Microbiology

Standard	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).	
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.	
Microbiology Sustaining Standard of Practice 1 (MB S1): Quality Control Stock Cultures The laboratory shall maintain stock cultures for all quality control procedures.	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.

Microbiology

Standard	Guidance
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 documentation on the media label, package insert, technical manual, or other document, that the manufacturer's quality control practices conform to specifications; and, ii. 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.	CLSI Approved Standard, Document M22 can be utilized to establish criteria for testing each lot or shipment of commercially prepared media. Copies of CLSI Approved Standard, Document M22 can be obtained from the Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087 or www.clsi.org. Users of commercially prepared media should continue to monitor each shipment of media deemed "non-exempt" in the latest version of the CLSI document M22. Media may be tested concurrent with initial use provided QC results are reviewed prior to release of patient results.
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.	Media may be tested concurrent with initial use provided results are reviewed prior to release of patient results.

Microbiology

Standard	Guidance
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.	c) This testing should be repeated when changing vendors.
Microbiology Sustaining Standard of Practice 5 (MB S5): Media for Satellite Locations 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.	In compliance with Reagents Sustaining Standard of Practice 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.	

Microbiology

Standard	Guidance
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 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.	The protocol should be at least as stringent as that outlined in CLSI M50-A. 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. a) A review of historical data may be used for the verification study. Refer to Reagents Sustaining Standard of Practice 2 (REAG S2) and Reagents Sustaining Standard of Practice 3 (REAG S3) for quality control requirements for all other reagents.

Microbiology

Standard	Guidance
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.	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.
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, d) a recommendation for follow-up testing, if appropriate.	These requirements are in addition to those required by Reporting Sustaining Standard of Practice 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.

Microbiology

Standard	Guidance
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; 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.	Laboratories holding a clinical laboratory permit in either Bacteriology – Comprehensive or Virology –Comprehensive 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 (866) 325-7743. Laboratories serving NYC should also access the NYC Department of Health and Mental Hygiene's Health Alert Network (HAN); for information, contact 1-888-NYCMED9 or nycmed@health.nyc.gov. 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 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 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 www.cdc.gov. Laboratories must comply with infectious disease reporting requirements as outlined in the Public Health SS1: Reporting standard. 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)).

Microbiology

Standard	Guidance
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 documentation of its transfer including record of appropriate packing and shipping or destruction within seven days must be completed.	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.

Microbiology
Nucleic Acid Amplification (MNAA) Assay

Standard	Guidance
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/labcert/TestApproval/index.htm. These standards apply to all microbial agents including HIV and supplement the general systems standards that pertain to molecular test methods.	
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.	
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 e) include documentation of annual competency assessments for each individual performing molecular assays.	This standard includes requirements in addition to those stated in Human Resources Sustaining Standard of Practice 6 (HR S6) and Human Resources Sustaining Standard of Practice 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 of Practice 3 (QA S3).

Microbiology
Nucleic Acid Amplification (MNAA) Assay

Standard	Guidance
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 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.	**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 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 rooms for pre- and post-amplification activities. 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.

Microbiology
Nucleic Acid Amplification (MNAA) Assay

Standard	Guidance
Microbiology Nucleic Acid Amplification Assays Sustaining Standard of Practice 2 (MNA S2): Prevention and Remediation of Nucleic Acid Contamination - continued	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 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.	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.

Microbiology
Nucleic Acid Amplification (MNAA) Assay

Standard	Guidance
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.	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 (MNAA) Assay

Standard	Guidance
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 NYS-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 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.	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. 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/labcert/TestApproval/index.htm) for additional guidance related to assay control ranges or exemptions from use of inhibition controls.

Microbiology
Nucleic Acid Amplification (MNAA) Assay

Standard	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.	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 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/labcert/TestApproval/index.htm) for further information.

Microbiology
Nucleic Acid Amplification (MNAA) Assay

Standard	Guidance
Microbiology Nucleic Acid Amplification Assay Sustaining Standard of Practice 7 (MNA S7): Reports for Laboratory Developed and Modified FDA Approved Sequence-based Assays Reports shall describe the relationship between the observed result and the predicted phenotype.	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 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 available at http://www.wadsworth.org/labcert/TestApproval/index.htm for additional requirements.
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.	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 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.

Microbiology
Nucleic Acid Amplification (MNAA) Assay

Standard	Guidance
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) 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.	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.

Microbiology
Bacteriology

Standard	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 effective July 14, 2014.	Reference: Clinical Microbiology Procedures Handbook, Second Edition Update, 2007.
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.	c) Polyvalent antisera should be tested with at least one organism from each polyvalent group.
Bacteriology Standard of Practice 2 (BT S2): Urine Loops Non-disposable urine loops shall be calibrated monthly.	Calibration may be performed using a blue-dye methodology or by using a calibrated drill bit.
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.	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.

Microbiology
Bacteriology

Standard	Guidance
Bacteriology Standard of Practice 4 (BT S4): Aerobic Blood Cultures Macroscopically negative aerobic blood cultures shall be subcultured at some point before discarding.	Subcultures need not be done on blood cultures performed by radiometric methods or automated non-radiometric methods if the bottles are monitored for five days.
ANTIMICROBIAL SUSCEPTIBILITY TESTING: DISK DIFFUSION AND MINIMAL INHIBITORY CONCENTRATION	
Bacteriology Standard 5 (BT S5): Defining Antibiotic Panels Antibiotic panels appropriate to the specimen source and organism isolated shall be defined.	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.
Disk diffusion methods (Standards 6-9)	It is recommended that laboratories performing disk diffusion testing have access to the latest CLSI guidelines (M02) and performance standards (M100).
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.	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 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.	If performed concurrently with patient testing, QC results should be reviewed prior to release of patient results.

Microbiology
Bacteriology

Standard	Guidance
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.	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 used.
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.	c) Zone sizes may be measured using a ruler, sliding calipers, templates, or other appropriate measurement devices prepared for this purpose. d) The laboratory may establish zone diameter ranges or may use the zone diameter ranges provided in the current CLSI Approved Standards, Performance Standards for Antimicrobial Susceptibility Testing.

Microbiology
Bacteriology

Standard	Guidance
Minimal inhibitory concentration methods (MIC) (standards 10-11)	It is recommended that laboratories performing MIC testing have access to the latest CLSI guidelines (M07) and performance standards (M100).
Bacteriology Standard of Practice 10 (BT S10): Reagent QC for MIC methods Each batch of macrodilution tubes, microdilution plates, concentration strips, and agar dilution plates used for MIC antimicrobial susceptibility testing shall be checked with the appropriate control strains before, or concurrent with, initial use using approved reference organisms.	If performed concurrently with patient testing, QC results should be reviewed prior to release of patient results.
Bacteriology Standard of Practice 11 (BT S11): MIC Quality Control Frequency, Assessment and Recording For MIC antimicrobial susceptibility 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 or by satisfactory testing of control strains each day of use for at least 20 days; c) record the actual MIC obtained during quality control testing; and d) verify quality control results are within established acceptable ranges.	a) Consult CLSI documents for appropriate quality control strains and acceptable guidelines for weekly QC testing. d) The established MIC range is the acceptable interpretive criteria for that drug-microorganism combination used for QC. The laboratory may establish MIC ranges or the laboratory may use the MIC ranges provided in the current CLSI Approved Standards, Performance Standards for Antimicrobial Susceptibility Testing.

Microbiology
Mycobacteriology

Standard	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 June 27, 2012, TB S5 revised and effective July 14, 2014	
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.	Operational guidelines for biological safety cabinets can be found in the Safety Standards of the General Systems Standards Part 1.
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.	

Microbiology
Mycobacteriology

Standard	Guidance
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 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.	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 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.	

Microbiology
Mycobacteriology

Standard	Guidance
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.	Carbol fuchsin stain is the preferred method 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.	a) Reporting time should be periodically monitored to ensure compliance. b) Culture is necessary due to the limited sensitivity of microscopy.
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.	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 Mycobacteriology Sustaining Standard of Practice 15 (TB S15). b) The patient smear history can be reviewed in the LIMS system of the referring laboratory.

Microbiology
Mycobacteriology

Standard	Guidance
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.	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.
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.	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 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).	

Microbiology
Mycobacteriology

Standard	Guidance
Mycobacteriology Sustaining Standard of Practice 12 (TB S12): 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 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.	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 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 molecular methods to identify *M. avium* complex and *M. gordonae*.	Identification of *M. avium* complex or *M. gordonae* by biochemical methods is not satisfactory. However, mass spectroscopy, HPLC, and new technologies are acceptable as long as they are appropriately validated and approved by NYS Clinical Laboratory Reference System or cleared by the FDA.

Microbiology
Mycobacteriology

Standard	Guidance
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.	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 (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/labcert/regaffairs/clinical/commdiseaseguide.pdf 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.

Microbiology
Mycobacteriology

Standard	Guidance
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.	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 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.	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 against the following first-line tuberculosis drugs: - Rifampin (RMP) - Isoniazid (INH) - Pyrazinamide (PZA) - Ethambutol (EMB)	For all isolates identified as *M. tuberculosis* complex: If the laboratory does not perform pyrazinamide (PZA) susceptibility testing, the isolate should be submitted within 24 hours to a New York State permitted laboratory for PZA testing.

Microbiology
Mycobacteriology

Standard	Guidance
Mycobacteriology Sustaining Standard of Practice 19 (TB S19): Verification of Reagents for Susceptibility Testing For anti-mycobacterial susceptibility tests, the laboratory 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.	It is recommended that laboratories performing susceptibility testing have access to the latest CLSI guideline, M24.
Mycobacteriology Sustaining Standard of Practice 20 (TB S20): Identification of the members of the *M. tuberculosis* complex Laboratories performing susceptibility testing of *M. tuberculosis* complex for first-line tuberculosis drugs shall ensure a final identification of all members of the *M. tuberculosis* complex.	The requirement for identification can be met by in-house testing or by submission of the isolate to an appropriate New York State permitted laboratory.

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Mycobacteriology

Standard	Guidance
Mycobacteriology Sustaining Standard of Practice 21 (TB S21): Second-Line Drugs Additional susceptibility testing shall be performed using second-line drugs for all initial positive cultures of *M. tuberculosis* complex from newly diagnosed patients if resistance is detected for one or more first-line drugs, with the exception of mono-PZA resistance. 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.	Whenever possible, the initial positive culture (i.e., 3 ml broth aliquot or slant) should be immediately submitted and a subculture should be retained in the originating laboratory.
Mycobacteriology Sustaining Standard of Practice 22 (TB S22): Reporting First-Line Drugs Susceptibility test results for first-line drugs shall be reported within 24 hours of findings. If applicable, the report shall specify that second line drug susceptibility testing is being performed.	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 The turn-around time between receipt of the primary specimen and reporting of susceptibility results for first line drugs shall not exceed 28 days for 80% of such specimens.	The lab receiving the primary specimen is responsible for ensuring that the turn around time requirement is met. This standard applies to laboratories performing smear only and laboratories performing susceptibility testing.

Microbiology
Mycology

Standard	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. Effective July 14, 2014	
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.	Additional required use of the BSC should be established by the laboratory director based on an infectious agent risk assessment (refer to Safety Standards).
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
Mycology

Standard	Guidance
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.	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 Sustaining Standard of Practice 4 (MYS4): Culture Incubation The mycology laboratory shall: a) incubate cultures at 30 ± 2° C; and b) provide sufficient humidity to prevent drying of inoculated plates.	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.	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. e) CLSI M54 provides additional information regarding appropriate media selection.

Microbiology
Mycology

Standard	Guidance
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).	
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.	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.
Mycology Sustaining Standard of Practice 8 (MY S8): Reference Material 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	
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.	The laboratory should establish frequency of QC checks based upon published guidelines from consensus organizations such as CLSI M54-A or other professional organizations.

Microbiology
Mycology

Standard	Guidance
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 c) verify that the results for the control organism(s) are within established limits before reporting patient results.	Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) approved methods include guidelines for appropriate quality control strains.
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.	Interpretative guidelines based upon the CLSI or EUCAST documents or FDA approved commercial products may be used.

Microbiology
Parasitology

Standard	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. Effective July 25, 2012	
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.	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, refrigerated or in Cary-Blair transport medium depending on the assay utilized. Consult the package insert or NYS-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.	Controls may be obtained commercially, previously tested patients or proficiency-testing samples.

Microbiology
Parasitology

Standard	Guidance
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 control and one negative control each time the test is performed.	Positive and negative controls supplied with commercially available tests are acceptable. Minimal control requirements for single use devices are described in the Quality Control Design and Process Quality Control Standards in Part 1 of the General Systems.
Parasitology Sustaining Standard of Practice 4 (PS4): Ocular Micrometer Calibration Laboratories shall calibrate ocular micrometers: a) annually for each objective; and b) with any change in objective or eyepiece.	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 Sustaining Standard of Practice 5 (PS5): Ova and Extracellular Parasite Measurement 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.	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.

Microbiology
Parasitology

Standard	Guidance
Parasitology Sustaining Standard of Practice 6 (PS6): Examination of Blood Smears The SOP for identification of blood borne parasites should 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.	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 (PS7): 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.	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*.	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.

Microbiology
Parasitology

Standard	Guidance
Parasitology Sustaining Standard of Practice 9 (PS9): 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.	It is recommended that all specimens be confirmed by another method.
Parasitology Sustaining Standard of Practice 10 (PS10): 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.	Many parasites can be easily missed if a single 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.
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.	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.

Microbiology
Virology

Standard	Guidance
These standards apply to **conventional tube culture** and to **centrifugation-enhanced rapid** methods unless otherwise specified. All laboratories performing viral culture using conventional tube cultures and/or rapid culture modifications (e.g. shell vials), shall additionally comply with the applicable requirements contained in the General Systems Standards and with designated Microbiology Standards.	
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.	
Virology Sustaining Standard of Practice 1 (VR S1): Cell Culture Systems The laboratory shall utilize cell cultures and methods appropriate for the isolation and/or detection of the viral agents specified in its test menu.	Several texts and resources are available including: CLSI M41-A, 26 (35): Viral Culture; Approved Guideline
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.	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 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.

Microbiology
Virology

Standard	Guidance
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.	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 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.	Documentation by the vendor that the cells are free of mycoplasma contamination is acceptable for commercially supplied cell lines.
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.	The type of medium refers to its formulation, e.g. Eagle's MEM with 2% FBS.

Microbiology
Virology

Standard	Guidance
Virology Sustaining Standard of Practice 6 (VR S6): Cell Culture Medium Quality Control 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.	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.
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.	Refer to Safety Sustaining Standard of Practice 6 (Safety S6) for operational guidelines.
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.	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).

Microbiology
Virology

Standard	Guidance
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.	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 of Practice 1 (QMS S1)(t) and Reagents Sustaining Standard of Practice 4 (Reagent S4).

Microbiology
Virology

Standard	Guidance
Virology Sustaining Standard of Practice 10 (VR S10): Specimen Criteria The SOPM shall define the remedial action that may be employed for specimens that exhibit contamination or toxicity.	Remedial action for specimens causing adverse culture events (e.g. contamination, toxicity) may include reinoculation, sub-passage, filtration, recollection).
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)).	a) Inoculation includes specimen adsorption conditions, if applicable. c) Observation includes the microscopic examination of 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.

Microbiology
Virology

Standard	Guidance
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.	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 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 of Practice 6 (VR S6)).

Microbiology
Virology

Standard	Guidance
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.	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 of Practice 20 (VR S20)). 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 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).
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.	
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.	Negative RBC controls are intended to determine whether the RBCs react with uninoculated cell culture monolayers. Virology Sustaining Standard of Practice 12 (VRS 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.

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Virology

Standard	Guidance
Virology Sustaining Standard of Practice 16 (VR S16): Confirmation and Identification of Cultured Viruses Each run shall include a: a) positive control; b) lot-matched negative (uninoculated) cell culture control.	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 uninoculated cells (see Virology Sustaining Standard of Practice 12 (VR S12)) 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.

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Virology

Standard	Guidance
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.	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 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 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.	c) Temperature-controlled centrifuges are preferred for these 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.

Microbiology
Virology

Standard	Guidance
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.	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 21 (VR S21): Reporting Criteria 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.	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 Virology Sustaining Standard of Practice 11 (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.