a minimum of one year: a) permanently stained slides; or, b) a portion of the specimen, properly preserved and stored. **Guidance –** a) The staining method used is the choice of the laboratory, but the stain should be appropriate for the organism. Common permanent stains include modified acid-fast, trichrome, and Giemsa.	**Standard deleted**

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New Guidance	These standards apply to **conventional tube culture** and to **centrifugation-enhanced rapid** methods unless otherwise specified. These standards also apply to culture for *Chlamydia.*
The following specialty sustaining standards of practices shall be incorporated into the laboratory's quality management system, where applicable to the scope of services provided.	**Deleted**
Virology Sustaining Standard of Practice 1 (VR S1): Cell Culture Systems The laboratory must utilize cell cultures and methods appropriate for the isolation and/or detection of the viral agents specified in its test menu.	**Virology Standard of Practice 1 (VR S1): Cell Culture Systems** The laboratory must utilize cell cultures and methods appropriate for the isolation and/or detection of the viral agents specified in its test menu.
Virology Sustaining Standard of Practice 2 (VR S2): Cell Culture Records The laboratory shall maintain records for each commercial cell culture lot received including: cell culture type and vendor; lot number; passage level (if appropriate); date of receipt; condition at the time of receipt including the percentage of monolayer confluence; and any corrective action taken for cell culture lots that are of questionable or unsatisfactory condition at the time of receipt. Similar records shall be maintained for cell cultures prepared in house. **Guidance –** The SOPM should include the criteria for assessing the quality of cell cultures at receipt (e.g. degree of monolayer confluence, adverse conditions such as cell	**Virology Sustaining Standard of Practice 2 (VR S2): Cell Culture Records** In addition to the requirements for Test Procedure Content Standard of Practice 1, the laboratory must have standard operating procedures that include the criteria for assessing the quality of cell cultures at receipt. The laboratory must maintain records for each commercial cell culture lot received including: cell culture type and vendor; lot number; passage level (if appropriate); date of receipt; cell quality at the time of receipt; and any corrective action taken for cell culture lots that are of questionable or unsatisfactory condition at the time of receipt. Similar records must be

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rounding, detached monolayers, pH extremes or microbial contamination). Date of seeding rather than date of receipt should be recorded for cell cultures prepared in-house.	maintained for cell cultures prepared in house. Date of seeding must be recorded for cell cultures prepared in-house. Date of seeding or expiration must be recorded for commercially acquired cell cultures, if provided by the vendor **Guidance –** Documentation of the assessment of the quality of cultures includes criteria such as (percentage of monolayer confluence and adverse conditions such as cell rounding, excessive vacuolation, detached monolayers, pH extremes or microbial contamination).
Virology Sustaining Standard of Practice 3 (VR S3): Cell Culture Shelf Life and Condition Cell cultures used for testing patient specimens shall be examined microscopically and confirmed for acceptability as defined in the SOPM on the day of inoculation and used within: a) the expiration date, if provided by the vendor or b) 10 days after the monolayers become 100% confluent or c) 10 days of receipt, if monolayers were 100% confluent when received. **Guidance –** Slightly sub-confluent monolayers are optimal for viral culture. Crowded, overgrown, or aged cultures may result in decreased virus susceptibility.	**Virology Sustaining Standard of Practice 3 (VR S3): Cell Culture Shelf Life and Condition** In addition to the requirements of Test Procedures Content Standard of Practice 1, cell cultures used for testing patient specimens must be examined microscopically and confirmed for acceptability as defined in the standard operating procedure on the day of inoculation and used within: a) the expiration date, if provided by the vendor; or b) seven (7) days after the monolayers become one hundred (100) percent confluent; or c) seven (7) days of receipt, if monolayers were one hundred (100) percent confluent when received. **Guidance –** Slightly sub-confluent monolayers are optimal for viral culture. Crowded, overgrown, or aged cultures may result in decreased

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	virus susceptibility.
Virology Sustaining Standard of Practice 4 (VR S4): Mycoplasma Screening of Cell Cultures Diploid and continuous cell lines shall be tested at least every 6 months for *Mycoplasma* contamination while the cell lines are in use. **Guidance –** Documentation by the vendor that the cells are free of mycoplasma contamination is acceptable for commercially supplied cell lines.	**Virology Standard of Practice 4 (VR S4): Mycoplasma Screening of Cell Cultures** All cell lines prepared and maintained in house must be tested for *Mycoplasma* contamination after receipt of new seed stock from an external supplier before being implemented into routine use and at least every six (6) months thereafter while the cell lines are in use. For commercially acquired cell lines, laboratories must procure documentation certifying that cell lines are tested for *Mycoplasma* contamination at least every six (6) months that the cell lines are in use.
Virology Sustaining Standard of Practice 5 (VR S5): Cell Culture Medium The laboratory shall maintain records for each lot of cell culture maintenance and growth medium including the type, vendor, lot number and the receipt and expiration dates. Similar records shall be maintained for culture media prepared in-house and for medium components added by the laboratory. **Guidance –** The type of medium refers to its formulation, e.g. Eagle's MEM with 2% FBS.	**Standard deleted** **Required under Reagent and Media Standard of Practice 1 (RGM S1): Reagent and Media Records**
Virology Sustaining Standard of Practice 6 (VR S6): Cell Culture Medium Quality Control	**Virology Standard of Practice 5 (VR S5): Cell Culture Medium Quality Control**

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Quality control of cell culture medium shall be documented and minimally ensure that cell culture media have been tested for sterility and toxicity prior to use and have an acceptable pH at the time of use. **Guidance –** Documentation by the vendor that appropriate sterility, toxicity and other quality control testing has been performed on commercially supplied medium is acceptable. However, appropriate in-house testing must be performed if components are added by the laboratory. Sterility testing, if necessary, should be performed using bacterial culture techniques. Visual inspection to confirm an acceptable pH range for medium containing a colorimetric pH indicator is acceptable. The possibility of viral inhibition by a medium component (e.g. serum) should be considered and investigated if there is evidence to suggest a decrease in viral culture sensitivity.	Quality control of cell culture medium must be documented and minimally ensure that cell culture media have been tested for sterility with bacterial and fungal techniques, as well as cell toxicity on the cell lines they will be use on, prior to use. The laboratory must: a) perform in-house sterility and toxicity testing on commercially acquired media prior to use, if components are added to the media; b) visually inspect media containing a colorimetric pH indicator to confirm an acceptable pH range; c) investigate and document viral growth inhibition by a media component if decreased viral sensitivity is observed; and d) for commercially procured media, retain vendor documentation of sterility, toxicity and other quality control testing (for the lifetime of the media and two years after discontinuing use.
Virology Sustaining Standard of Practice 7 (VR S7): Biological Safety Cabinet (BSC) Use A class II or higher BSC shall be used for inoculating cell cultures with clinical specimens and for all procedures involving the maintenance and processing of inoculated cell cultures and culture-amplified materials. **Guidance –** Refer to Safety Sustaining Standard of Practice 6 (Safety S6) for operational guidelines.	**Standard deleted** **Required under Microbiology Standard of Practice 1 (MB S1): Biological Safety Cabinet**

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Virology Sustaining Standard of Practice 8 (VR S8): Specimen Processing Procedures The SOPM shall include specimen processing and inoculation procedures for each type (e.g. swab, tissue, blood) of specimen. **Guidance –** Processing procedures include dilution, fractionation, centrifugation, decontamination, homogenization or other treatments utilized for rendering specimens suitable for cell culture inoculation. Inoculation procedures primarily refer to inoculum volume and adsorption steps (if performed).	**Standard deleted** **Required under Test Procedure Content Standard of Practice 1 (TPC S1): Test Procedure Content**
Virology Sustaining Standard of Practice 9 (VR S9): Cell Culture and Culture Medium Lot Numbers Dates of culture manipulations and microscopic observations shall be recorded on each specimen worksheet. **Guidance –** Culture manipulations include all processing steps such as inoculation, medium changes, subpassage and harvesting. This standard is consistent with the requirement for recreating the test process as stated in standards Quality Management System Sustaining Standard 1 (QMS S1)(t) and Reagents Sustaining Standard 4 (Reagent S4).	**Virology Standard of Practice 6 (VR S6): Cell Culture and Culture Medium Lot Numbers** Dates and activities of all culture manipulations and microscopic observations must be recorded **Guidance –** Culture manipulations includeinoculation, medium changes, subpassage and harvesting.
Virology Sustaining Standard of Practice 10 (VR S10):	**Standard deleted**

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<table>
<tr><th colspan="2">Microbiology</th></tr>
<tr><th colspan="2">Virology</th></tr>
<tr><th>Former Standard and Guidance</th><th>Proposed Standard and Guidance</th></tr>
<tr><td>Specimen Criteria
The SOPM shall define the remedial action that may be employed for specimens that exhibit contamination or toxicity.
Guidance – Remedial action for specimens causing adverse culture events (e.g. contamination, toxicity) may include reinoculation, sub-passage, filtration, recollection).</td><td>Required under Test Procedure Content Standard of Practice 1 (TPC S1): Test Procedure Content</td></tr>
<tr><td>Virology Sustaining Standard of Practice 11 (VR S11): Viral Culture Criteria and Timepoints
The SOPM shall define culture criteria including:
a) inoculation and incubation conditions (e.g. temperature);
b) minimal culture duration;
c) minimally acceptable culture observation and documentation schedules;
d) maintenance/processing schedules (e.g. media changes, hemadsorption [HAd], staining);
e) conditions that render a culture unacceptable or uninterpretable, including an unacceptable monolayer condition at completion (see Virology Sustaining Standard 21 (VR S21)).
Guidance –
a) Inoculation includes specimen adsorption conditions, if applicable.
c) Observation includes the microscopic examination of</td><td>Virology Standard of Practice 7 (VR S7): Viral Culture Criteria and Timepoints
In addition to the requirements in Test Procedure Content Standard of Practice 1, the standard operating procedure must define all aspects of culture including:
a) inoculation and incubation conditions (e.g., temperature);
b) minimal culture duration;
c) minimally acceptable culture observation (every three (3) days for conventional culture) and documentation schedules;
d) maintenance/processing schedules; and
e) remedial action needed for specimens exhibiting contamination or toxicity.
Guidance –
a) Inoculation includes specimen adsorption conditions, if applicable.
c) Observation includes the microscopic examination of</td></tr>
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monolayers for evidence of cytopathic effect (CPE), toxicity and contamination. c) The minimal frequency for microscopically observing cultures and recording results is every other day during the first week of incubation and then every 2 to 3 days thereafter. c) Optimally, cultures should be observed on the first day after specimen inoculation in order to initiate timely remediation of adverse events (e.g. toxicity, contamination) and to detect rapidly growing agents. The SOPM should define exceptions and describe remedial action for handling of potentially compromised cultures.	monolayers for evidence of cytopathic effect (CPE), toxicity and contamination. c) Optimally, cultures should also be observed on the first day after specimen inoculation in order to initiate timely remediation of adverse events (e.g. toxicity, contamination) and to detect rapidly growing viruses. d) Maintenance and processing include media changes, hemadsorption, harvesting, and staining procedures. e) Remedial action for specimens causing adverse culture events (e.g. contamination, toxicity) may include re-inoculation, sub-passage, filtration, or recollection).
Virology Sustaining Standard of Practice 12 (VR S12): Negative Cell Culture Controls Uninoculated (negative) lot-matched cell culture controls shall be processed, incubated, maintained and observations recorded in parallel with patient specimens for the entire duration that each lot is in use. **Guidance –** Uninoculated (negative) controls are observed for evidence of unacceptable occurrences such as monolayer deterioration, CPE, HAd and other conditions that should not occur in uninoculated cell cultures. Troubleshooting and corrective action are based on adverse findings as described in the SOPM. Maintenance of controls in parallel with patient specimens requires that the same lot of cell culture medium being used for	**Virology Sustaining Standard of Practice 8 (VR S8): Negative Cell Culture Controls** Uninoculated (negative) lot-matched cell culture controls must be processed, incubated, maintained and observations recorded in parallel with patient specimens while each lot is in use. **Guidance –** Uninoculated (negative) controls are observed for evidence of unacceptable occurrences such as monolayer deterioration, CPE, HAd and other conditions that that may adversely affect viral growth or the ease of CPE-recognition. Troubleshooting and corrective action are based on adverse findings as described in the SOPM. Maintenance of controls in parallel with patient specimens

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medium changes on patient specimens is used for the controls. When using centrifugation-enhanced rapid methods, processing includes the centrifugation step. The possibility that a defective lot of culture medium may be the source of deterioration or contamination in uninoculated (negative) controls should be considered. The need to repeat medium quality control testing should be determined (see Virology Sustaining Standard 56 (VR S56): Cell Culture Medium Quality Control).	requires that the same lot of cell culture medium being used for medium changes on patient specimens is used for the controls. When using centrifugation-enhanced rapid methods, processing includes the centrifugation step.
Virology Sustaining Standard of Practice 13 (VR S13): Positive Live Virus Culture Controls Live virus-inoculated (positive) culture controls shall be employed as deemed necessary with the exception of genetically engineered cell lines where they shall be included for every run. **Guidance –** With the exception of genetically engineered cell lines, virus- inoculated (positive) culture controls need be employed only as necessary (e.g. suspected performance problems; assessing a new cell culture type or source; training and remediation activities). In lieu of the routine use of positive live virus controls, the laboratory should identify appropriate indicators that would be useful in identifying cell culture sensitivity problems (see Virology Sustaining Standard 20 (VR S20): Viral Culture Performance Monitoring). Virus-inoculated positive controls are included in every run when using genetically engineered cell lines to	**Virology Standard of Practice 9 (VR S9): Positive Live Virus Culture Controls** For non-genetically engineered cell lines, the laboratory must: a) identify and monitor appropriate quality indicators for identifying cell culture performance; and b) employ live-virus positive controls to check culture sensitivity if quality indicators suggest an otherwise unexplained decreased in virus detection. **Guidance –** See Virology Standard of Practice 14 (VR S14): Viral Culture Performance Monitoring for additional information. Virus-inoculated positive controls are included in every run when using genetically engineered cell lines to confirm the stability and activity of the transfected plasmid as well as the reactivity of the enzyme and substrate. Virus-inoculated (positive) culture controls need to be

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confirm the stability and activity of the transfected plasmid as well as the reactivity of the enzyme and substrate. Virus preparations used for inoculating positive controls should optimally consist of low-passage material derived from clinical isolates rather than laboratory-adapted strains and should be inoculated at a low multiplicity of infection (m.o.i).	employed for conventional virus culture when: • performance /sensitivity/susceptibility issues are observed or suspected; • assessing a new cell culture type or source; and • training and remediation activities. Highly passaged (more than five (5) times), laboratory adapted strains or high titer virus cultures are not suitable as positive controls for virus culture.
Virology Sustaining Standard of Practice 14 (VR S14): RBC Suspensions The laboratory shall maintain records for each lot of red blood cells (RBC) used for viral detection/identification procedures and shall include the RBC type, vendor, lot number and date of expiration.	**Standard deleted** **Required under Reagents and Media Standard of Practice 1 (RGM S1): Reagent and Media Records**
Virology Sustaining Standard of Practice 15 (VR S15): RBC Controls for Hemadsorption (HAd) Assays RBC Controls in each HAd run shall include: a) a negative control that is an uninoculated cell culture monolayer that is lot-matched to that used for patient specimens; and, b) at least one positive control per lot number of RBCs. **Guidance –** Negative RBC controls are intended to determine whether the RBCs react with uninoculated cell	**Virology Sustaining Standard of Practice 10 (VR S10): RBC Controls for Hemadsorption (HAd) Assays** Red Blood Cell Controls in each HAd run must include: a) a negative control that is an uninoculated cell culture monolayer that is lot-matched to that used for patient specimens; and b) at least one positive control per lot number of RBCs. **Guidance –** Negative RBC controls are intended to determine whether the

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culture monolayers. Virology S12 requires that negative HAd controls be performed in parallel with HAd assays performed on patient inoculated cell cultures. Positive RBC controls should confirm that the RBCs react in the expected manner with virus-inoculated monolayers.	RBCs react with uninoculated cell culture monolayers. Virology Standard of Practice 8 requires that negative HAd controls be performed in parallel with HAd assays performed on patient inoculated cell cultures. Positive RBC controls should confirm that the RBCs react in the expected manner with virus-inoculated monolayers.
Virology Sustaining Standard of Practice 16 (VR S16): Confirmation and Identification of Cultured Viruses Each run shall include a: a) positive control; b) ot-matched negative (uninoculated) cell culture control. **Guidance –** a) The positive control is intended to confirm detection system reactivity. This standard does not require the positive control to be lot-matched to patient specimens and allows the use of appropriate commercial controls. The optional use of lot- matched virus-inoculated positive controls would serve to monitor cell culture sensitivity. If live virus preparations are used for inoculating positive controls, they should optimally consist of low-passage material derived from clinical isolates rather than laboratory-adapted material and should be inoculated at a low m.o.i. b) The negative cell culture control reveals background or non- specific reactivity of the detection system and serves to assist with reading. Using lot-matched	**Virology Standard of Practice 11 (VR S11): Confirmation and Identification of Cultured Viruses** When cytopathic effect in culture is observed, virus confirmation or identification must include a: a) positive control; and b) lot-matched negative (uninoculated) cell culture control. **Guidance –** a) The positive control is intended to confirm detection system reactivity. b) The negative cell culture control reveals background or non-specific reactivity of the detection system and serves to assist with reading. Using lot-matched uninoculated cells (see Virology Standard of Practice 8 (VR S8): Negative Cell Culture Controls) also serves to identify problems that may be associated with the specific lot of cell cultures used for specimen inoculation. When using an indirect antibody staining format, additional controls may be incorporated to rule out reactivity of the conjugate with the cell substrate. Alternatively, these may be

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uninoculated cells (see Virology Sustaining Standard 12 (VR S12): Negative Cell Culture Controls) also serves to identify problems that may be associated with the specific lot of cell cultures used for specimen inoculation. When using an indirect antibody staining format, additional controls may be incorporated to rule out reactivity of the conjugate (labeled secondary antibody) with the cell substrate. Alternatively, these may be utilized as needed for troubleshooting purposes if, for example, the negative control (b) yields positive or unacceptable results. In this case, diluent or an irrelevant antibody would be substituted for the primary unlabeled antibody.	utilized as needed for troubleshooting purposes if, for example, the negative control yields positive or unacceptable results.
Virology Sustaining Standard of Practice 17 (VR S17): Viral Neutralization and Hemagglutination-Inhibition (HI) Assays For neutralization and HI assays, the laboratory shall: a) use an established optimal concentration of antibodies or antisera; and, b) use the proper concentration (e.g. TC_{ID50} or hemagglutinating units) of test and control viruses. **Guidance –** Optimal concentrations of control virus material and antibody preparations may need to be re-established or confirmed with prolonged storage. Repeated freeze-thaw cycles should be avoided.	**Virology Standard of Practice 12 (VR S12): Viral Neutralization and Hemagglutination-Inhibition Assays** For neutralization and hemagglutination-inhibition (HI) assays, the laboratory must establish and use: a) optimal concentrations of antibodies or antisera; and b) appropriate concentrations (e.g. TCR_{ID50} or hemagglutinating units) of test and control viruses. **Guidance –** Optimal concentrations of control virus material and antibody preparations may need to be re-established or confirmed after prolonged storage (more than ten (10) years). Repeated freeze-thaw cycles should be avoided.

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Virology Sustaining Standard of Practice 18 (VRS18): Viral Neutralization and Hemagglutination-Inhibition (HI) Assay Controls For neutralization and HI assays, the laboratory shall include appropriate controls in each run (e.g. matched virus-antibody control; virus-diluent control; unmatched virus-antibody control; RBC controls; viral dose control).	**Virology Standard of Practice 13 (VRS13): Viral Neutralization and Hemagglutination-Inhibition Assay Controls** For neutralization and hemagglutination-inhibition (HI) assays, the laboratory must include the following controls in each run: a) matched virus-antibody control; b) virus-diluent control; c) unmatched virus-antibody control; d) RBC controls; and e) viral dose control
Virology Sustaining Standard of Practice 19 (VR S19): Rapid Centrifugation-enhanced Virus Culture Methods The laboratory shall: a) perform centrifugation using closed or sealed buckets or rotor; b) include in the SOPM the speed required to achieve the appropriate g-force for each type of rotor in use and record the centrifuge speed setting at the beginning of each run; c) include in the SOPM the acceptable temperature range for the centrifugation step. **Guidance –** c) Temperature-controlled centrifuges are preferred for these	**Standard deleted** **Microbiology Standard of Practice 2 (MB S2): Centrifugation Safety**

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procedures. Laboratories not using a temperature-controlled centrifuge should be aware of the potential for detrimental effects on culture monolayers and culture sensitivity resulting from over-heating of the centrifuge chamber. Laboratories using temperature-controlled centrifuges should not begin culture centrifugation until an appropriate temperature has been reached.	
Virology Sustaining Standard of Practice 20 (VR S20): Viral Culture Performance Monitoring The laboratory shall monitor key indicators of viral culture performance as defined in the QA Program and shall review these monthly and implement timely troubleshooting and remediation activities as necessary. **Guidance –** Appropriate key indicators of viral culture performance should be selected based on the scope of testing and the methods performed by the laboratory. Examples include: unacceptable observations/results with negative and positive controls; unanticipated variations in isolation rates; discrepancies between different methods; discrepancies with results obtained by other laboratories; shift or staff-related performance variations; rate of unsatisfactory or incomplete specimens; and, rate of contaminated specimens.	**Virology Sustaining Standard of Practice 14 (VR S14): Viral Culture Performance Monitoring** The laboratory must monitor key indicators of viral culture performance according to Quality Management System Standard of Practice 3 and must review these at least monthly and implement timely troubleshooting and remediation activities as necessary. **Guidance –** Appropriate quality indicators of viral culture performance should be selected based on the scope of testing and the methods performed by the laboratory. Examples include: unacceptable observations/results with negative and positive controls; unanticipated variations in isolation rates; discrepancies between different methods; discrepancies with results obtained by other laboratories; shift or staff-related performance variations; rate of unsatisfactory or incomplete specimens; and, rate of contaminated specimens.
Virology Sustaining Standard of Practice 21 (VR S21): Reporting Criteria	**Standard deleted** **Required under Microbiology Standard of Practice 6 (MB**

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Reports shall include qualifiers for cultures: a) that are incomplete or uninterpretable; b) which the isolate identification is considered presumptive, i.e. an isolate is not confirmed by a specific viral identification system. **Guidance –** a) A viral culture result cannot be interpreted as negative unless at least 50% of the expected area of monolayer coverage is still present (on the side of the tube, surface of the coverslip, or bottom of the well) and is of normal morphology at the end of the minimal incubation period (See VR S11). Negative culture results should include a qualifier in cases where optimal specimen transport time/conditions have not been met. b) Specific viral identification systems include immunologic or nucleic acid detection assays as well as genetically engineered cell line systems designed for the detection of viral agents.	**S6): Reports**

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