# *Microbiology Categories*

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| **Microbiology Standard of Practice 1 (MB S1): Biological Safety Cabinet**<br>A class II or higher biological safety cabinet (BSC) must be used when:<br>a) processing specimens submitted for mycobacteriological testing, including slide preparation or handling unsealed mycobacteriology cultures;<br>b) processing patient specimens submitted for isolation of pathogenic fungi or handling cultures of pathogenic fungi;<br>c) inoculating cell cultures with clinical specimens and for all procedures involving the maintenance and processing of inoculated cell cultures and culture-amplified materials; or<br>d) performing any other procedures that have the potential to create infectious aerosols. | 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. |
| **Microbiology Standard of Practice 2 (MB S2): Centrifugation Safety**<br>For all mycobacteriology, mycology and virology procedures involving centrifugation of potentially infectious materials:<br>a) aerosol-free centrifuge cups must be used; and<br>b) centrifuge cups must be opened in a class II or higher | Proper safety practices are important for centrifugation of shell vial cultures. |

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| biological safety cabinet (BSC). | |
| **Microbiology Standard of Practice 3 (MB S3): Quality Control Stock Cultures**<br>The laboratory must have quality control procedures for all stocks cultures to minimize contamination or alteration of relevant characteristics. | 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.<br>Validated patient isolates, proficiency testing specimens, or commercially prepared controls may be used unless otherwise required by manufacturer. |
| **Microbiology Standard of Practice 4 (MB S4): Microbial Growth Medium**<br>Each lot or shipment of commercially prepared or in-house prepared media must be tested:<br>a) on-site for growth, selectivity, and/or inhibition and biochemical responses; or<br>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:<br>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 | Media may be tested concurrent with initial use provided QC results are reviewed prior to release of patient results. |

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| ii. the laboratory documents receipt and condition of each shipment or lot of media, and notifies the media manufacturer or in-house preparer of:<br>- cracked Petri dishes;<br>- unequal filling of plates;<br>- cracked media in plates;<br>- hemolysis;<br>- freezing;<br>- excessive number of bubbles; or<br>- contamination. | |
| **Microbiology Standard of Practice 5 (MB S5): Expiration Date Prepared In-House**<br>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 Standard of Practice 6 (MB S6): Reports**<br>In addition to the requirements in Reporting Standard of Practice 2, test reports must include:<br>a) the test method;<br>b) qualifiers for viral cultures that are incomplete or uninterpretable or when isolate identification is | a) Examples of assay methodology include culture, EIA, PCR, etc. Specific test systems are not required to be listed on a test report. |

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| considered presumptive, i.e., an isolate is not confirmed by a specific viral identification system; and<br>c) a recommendation for follow-up testing, if appropriate. | |
| **Microbiology Standard of Practice 7 (MB S7): Laboratory Response Network**<br>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:<br>a) maintaining updated guidelines and protocols related to the testing, identification and reporting of select and emerging infectious agents including information regarding special handling and safety practices to be employed;<br>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;<br>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<br>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 | 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.<br>Information regarding laboratory testing for select and emerging infectious agents is available to all laboratories on the American Society of Microbiology website.<br>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 posts these announcements on the HCS.<br>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.<br>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.<br>Laboratories must comply with infectious disease reporting |

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| handling is suspected. | requirements as outlined in the Public Health Reporting Standards of Practice 1 and 2. |
| **Microbiology Standard of Practice 8 (MB S8): Select Agent Inventory**<br>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 its transfer including record of appropriate packing and shipping or destruction within seven (7) 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.<br>Laboratories must comply with pertinent items of the Select Agent Rule (e.g., disposal/transfer of select agents)<br>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. |

# *Microbiology Nucleic Acid (MNA) Amplification Assay*

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| **Microbiology Nucleic Acid Amplification Assay Standard of Practice 1 (MNA S1): Quality Control Samples for Laboratory Developed Tests**<br><br>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:<br><br>a) one (1) control capable of detecting amplification inhibition by patient specimens unless the LDT approved by the Department exempts the requirement;<br><br>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);<br><br>c) for qualitative multi-target assays at least one (1) specific target positive control;<br><br>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<br><br>e) additional negative controls in laboratories that | This standard applies to controls to be used with laboratory developed tests (LDTs).<br><br>Information on Departmental approval of LDTs is available at:<br><br>https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.<br><br>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.<br><br>For laboratories preparing mastermix to be used on multiple instruments, the template-free mastermix control should be utilized for each run of each instrument.<br><br>a) Inhibition controls may be excluded if there are sufficient 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.<br><br>a) Inhibition controls are not required if the run includes isolates only and not patient specimens.<br><br>b, c)<br>A low-range positive is defined as having a value of not |

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| manipulate amplicon but do not use separate rooms for pre- and post-amplification processes. | more than ten (10) fold above the assay detection limit.<br>b, c)<br>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.<br>d) Processes that involve manipulation of amplicon include conventional PCR and nucleic acid sequencing.<br>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). |
| **Microbiology Nucleic Acid Amplification Assay Standard of Practice 2 (MNA S2): Quality Control Samples for Sequencing Assays**<br>Each sequencing assay must include a:<br>a) negative amplification control;<br>b) negative sequencing control;<br>c) positive sequencing control; and<br>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 | Information on Departmental approval of LDTs is available at:<br>https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.<br>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.<br>a, b)<br>The negative amplification control may also be used as the negative sequencing control.<br>c) 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 |

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| developed test (LDT). | sequencing controls.<br>d) Positive amplification controls and inhibition controls are not necessary when performing sequencing on clinical isolates.<br>d) Positive amplification and inhibition controls may be omitted when testing primary specimens for genotyping assays for prognostic purposes. |
| **Microbiology Nucleic Acid Amplification Assay Standard of Practice 3 (MNA S3): Reports for Laboratory Developed Sequence-based Assays**<br>In addition to the requirements in Reporting Standard of Practice 2, reports must include disclaimers on all infectious agent-based sequencing tests from primary specimens that have no amplification or genotype results. | Positive amplification and inhibition controls may be omitted when testing primary specimens for genotyping assays for prognostic purposes. For assays that lack positive amplification and inhibition controls, a disclaimer on the report is needed to document that the result may be due to the following: infectious agent was below the limit of detection in the sample, mutations were present in the genome, or inhibitors were present in the sample that prevented amplification. |
| **Microbiology Nucleic Acid Amplification Assay Standard of Practice 4 (MNA S4): Task Separation for FDA-Approved Closed System Amplification Test**<br>For a closed system amplification test (CSAT), the laboratory must:<br>a) handle, process, and store clinical specimens, reagents and supplies in a manner that prevents exposure to amplicon, plasmids, and culture-amplified materials; and | 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, and then the testing process is initiated with no additional external manipulation or addition of reagents unless approval is received by the Department.<br>CSAT instrumentation should be segregated from areas in which specimens are routinely processed in order to avoid cross-contamination. |

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| b) locate the instrument in an area free of open amplicon systems. | 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. |

## *Bacteriology*

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| Laboratories that perform testing for *Chlamydia* culture must follow applicable Virology Culture standards. | |
| **Bacteriology Standard of Practice 1 (BT S1): Reagent Quality Control**<br>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:<br>a) each day of use for beta-lactamase and all stains other than Gram stain;<br>b) each week of use for Gram stain; and<br>c) every six (6) months for antisera. | c) Polyvalent antisera should be tested with at least one (1) organism from each polyvalent group. |
| **Bacteriology Standard of Practice 2 (BT S2): Urine Culture Inoculating Loops**<br>Verification of calibration of non-disposable urine culture inoculating loops must be performed monthly or done as specified in manufacturer instructions. | 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**<br>The environmental conditions of anaerobic bags, jars, and glove boxes must 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. |

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| **Bacteriology Standard of Practice 4 (BT S4): Aerobic Blood Cultures**<br>Macroscopically negative aerobic blood cultures must be subcultured before discarding. | Subcultures need not be done on blood cultures if the bottles are monitored for five (5) days. |
| ***Antimicrobial susceptibility Testing: Disk diffusion and minimal inhibitory concentration*** | |
| **Bacteriology Standard 5 (BT S5): Defining Antibiotic Panels**<br>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. | 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)** | |
| **Bacteriology Standard of Practice 6 (BT S6): Media Quality Control for Disk Diffusion Methods**<br>Each batch of media used for antimicrobial susceptibility testing must be verified with the appropriate reference organisms strains before, or concurrent with, initial use. | If performed concurrently with patient testing, quality control (QC) results must be reviewed prior to release of patient results. |
| **Bacteriology Standard of Practice 7 (BT S7): Antibiotic Quality Control for Disk Diffusion Methods**<br>Each new lot of antimicrobial disks must be verified with the appropriate reference organisms before, or concurrent with, initial use. | If performed concurrently with patient testing, quality control (QC) results must be reviewed prior to release of patient results. |

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| **Bacteriology Standard of Practice 8 (BT S8): Antibiotic Disk Distribution**<br>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. | 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*), 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. |
| **Bacteriology Standard of Practice 9 (BT S9): Disk Diffusion Quality Control Frequency, Assessment and Recording**<br>For antimicrobial susceptibility disk diffusion testing, the laboratory must:<br>a) use the appropriate control organism(s) each day of testing; and<br>b) record zone sizes for each antimicrobial quality control test; or<br>c) meet the requirements of Quality Control Standard of Practice 2, 3 and 4; and<br>d) document that quality control results are within established zone diameter ranges. | b) Zone sizes may be measured using a ruler, sliding calipers, templates, or other appropriate measurement devices.<br>d) The laboratory may establish zone diameter ranges using relevant references. |

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| **Minimal inhibitory concentration methods (MIC) (standards 10-11)** | |
| **Bacteriology Standard of Practice 10 (BT S10): Reagent Quality Control for Minimum Inhibitory Concentration Methods**<br>Each batch of materials and reagents used for minimum inhibitory concentration (MIC) antimicrobial susceptibility testing must be verified with the appropriate reference organisms before, or concurrent with, initial use. | |
| **Bacteriology Standard of Practice 11 (BT S11): Minimum Inhibitory Concentration Quality Control Frequency, Assessment and Recording**<br>For minimum inhibitory concentration (MIC) antimicrobial susceptibility testing, the laboratory must:<br>a) use the appropriate control organism(s) each day of testing; and<br>b) record the MIC values for each antimicrobial quality control test; or<br>c) meet the requirements of Quality Control Standard of Practice 2, 3, and 4; and<br>d) document that quality control results are within established MIC ranges. | d) The established MIC range is the acceptable interpretive criteria for that drug-microorganism combination used for quality control. |

# *Mycobacteriology*

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| **Mycobacteriology Standard of Practice 1 (TB S1): Specimen Centrifugation**<br>Specimens must be centrifuged for a minimum of fifteen (15) minutes at greater than or equal to 3,000 x g. | |
| **Mycobacteriology Standard of Practice 2 (TB S2): Cross-Contamination**<br>The laboratory must design procedures that minimize the possibility of cross-contamination including, but not limited to:<br>a) opening and manipulating only one (1) patient specimen at a time in a biological safety cabinet (BSC); and<br>b) excluding positive control organisms from the BSC while patient specimens are being processed. | Batch staining with jars or dishes should not be utilized. This does not apply to automated staining systems. |
| **Mycobacteriology Standard of Practice 3 (TB S3): Staining Quality Control**<br>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 Standard of Practice 4 (TB S4): Fluorochrome Stains**<br>Laboratories using fluorochrome staining must confirm positive | Carbol fuchsin is the preferred stain for confirmation in newly diagnosed patients. |

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| results in newly diagnosed patients by:<br>a) carbol fuchsin stain; or<br>b) independent evaluation by a second person. | |
| **Mycobacteriology Standard of Practice 5 (TB S5): Reporting Smear Results**<br>In addition to report requirements in Reporting Standard of Practice 2, reports of all smear stain results must:<br>a) be communicated to the ordering physician or other authorized person within thirty (30) hours of the receipt of the specimen; and<br>b) indicate that culture is being performed. | a) Reporting time should be periodically monitored to ensure compliance. |
| **Mycobacteriology Standard of Practice 6 (TB S6): Laboratories testing only Smears - Specimen Submission and Result Notification**<br>Laboratories testing only smears must:<br>a) refer all specimens for culture to a laboratory holding a valid New York State clinical laboratory permit in the category of Mycobacteriology; and<br>b) notify the reference laboratory if the specimen being sent is the first smear positive specimen from the patient. | b) This notification is essential so that the reference laboratory can comply with Mycobacteriology Sustaining Standard of Practice 13 (TB S13).<br>b) The patient smear history can be reviewed in the laboratory information system of the referring laboratory. |

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| **Mycobacteriology Standard of Practice 7 (TB S7): Retention of Stained Slides**<br>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. | 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 Standard of Practice 8 (TB S8): Nucleic Acid Amplification**<br>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.<br>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. | Non-amplified nucleic acid assays do not satisfy this standard.<br>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 Standard of Practice 9 (TB S9): Media**<br>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). | |

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| **Mycobacteriology Standard of Practice 10 (TBS 10): Culture Purity**<br>To ensure that positive liquid cultures are not contaminated, the laboratory must perform:<br>a) acid fast staining microscopy; and<br>b) plating on enriched, non-selective culture media (e.g., Chocolate agar). | |
| **Mycobacteriology Standard of Practice 11 (TBS 11): Retention of Isolates**<br>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. | Multiple isolates may be requested from the same patient for public health investigation.<br>Isolates may be retained on appropriate media and stored at -70 to -80 degrees Celsius. |
| **Mycobacteriology Standard of Practice 12 (TB S12): Identifying *M. avium* complex and *M. gordonae***<br>Detection of *M. avium* complex and *M. gordonae* by biochemical methods must be confirmed by another method. | |
| **Mycobacteriology Standard of Practice 13 (TB S13): Submission of Isolates to a Public Health Laboratory**<br>Laboratories must submit to either the Wadsworth Center or the New York City (NYC) Public Health Laboratory: | Isolates recovered from patients residing in New York City (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 |

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| 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<br>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. | Submission Requirements for Communicable Diseases available at http://www.wadsworth.org/regulatory/clep/laws.<br>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.<br>b) A change in drug susceptibility may be identified by the health care provider or through the patient's history. |
| **Mycobacteriology Standard of Practice 14 (TB S14): Referral of Positive Isolates for Susceptibility Testing**<br>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. | Whenever possible, the initial positive culture (i.e., equal to or greater than one (1) ml broth aliquot or slant) should be submitted and a subculture should be retained in the originating laboratory<br>The submitting laboratory should provide the date of identification to the reference laboratory.<br>For laboratories not performing susceptibility testing in-house, the submitting laboratory must periodically monitor whether the interval between the initial identification and the receipt of culture by the reference laboratory is acceptable. |

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| **Mycobacteriology Standard of Practice 15 (TB S15): Culture-Based Susceptibility Testing**<br>Susceptibility testing must 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 Standard of Practice 16 (TB S16): First-Line Tuberculosis Drugs**<br>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.<br>All isolates predicted to be resistant by nucleic acid-based methods must be confirmed by culture-based susceptibility testing.<br>All isolates predicted to be sensitive by nucleic acid-based methods other than whole genome sequencing must be confirmed by culture-based susceptibility testing.<br>If the laboratory does not perform pyrazinamide susceptibility testing, the isolate must be submitted within one (1) business day for pyrazinamide testing to a laboratory holding a valid New York State clinical laboratory permit in the category of Mycobacteriology. | |

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| **Mycobacteriology Standard of Practice 17 (TB S17): Verification of Reagents for Culture-Based Susceptibility Testing**<br>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:<br>a) establish limits for acceptable control results;<br>b) use the appropriate control organism(s) to check the procedure each week tests are performed;<br>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;<br>d) verify that the results for the control organism(s) are within established performance specifications before reporting patient results; and<br>e) document the results of all control procedures performed. | |

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| **Mycobacteriology Standard of Practice 18 (TB S18): Second-Line Drugs**<br>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.<br>All isolates predicted to be resistant by nucleic acid-based methods must be confirmed by culture-based susceptibility testing.<br>Isolates predicted to be sensitive by nucleic acid-based methods, other than whole genome sequencing, must be confirmed by culture-based susceptibility testing. | |
| **Mycobacteriology Standard of Practice 19 (TB S19): Reporting Susceptibility Test Results**<br>All susceptibility test results must be reported within one (1) business day of findings. | Test results for susceptibility to first line drugs should not be held pending the results of the additional testing. |

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| **Mycobacteriology Standard of Practice 20 (TB S20): Turnaround Time for Susceptibility Testing**<br>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.<br>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. | **Mycobacteriology Standard of Practice 20 (TB S20): Turnaround Time for Susceptibility Testing**<br>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.<br>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. |

# *Mycology*

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| **Mycology Standard of Practice 1 (MY S1): Microscopy of Primary Specimens**<br>If primary specimens are used for the detection of fungal elements by microscopy, it must include appropriate mounting medium or stain. | 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 Standard of Practice 2 (MYS2): Culture Incubation**<br>The mycology laboratory must:<br>a) incubate cultures at 30 ± 2 degrees Celsius unless otherwise instructed by the manufacturer; and<br>b) provide sufficient humidity to prevent drying of inoculated plate. | b) Insufficient humidity is evident when agar is cracked before the end of the incubation period. |
| **Mycology Standard of Practice 3 (MYS3): Culture Media for Isolation of Molds and Yeasts**<br>Laboratories that perform culture must 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):<br>• general purpose media such as: Sabouraud dextrose agar--no antibacterial or antifungal agents;<br>• general purpose media with cycloheximide such as: Sabouraud dextrose agar - with antibacterial agents and cycloheximide;<br>• enriched media such as: BHI agar, SABHI agar; and |

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| | • 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 4 (MY S4): Identification of Pathogenic Molds**<br>Methods for identification of pathogenic molds must include:<br>a) a medium to stimulate production of characteristic spores and biochemical tests to differentiate fungi; or<br>b) a nucleic acid method; or<br>c) a MALDI-TOF mass spectrometry method. | |
| **Mycology Standard of Practice 5 (MY S5): Identification of Pathogenic Yeasts**<br>Methods for identification of pathogenic yeasts must include:<br>a) media for phenotypic tests; or<br>b) a nucleic acid method; or<br>c) a MALDI-TOF mass spectrometry method. | 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. |

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| **Mycology Standard of Practice 6 (MY S6): Reference Material**<br>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. | |
| **Mycology Standard of Practice 7 (MY S7): Quality Control of Stains**<br>The laboratory must check the reactivity of each batch (prepared in-house), lot number (commercially prepared), and shipment when prepared or opened by using:<br>a) a positive control for lactophenol cotton blue;<br>b) positive and negative control organisms for all stains. | |
| **Mycology Standard of Practice 8 (MY S8): Antifungal Susceptibility Testing Quality Control**<br>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:<br>a) utilize antibiotic panels appropriate to the specimen source and organism isolated;<br>b) verify manufacturer's limits or establish limits for acceptable control results; | |

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| c) verify each batch of media used for antimicrobial susceptibility testing with the appropriate reference organism strains before, or concurrent with, initial use; and<br>d) document that quality control (QC) results are within established limits before reporting patient results. | |

# *Parasitology*

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| **Parasitology Standard of Practice 1 (PS S1): Stool Specimen Preservation for Morphological Examination**<br>Stool specimens to be used for parasitological identification based on morphology must be:<br>a) examined within one (1) hour of collection; or<br>b) preserved within one (1) hour of collection using the fixative appropriate for the test being ordered; or<br>c) refrigerated for no more than three (3) hours to preservation. | 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.<br>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. |
| **Parasitology Standard of Practice 2 (PS S2): Quality Controls for Staining**<br>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. | Controls may be obtained commercially, previously tested patients or proficiency-testing samples as long as the sample used demonstrates the characteristics of the stain. |

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| **Parasitology Standard of Practice 3 (PS S3): Ocular Micrometer Calibration**<br>Laboratories must calibrate ocular micrometers:<br>a) annually for each objective; and<br>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.<br>a) The standard operating procedure should contain instructions for calibration and examples to show how each objective is calibrated. |
| **Parasitology Standard of Practice 4 (PS S4): Ova and Extracellular Parasite Measurement**<br>Using a calibrated ocular micrometer, laboratories must:<br>a) measure and document the size of all ova and extracellular protists; and<br>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. *Cryptosporidium* and *Cyclospora*).<br>a) Documentation may be on worksheets or electronic records.<br>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 Standard of Practice 5 (PS S5): Examination of Blood Smears**<br>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:<br>a) a description of the number of fields that need to be examined for both positive and negative results; and | Giemsa stain is recommended; however, Wright's stain or a Wright-Giemsa combination stain may also be used.<br>a) an adequate number of fields [e.g., 300 oil immersion fields (10x100)] should be examined under oil immersion before calling a specimen negative. |

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| b) examination at 10X for the detection of filarids, if such testing is indicated. | |
| **Parasitology Standard of Practice 6 (PS S6): Reference Material**<br>A reference collection of slides, photographs, or gross specimens of identified parasites must be readily available in the laboratory for comparison with diagnostic specimens. | 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 Standard of Practice 7 (PS S7): Report Content**<br>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 *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 most common parasites in New York State are not tested for when an Ova and Parasite test is performed.<br>If a test for a specific organism is ordered, (e.g. Giardia antigen detection) this standard does not apply. |
| **Parasitology Standard of Practice 8 (PS S8): Single-Use Antigen Assays**<br>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. | It is recommended that all specimens be confirmed by another method. |

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| **Parasitology Standard of Practice 9 (PS S9): Reporting Negative Results**<br>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 parasitic infection. | 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. |

# *Virology*

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| **Virology Standard of Practice 1 (VR S1): Cell Culture Systems**<br>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 2 (VR S2): Cell Culture Records**<br>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.<br>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 maintained for cell cultures prepared in house.<br>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. | 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). |

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| **Virology Standard of Practice 3 (VR S3): Cell Culture Shelf Life and Condition**<br>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:<br>a) the expiration date, if provided by the vendor; or<br>b) seven (7) days after the monolayers become one hundred (100) percent confluent; or<br>c) seven (7) days of receipt, if monolayers were one hundred (100) percent confluent when received. | Slightly sub-confluent monolayers are optimal for viral culture. Crowded, overgrown, or aged cultures may result in decreased virus susceptibility. |
| **Virology Standard of Practice 4 (VR S4): Mycoplasma Screening of Cell Cultures**<br>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.<br>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. | |

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| **Virology Standard of Practice 5 (VR S5): Cell Culture Medium Quality Control**<br>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.<br>The laboratory must:<br>a) perform in-house sterility and toxicity testing on commercially acquired media prior to use, if components are added to the media;<br>b) visually inspect media containing a colorimetric pH indicator to confirm an acceptable pH range;<br>c) investigate and document viral growth inhibition by a media component if decreased viral sensitivity is observed; and<br>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 Standard of Practice 6 (VR S6): Cell Culture and Culture Medium Lot Numbers**<br>Dates and activities of all culture manipulations and microscopic observations must be recorded. | Culture manipulations include inoculation, medium changes, subpassage and harvesting. |

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| **Virology Standard of Practice 7 (VR S7): Viral Culture Criteria and Timepoints**<br>In addition to the requirements in Test Procedure Content Standard of Practice 1, the standard operating procedure must define all aspects of culture including:<br>a) inoculation and incubation conditions (e.g., temperature);<br>b) minimal culture duration;<br>c) minimally acceptable culture observation (every three (3) days for conventional culture) and documentation schedules;<br>d) maintenance/processing schedules; and<br>e) remedial action needed for specimens exhibiting contamination or toxicity. | a) Inoculation includes specimen adsorption conditions, if applicable.<br>c) Observation includes the microscopic examination of monolayers for evidence of cytopathic effect (CPE), toxicity and contamination.<br>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.<br>d) Maintenance and processing include media changes, hemadsorption, harvesting, and staining procedures.<br>e) Remedial action for specimens causing adverse culture events (e.g. contamination, toxicity) may include re-inoculation, sub-passage, filtration, or recollection). |
| **Virology Standard of Practice 8 (VR S8): Negative Cell Culture Controls**<br>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. | Uninoculated (negative) controls are observed for evidence of unacceptable occurrences such as monolayer deterioration, cytopathic effect (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.<br>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. |

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| | When using centrifugation-enhanced rapid methods, processing includes the centrifugation step. |
| **Virology Standard of Practice 9 (VR S9): Positive Live Virus Culture Controls**<br>For non-genetically engineered cell lines, the laboratory must:<br>a) identify and monitor appropriate quality indicators for identifying cell culture performance; and<br>b) employ live-virus positive controls to check culture sensitivity if quality indicators suggest an otherwise unexplained decreased in virus detection. | See Virology Standard of Practice 14 (VR S14): Viral Culture Performance Monitoring for additional information.<br>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.<br>Virus-inoculated (positive) culture controls need to be employed for conventional virus culture when:<br>• performance /sensitivity/susceptibility issues are observed or suspected;<br>• assessing a new cell culture type or source; and<br>• training and remediation activities.<br>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 Standard of Practice 10 (VR S10): RBC Controls for Hemadsorption (HAd) Assays**<br>Red Blood Cell (RBC) controls in each HAd run must include:<br>a) a negative control that is an uninoculated cell culture monolayer that is lot-matched to that used for patient | Negative RBC controls are intended to determine whether the 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.<br>Positive RBC controls should confirm that the RBCs react in |

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| specimens; and<br>b) at least one (1) positive control per lot number of RBCs. | the expected manner with virus-inoculated monolayers. |
| **Virology Standard of Practice 11 (VR S11): Confirmation and Identification of Cultured Viruses**<br>When cytopathic effect in culture is observed, virus confirmation or identification must include a:<br>a) positive control; and<br>b) lot-matched negative (uninoculated) cell culture control. | a) The positive control is intended to confirm detection system reactivity.<br>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.<br>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 utilized as needed for troubleshooting purposes if, for example, the negative control yields positive or unacceptable results. |
| **Virology Standard of Practice 12 (VR S12): Viral Neutralization and Hemagglutination-Inhibition Assays**<br>For neutralization and hemagglutination-inhibition (HI) assays, the laboratory must establish and use:<br>a) optimal concentrations of antibodies or antisera; and<br>b) appropriate concentrations (e.g. $TCR_{ID50}$ 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 after prolonged storage (more than ten (10) years). Repeated freeze-thaw cycles should be avoided. |

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| **Virology Standard of Practice 13 (VRS13): Viral Neutralization and Hemagglutination-Inhibition Assay Controls**<br>For neutralization and hemagglutination-inhibition (HI) assays, the laboratory must include the following controls in each run:<br>a) matched virus-antibody control;<br>b) virus-diluent control;<br>c) unmatched virus-antibody control;<br>d) RBC controls; and<br>e) viral dose control. | |
| **Virology Standard of Practice 14 (VR S14): Viral Culture Performance Monitoring**<br>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. | 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. |