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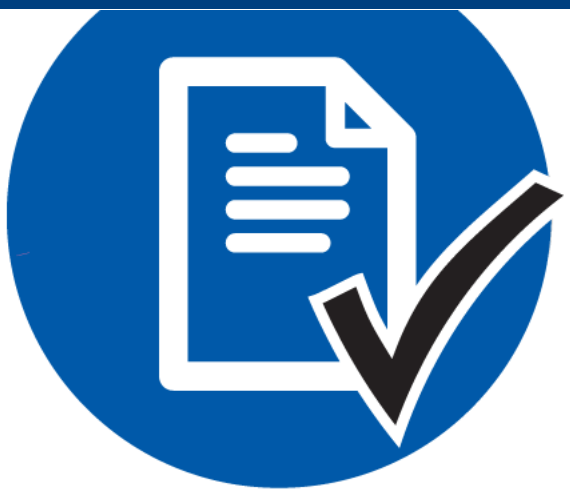
Providence Sacred Heart Medical Center
Main Laboratory

Microbiology Checklist

CAP Accreditation Program



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07.11.2011

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Microbiology Checklist

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SUMMARY OF CHECKLIST EDITION CHANGES
Microbiology Checklist
07/11/2011 Edition

The following requirements have been added, revised, or deleted in this edition of the checklist, or in the two editions immediately previous to this one.

If this checklist was created for a reapplication, on-site inspection or self-evaluation it has been customized based on the laboratory's activity menu. The listing below is comprehensive; therefore some of the requirements included may not appear in the customized checklist. Such requirements are not applicable to the testing performed by the laboratory.

Note: For revised checklist requirements, a comparison of the previous and current text may be found on the CAP website. Click on Laboratory Accreditation, Checklists, and then click the column marked Changes for the particular checklist of interest.

NEW Checklist Requirements

Requirement	Effective Date
MIC.13275	06/17/2010
MIC.16275	06/17/2010
MIC.21530	07/11/2011
MIC.21815	07/11/2011
MIC.21944	07/11/2011

REVISED Checklist Requirements

Requirement	Effective Date
MIC.10060	06/17/2010
MIC.11020	07/11/2011
MIC.11027	06/17/2010
MIC.11040	06/17/2010
MIC.11100	07/11/2011
MIC.13250	06/17/2010
MIC.14075	06/17/2010
MIC.14550	07/11/2011
MIC.14575	07/11/2011
MIC.14583	07/11/2011
MIC.16500	06/17/2010
MIC.19840	07/11/2011
MIC.21010	06/17/2010
MIC.22500	07/11/2011
MIC.32250	06/17/2010
MIC.41330	06/17/2010
MIC.41345	07/11/2011
MIC.43250	07/11/2011
MIC.43350	07/11/2011
MIC.48450	07/11/2011
MIC.52200	06/17/2010
MIC.63262	07/11/2011
MIC.63274	06/17/2010
MIC.63276	07/11/2011
MIC.63328	07/11/2011
MIC.63350	07/11/2011

MIC.63575	07/11/2011
MIC.64025	06/17/2010
MIC.64730	07/11/2011
MIC.64810	06/17/2010
MIC.64874	07/11/2011
MIC.64884	06/17/2010
MIC.64926	07/11/2011

DELETED Checklist Requirements

Requirement	Effective Date
MIC.00005	07/10/2011
MIC.00010	07/10/2011
MIC.00130	07/10/2011
MIC.00250	07/10/2011
MIC.00425	07/10/2011
MIC.00550	07/10/2011
MIC.00650	07/10/2011
MIC.10050	06/16/2010
MIC.11150	07/10/2011
MIC.12050	07/10/2011
MIC.12110	07/10/2011
MIC.12115	07/10/2011
MIC.12120	07/10/2011
MIC.12130	07/10/2011
MIC.12140	07/10/2011
MIC.12150	07/10/2011
MIC.13150	07/10/2011
MIC.14650	07/10/2011
MIC.15150	07/10/2011
MIC.15200	07/10/2011
MIC.16050	07/10/2011
MIC.16175	07/10/2011
MIC.18000	07/10/2011
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MIC.18700	07/10/2011
MIC.18750	07/10/2011
MIC.18800	07/10/2011
MIC.18850	07/10/2011
MIC.18900	07/10/2011
MIC.18920	07/10/2011
MIC.18940	07/10/2011
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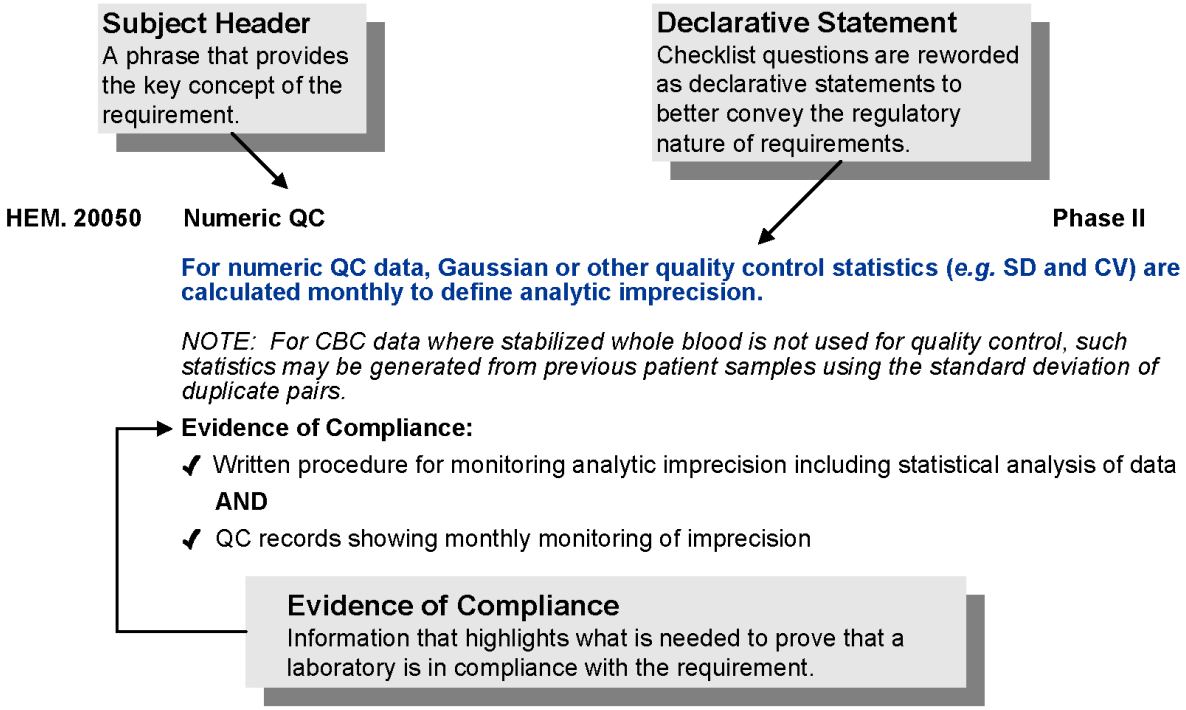
MIC.41230	07/10/2011
MIC.52280	07/10/2011
MIC.61390	07/10/2011
MIC.64636	06/16/2010
MIC.64740	07/10/2011

MIC.64878	07/10/2011
MIC.64928	07/10/2011

UNDERSTANDING THE CAP ACCREDITATION CHECKLIST COMPONENTS

To provide laboratories with a better means to engage in and meet their accreditation requirements, the CAP has enhanced the checklist content and updated its design. New components containing additional information for both the laboratory and inspectors include Subject Headers, Declarative Statements and Evidence of Compliance. See below for a definition of each new feature as an example of how they appear in the checklists.



USING EVIDENCE OF COMPLIANCE (EOC)

This component, which appears with several checklist requirements, is intended to:

1. Assist a laboratory in preparing for an inspection and managing ongoing compliance
2. Drive consistent understanding of requirements between the laboratory and the inspector
3. Provide specific examples of acceptable documentation (policies, procedures, records, reports, charts, etc.)

In addition to the Evidence of Compliance listed in the checklist, other types of documentation may be acceptable. Whenever a policy/procedure/process is referenced within a requirement, it is only repeated in the Evidence of Compliance if such statement adds clarity. All policies/procedures/processes covered in the CAP checklists must be documented. A separate policy is not needed for each item listed in EOC as it may be referenced in an overarching policy.

INTRODUCTION

An inspection of a laboratory section, or department will include the discipline-specific checklist(s), the Laboratory General Checklist, and the All Common Checklist.

In response to the ongoing request to reduce the redundancy within the Accreditation Checklists, the CAP accreditation program is introducing the All Common Checklist (COM).

The purpose of the All Common Checklist is to group together those requirements that were redundant in Laboratory General and the discipline-specific checklists. Therefore, the CAP centralized all requirements regarding: proficiency testing, procedure manuals, test method validations, and critical results into one checklist, the COM checklist.

Note for non-US laboratories: Checklist requirements apply to non-US laboratories unless the checklist items contain a specific disclaimer of exclusion.

GENERAL MICROBIOLOGY

Requirements in this section apply to ALL of the subsections in the microbiology laboratory (bacteriology, mycobacteriology, mycology, parasitology, molecular microbiology, and virology). When the microbiology department is inspected by a team, each member of the team must survey individual subsections for compliance with requirements in this General Microbiology section. The team leader is then responsible for completing this section at the conclusion of the inspection.

PROFICIENCY TESTING

MIC.00350 PT Extent of Testing **Phase II**

Organisms in proficiency testing specimens are identified to the same level as those from patient samples.

NOTE: If the laboratory's proficiency testing reports include incomplete identifications (e.g. "Gram positive cocci" or "Mycobacterium species, not tuberculosis"), it must document that this matches the information produced by the laboratory's internal capabilities in patient reports. In other words, patient reports cannot be more specific than the identification level reporting in proficiency testing, unless the former contain more specific information provided by reference laboratories.

QUALITY MANAGEMENT AND QUALITY CONTROL

WAIVED TESTS

***REVISED** 06/17/2010*
MIC.10060 Documented QC Results - Waived Tests **Phase II**

Control results are documented for quantitative and qualitative tests, as applicable.

NOTE: Quality control must be performed according to manufacturer instructions. To detect problems and evaluate trends, testing personnel or supervisory staff must review quality control data on days when controls are run. The laboratory director or designee must review QC data at least monthly. Because of the many variables across laboratories, the CAP makes no specific recommendations on the frequency of any additional review of QC data.

*With respect to internal controls, acceptable control results must be documented, at a minimum, once per day of patient testing for each device.**

All unacceptable control results must be documented (see below).

**Acceptable internal control results need not be documented, if (and only if) an unacceptable instrument control automatically locks the instrument and prevents release of patient results.*

MIC.10070 QC Corrective Action - Waived Tests **Phase II**

There is documentation of corrective action when quality control results exceed the acceptable range.

MIC.10080 QC Verification- Waived Tests **Phase II**

The results of controls are verified for acceptability before reporting results.

Evidence of Compliance:

- ✓ Records showing verification of acceptability of QC

NOTE: The remaining requirements in this checklist on controls, calibration, reportable range, and interinstrument comparisons do not apply to waived tests.

GENERAL ISSUES

MIC.11010 Documented QM/QC Plan **Phase II**

The microbiology laboratory has a written quality management/quality control (QM/QC program.

NOTE: The program must ensure quality throughout the pre-analytic, analytic and post-analytic (reporting) phases of testing, including patient identification and preparation; specimen collection, identification, preservation, transportation, and processing; and accurate, timely result reporting. The program must be capable of detecting problems in the laboratory's systems, and identifying opportunities for system improvement. The laboratory must be able to develop plans of corrective/preventive action based on data from its QM system.

All QM requirements in the Laboratory General Checklist pertain to the microbiology laboratory.

MIC.11015 QC Handling **Phase II**

Control specimens are tested in the same manner and by the same personnel as patient samples.

NOTE: QC specimens must be analyzed by personnel who routinely perform patient testing. This does not imply that each operator must perform QC daily, so long as each instrument and/or test system has QC performed at required frequencies, and all analysts participate in QC on a regular basis. To the extent possible, all steps of the testing process must be controlled, recognizing that preanalytic and postanalytic variables may differ from those encountered with patients.

Evidence of Compliance:
- ✓ Records reflecting that QC is run by the same personnel performing patient testing

***REVISED** 07/11/2011*
MIC.11020 Monthly QC Review **Phase II**

Quality control data are reviewed and assessed at least monthly by the laboratory director or designee.

NOTE: The QC data for tests performed less frequently than once per month should be reviewed when the tests are performed.

Evidence of Compliance:
- ✓ Records of QC review with documented follow-up for outliers, trends or omissions

MIC.11025 Verification of Accuracy **Phase II**

If the laboratory performs test procedures for which calibration and control materials are not available, procedures have been established to verify the accuracy of patient test results.

***REVISED** 06/17/2010*
MIC.11027 Comparability of Instrument **Phase II**

If the laboratory uses more than one instrument to test for a given analyte, the instruments are checked against each other at least twice a year for correlation of results.

NOTE: This requirement applies to two instruments manufactured by the same or different manufacturers that detect the same component of a pathogen. This requirement applies only to instruments accredited under a single CAP number. The laboratory director is responsible for establishing the details of the protocol for this check.

Two or more detectors or incubation cells connected to a single data collection, analysis and reporting computer need not be considered separate systems. Examples of such detectors/cells might be multiple incubation and monitoring cells in a continuous monitoring blood culture instrument or multiple thermocycler cells in a real time polymerase chain reaction instrument. This checklist requirement does not apply to multiple analytical methods designed to detect different components of a pathogen, since such methods are not detecting the same analyte.

Quality control data may be used for this comparison for tests performed on the same instrument platform, with control materials of the same manufacturer and lot number.

Evidence of Compliance:
- ✓ Written procedure for performing instrument correlation including criteria for acceptability **AND**
- ✓ Records of correlation studies reflecting performance at least twice per year with appropriate specimen types

MIC.11030 Specimen Collection Manual **Phase II**

There is a documented procedure describing methods for patient identification, patient preparation, specimen collection and labeling, specimen preservation, and conditions for transportation, and storage before testing, consistent with good laboratory practice.

***REVISED** 06/17/2010*
MIC.11040 Instrument Maintenance Evaluation **Phase II**

There is documentation of monthly evaluation of instrument maintenance and function, including temperatures of refrigerators/freezers in which reagents or patient specimens are kept.

***REVISED** 07/11/2011*

MIC.11100 Unusual Laboratory Results **Phase II**

There is a documented system in operation to detect and correct significant clerical and analytical errors, and unusual laboratory results, in a timely manner.

NOTE: One common method is review of results by a qualified person (technologist, supervisor, pathologist, laboratory director) before release from the laboratory, but there is no requirement for supervisory review of all reported data for single analyte tests that do not include interpretation. All tests that include an interpretation must be reviewed by the laboratory director or qualified designee before release from the laboratory. In computerized laboratories, there should be automatic "traps" for improbable results. The system for detecting clerical errors, significant analytical errors, and unusual laboratory results must provide for timely correction of errors, i.e. before results become available for clinical decision making. For confirmed errors detected after reporting, corrections must be promptly made and reported to the ordering physician or referring laboratory, as applicable.

Each procedure must include a listing of common situations that may cause analytically inaccurate results, together with a defined protocol for dealing with such analytic errors or interferences. This may require alternate testing methods; in some situations, it may not be possible to report results for some or all of the tests requested.

The intent of this requirement is NOT to require verification of all results outside the reference (normal) range.

Evidence of Compliance:
- ✓ Records of review of results **OR** records of consistent implementation of the error detection system(s) defined in the procedure **AND**
- ✓ Records of timely corrective action of identified errors

MIC.11300 Supervisory Result Review **Phase II**

In the absence of on-site supervisors, results of tests performed by personnel are reviewed by the laboratory director or general supervisor within 24 hours.

NOTE: The CAP does NOT require supervisory review of all test results before or after reporting to patient records. Rather, this requirement is intended to address only that situation defined under CLIA for "high complexity testing" performed by trained high school graduates qualified under 42CFR493.1489(b)(5) when a qualified general supervisor is not present.

Evidence of Compliance:
- ✓ Written policy defining the review process and personnel whose results require review **AND**
- ✓ Records of result review for specified personnel

MIC.11350 Morphologic Observation Assessment **Phase II**

The microbiology laboratory at least annually assesses morphologic observations among personnel performing gram, trichrome and other organism stains, to ensure consistency.

NOTE: Suggested methods to accomplish this include:

1. *Circulation of organisms with defined staining characteristics, and/or*
2. *Multi-headed microscopy, and/or*
3. *Use of photomicrographs with referee and participant identifications (e.g. former CAP microbiology Surveys or other photomicrographs from teaching collections)*
4. *Use of digital images*

Evidence of Compliance:
- ✓ Written procedure defining the method and criteria used for evaluation of consistency **AND**
- ✓ Employee records documenting morphology assessment

SPECIMEN COLLECTION AND HANDLING

Culture specimens are often collected by nurses or others outside the laboratory. An important aspect of quality control is the provision of adequate instructions to ensure proper collection and handling of specimens before they are received by the laboratory.

MIC.13100 Specimen Acceptability Criteria **Phase II**

There are criteria for establishing specimen acceptability.

NOTE: This could include important issues such as absence of gross external contamination, adequate specimen type/quantity, suitable preservation, prevention of dried swabs, and correct use of transport media when required.

Evidence of Compliance:
- ✓ Records of rejected specimens

MIC.13200 Culture Requisitions **Phase I**

Requests for isolation include source of specimen and, when appropriate, type of infection and/or organism expected.

*****REVISED** 06/17/2010***
MIC.13250 Specimen Collection/Handling **Phase II**

There are documented instructions for microbiology specimen collection and handling that include all of the following.

1. **Method for proper collection of culture specimens from different sources**
2. **Proper labeling of culture specimens**
3. **Use of transport media when necessary**
4. **Procedures for safe handling of specimens (tightly sealed containers, no external spillage)**
5. **Need for prompt delivery of specimens to ensure minimum delay and processing (e.g. CSF, wound cultures, anaerobes)**
6. **Method for preservation of specimens if processing is delayed (e.g. refrigeration of urines)**

NOTE: Manufacturer's recommendations must be followed when there is a delay in delivery or processing of specimens for automated instruments (e.g. blood culture instruments).

*****NEW** 06/17/2010***
MIC.13275 Specimens for Molecular Amplification **Phase II**

The laboratory has procedures for the handling of specimens that will also be tested using molecular amplification methods.

NOTE: Special precautions must be taken to avoid sample cross-contamination that may not affect culture-based methods but may lead to false positive results when tested using molecular amplification methods. For example, proper procedures to prevent cross-contamination must be used when samples are processed in the same biohazard hood in which virus cultures are manipulated post-inoculation. Please refer to the Molecular Microbiology section of this checklist.

REAGENTS - GENERAL

***REVISED** 06/17/2010*

MIC.14075 Reagent Handling/Storage - Waived Tests **Phase II**

For waived tests, the laboratory follows manufacturer instructions for handling and storing reagents, cartridges, test cards, etc.

NOTE: If the manufacturer defines a required storage temperature range, the temperature of storage areas must be monitored and recorded daily. The two acceptable ways of recording temperatures are: 1) recording the numerical temperature, or 2) placing a mark on a graph that corresponds to a numerical temperature (either manually, or using a graphical recording device). The identity of the individual recording the temperatures(s) must be documented (recording the initials of the individual is adequate).

The use of automated (including remote) temperature monitoring systems is acceptable, providing that laboratory personnel have ongoing immediate access to the temperature data, so that appropriate corrective action can be taken if a temperature is out of the acceptable range. The functionality of the system must be documented daily.

Evidence of Compliance:

- ✓ Written procedure consistent with manufacturer instructions for each waived test

NOTE: The remaining requirements on reagents in this checklist do not apply to waived tests.

The following generic requirements apply to all subsections of the Microbiology Laboratory.

MIC.14350 Reagent Labeling **Phase II**

Reagents and solutions are properly labeled, as applicable and appropriate, with the following elements.

1. **Content and quantity, concentration or titer**
2. **Storage requirements**
3. **Date prepared or reconstituted by laboratory**
4. **Expiration date**

NOTE: The above elements may be recorded in a log (paper or electronic), rather than on the containers themselves, providing that all containers are identified so as to be traceable to the appropriate data in the log. While useful for inventory management, labeling with "date received" is not routinely required. There is no requirement to routinely label individual containers with "date opened"; however, a new expiration date must be recorded if opening the container changes the expiration date, storage requirement, etc.

Evidence of Compliance:

- ✓ Written policy defining elements required for reagent labeling

MIC.14450 Reagent/Media Storage **Phase II**

All reagents and media are stored as recommended by the manufacturer.

NOTE: If ambient temperature is indicated, there must be documentation that the defined ambient temperature is maintained and corrective action is taken when tolerance limits are exceeded.

Evidence of Compliance:

- ✓ Records of reagent/media storage consistent with manufacturer's instructions, including refrigerator, freezer and room temperature monitoring, as applicable

***REVISED** 07/11/2011**

MIC.14550 Reagent Expiration Date **Phase II**

All reagents and media are used within their indicated expiration date.

NOTE: The laboratory must assign an expiration date to any reagents that do not have a manufacturer-provided expiration date. The assigned expiration date should be based on known stability, frequency of use, storage conditions, and risk of deterioration.

For laboratories not subject to US regulations, expired reagents may be used only under the following circumstances: 1. The reagents are unique, rare or difficult to obtain; or 2. Delivery of new shipments of reagents is delayed through causes not under control of the laboratory. The laboratory must document validation of the performance of expired reagents in accordance with written laboratory policy. Laboratories subject to US regulations must not use expired reagents.

Evidence of Compliance:

- ✓ Written policy for evaluating reagents lacking manufacturer's expiration date

***REVISED** 07/11/2011**

MIC.14575 New Reagent Lot Verification **Phase II**

New reagent lots and/or shipments are checked against old reagent lots or with suitable reference material before or concurrently with being placed in service and results are documented.

NOTE: Minimum crosschecking includes testing at least one known positive and one known negative sample with the new reagent lot or shipment. It is often preferable to do these validations with patient samples that have been tested previously, or can be tested simultaneously with the old reagent lot.

Although patient specimens are preferred, it is acceptable to use external quality control materials to check new reagent lots or shipments if the quality control material has been previously verified and exhibits no matrix effects.

For reagents used to detect or evaluate cultured microorganisms, suitable reference material may consist of control strains of organisms, or previously identified organisms.

Evidence of Compliance:

- ✓ Written procedure for the verification of new lots and shipments prior to use **AND**
- ✓ Records of verification of new reagents/shipments

***REVISED** 07/11/2011**

MIC.14583 Direct Antigen Test QC **Phase II**

For direct antigen tests on patient specimens that DO include internal controls, a positive and negative external control are tested and documented with each new kit lot number or separate shipments of a given lot number.

NOTE: Internal controls may be used for daily quality control, providing that the following requirements are met:

1. *Prior to initiating patient testing, the internal controls are validated against external controls. Validation studies must include daily comparison of external controls to built-in controls for at least 20 consecutive days when patient samples are tested. Acceptable validation is required before daily quality control can be limited to built-in controls. The laboratory director is responsible for determining criteria for acceptability, and other details of validation. Validation records must be retained while an instrument method is in service, and for two years afterwards.*
2. *A positive and negative external control (organism or antigen extract) is tested and documented with each new kit lot number or separate shipments of a given lot number.*

3. *Manufacturers' recommendations are followed. "Flow" or "procedural" controls qualify as internal controls. For panels or batteries, controls must be employed for each antigen sought. For tests classified as "high complexity" under CLIA, the system must be checked each day of use with a positive external control (organism or antigen).*
4. *External surrogate sample controls are run as frequently as recommended by the test manufacturer, or every 30 days, whichever is more frequent.*

Evidence of Compliance:
- ✓ Written QC procedures for each test consistent with the manufacturer's instructions **AND/OR** records documenting in-house validation of internal control systems

MIC.14616 **Direct Antigen Test QC** **Phase II**

For direct antigen tests on patient specimens that do NOT include internal controls, a positive and negative control are tested and documented each day of patient testing.

NOTE: For panels or batteries, controls must be employed for each antigen sought in patient specimens. For each test system that requires an antigen extraction phase, the system must be checked with an appropriate positive control that will detect problems in the extraction process.

REPORTING OF RESULTS

MIC.15000 **Preliminary Reports** **Phase I**

When indicated, preliminary reports are promptly generated.

Evidence of Compliance:
- ✓ Written procedure(s) defining when preliminary results are issued

INSTRUMENTS AND EQUIPMENT

All instruments and equipment should be properly operated, maintained, serviced, and monitored to ensure that malfunctions of these instruments and equipment do not adversely affect the analytical results.

MIC.16000 **Instrument Maintenance Schedule** **Phase II**

Instruments (microscopes, centrifuges, *etc.*) are on a regular instrument maintenance schedule and records of function checks are maintained.

MIC.16100 **Instrument Service Records** **Phase II**

Instrument maintenance, service and repair records (or copies) are promptly available to, and usable by, the technical staff operating the equipment.

NOTE: Effective utilization of instruments by the technical staff depends upon the prompt availability of maintenance, repair, and service documentation (copies are acceptable). Laboratory personnel are responsible for the reliability and proper function of their instruments and must have access to this information. Off-site storage, such as with centralized medical maintenance or computer files, is not precluded if the inspector is satisfied that the records can be promptly retrieved.

MIC.16150 **Pipettors and Diluters** **Phase II**

Pipettes, microtiter diluters or automatic dispensers that are used for quantitative dispensing of material are checked for accuracy and reproducibility at specified intervals, with results documented.

NOTE: This requirement is not applicable for precalibrated inoculation loops that are used in the direct plating of clinical specimens such as urine cultures.

Evidence of Compliance:
- ✓ Written procedure detailing method for checking the accuracy and reproducibility of automatic pipettes

MIC.16200 Thermometric Standard Device **Phase II**

An appropriate thermometric standard device of known accuracy is available (guaranteed by manufacturer to meet NIST standards).

NOTE: Thermometers should be present on all temperature-controlled instruments and environments and checked daily. Thermometric standard devices should be recalibrated or recertified prior to the date of expiration of the guarantee of calibration.

Evidence of Compliance:
- ✓ Thermometer certificate of accuracy

MIC.16250 Non-Certified Thermometers **Phase II**

All non-certified thermometers in use are checked against an appropriate thermometric standard device before being placed in service.

Evidence of Compliance:
- ✓ Written procedure defining criteria for verification of non-certified thermometers **AND**
- ✓ Records of verification prior to being placed in service

*****NEW** 06/17/2010***
MIC.16275 Microscopes **Phase I**

Microscopes used for immunofluorescent testing contain the appropriate filter(s) recommended by the manufacturer.

NOTE: The use of filters not recommended by the manufacturer can lead to erroneous results.

TEMPERATURE-DEPENDENT EQUIPMENT

MIC.16300 Temperature-Dependent Equipment **Phase II**

Thermometers are placed in, or integrated in all of the following equipment.

1. **Refrigerators**
2. **Incubators**
3. **Water baths**
4. **Heating blocks**
5. **Freezers**

*****REVISED** 06/17/2010***
MIC.16500 Temperature Checks **Phase II**

Temperatures are checked and recorded daily.

NOTE: Temperature-dependent equipment containing reagents and patient specimens must be monitored daily, as equipment failures could affect accuracy of patient test results. Items such as water baths and heat blocks used for procedures need only be checked on days of patient testing.

The two acceptable ways of recording temperatures are: 1) recording the numerical temperature, or 2) placing a mark on a graph that corresponds to a numerical temperature (either manually, or using a graphical recording device). The identity of the individual recording the temperature(s) must be documented (recording the initials of the individual is adequate).

The use of automated (including remote) temperature monitoring systems is acceptable, providing that laboratory personnel have ongoing immediate access to the temperature data, so that appropriate corrective action can be taken if a temperature is out of the acceptable range. The functionality of the system must be documented daily.

MIC.16550 Adequate Incubators **Phase I**

There are sufficient, clean, and well-maintained incubators available at specified temperature ranges.

PERSONNEL

MIC.17000 Personnel - Technical Operations **Phase II**

The person(s) in charge of technical operations in microbiology has education in microbiology equivalent to an MT(ASCP) and at least 4 years experience (one of which is in microbiology) under a qualified laboratory director.

Evidence of Compliance:

- ✓ Records of qualifications including degree or transcript, certification/registration, current license (if required) and work history in related field

MIC.17050 Visual Color Discrimination **Phase I**

Personnel working in microbiology are checked for visual color discrimination.

NOTE: Testing is not required for personnel who do not perform laboratory tests requiring color discrimination. This does not mean that visually color-impaired technical personnel cannot be employed, only that they be tested, with job assignments and responsibilities evaluated accordingly.

Evidence of Compliance:

- ✔ Record of color discrimination testing **OR** functional assessment, if indicated

BIOSAFETY

Items in this section apply to ALL areas of the microbiology laboratory. Additional items for specific subsections (bacteriology, mycobacteriology, mycology, parasitology, and virology) are found under the Laboratory Safety subsections for each of those areas.

MIC.18968 Agents of Bioterrorism **Phase I**

The microbiology laboratory has policies and procedures for the recognition of isolates that may be used as agents of bioterrorism.

NOTE: Microorganisms likely to be utilized as biological weapons include Bacillus anthracis (anthrax), Brucella species (brucellosis), Clostridium botulinum (botulism), Francisella tularensis (tularemia), Yersinia pestis (plague) and variola major (smallpox).

As part of an institution-wide plan to prepare and respond to a bioterrorism event, the microbiology laboratory should have policies and procedures for the recognition of isolates that may be used as agents of bioterrorism.

MIC.18976 **Bioterrorism Response Plan** **Phase I**

The laboratory participates in the institution's bioterrorism response plan.

Evidence of Compliance:
- ✓ Organizational bioterrorism plan describing the role of the laboratory

MIC.18985 **Spill Handling** **Phase II**

There are documented policies for handling spills of contaminated materials.

MIC.19010 **Bench Top Decontamination** **Phase II**

There is documentation of daily decontamination of bench tops.

MIC.19035 **Safe Specimen Processing** **Phase II**

There are documented policies and procedures for the safe handling and processing of specimens.

NOTE: Suggested topics to be considered in the policies and procedures for the safe handling and processing of specimens include the need for tight sealing of containers, avoiding spills of hazardous materials, requirements for wearing gloves, the need for respirator protection, availability and use of vaccinations, and the potential hazards of sniffing plates.

MIC.19060 **Biosafety Levels** **Phase II**

Policies and procedures have been developed to minimize the occupational risk of exposure to infectious agents handled in the microbiology laboratory, in accordance with current recommendations regarding the biosafety levels for working with different organisms.

NOTE: The four biosafety levels (BSL) for working with infectious agents are described in the CDC-NIH guidelines (Biosafety in Microbiological and Biomedical Laboratories, US Dept of Health and Human Services, fifth edition, 2007). Each BSL consists of combinations of equipment, procedures and techniques, and laboratory design that are appropriate for the type of laboratory and infectious agent handled. The laboratory director is responsible for the maintenance of precautions in the laboratory to minimize the risk of personnel infection. Precautions must be appropriate for the types of organisms tested and the nature of the studies performed.

MIC.19160 **Biosafety Levels** **Phase II**

Engineering and work practice controls appropriate to the Biosafety level of the laboratory are defined and implemented.

NOTE: Each increasing BSL number (1 to 4) implies increased occupational risk from exposure to an agent or performance of a procedure, and therefore is associated with more stringent control and containment practices.

***REVISED** 07/11/2011*

MIC.19840 Biological Safety Cabinet **Phase II**

A biological safety cabinet (BSC) or hood is available for handling specimens or organisms considered highly contagious by airborne routes.

Evidence of Compliance:
- ✓ Maintenance schedule of BSC function checks **AND**
- ✓ Records of testing and certification

MIC.20520 Biological Safety Cabinet **Phase II**

The biological safety cabinet (BSC) is certified at least annually to ensure that filters are functioning properly and that airflow rates meet specifications.

Evidence of Compliance:
- ✓ Maintenance schedule of BSC function checks **AND**
- ✓ Records of testing and certification

BACTERIOLOGY

QUALITY CONTROL

***REVISED** 06/17/2010*

MIC.21010 QC Verification **Phase II**

The results of controls are reviewed for acceptability before reporting patient results.

Evidence of Compliance:
- ✓ Written policy/procedure stating that controls are reviewed and acceptable prior to reporting patient results **AND**
- ✓ Evidence of corrective action taken when QC results are not acceptable

MIC.21040 QC Corrective Action **Phase II**

There is documentation of corrective action when control results exceed defined acceptability limits.

NOTE: Patient/client test results obtained in an analytically unacceptable test run or since the last acceptable test run must be re-evaluated to determine if there is a significant clinical difference in patient/client results. Re-evaluation may or may not include re-testing patient samples, depending on the circumstances.

Even if patient samples are no longer available, test results can be re-evaluated to search for evidence of an out-of-control condition that might have affected patient results.

MEDIA

The laboratory has the responsibility for ensuring that all media used, whether purchased or prepared by the laboratory, are sterile, able to support growth appropriately and are appropriately reactive biochemically. This will ordinarily require that the laboratory maintain a stock of reference organisms and test the media before or concurrent with use. Explicit documentation of such testing is essential.

For prepared, purchased media the laboratory must have explicit documentation that each lot of purchased medium has been tested for sterility, ability to support growth of appropriate organisms and biochemical reactivity at the time of preparation or concurrent with use in the laboratory. The recipient laboratory must have a copy of National Committee for Clinical Laboratory Standards (NCCLS) Document Number M22-A3 (Quality Assurance for Commercially Prepared Microbiological Culture Media) as a reference source. The manufacturer or preparer must document to the user that their quality control activities meet the NCCLS guidelines, or are otherwise equivalent. The laboratory director may wish to have a signed contractual arrangement with his/her selected manufacturer to cover all expected quality control and documentation. For each lot the preparer will certify that quality control performance was acceptable and maintain a record of test results and the lot numbers for ALL media for at least 2 years. The user laboratory may record that fact in place of the more detailed documentation of media performance. The user must visually examine each shipment for breakage, contamination, appearance, or evidence of freezing or overheating. Transportation of media/reagents under unfavorable environmental conditions may adversely affect product performance.

The user laboratory must continue to test each lot of media except those listed as being exempt from such testing in the tables in M22-A3, using quality control methods employed for media manufactured in-house (which are also listed in the M22 tables). In addition, each shipment of a commercial identification system must be tested for appropriate performance. If more than one lot number is received per shipment, each lot number must be tested.

The user must control media for critical reactions that are not tested by the preparer, even though the media may be exempted from testing for other purposes, as specified in M22-A3 tables. The director is responsible for the quality and performance of media and must document all media failures and the resultant corrective action taken.

MIC.21200 Media Supplier **Phase II**

The laboratory has documentation that its media supplier carries out the quality assurance guidelines enumerated in NCCLS Document M22-A3.

NOTE: The laboratory has the responsibility for ensuring that all media used, whether purchased or prepared by the laboratory, are sterile, able to support growth appropriately and are appropriately reactive biochemically. This will ordinarily require that the laboratory maintain a stock of reference organisms and test the media before or concurrent with use. Explicit documentation of such testing is essential.

For prepared, purchased media, the laboratory must have explicit documentation that each lot of purchased medium has been tested for sterility, ability to support growth of appropriate organisms and biochemical reactivity at the time of preparation or concurrent with use in the laboratory. The recipient laboratory must have a copy of the NCCLS document number M22-A3 (Quality assurance for commercially prepared microbiological culture media) as a reference source. The manufacturer or preparer must document to the user that their quality control activities meet the NCCLS guidelines, or are otherwise equivalent. The laboratory director may wish to have a signed contractual arrangement with his/her selected manufacturer to cover all expected quality control and documentation thereof. For each lot, the preparer will certify that quality control performance was acceptable, and maintain a record of test results and the lot numbers for all media for at least 2 years. The user laboratory may record that fact in place of the more detailed documentation of media performance. The user must visually examine each shipment for breakage, contamination, appearance, or evidence of freezing or overheating. Transportation of media/reagents under unfavorable environmental conditions may adversely affect product performance.

The user laboratory must continue to test each lot of media except those listed as being exempt from such testing in the tables in M22-A3, using quality control methods that are used for media manufactured in-house. In addition, each shipment or lot, if more than one lot number is received per shipment of a commercial identification system must be tested for appropriate performance.

The user must control media for critical reactions that are not tested by the preparer, even though the media may be exempted from testing for other purposes, as specified in M22-A3 tables. The

director is responsible for the quality and performance of media, and must document all media failures and the resultant corrective action taken.

MIC.21220 Media Visual Inspection **Phase I**

The laboratory has documentation that each shipment of purchased media is examined for breakage, contamination, appearance, and evidence of freezing or overheating.

MIC.21240 Media QC **Phase II**

The laboratory has documentation that an appropriate sample of each purchased medium that is not listed in M22-A3 as exempt from testing is checked for each of the following.

1. **Ability to support growth (where applicable) by means of stock cultures or by parallel testing with previous batches**
2. **Biochemical reactivity, where appropriate**

MIC.21300 Media QC **Phase II**

For microbiology media prepared in-house, there is documentation that an appropriate sample of each medium prepared by the laboratory is checked for each of the following.

1. **Sterility (following introduction of additives after sterilization)**
2. **Ability to support growth (where applicable) by means of stock cultures or by parallel testing with previous batches**
3. **Biochemical reactivity (where appropriate)**

Evidence of Compliance:

- ✓ Written procedure for testing media prepared in-house

MIC.21420 Media Visual Examination **Phase II**

All media are in visibly satisfactory condition (with expiration date, plates smooth, adequately hydrated, uncontaminated, appropriate color and thickness, tubed media not dried or loose from sides).

MIC.21460 Reference Organisms **Phase II**

Reference organisms are used to check stains, reagents and susceptibility test methods.

STAINS

*****NEW** 07/11/2011***

MIC.21530 Direct Gram Stains **Phase I**

The laboratory has protocols in place to use gram stain results to provide a preliminary identification of organisms, evaluate specimen quality when appropriate, and to guide work-up of cultures.

NOTE: The laboratory should have guidelines for the interpretation of the gram stain reaction of the organism, morphology of the organism, and the quantification of organisms and cells. The protocol should address correlation of direct gram stain results with final culture results.

Evidence of Compliance:
- ✓ Written procedure for gram stain

MIC.21540 **Gram Stain QC** **Phase II**

The gram staining procedure is checked and recorded for each new batch of stains and at least weekly against known gram-positive and gram-negative control organisms.

NOTE: Personnel who perform gram stains infrequently should run a gram-positive and gram-negative control each day of testing.

Evidence of Compliance:
- ✓ Written procedure for gram stain QC

MIC.21560 **Non-Immunofluorescent Stain QC** **Phase II**

All non-immunofluorescent, non-immunologic-based stains (other than Gram stains) are checked with a positive control and negative control for intended reactivity each day of use, and for each new batch, lot number and shipment.

Evidence of Compliance:
- ✓ Written procedure for non-IF stains QC

MIC.21570 **Fluorescent Stain QC** **Phase II**

Fluorescent stains are checked for positive and negative reactivity each time of use.

Evidence of Compliance:
- ✓ Written procedure for fluorescent stain QC

REAGENTS

The laboratory has the responsibility for ensuring that all reagents used, whether purchased or prepared by the laboratory, are appropriately reactive. The verification of reagent performance is required and must be documented. Any of several methods may be appropriate, such as direct analysis with reference materials, parallel testing of old vs. new reagents, and checking against routine controls. The intent of the requirements is for new reagents to be checked by an appropriate method and the results recorded before patient results are reported. Where individually packaged reagents/kits are used, there should be criteria established for monitoring reagent quality and stability, based on volume of usage and storage requirements. Processing of periodic "wet controls" to validate reagent quality and operator technique is a typical component of such a system.

MIC.21624 **Reagent QC** **Phase II**

Positive and negative controls are tested and results recorded for each new batch, lot number, and shipment of reagents, disks and stains.

NOTE: Reagents subject to this requirement include (but are not limited to) catalase, coagulase (including latex methods), oxidase and indole reagents; bacitracin, optochin, Streptococcal grouping reagents, ONPG, X, V, and XV disks.

MIC.21626 **Identification System QC** **Phase II**

Each new lot number and shipment of reagents used in bacterial identification systems are tested with positive and negative organisms.

NOTE: Streamlined QC may be performed, as specified by the manufacturer, for commercial microbial identifications systems (MIS) if the systems and streamlined QC protocols are used according to the manufacturer's instructions without modification. The laboratory may use additional QC organisms in addition to those required for the streamlined QC. In order to qualify for streamlined QC, the user must fulfill initial and ongoing requirements as defined by the manufacturer and CLSI document M50-A.

For user-developed identification systems, commercial systems which have not developed a streamlined QC process, or any commercial system whose use is altered in any way from the manufacturer's instructions all biochemical tests in each new lot number and shipment must be evaluated with a known positive and negative organism, to assure appropriate reactivity.

If streamlined QC is used, it is critical for laboratories to keep documentation of the test system verification and historical QC review as long as the streamlined QC is used, but in no case for less than two years.

Any test (e.g. oxidase test) required for interpretation of MIS results which is not part of the MIS cannot be included in MIS streamlined QC protocols. QC requirements for such tests, including the use of positive and negative controls for each new batch, lot number and shipment are given in MIC.21624.

Evidence of Compliance:

- ✓ Written procedure for QC on new lots or shipment of reagents for each MIS using the conventional QC method (positive and negative for each substrate) **OR** a written procedure for streamlined QC **AND**
- ✓ Records of test system verification or historical QC review used to qualify for streamlined QC, if applicable

MIC.21628 **Antisera QC** **Phase I**

Positive and negative controls are tested and results recorded for each new batch, lot number and shipment of antisera when prepared or opened and once every 6 months thereafter.

MIC.21632 **Beta-Lactamase QC** **Phase II**

Positive and negative controls are tested and results recorded for beta-lactamase (other than Cefinase ®) on each day of use.

NOTE: Beta lactamase tests using Cefinase ® need be checked only with each batch, shipment or lot number.

MIC.21800 **Serologic QC** **Phase II**

Results of serologic control tests are recorded.

MIC.21810 **Reagent Kit Components** **Phase II**

If there are multiple components of a reagent kit, the laboratory uses components of reagent kits only within the kit lot, unless otherwise specified by the manufacturer.

Evidence of Compliance:

- ✓ Written documentation defining allowable exceptions for mixing kit components from different lots

MIC.21812 **Anaerobic Conditions QC** **Phase II**

There is documentation that anaerobic systems (*e.g.* jars, chambers, bags) are checked for adequate anaerobic conditions with methylene blue strips, fastidious anaerobic organisms or other appropriate procedures.

MIC.21813 CO_2 Incubator Levels **Phase I**

CO_2 incubators are checked daily for adequate CO_2 levels, with recording of results.

NOTE: Some organisms require CO_2 to grow sufficiently to form visible colonies. CO_2 monitoring is required in all CO_2 incubators, including those that adjust gas flow to maintain a set CO_2 level, to ensure that the environment is within an acceptable range for CO_2 content. It is acceptable to monitor and record CO_2 levels from digital readouts; however, the laboratory must verify that the readout is accurate (by initial calibration or Fyrite). The frequency of verification must be defined in the laboratory's equipment quality control procedure and should be performed, at minimum, at the frequency recommended by the manufacturer.

*****NEW** 07/11/2011***
MIC.21815 Campylobacter Incubation Conditions QC **Phase I**

Campylobacter incubation conditions (e.g. jars, bags) are checked each time of use with QC organisms or other appropriate methods of validation to ensure adequate environmental conditions to support growth of Campylobacter.

ANTIMICROBIAL SUSCEPTIBILITY TESTING, QC REQUIREMENTS, AND RESULTS REPORTING

MIC.21820 Pure Culture - Susceptibility Testing **Phase II**

Only single isolates or pure cultures are only used for final performance of antimicrobial susceptibility testing (*i.e.* no mixed susceptibilities).

Evidence of Compliance:
- ✓ Written procedure describing the use of pure isolates for susceptibility testing, including the use of purity plate verification

MIC.21840 Susceptibility Disk QC **Phase II**

Each new lot of susceptibility disks are checked for activity before or concurrent with initial use.

Evidence of Compliance:
- ✓ Records of new lot susceptibility disk QC

MIC.21910 Susceptibility QC **Phase II**

For antimicrobial susceptibility testing of either disk or dilution type, control organisms are tested with each new lot or batch of antimicrobials or media, and each day the test is performed thereafter.

NOTE: For antimicrobial susceptibility testing, control organisms must be tested with each new lot or batch of antimicrobials or media, and daily thereafter. However, the frequency of test monitoring may be reduced to weekly (including the testing of new lots or batches of antimicrobials or media)

if the laboratory can document satisfactory performance with daily control tests as suggested by CLSI guidelines. For this purpose, satisfactory performance is defined as follows:

1. *There is documentation that all reference strains were tested for 20 or 30 consecutive test days, and*
2. *For each drug/microorganism combination, no more than 1 of 20 or 3 of the 30 values (zone diameter or MICs) may be outside the accuracy ranges. These limits may be established by the laboratory or CLSI Guidelines M02-A10 or M07-A8*

Evidence of Compliance:

✓ Records of susceptibility QC results documented at defined frequency and meeting defined acceptability criteria

MIC.21930 Susceptibility Test System **Phase II**

For antimicrobial susceptibility testing systems, there are documented criteria for interpretation of the endpoint or zone size.

NOTE: There must be stated criteria to determine the presence of an endpoint or zone size in the antimicrobial susceptibility testing system. The laboratory may use CLSI (NCCLS) criteria, but the use of other validated criteria is acceptable.

MIC.21940 Standardized Inoculum **Phase I**

The inoculum used for antimicrobial susceptibility testing (*i.e.* inoculum size) is controlled using a turbidity standard or other acceptable method.

NOTE: Antibiotic susceptibility may be substantially affected by inoculum size.

Evidence of Compliance:

✓ Written procedure for standardizing susceptibility inoculum

MIC.21943 Antibiotic Reporting Guidelines **Phase I**

Guidelines are established for the number and type of antibiotics reported for organisms isolated from different sites of infection.

NOTES: The microbiology department should consult with the medical staff and pharmacy to develop a list of antibiotics to be reported for organisms isolated from different sites. These lists may be based on the guidelines developed by the CLSI table to report routinely (Group A) and which might be reported only selectively (Group B). Limiting the number of antimicrobial agents reported, referred to as "cascade reporting," usually means reporting no more than four potential agents to which in at least one instance the isolate in question is susceptible. Selective reporting should help improve the clinical relevance of test reporting and help minimize the selection of multi resistant nosocomial strains by overuse of broad-spectrum agents. Laboratories should also only report those antimicrobial agents that are effective at the site from which the organism was isolated. Documentation of agreed upon policies should be available in the laboratory.

*****NEW** 07/11/2011***

MIC.21944 Supplemental Antimicrobial Agents **Phase I**

There are protocols for testing supplemental agents on isolates resistant to routinely tested antimicrobial agents, as needed.

NOTE: The protocol may include submission of isolates to an outside reference laboratory if testing is not performed onsite.

MIC.21946 Cumulative Susceptibility Data **Phase I**

For hospital based microbiology laboratories, cumulative antimicrobial susceptibility test data are maintained and reported to the medical staff at least yearly.

MIC.21950 Inconsistent Antimicrobial Results **Phase I**

The procedure manual addresses unusual or inconsistent antimicrobial testing results.

NOTE: Acceptable results derived from testing QC strains does not guarantee accurate results with all patient isolates. When unusual or inconsistent results are encountered with patient isolates, the results should be investigated further to ensure accuracy. For example, repeat testing and/or repeat identification procedures using an alternative method (if possible) should be performed in an effort to ensure accurate results. Some examples include:

1. *Escherichia coli that appears resistant to imipenem*
2. *Klebsiella spp. susceptible to ampicillin*
3. *Proteus mirabilis resistant to ampicillin*
4. *Staphylococcus aureus resistant to vancomycin*

Evidence of Compliance:

- ✓ Records of investigation for unusual/inconsistent results

PROCEDURES AND TESTS

ROUTINE PROCEDURES: The following requirements define minimum standards for evaluation of routine cultures. This does not preclude the use of screening cultures (limited studies) and should not be construed to mean that all routine cultures require special media. Special media should be available if needed.

RESPIRATORY CULTURES

Potential pathogens may be part of the oral flora of patients and may not require full identification or susceptibility testing, if there is evidence of heavy contamination of the sputum with saliva. Attempts should be made to correlate culture results (e.g. the presence of a single species in large numbers) with gram-stained smear results (e.g. the presence of single morphotype in large numbers) and whenever possible with clinical information obtained from the physician concerning objective evidence of pneumonia.

Routine procedures from acceptable sputum cultures should allow the isolation of pneumococci, Staphylococcus aureus, Pseudomonas aeruginosa, and Enterobacteriaceae. Isolation, identification, and reporting of Hemophilus species, Neisseria meningitidis, and Moraxella catarrhalis should be reserved for those instances in which there is a predominance of morphotypes resembling these organisms on the gram-stained smears.

MIC.22100 Sputa Gram Stain **Phase I**

A gram-stained smear is performed routinely on expectorated sputa to determine acceptability of a specimen for bacterial culture or the extent of culture workup.

MIC.22110 Unacceptable Sputa Specimens **Phase I**

Unacceptable sputum samples are not cultured (or cultured only by special request) and the health care provider or submitting laboratory is notified so another specimen can be collected without delay, if clinically indicated.

NOTE: It is suggested that the laboratory notify an appropriate caregiver about an inadequate specimen even in an outpatient setting, such as a reference laboratory. Notification can be by phone or computer report. The laboratory may implement written agreements with particular providers or submitting laboratories defining protocols for handling sputum samples.

Evidence of Compliance:
- ✓ Records of specimen rejection such as rejection log or patient report

URINE CULTURES

MIC.22200 **Urine Colony Count** **Phase II**

Quantitative cultures (colony counts) are performed.

NOTE: The minimal standards for evaluation of urine cultures should include an estimate of number of organisms and identification of gram-positive and gram-negative organisms.

Evidence of Compliance:
- ✓ Written procedure for colony counts

MIC.22210 **Urine Culture Procedure** **Phase II**

The media and procedures used permit the isolation and identification of both gram-positive and gram-negative bacteria.

GENITAL CULTURES

MIC.22273 **Streptococcus Screen** **Phase I**

Group B streptococcus screens from pregnant women are collected and cultured in accordance with the current guidelines.

NOTE: In 2002, the CDC released revised guidelines for the prevention of perinatal Group B streptococcal disease. The new guidelines recommend universal prenatal screening for vaginal and rectal Group B streptococcal (GBS) colonization of all pregnant women at 35-37 weeks gestation. Procedures for collecting and processing clinical specimens for GBS culture and performing susceptibility testing to clindamycin and erythromycin are also included in the guidelines.

STOOL CULTURES

MIC.22336 **Stool Culture Reporting** **Phase I**

The final report for routine bacterial stool cultures lists the organisms for which the specimen was cultured (*e.g.* Salmonella, Shigella, Vibrio, *etc.*).

NOTE: It is inappropriate to report "No enteric pathogens isolated." The report should list the organisms whose presence was sought by culture (e.g. No Salmonella, Shigella, or Campylobacter, etc. isolated).

MIC.22400 Enteric Pathogen Isolation **Phase II**

Routine procedures permit isolation and identification of enteric pathogens in patients with diarrhea.

MIC.22410 Stool Enrichment/Selective Media **Phase II**

Enrichment procedures or selective media are used routinely to permit recovery of small numbers of enteric pathogens in asymptomatic carriers.

MIC.22440 Stool Number/Timing **Phase I**

The laboratory has guidelines (developed with clinicians) for the number and/or timing of collection of stool specimens submitted for routine bacterial testing.

NOTE: The laboratory should consider developing guidelines with its clinicians for the number and/or timing of collection of stool specimens submitted for routine bacterial testing. Suggestions made by the authors of a 1996 CAP Q-Probes study (Valenstein et al) include:

1. *Accept no more than 2 specimens/patient without prior consultation with an individual who can explain the limited yield provided by additional specimens*
2. *Do not accept specimens from inpatients after the third hospital day, without prior consultation*
3. *Test stool for Clostridium difficile toxin for all patients over 6 months of age with clinically significant diarrhea and a history of antibiotic exposure. Consider C. difficile testing as an alternative to routine microbiologic studies for inpatients over 6 months of age who have test requests for routine enteric pathogens*

These recommendations are for diagnostic testing. Different guidelines may apply to tests ordered for follow-up.

CEREBROSPINAL FLUID CULTURES

*****REVISED** 07/11/2011***
MIC.22500 CSF Processing **Phase II**

CSF samples for culture are processed immediately on receipt.

NOTE: Bacterial meningitis is a critical condition that requires immediate attention. Samples must be processed upon receipt when meningitis is suspected. The laboratory may choose to handle surveillance cultures, e.g. involving neurosurgical implants, differently.

Evidence of Compliance:
- ✓ Policy and procedure for CSF processing **AND**
- ✓ Culture log or patient records

MIC.22520 CSF Media/Incubation **Phase II**

The procedure (media and incubation conditions) permits recovery of fastidious bacteria expected in this type of specimen (*N. meningitidis, H. influenzae, etc.*).

MIC.22550 CSF Back-Up Cultures **Phase II**

If antigen-detection methods are used, back-up cultures are performed on both positive and negative CSF specimens.

NOTE: Total dependence on a bacterial antigen test for the diagnosis of bacterial meningitis does NOT meet accreditation requirements. The sensitivity and, especially in urine, the specificity of such tests are not adequate. In addition, meningitis may be caused by bacteria not covered by the antigen tests. Thus, culture is essential for evaluation of bacterial meningitis, and must be performed on the patient specimen - if not performed by the present laboratory, the inspector must seek evidence that culture has been performed elsewhere.

Evidence of Compliance:

- ✓ Written procedure stating that CSF cultures are performed in conjunction with bacterial antigen tests **OR** procedure describing testing at another location **AND**
- ✓ Records of back-up CSF cultures performed on-site **OR** records indicating that cultures are performed at another location **OR** documentation that order for CSF bacterial antigen was blocked by the computer due to no order for a culture

BLOOD CULTURES

MIC.22600 Blood Culture System **Phase II**

The blood culture system in use is designed to recover both aerobic and, when indicated or if intended to be part of the routine procedure, anaerobic organisms.

MIC.22610 Manual Blood Culture Systems **Phase II**

For non-automated systems, macroscopically negative aerobic blood cultures are stained and/or subcultured at some point before discarding.

NOTE: Subcultures and/or stains need not be done on blood cultures performed by automated methods if bottles are monitored for at least 5 days.

Evidence of Compliance:

- ✓ Records of staining and/or subculture of macroscopically negative cultures

MIC.22620 Blood Culture Examination **Phase II**

Blood cultures are examined for evidence of growth at least twice daily for the first two days of incubation, then at least daily for the remainder of the incubation period.

NOTE: The time to detection of positive blood cultures, whether processed by manual or automated methods, depends on the schedule of inspection for evidence of growth. The means of the inspection may include visual examination, gram staining, subculturing, or electronic analysis by continuous monitoring instruments. Because most significant positive blood cultures may be detected within 48 hours of incubation, it is recommended that blood cultures be examined for evidence of growth at least two times on the first two days of incubation, then at least once daily through the remainder of the laboratory's routine incubation period.

Evidence of Compliance:

- ✓ Patient records/worksheet with result of examination for manual methods documented at defined frequency

MIC.22630 Blood Culture Collection **Phase II**

Sterile techniques for drawing and handling of blood cultures are defined, made available to individuals responsible for specimen collection and practiced.

NOTE: It is recommended that blood culture statistics, including number of contaminated cultures, be maintained and reviewed regularly by the laboratory director. The laboratory should establish a threshold for an acceptable rate of contamination. Tracking the contamination rate and providing feedback to phlebotomists or other persons drawing cultures has been shown to reduce contamination rates. Other measures to monitor include types of skin disinfection, volume of blood drawn, number of culture sets drawn, number of single cultures and line draws.

MIC.22640 Blood Culture Volume **Phase I**

Recommendations for the appropriate volume of blood per culture are available to those collecting the specimens.

NOTE: In adults, optimally 20 mL of blood per culture set (2 bottles) should be collected for culture. Larger volumes of blood increase the yield of true positive cultures. The laboratory should periodically monitor collected blood volumes and provide feedback to clinical staff. Automated blood culture systems approved or cleared by the FDA may use smaller volumes per culture set and are acceptable.

WOUND CULTURES

MIC.22700 Wound/Anaerobic Cultures **Phase II**

Special procedures are defined to culture anaerobic organisms when indicated.

NOTE: The minimum standards for the evaluation of deep wound cultures require adequate procedures for the collection, recovery and identification of clinically relevant pathogens, including aerobic, facultatively anaerobic, and strictly anaerobic organisms, when indicated and when the submitted material is of sufficient quality to provide an interpretable result. Suggested media for anaerobes include an anaerobic blood agar plate, a medium that inhibits gram-positive and facultative gram-negative bacilli such as KV blood agar, a differential or selective medium such as BBE (Bacteroides bile-esculin), and a gram-positive selective medium (colistin-nalidixic acid blood agar or phenylethyl alcohol blood agar). Provisions for adequate anaerobic incubation, with monitoring of the anaerobic environment, must be available. If the laboratory is not equipped to handle anaerobic incubation, there must be a procedure to refer the specimen to a reference laboratory in an expeditious fashion using a satisfactory transport system.

MIC.22710 Direct Smear Gram Stain **Phase I**

Gram stains of direct smears are examined and results reported, when indicated.

NOTE: Gram stains are recommended to evaluate specimen quality and guide the work-up of the specimen. Examination of the smear may reveal morphotypes of the organisms present, acute inflammatory cells and squamous epithelial cells.

LABORATORY SAFETY

NOTE TO THE INSPECTOR: The inspector should review relevant requirements from the Safety section of the Laboratory General checklist, to assure that the bacteriology laboratory is in compliance. Please elaborate upon the location and the details of each deficiency in the Inspector's Summation Report.

The following requirement pertains specifically to the bacteriology laboratory.

MIC.23200 Hazardous Waste Disposal **Phase II**

Microbiology specimen residuals and contaminated media are disposed of in a manner to minimize hazards to all personnel handling the material.

NOTE: Sterilization or decontamination within the microbiology section before disposal is preferred. If such material is transported before treatment, it must be placed into a leak-resistant rigid container, and appropriately labeled.

Evidence of Compliance:
- ✓ Written procedure for the handling and disposal of microbiology waste

MYCOBACTERIOLOGY

QUALITY CONTROL

SPECIMEN HANDLING

MIC.31100 Specimen Collection/Transport **Phase I**

Specimens for mycobacterial culture are collected appropriately and transported to the laboratory without delay.

NOTE: The laboratory should recommend collecting 3 sputum specimens for acid-fast smears and culture in patients with clinical and chest x-ray findings compatible with tuberculosis. These three samples should be collected at 8-24 hour intervals (24 hours when possible) and should include at least one first morning specimen. Specimens must be delivered to the laboratory promptly; specimens that cannot be processed within one hour of the time of collection should be refrigerated during transport to and storage in the laboratory prior to processing. This will decrease overgrowth with contaminating organisms likely to be present.

Laboratories are encouraged to process acid-fast specimens in their laboratory or obtain results from referral laboratories as soon as possible so that smear results can be available within 24 hours of collection (see MIC.31200 below).

Evidence of Compliance:
- ✓ Written procedure describing specimen collection and handling requirements

REPORTING OF RESULTS

MIC.31200 Acid Fast Stain Results **Phase I**

When clinically indicated, results of acid-fast stains are reported within 24 hours of specimen receipt by the testing laboratory.

Evidence of Compliance:
- ✓ Written procedure defining turnaround time for reporting acid-fast stain results

MIC.31220 Mtb Susceptibility Test Results **Phase I**

Susceptibility test results for *M. tuberculosis* are available in a timely manner.

NOTE: The rapid recognition of drug-resistant organisms is essential to the control of multidrug-resistant tuberculosis. For isolates of M. tuberculosis complex, the CDC and Prevention

Laboratory work group recommends that laboratories use methods that may allow susceptibility test results to be available within 28 days of specimen receipt. From a CAP accreditation perspective, 28 days is a goal, not a requirement.

MEDIA

NOTE: See QUALITY CONTROL text preceding requirement MIC.21200 concerning requirements for commercially prepared media.

MIC.31400 Media QC **Phase II**

An appropriate sample of each medium and additive prepared by the laboratory is checked for all of the following elements.

1. **Sterility (if additives are introduced after initial sterilization)**
2. **Ability to support growth (when applicable) by means of stock cultures or by parallel testing with previous batches**
3. **Biochemical reactivity (where appropriate)**

NOTE: This checklist requirement does not apply to commercially prepared additives that are reconstituted when added to mycobacterial media.

Evidence of Compliance:
- ✓ Records of media QC for laboratory-prepared media and additives

MIC.31460 Media Visual Examination **Phase II**

All media are in satisfactory condition (adequately hydrated, tubed media not dried or loose from sides).

CONTROLS AND STANDARDS

MIC.31630 QC Verification **Phase II**

The results of controls are reviewed for acceptability before reporting patient results.

Evidence of Compliance:
- ✓ Records showing verification of acceptability of QC

MIC.31635 QC Corrective Action **Phase II**

There is documentation of corrective action when control results exceed the acceptability limits.

NOTE: Patient test results obtained in an analytically unacceptable test run or since the last acceptable test run must be re-evaluated to determine if there is a significant clinical difference in patient/client results. Re-evaluation may or may not include retesting samples, depending on the circumstances.

MIC.31640 AFB Stain QC **Phase II**

AFB stains are checked each day of use with appropriate positive and negative controls, and results documented.

MIC.31650 Fluorescent Stain QC **Phase II**

Fluorescent stains are checked with positive and negative controls each time of use and results documented.

MIC.31660 NAP Test QC **Phase I**

A known strain of *M. tuberculosis* is tested whenever the NAP test is performed.

MIC.31670 Nucleic Acid Probe QC **Phase II**

If nucleic acid probes are used for identification of mycobacteria grown in culture, appropriate positive and negative controls are tested on each day of use.

Evidence of Compliance:

- ✓ Records of nucleic acid probe QC documented at defined frequency

MIC.31680 M.tb Susceptibility QC **Phase II**

If the laboratory performs susceptibility testing of *M. tuberculosis*, a control strain sensitive to all antimycobacterial agents is run each week of patient testing, and with each new batch/lot number of media and antimicrobial agents.

Evidence of Compliance:

- ✓ Records of routine and new lot M.tb QC results documented at defined frequency

PROCEDURES AND TESTS

RAPID METHODS

The College of American Pathologists encourages laboratories in areas of the country where the incidence of tuberculosis has increased over the past several years and laboratories in other parts of the country that have experienced an increased rate of recovery of mycobacteria to utilize the most rapid and reliable methods available for detection and identification of mycobacteria, especially M. tuberculosis, and the most rapid and reliable methods available for susceptibility testing of isolates of M. tuberculosis.

MIC.32100 Fluorochrome Stain **Phase II**

Fluorochrome staining is performed on mycobacterial smears prepared from primary specimens, either in the laboratory or by the reference laboratory.

NOTE: Such smears are easier to read than those stained with a conventional carbol-fuchsin based stain. Fluorescing organisms stand out prominently against the background of the smear, and the smears can be examined at a lower power than conventionally-stained smears, so that a larger amount of material can be examined in a given period of time. As with the interpretation of Ziehl-Neelsen- and Kinyoun-stained smears, expertise is needed for interpretation of smears stained with a fluorescent stain; not everything that fluoresces in such a stain is necessarily a mycobacterium. Particularly when only a few organism-like structures are seen, it is important to pay careful attention to their morphology before interpreting them as Mycobacteria.

Evidence of Compliance:

- ✓ Written procedure for including fluorochrome staining on primary specimens for mycobacterial culture **OR** written policy for referral of specimens to a reference laboratory for fluorochrome staining **AND**

- ✓ Patient reports/worksheets with fluorochrome stain results **OR** reference laboratory reports with results

MIC.32140 Rapid Method **Phase I**

Nucleic acid probes, chromatography, the NAP test, or other rapid method (*e.g.* nucleic acid amplification or sequencing) is employed for identification of mycobacterial isolates.

Evidence of Compliance:
- ✓ Written procedure defining method(s) in use for identification of mycobacterial isolates

CONCENTRATION, INOCULATION, INCUBATION

MIC.32200 Concentration Methods **Phase II**

Certain specimens (*e.g.* sputum) are concentrated before AFB smear examination and culture.

Evidence of Compliance:
- ✓ Documentation of specimens requiring concentration

*****REVISED** 06/17/2010***
MIC.32250 Specimen Inoculation **Phase I**

Specimens (other than blood) are routinely inoculated on media that support optimal growth of the majority of clinically relevant mycobacterial species.

NOTE: The use of two types of media (for specimens other than blood), including one liquid medium (when possible) or a comparable culture method, is recommended for optimal isolation of mycobacteria.

CULTURES

Laboratories providing complete identification must provide a sufficient variety of differential tests to accurately identify and differentiate the different types of mycobacteria, including temperature growth requirements and photoreactivity studies. Laboratories not providing complete identification are encouraged to at least provide photoreactivity studies.

MIC.32320 Incubation Temperature **Phase II**

Mycobacterial cultures are maintained at 35-37°C.

NOTE: The optimal incubation temperature for most mycobacterial specimens is 35 to 37° C. Exceptions to this include specimens obtained from skin or soft tissue suspected to contain M. marinum (incubate at 30-32° C) or M. xenopi (incubate at 42° C). These specimens should be held at 35 – 37° C in addition to the lower or higher temperature.

Evidence of Compliance:
- ✓ Temperature records

DIFFERENTIAL BIOCHEMICAL PROCEDURES

MIC.32420 Differential Biochemical Test **Phase II**

Differential biochemical tests are appropriate for the extent and manner of mycobacterial identification.

NOTE: The number and types of biochemical tests needed depend upon (a) the extent to which mycobacteria are identified (e.g. "Mycobacterium kansasii" or "photochromogen"), (b) the particular species which a laboratory attempts to identify (e.g. does it attempt to identify Mycobacterium terrae complex, or the species and subspecies of the Mycobacterium chelonae-Mycobacterium fortuitum complex), and (c) the degree to which biochemical testing is ancillary to other methods such as nucleic acid probes and HPLC. Useful biochemical tests include, but are not limited to, arylsulfatase, 68° C catalase, semiquantitative catalase, iron uptake, MacConkey agar, 5% NaCl, niacin accumulation, nitrate reductase, Tween 80 hydrolysis, and urease. These tests are particularly useful for the following identifications and discriminations:

TEST	*UTILITY*
Arylsulfatase	*Helps distinguish pathogenic from non-pathogenic rapid growers; also useful for M. marinum, M. szulgai, M. xenopi, M. triviale.*
68°C catalase	*Helpful for identification of M. tuberculosis*
Semiquantitative catalase	*Helpful in certain circumstances. M. tuberculosis complex, MAC, M. xenopi, and a few other species produce <45 mm of bubbles.*
Iron uptake	*Helps distinguish M. chelonae from M. fortuitum.*
MacConkey agar	*Helps with identification of rapid growers.*
5% NaCl	*Helps with identification of rapid growers and M. triviale.*
Niacin accumulation	*Helps with identification of M. tuberculosis, M. simiae, some strains of M. bovis.*
Nitrate reductase	*Helpful in identifying many mycobacterial species.*
Tween 80 hydrolysis	*Helps distinguish some usually pathogenic from some usually non-pathogenic mycobacterial species.*
Urease	*Helpful in identifying many mycobacterial species.*

Evidence of Compliance:

✓ Written procedure detailing tests performed and identification scheme appropriate for the extent of testing

MIC.32480 Biochemical Test QC **Phase II**

All biochemical tests employed are checked each day of use with appropriate positive and negative controls and results recorded.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) FOR MICROBIAL IDENTIFICATION

MIC.32518 HPLC Calibrators/Standards **Phase II**

Appropriate calibrators or standards are run with each analytic batch.

NOTE: Either calibration standards or organisms of known identity must be run with each analytic batch, and criteria must exist for acceptance of runs based on mobility of internal standards, ability to identify significant peaks, baseline noise, peak symmetry of internal standards, detection of low-quantity peaks, and similar criteria.

Evidence of Compliance:

- ✓ Written procedure defining calibrators/standards appropriate for the test system used **AND**
- ✓ Records of calibration/calibration verification with each batch

MIC.32556 **HPLC Controls** **Phase II**

Appropriate controls are extracted and run through the entire procedure.

NOTE: Control organisms must be extracted and carried through the entire procedure with each run or batch. Appropriate positive (e.g. mycobacterial) and negative controls (organisms such as Candida from which no mycolic acids are expected) must be included with each run.

Evidence of Compliance:

- ✓ Written procedure defining QC requirements **AND**
- ✓ QC records documented at defined frequency

MIC.32594 **Chromatogram Controls** **Phase I**

External chromatogram pattern controls are available.

NOTE: Patterns for known strains should be established in those laboratories using HPLC. In addition laboratories should have access to the standard method manuals containing comparable chromatographic patterns for comparison.

MIC.32632 **Column Verification** **Phase II**

New columns are verified for performance before use.

NOTE: Column verification must include assessment of flow, consistency, and carryover. If the HPLC-method interpretive software uses a peak-naming table, it must be calibrated with each change of column. Generally the basic performance of new columns is certified by the manufacturer. HPLC analysis requires columns be equilibrated with about 10 column volumes of solvent followed with a blank run to test pressure and solvent flow.

Evidence of Compliance:

- ✓ Written procedure for column verification **AND**
- ✓ Records of column verification

MIC.32670 **Column/Detector Monitoring** **Phase II**

The performance of the column and detector are monitored on each day of use.

NOTE: Unextracted standard organisms and extracted calibrators or controls, typically containing a range of mycolic acids (or other appropriate targets) of known relative retention times, may be analyzed to monitor critical aspects of HPLC performance. Appropriate criteria for evaluating such parameters as retention time of specific standards, relative retention compounds time, separation of closely eluting peaks of interest, detection of known low-quantity peaks, column pressure, chromatography quality and detector response should be established and monitored. Column temperatures and pump pressures are monitored with each run to ensure they met specified criteria for analysis. The column and detector operations are monitored with a blank run prior to use and during batch runs. Positive and negative control samples supplement the blank run when samples are analyzed.

Evidence of Compliance:
- ✓ Records for column and detector monitoring documented at defined frequency

MIC.32708 **Carryover Detection** **Phase II**

There is a procedure for the detection and evaluation of potential carryover.

NOTE: No matter what type of injection is used, the procedure must address criteria for the evaluation of potential carryover from a preceding elevated (high concentration) sample to the following sample, either periodically, or in each analytical batch analysis.

Evidence of Compliance:
- ✓ Records of reassessment of samples with potential carryover

MIC.32746 **HPLC Growth Media** **Phase II**

The laboratory procedures define which growth media may be used for organisms to be analyzed by HPLC.

NOTE: Final results can be influenced by conditions of growth. For reliable results, standard conditions of analysis must be met, including growth media.

MIC.32784 **Peak Verification** **Phase II**

There is a procedure for verifying calibration of the peak-naming table, if used.

NOTE: In order to insure that peaks are correctly identified by interpretive software, the table must be validated at least annually with standard materials or organisms with known characteristics.

MIC.32822 **HPLC Method Validation** **Phase II**

The HPLC method has been validated using known strains of bacteria, including strains expected to be encountered in routine clinical use.

Evidence of Compliance:
- ✓ Record of method validation with appropriate strains

MIC.32860 **HPLC Result Review** **Phase II**

There is a procedure for review of HPLC results in conjunction with other laboratory data prior to reporting results.

NOTE: HPLC is only one tool for microbial identification. When results of HPLC analysis conflict with growth characteristics, pigmentation, or the results of biochemical or molecular testing, identification decisions must be based on all the information available.

MIC.32898 **HPLC Analysis - Pure Isolates** **Phase II**

There are procedures to verify the purity of cultures used as a source for HPLC analysis.

NOTE: Results of HPLC analysis may be unreliable if mixed cultures are tested. If HPLC is performed on an isolate from liquid culture and an interpretable chromatogram is obtained, it is not necessary to await the results of the purity check before reporting results, but a purity check must still be performed.

MIC.32936 **HPLC Reagent Storage/Grade** **Phase II**

Reagents and solvents are stored correctly and of appropriate grade, and solvent purity is assessed when needed.

NOTE: Only HPLC grade solvents are recommended for this procedure. Degradation begins once ultra-pure solvents are opened. Degradation can be slowed by storing solvents in tightly capped, amber bottles in the dark. Solvent purity verification is suggested when a degradation-related problem is suspected.

Evidence of Compliance:
- ✓ Reagent logs

MIC.32974 **Instrument Operation** **Phase II**

Procedures are documented for operation, calibration, and maintenance.

NOTE: Basic principles of HPLC analysis require continual monitoring of analysis conditions, including maintenance, standard operating procedures, and system calibration. System problems and corrective actions must be appropriately documented.

MIC.33012 **Instrument Performance** **Phase II**

Instrument performance (e.g. retention times, detector response) is checked after major instrument maintenance.

NOTE: Instrument performance must be verified by control runs after major maintenance.

Evidence of Compliance:
- ✓ Instrument performance records

LABORATORY SAFETY

NOTE TO THE INSPECTOR: The inspector should review relevant requirements from the Safety section of the Laboratory General checklist, to assure that the mycobacteriology laboratory is in compliance. Please elaborate upon the location and the details of each deficiency in the Inspector's Summation Report.

The following requirements pertain specifically to the mycobacteriology laboratory.

MIC.33050 **Specimen Collection** **Phase II**

All specimens for mycobacterial culture are collected and/or received in sealed leak-proof containers.

MIC.33100 **Centrifuge Safety** **Phase II**

In centrifuging specimens, sealed screw-capped tubes are enclosed in sealed safety centrifuge carriers (*i.e.* a double closure system) used to minimize aerosol hazards.

MIC.33300 **Biological Safety Cabinet** **Phase II**

The biological safety cabinet meets minimum requirements for mycobacterial work.

NOTE: Exhaust air from a class I or class II biological safety cabinet must be filtered through HEPA filters. Air from Class I and IIB cabinets is hard-ducted to the outside. Air from Class IIA cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every 12 months. It may be exhausted through a dedicated stack that protects against backflow of air from adverse weather conditions or through the building exhaust air system in a manner (e.g. thimble connection) that avoids any interference with the air balance of the biological safety cabinet or building exhaust system.

Evidence of Compliance:
- ✓ Written procedure defining the types of safety cabinets, filtration systems and exhaust systems used **AND**
- ✓ Maintenance schedule of BSC function checks **AND**
- ✓ Records of testing and certification **AND**
- ✓ Records of HEPA filters used for filtration of all BSC classes **AND**
- ✓ Records of exhaust mechanism **OR** recirculation, if appropriate

MYCOLOGY

QUALITY CONTROL

MEDIA

NOTE: See QUALITY CONTROL text preceding Requirement MIC.21200 concerning requirements for commercially prepared media.

✓ **MIC.41200 Media QC** **Phase II**

An appropriate sample of each medium prepared by the laboratory or purchased but not excluded from testing in NCCLS M22-A3 is checked for each of the following.

1. **Sterility (following introduction of additives after sterilization)**
2. **Ability to support growth and biochemical reactivity (where applicable) by means of stock cultures or by parallel testing with previous batches**

Evidence of Compliance:
- ✓ Records of media QC for laboratory-prepared or non-exempt purchased media

CONTROLS AND STANDARDS

Good laboratory practice includes checking all media either at the time of receipt or concurrently with use. This applies to purchased media as well as media prepared by the laboratory. See text preceding MIC.21200 concerning requirements for commercially prepared media.

✓ **MIC.41250 Reference Organisms** **Phase II**

Reference cultures are used to check stains and reagents at appropriate intervals.

✗ **MIC.41270 Nucleic Acid Probe/Exo-antigen QC** **Phase II**

If nucleic acid probes or exo-antigen tests are used for identification of fungi isolated from culture, appropriate positive and negative controls are tested on each day of use.

Evidence of Compliance:
- ✓ Written procedure defining QC for nucleic acid probe or exo-antigen tests **AND**
- ✓ Records of nucleic acid probe or exo-antigen QC documented at defined frequency

*****REVISED** 06/17/2010***

MIC.41330 QC Verification **Phase II**

The results of controls are reviewed for acceptability before reporting patient results.

Evidence of Compliance:
- ✓ Written policy/procedure stating that controls are reviewed and acceptable prior to reporting patient results **AND**
- ✓ Evidence of corrective action taken when QC results are not acceptable

***REVISED** 07/11/2011*
MIC.41345 QC Corrective Action **Phase II**

There is documentation of corrective action when quality control results exceed the acceptability limits.

NOTE: Patient test results obtained in an analytically unacceptable test run or since the last acceptable test run must be re-evaluated to determine if there is a significant clinical difference in patient results. Re-evaluation may or may not include retesting samples, depending on the circumstances.

MIC.41370 Direct Smear Stain QC **Phase II**

Direct patient specimen stains (*e.g.* acid fast, PAS, Giemsa, Gomori's methenamine silver, India ink) are checked with positive and negative controls on each day of patient sample testing.

NOTE: For certain stains such as GMS and Giemsa, the slide itself serves as the negative control. Controls for KOH preparations are not required.

Evidence of Compliance:
- ✓ Records of stain QC documented at defined frequency

MIC.41390 Fluorescent Stain QC **Phase II**

Fluorescent stains (such as calcofluor white) are checked with positive and negative controls each time of use and results documented.

PROCEDURES AND TEST

The intent of this series of requirements is to ensure the use of an appropriate variety of media and growth conditions to isolate the significant pathogens with minimal interference from contaminants.

MIC.42000 Preliminary Screen Procedures **Phase II**

Preliminary screening procedures, such as direct wet mount preparations and stains are performed when indicated (*e.g.* 10% KOH, India ink, Giemsa).

Evidence of Compliance:
- ✓ Written procedures defining specimens for which direct wet prep and staining are required

MIC.42050 Selective Media **Phase II**

Suitable selective media are used for the growth and isolation of dermatophytes and/or systemic fungi.

Evidence of Compliance:
- ✓ Written procedure for mycology culture defining the media used for growth and isolation

MIC.42100 **Selective Media** **Phase II**

Media with antimicrobial agents are used to suppress the growth of contaminants.

NOTE: Antimicrobial agents may inhibit some yeasts and the yeast phase of dimorphic organisms. Both types of media (with and without antimicrobials) should be available and used when indicated.

Evidence of Compliance:
- ✓ Written procedure for mycology culture defining the use of media to suppress contaminants

MIC.42150 **Incubation Temperature** **Phase II**

Incubation temperatures for the growth and isolation of dermatophytes and systemic fungi are defined and followed under culture conditions.

Evidence of Compliance:
- ✓ Temperature records

MIC.42200 **Incubation Temperature** **Phase II**

If cultures are incubated at room temperatures, actual ambient temperature (22-26° C) is checked daily to determine if proper growth conditions are being maintained.

MIC.42250 **Differential Tests** **Phase II**

Procedures for the differentiation and identification of fungi (differential tests) are adequate for the needs of the laboratory.

NOTE: Laboratories offering full identification must have sufficient procedures to do so. Smaller laboratories with limited services should have an arrangement with an approved reference laboratory for back-up and complete identification of mycology specimens.

Evidence of Compliance:
- ✓ Written procedure detailing tests performed and identification scheme appropriate for the extent of testing

MIC.42350 **Differential Tests** **Phase II**

Differential tests include biochemical tests (*e.g.* urease, carbohydrate assimilation and/or fermentation).

MIC.42400 **Differential Tests** **Phase I**

Differential tests include slide cultures (when appropriate).

MIC.42450 **Differential Tests** **Phase I**

Differential tests include nutritional studies for dermatophytes when identification is carried to the species level.

MIC.42550 **Dimorphic Fungi** **Phase I**

The identification of dimorphic fungal isolates is confirmed by exo-antigen, molecular, yeast-mold conversion or tissue phase detection tests.

NOTE: Exo-antigen tests, DNA probes, or DNA sequencing are recommended.

LABORATORY SAFETY

NOTE TO THE INSPECTOR: The inspector should review relevant requirements from the Safety section of the Laboratory General checklist, to assure that the mycology laboratory is in compliance. Please elaborate upon the location and the details of each deficiency in the Inspector's Summation Report.

The following requirements pertain specifically to the mycology laboratory.

MIC.43050 Safety Precautions **Phase II**

If plate culture media is used in mycology, appropriate safety precautions are taken (such as taping lid to plate on both sides when not in use or other appropriate measures) to prevent the accidental opening of a plate.

NOTE: Some authorities recommend the transfer of growing colonies from plate to tubed media, if the former is routinely used for initial inoculation.

Evidence of Compliance:
- ✓ Written procedure defining safety precautions for handling mycology culture plates

MIC.43100 Safety Precautions **Phase II**

When working with a colony exhibiting mycelial growth, transfers are performed within a biological safety cabinet.

MIC.43150 Safety Precautions **Phase II**

The use of slide culture techniques is limited, where possible, to work with low virulence organisms; or if used for dimorphic fungi, special safety precautions are defined and rigidly adhered to.

MIC.43200 Safety Precautions **Phase II**

When preparing teased preparations or "scotch" tape preps, mycelia are always submerged in some liquid medium (such as lactophenol cotton blue).

*****REVISED** 07/11/2011***
MIC.43250 Biological Safety Cabinet **Phase II**

A biological safety cabinet (BSC) or hood is available for handling specimens or organisms considered to be highly contagious by airborne routes.

Evidence of Compliance:
- ✓ Maintenance schedule of BSC function checks **AND**
- ✓ Records of testing and certification

MIC.43300 Biological Safety Cabinet **Phase II**

The biological safety cabinet (BSC) is certified annually to ensure that filters are functioning properly and that airflow rates meet specifications.

Evidence of Compliance:
- ✓ Maintenance schedule of BSC function checks **AND**
- ✓ Records of testing and certification

***REVISED** 07/11/2011*
MIC.43350 Biological Safety Cabinet **Phase II**

The BSC meets minimum requirements for mycologic work.

NOTE: Exhaust air from a class I or class II BSC must be filtered through HEPA filters. Air from Class I and IIB is hard ducted to the outside. Air from Class IIA cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every 12 months. It may be exhausted through a dedicated stack that protects against backflow of air from adverse weather conditions or through the building exhaust air system in a manner (e.g. thimble connection) that avoids any interference with the air balance of the BSC or building exhaust system.

Evidence of Compliance:
- ✓ Written procedure defining the types of safety cabinets, filtration systems and exhaust systems used **AND**
- ✓ Maintenance schedule of BSC function checks **AND**
- ✓ Records of testing and certification **AND**
- ✓ Records of HEPA filters used for filtration of all BSC classes **AND**
- ✓ Records of exhaust mechanism **OR** recirculation, if appropriate

PARASITOLOGY

QUALITY CONTROL

MIC.45900 QC Verification **Phase II**

The results of controls are reviewed for acceptability before reporting patient results.

Evidence of Compliance:
- ✓ Written policy/procedure stating that controls are reviewed and acceptable prior to reporting patient results **AND**
- ✓ Evidence of corrective action taken when QC results are not acceptable

***REVISED** 07/11/2011*
MIC.48450 QC Corrective Action **Phase II**

There is documentation of corrective action when control results exceed the acceptability limits.

NOTE: Patient test results obtained in an analytically unacceptable test run or since the last acceptable test run must be re-evaluated to determine if there is a significant clinical difference in patient/client results. Re-evaluation may or may not include retesting samples, depending on the circumstances.

MIC.51000 Reference Materials **Phase II**

Reference materials, such as permanent mounts, photomicrographs, NCCLS documents M15-A and M28-A2, or printed atlases are available at the work bench to assist with identifications.

REAGENTS

MIC.51120 **Reagents** **Phase II**

If zinc sulfate is used, the solution is checked for specific gravity (1.18 for fresh specimens and 1.20 for formalin-fixed specimens) with a hydrometer whose scale is large enough to differentiate the two values.

Evidence of Compliance:
- ✓ Records for specific gravity checks on the zinc sulfate solution

MIC.51140 **Reagent Storage** **Phase I**

The zinc sulfate flotation solution is stored in a tightly-stoppered bottle.

MIC.51160 **Permanent Stain QC** **Phase II**

All permanent parasitology stains are checked for intended reactivity with controls or reference materials at least monthly (or with each test if performed less frequently than every month).

NOTE: PVA fixative solutions thoroughly mixed with fresh fecal material that has been seeded with buffy coat leukocytes usually provides reliable controls for permanent stains.

Evidence of Compliance:
- ✓ Records of permanent stain QC documented at defined frequency

MIC.51170 **Special Stain QC** **Phase II**

Stains that are used to detect specific parasites (*e.g.* acid fast, fluorescent) are checked with appropriate control organisms each time that stain is used.

Evidence of Compliance:
- ✓ Records of special stain QC each time of use

INSTRUMENTS AND EQUIPMENT

MIC.51210 **Ocular Micrometer** **Phase II**

An ocular micrometer is available for determining the size of eggs, larvae, cysts, trophozoites, and microfilaria or other bloodborne parasites.

MIC.51220 **Calibration/Recalibration - Ocular Micrometer** **Phase II**

The ocular micrometer has been calibrated for the microscope(s) in which it is used and it is recalibrated each time the eyepieces or objectives are changed.

NOTE: Calibrations can be checked against a micrometer or other objects of known dimensions. If there are no changes to a particular microscope's optical components, there is no need to recheck calibration.

Evidence of Compliance:
- ✓ Records of initial calibration and recalibration if applicable

PROCEDURES AND TESTS

STOOLS FOR OVA AND PARASITES

MIC.52100 Ova/Parasite Exam **Phase II**

The microscopic examination of all stools submitted for an ova and parasite (O&P) examination includes a concentration procedure and a permanent stain.

NOTE: When a stool specimen is submitted fresh, the usual approach would be to perform a direct wet preparation (looking for motility), a concentration (helminth eggs/larvae/protozoan cysts), and the permanent stained smear (identification of protozoa missed by concentration and confirmation of suspect organisms). As a minimum (and certainly if the stool is submitted in preservatives), the standard O&P examination would include the concentration procedure and a permanent stained smear. The main point is to ensure that the permanent stained smear is performed on all stool specimens, regardless of what was or was not seen in the concentration wet preparation. Often, intestinal protozoa will be seen in the permanent stained smear, but may be missed in the concentration examination.

Evidence of Compliance:
- ✓ Written procedures for stool for O&P **AND**
- ✓ Patient reports/worksheets with concentration and permanent stain results

MIC.52160 Direct Wet Mount **Phase I**

The microscopic examination of liquid stools includes a direct wet mount if submitted fresh.

NOTE: Liquid stools contain the motile form of the intestinal protozoa (the trophozoite). Thus, in fresh stool, the examination of a direct saline preparation might allow one to see motile organisms. However, there are often significant delays between the time of stool passage and when the laboratory receives the specimen. Thus, the ability to see motile organisms may or may not be possible, depending on the condition of the specimen. The number of times motile protozoa are seen will vary tremendously.

Evidence of Compliance:
- ✓ Written procedure for O&P **AND**
- ✓ Patient reports/worksheet with direct wet mount results, as applicable

MIC.52190 Stool Number/Timing **Phase I**

The laboratory has guidelines (developed with clinicians) for the number and/or timing of collection of stool specimens submitted for routine parasitology testing.

NOTE: Suggestions made by the authors of a 1996 CAP Q-Probes study (Valenstein et al) include:

1. *Accept no more than 2 or 3 specimens/patients without prior consultation with an individual who can explain the limited yield provided by additional specimens*
2. *Do not accept specimens from inpatients after the fourth hospital day, without prior consultation*

These recommendations are for diagnostic testing. Different guidelines may apply to tests ordered for follow-up.

BLOOD FILMS FOR MALARIA AND OTHER PARASITES

MIC.52193 **Blood Parasite Detection** **Phase I**

The microscopic examination of blood films submitted for detection of blood parasites allows for detection of parasites responsible for malaria, babesiosis, trypanosomiasis and filariasis.

MIC.52195 **Percentage Parasitemia Reporting** **Phase I**

When blood films are positive for malaria parasites (Plasmodium spp.), the percentage parasitemia is reported along with the organism identification.

NOTE: It is important to report the percentage of parasitemia when blood films are reviewed and found to be positive for malaria parasites. Because of the potential for drug resistance in some of the Plasmodium species, particularly P. falciparum, it is important that every positive smear be assessed and the parasitemia reported exactly the same way on follow-up specimens as on the initial specimen. This allows the parasitemia to be followed after therapy has been initiated. The parasitemia will usually drop very quickly within the first 24 hours; however, in cases of drug resistance, the level may not decrease, but actually increase over time.

Evidence of Compliance:

- ✓ Written procedure for performing and reporting parasitemia percentage with identification

*****REVISED** 06/17/2010***

MIC.52200 **Thick and Thin Films** **Phase I**

Both thick and thin films (routine blood films and/or buffy coat films), or methods of equivalent sensitivity, are made to provide thorough examination for blood parasites.

MIC.52220 **Malaria Stain Procedure** **Phase I**

There is documentation that malaria stains are washed with a buffer of a pH appropriate for the stain used (*e.g.* pH 6.8-7.2 for Giemsa), or the range specified by the manufacturer.

MIC.52260 **Slide Review Procedure** **Phase I**

An adequate number of fields are examined under oil immersion using the 100X oil immersion objective (*e.g.* 300 fields).

Evidence of Compliance:

- ✓ Written procedure defining criteria for assessment of malaria slides including objective and number of fields examined

LABORATORY SAFETY

NOTE TO THE INSPECTOR: The inspector should review relevant requirements from the Safety section of the Laboratory General checklist, to assure that the parasitology laboratory is in compliance. Please elaborate upon the location and the details of each deficiency in the Inspector's Summation Report.

The following requirements pertain specifically to the parasitology laboratory.

MIC.53050 Formalin Safety **Phase II**

If a procedure uses formalin, formaldehyde vapor concentrations are maintained below the following maxima, expressed as parts per million.

NOTE: Formaldehyde vapor concentrations must be monitored in all areas where formalin is used. Initial monitoring involves identifying all employees who may be exposed at or above the action level or at or above the STEL and accurately determining the exposure of each employee identified. Once an initial monitoring procedure has been performed, further periodic formaldehyde monitoring is mandated if the initial monitoring result equals or exceeds 0.5 ppm (8 hr time-weighted exposure, the "action level") or 2.0 ppm (STEL). The laboratory may discontinue periodic formaldehyde monitoring if results from 2 consecutive sampling periods taken at least 7 days apart show that employee exposure is below the action level and the short-term exposure limit, and 1) no change has occurred in production, equipment, process or personnel or control measures that may result in new or additional exposure to formaldehyde, and 2) there have been no reports of conditions that may be associated with formaldehyde exposure.

Formaldehyde monitoring must be repeated any time there is a change in production, equipment, process, personnel, or control measures which may result in new or additional exposure to formaldehyde for an employee involved in the activity. If any personnel report signs or symptoms of respiratory or dermal conditions associated with formaldehyde exposure, the laboratory must promptly monitor the affected person's exposure.

8 hr Time-Weighted Exposure Limit	Action Level (8 hr Time-Weighted Exposure)	15 min Short-Term Exposure Limit (STEL)
0.75	0.5	2.0

Evidence of Compliance:

- ✓ Written safety procedure for formalin including action limits, criteria for discontinuation of monitoring and criteria for resumption of monitoring **AND**
- ✓ Record of initial formalin monitoring **AND**
- ✓ Records of resumption of formalin monitoring when action limits are exceeded

MIC.53150 Ether Safety **Phase II**

If a procedure uses ether, the diethyl ether is stored on open shelves in a well ventilated room using the smallest can feasible (as shipped by manufacturer).

NOTE: The use of concentration techniques other than those requiring the use of ether is recommended.

MOLECULAR MICROBIOLOGY

For guidance on which subsection of the Molecular part of the Microbiology checklist applies to specific types of tests, see table below. Note that the subsection on General Requirements applies to all of these tests.

Test Description	Examples	Required Checklist Section
FDA-cleared/approved non-amplification methods	PACE2 for detection of CT/GC, PNA FISH, AFFIRM	FDA-cleared/approved non-amplification methods
FDA-cleared/approved amplification methods	HIV-1 viral load, amplified CT/GC, HPV, HIV-1 genotyping	FDA-cleared/approved target & signal amplification methods & sequencing
FDA-cleared/approved amplification methods, using a different specimen type other than cleared/approved	Use of CSF specimens in HIV-1 viral load testing, use of rectal swab for CT testing	FDA-cleared/approved target & signal amplification methods & sequencing
FDA-cleared/approved amplification methods, using a collection device other than cleared/approved	Changing the collection device or swab for CT/GC testing, alternative swab or transport media for respiratory virus detection	FDA-cleared/approved target & signal amplification methods & sequencing
FDA-cleared/approved amplification methods with modification of a test component	Changing the extraction method for HIV-viral load testing, modification of interpretative criteria	Laboratory-developed or modified FDA-cleared/approved tests
Laboratory-developed tests	CMV viral load testing using an ASR, laboratory developed EBV viral load with custom synthesized primers and probes	Laboratory-developed or modified FDA-cleared/approved tests

This checklist section does not apply to tests using direct non-amplified nucleic acid probes to identify organisms from a positive culture. Such tests may be inspected with the Mycobacteriology, Mycology or other appropriate section of this checklist.

This checklist section applies to all other molecular microbiology tests, including FDA cleared/approved tests, as well as tests not approved/cleared by the FDA (including FDA cleared/approved tests modified by the laboratory, and laboratory-developed tests).

NOTES:

1. *When specimens are referred to outside reference laboratories for sequence analysis or other testing, such laboratories must meet the requirements in GEN.41350 and other applicable requirements in the "Reporting of Results" section of the Laboratory General checklist.*
2. *If the laboratory chooses to pool specimens for testing (e.g. Chlamydia trachomatis/Neisseria gonorrhoeae NAAT on pooled urine specimens), the testing procedure for pooled samples must be validated, including limit of detection, reproducibility, and accuracy (method comparison). As part of the method comparison, the protocol for pooled specimens must be compared to the single (non-pooled) testing protocol using an adequate number of clinical specimens covering the entire range of organism concentration seen in clinical specimens (i.e. low and high positive specimens).*
   - *The sensitivity of the method must equal the performance characteristics of the FDA-cleared/approved method, i.e. it must equal that of low positive, unspooled sample*
   - *The "laboratory-developed or modified FDA-cleared/approved test" section of the checklist must be used for the validation if the limit of detection differs from the licensed method.*

A database of FDA approved/cleared tests can be found at
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCLIA/Search.cfm

Laboratories that use this section of the checklist must also comply with all applicable requirements included in the General section of the Microbiology checklist.

GENERAL REQUIREMENTS

QUALITY MANAGEMENT

MIC.63252 Statistics **Phase I**

When appropriate, appropriate statistics (*e.g.* percentage of results that are positive for *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*) are maintained and monitored.

NOTE: An increase above the expected positive rate within a run or over multiple runs should prompt investigation for potential false positive results.

Evidence of Compliance:
- ✓ Written procedure for calculating statistics including thresholds **AND**
- ✓ Records of statistical data, evaluation and corrective action if indicated

MIC.63256 Turnaround Times **Phase I**

There is evidence that the laboratory monitors sample turnaround times and that they are appropriate for the intended purpose of the test.

NOTE: There are certain clinical situation in which rapid completion is essential. An example is detection of HSV in CSF.

Evidence of Compliance:
- ✓ Written policy defining turnaround time and mechanism for monitoring **AND**
- ✓ Records showing that times defined in the policy are routinely met

QUALITY CONTROL

Controls are samples that act as surrogates for patient/client specimens. They are processed like a patient/client sample to monitor the ongoing performance of the entire analytic process in every run.

Qualitative molecular tests typically include positive and negative controls and, in some instances, a sensitivity control to show that low level target is detectable. Quantitative tests typically include a negative control and at least two (2) levels of control at relevant decision points to verify that calibration status is maintained within acceptable limits.

*****REVISED** 07/11/2011***
MIC.63262 Daily QC **Phase II**

Controls are run daily for quantitative and qualitative tests.

Controls should verify assay performance at relevant clinical decision points. The selection of these points may be based on clinical or analytical criteria.

NOTE 1: Except for tests meeting the criteria in Note 2, below, external surrogate sample controls must be run as follows:*

- *For quantitative molecular tests, 3 controls must be run daily - a negative control, a low-positive control and a high-positive control, except where a specific exception is given in this checklist*

- *For qualitative tests, a positive and negative control must be run daily*

Control testing is not necessary on days when patient testing is not performed.

NOTE 2: Daily controls may be limited to electronic/procedural/built -in (e.g. internal, including built-in liquid) controls for tests meeting the following criteria:

1. *For quantitative tests, the test system includes 2 levels of electronic/procedural/built-in internal controls that are run daily*
2. *For qualitative tests, the test system includes an electronic/procedural /built-in internal control run daily*
3. *The system is FDA-cleared or approved, and not modified by the laboratory***
4. *The laboratory has performed studies to validate the adequacy of limiting daily QC to the electronic/procedural/built-in controls. Validation studies must include daily comparison of external controls to built-in controls for at least 20 consecutive days when patient samples are tested. For validation of multiple identical devices, the minimum of 20 consecutive daily comparisons applies to the initial device; the laboratory director is responsible for determining the extent of the validation studies for the other devices. Acceptable validation is required before daily quality control can be limited to built-in controls. The laboratory director is responsible for determining criteria for acceptability, and other details of the validation. Validation records must be retained while an instrument/method is in service, and for two years afterwards. The requirement for 20 consecutive daily comparisons is effective for validation studies performed after 1/31/2012. Corrective action must be taken if either the internal or external control is out of acceptable range during or after the evaluation process. Repeating controls or re-evaluation of the internal control system may be necessary to achieve acceptable results.*
5. *External surrogate sample controls are run for each new lot number or shipment of test materials; after major system maintenance; and after software upgrades.*** Regarding the positive external control for qualitative tests, best practice is to run a weak positive control, and in the case of drug testing, also a high negative control (e.g. 25% below cutoff) to maximize detection of problems with the test system..*
6. *External surrogate sample controls are run as frequently as recommended by the test manufacturer, or every 30 days, whichever is more frequent.*

NOTE 3:

- *Controls must assess adequacy of extraction and amplification, e.g. positive and negative controls that go through the entire testing process. For qualitative tests with an internal control that goes through the extraction and amplification steps, a target specific positive control(s) is still required.*

1. *If the internal control does not go through the extraction method, a separate extraction control is needed for each run (positive controls fulfill this requirement).*
2. *If the samples from an extraction batch are tested over multiple amplification runs, each amplification run (as defined by the laboratory) must have its own amplification control. A single extraction control need only be tested in one of the amplification runs.*
3. *If samples from multiple extraction batches are tested in a single amplification run, each extraction batch needs an extraction control. All extraction controls must be tested in a single amplification run. A single amplification control is sufficient.*

**A "surrogate sample" is a specimen designed to simulate a patient sample for quality control purposes. Traditional external liquid control materials are considered surrogate external surrogate sample controls. Some surrogate sample controls may not be external, but may be contained within an instrument (e.g. in a cartridge); systems using these built-in controls must meet the requirements in Note 2, above.*

***Sample types (or use of collection devices) not listed in manufacturer instructions are acceptable, if validated by the laboratory.*

****Repetition of the initial validation study is not required when running external surrogate sample controls with new lots/shipments of test materials, after system maintenance or software upgrades, or in accordance with paragraph 6 in the Note.*

Evidence of Compliance:

- ✓ Records of QC results including external and electronic/procedural/built-in control systems **AND**
- ✓ Records documenting in-house validation of electronic/procedural/built-in control systems, if used

MIC.63264 Multiplex QC **Phase II**

For multiplex tests, controls for each analyte are either included in each run or rotated so that all analytes are tested periodically.

Evidence of Compliance:

- ✓ Written procedure defining multiplex test QC **AND**
- ✓ Records of multiplex test QC

*****REVISED** 06/17/2010***

MIC.63274 QC Verification **Phase II**

Results of controls are reviewed for acceptability prior to reporting patient results.

NOTE: Conditions causing unacceptable control results must be investigated and corrective action must be documented.

Evidence of Compliance:

- ✓ Written policy/procedure stating that controls are reviewed and acceptable prior to reporting patient results **AND**
- ✓ Evidence of corrective action taken when QC results are not acceptable

MIC.63275 QC Acceptability Limits **Phase II**

Acceptability limits are defined for all control procedures, control materials, and standards.

NOTE: Acceptability limits must be defined for all control procedures, control materials, and standards. These controls must be appropriate for the range of sensitivities tested and should, ideally, focus on result ranges that are near clinical decision points.

Evidence of Compliance:

- ✓ Written QC procedure(s) defining acceptability limits

*****REVISED** 07/11/2011***

MIC.63276 QC Corrective Action **Phase II**

There is documentation of corrective action when control results exceed defined acceptability limits.

NOTE: Even if patient samples are no longer available, test results can be re-evaluated to search for evidence of an out-of-control condition that might have affected patient results. For example, evaluation could include comparison of patient means for the run in question to historical patient means, and/or review of selected patient results against previous results to see if there are consistent biases (all results higher or lower currently than previously for the test(s) in question). Re-evaluation may or may not include retesting samples, depending on the circumstances.

MIC.63277 QC Statistics **Phase II**

For QUANTITATIVE assays, quality control statistics are performed monthly to define analytic imprecision and to monitor trends over time.

NOTE: The laboratory must use statistical methods such as calculating SD and CV monthly to evaluate variance in numeric QC data.

Evidence of Compliance:
- ✓ Written procedure for monitoring of analytic imprecision including statistical analysis of data

✓ **MIC.63278** **Inhibition Assessment** **Phase II**

For assays without an internal control, the laboratory has a procedure to assess inhibition for each specimen type.

NOTE: Documentation of an acceptable inhibition rate may be provided by the manufacturer. If not, laboratories may test for inhibition by spiking an aliquot of the clinical specimen with target nucleic acid. This practice can be discontinued once the laboratory accumulates sufficient data showing that the inhibition rate falls within acceptable limits.

This requirement does not apply to probe-based solution hybridization methods (e.g. Gen-Probe AccuProbe) performed without nucleic acid amplification.

✓ **MIC.63282** **Equivocal QC** **Phase II**

If results of negative controls are positive or equivocal, the laboratory has a written procedure in place to investigate and resolve the problem.

NOTE: This checklist requirement does not apply to probe-based solution hybridization methods (e.g. Gen-Probe AccuProbe) performed without nucleic acid amplification.

PROCEDURE MANUAL

NOTE: The requirements in the Procedure Manual subsection of the General Microbiology section of this checklist are applicable to the Molecular Microbiology section.

✓ **MIC.63297** **Analytic Interpretation** **Phase II**

There are written guidelines for analytic interpretation of results, as applicable.

✗ **MIC.63298** **Calculating Quantitative Values** **Phase II**

For quantitative molecular tests, methods for calculating quantitative values are adequately described and units clearly documented.

SPECIMEN HANDLING & PROCESSING

✓ **MIC.63318** **Specimen Collection Manual** **Phase II**

Procedures are in place to prevent specimen loss, alteration, or contamination during collection, transport, processing and storage.

NOTE: Specimen collection, processing and storage must follow manufacturer instructions. For example, for amplified molecular testing using liquid based cervical cytology (LBC) specimens, there must be a procedure in place to ensure absence of cross contamination of samples during

the LBC processing steps; alternatively, an aliquot can be removed for amplified molecular testing prior to LBC processing.

MIC.63322 Specimen Aliquots **Phase II**

If aliquoting of specimens is performed, there is a written procedure to prevent any possible cross-contamination of the aliquot containers.

NOTE: Although in some cases it may be appropriate to aliquot a specimen, the laboratory must have a policy that no aliquot is ever returned to the original container.

MIC.63324 Residual Samples **Phase I**

If residual samples are used for amplification-based testing, policies and procedures ensure absence of cross-contamination of samples.

NOTE: An example of a residual sample is a liquid based cytology sample that is tested post-cytologic processing using amplified C. trachomatis or N. gonorrhoeae tests.

MIC.63327 Specimen ID **Phase II**

There is a system to positively identify all patient specimens, specimen types and aliquots through all phases of the analysis, including specimen receipt, nucleic acid extraction, nucleic acid quantification, hybridization, detection, documentation, and storage.

NOTE: Each specimen container must identify the patient uniquely. Identification may be text-based, numeric, bar-coded, etc. The form of this system is entirely at the discretion of each laboratory, so long as all primary collection containers and their aliquots have a unique label which one can trace back to full particulars of patient identification, collection date, specimen type, etc.

*****REVISED** 07/11/2011***
MIC.63328 Specimen Processing/Storage **Phase II**

Patient samples are processed promptly or stored appropriately to minimize degradation of nucleic acids.

NOTE: Frost-free freezers may not be used to store patient samples unless freezer temperature is monitored by a continuous monitoring system, or a maximum/minimum thermometer.

Evidence of Compliance:
- ✓ Written procedure for processing and storage of specimens

RESULTS REPORTING

MIC.63330 Final Report **Phase I**

The final report includes a summary of the test method and information regarding clinical interpretation if appropriate.

NOTE: For example, HIV-1 viral load results may vary significantly depending upon the test method used; including the test method in the report is important information for interpreting the results.

REAGENTS

The laboratory has the responsibility for ensuring that all reagents used are appropriately reactive biochemically. The verification of reagent performance is required and must be documented. Any of several methods may be appropriate, such as direct analysis with reference materials, parallel testing of old vs. new reagents, and checking against routine controls.

***REVISED** 07/11/2011*
MIC.63350 Reagent Storage **Phase II**

All test reagents and controls are stored properly and in a manner which minimizes target DNA/RNA contamination and degradation.

NOTE: Pre- and post-amplification reagents and controls should be stored under appropriate temperature and other conditions in designated pre- and post-amplification areas. Temperature-sensitive reagents and/or controls may not be stored in frost-free freezers, unless either of the following conditions are met: 1. Reagent/control materials are kept in thermal containers and the laboratory can demonstrate that the function of these materials is not compromised; or 2. Freezer temperature is monitored by a continuous monitoring system, or a maximum/minimum thermometer.

Patient samples may be stored in a frost free freezer only if the temperature is monitored in accordance with (2) , above.

Evidence of Compliance:
- ✓ Written procedure defining storage requirements for reagents and controls

***REVISED** 07/11/2011*
MIC.63575 New Reagent Lot Validation **Phase II**

New reagent lots and/or shipments are checked against old reagent lots with patient samples or with suitable reference material before or concurrently with being placed in service.

NOTE: New reagent lots and/or shipments must be tested for appropriate reactivity before or concurrently with being placed in service to ensure that the new lot of reagent maintains consistent results for patient specimens. Patient specimens should be used to compare a new lot against the old lot, when possible, since it is patient specimens that are tested. However, for comparison of a new shipment of reagent from the same lot as the reagent in current use, control material is adequate, as there should be no change in potential matrix interactions between QC material and different shipments of the same lot number of reagent.

For qualitative tests, minimum cross-checking includes retesting at least one known positive and one known negative patient sample against the new reagent lot. A weakly positive sample should also be used in systems where patient results are reported in that fashion. If it is not practical to use patient specimens for comparing the new lot to the old lot, reference materials or quality control materials (from the new or old lot) may be used.

The following comments apply to quantitative tests:

Minimum cross-checking includes retesting with the new lot/shipment at least one known negative sample, one sample in the low positive range and one sample in the mid-to-high positive range that were tested with the old lot/shipment.

Some method manufacturers provide reference materials or QC products specifically intended to validate successful calibration of their methods; these should be used when available. Such materials have method-specific, and, where appropriate, reagent-lot-specific, target values. Thus these materials should be used only with the intended methods.

Proficiency testing materials with peer group established means and QC materials are acceptable alternatives for validating new reagent lots. However, the laboratory should be aware that PT and QC materials may be affected by matrix interference between different reagent lots. Thus, even if results show no change following a reagent lot change, a calibration inconsistency for patient specimens could exist nonetheless, masked by matrix interference affecting the PT or QC material. It is for this reason--to confirm the absence of matrix interference--that the use of patient samples is recommended.

If QC material is used, the material should have a peer group established mean value based on interlaboratory comparison that is method specific and includes data from at least 10 different laboratories.

Third party general purpose reference materials may be suitable for validation of calibration following reagent lot changes if the material is documented in the package insert or by the method manufacturer to be commutable with patient specimens for the method. A commutable reference material is one that gives the same numeric result as would a patient specimen containing the same quantity of analyte in the analytic method under discussion; e.g., matrix effects are absent. Commutability between a reference material and patient specimens can be demonstrated using the protocol in CLSI EP14-A2.

Evidence of Compliance:
- ✓ Written policy for the validation of new lots and shipments prior to use **AND**
- ✓ Records of validation of new reagents lots/shipments

MIC.63580 New Reagent Lot - Multiplex Tests **Phase II**

For multiplex tests, all analytes detected by the assay are individually verified for each new shipment and/or lot.

NOTE: Verification of new shipments and/or lots may be difficult for rare organisms or subtypes. In these situations, verification may be performed annually.

Evidence of Compliance:
- ✓ Written procedure for new lot/shipment validation of all analytes detected by each multiplex assay **AND**
- ✓ Records of new lot/shipment validation

PROCEDURES & TESTS

MIC.63800 Carryover **Phase II**

Nucleic acid amplification procedures (*e.g.* PCR) are designed to minimize carryover (false positive results) using appropriate physical containment and procedural controls.

NOTE: This item is primarily directed at ensuring adequate physical separation of pre- and post-amplification samples to avoid amplicon contamination. The extreme sensitivity of amplification systems requires that the laboratory take special precautions. For example, pre- and post-amplification samples should be manipulated in physically separate areas; gloves must be worn and frequently changed during processing; dedicated pipettes (positive displacement type or with aerosol barrier tips) must be used; and manipulations must minimize aerosolization. Enzymatic destruction of amplification products is often helpful, as is real-time measurement of products to avoid manual manipulation of amplification products.

Evidence of Compliance:
- ✓ Written procedure that defines the use of physical containment and procedural controls as applicable to minimizing carryover

***REVISED** 06/17/2010*

MIC.64025 Isolation/Preparation **Phase II**

The adequacy of nucleic acid isolation/preparation procedures are evaluated.

NOTE: Adequacy of nucleic acid isolation/preparation procedures (manual or automated) must be evaluated with each assay by the use of positive and negative controls run in parallel with patient samples. To the extent possible, controls must be processed through all steps of the assay, including the extraction phase.

Evidence of Compliance:
- ✓ Written procedure for evaluating adequacy of nucleic acid **AND**
- ✓ Records of controls used to assess adequacy

MIC.64350 Temperature Range Defined **Phase II**

For each step of the procedure all incubation temperatures are defined and documented.

NOTE: For some instruments this function is performed automatically by software provided by the manufacturer.

MIC.64450 Incubations - Manufacturer Specifications **Phase I**

Incubations (reactions) performed using baths/blocks/instruments meet manufacturer specifications.

NOTE: Bath/blocks/instruments must be able to maintain the appropriate temperature throughout the incubation (reaction) within the range specified by the manufacturer of the assay.

Evidence of Compliance:
- ✓ Written procedure for incubation performance consistent with manufacturer specifications

MIC.64550 Temperature Corrective Action **Phase II**

If any incubation temperature is out of range, the deviation is reported to the supervisor or designee and corrective action documented.

INSTRUMENTS

MIC.64614 Thermocycler Temperature Checks **Phase I**

Individual wells (or a representative sample thereof) of thermocyclers are checked for temperature accuracy before being placed in service and at least annually thereafter.

NOTE: A downstream measure of well-temperature accuracy (such as productivity of amplification) may be substituted to functionally meet this requirement. For closed systems this function should be performed as a component of the manufacturer-provided preventative maintenance.

Evidence of Compliance:
- ✓ Written procedure for verification of thermocycler accuracy **AND**
- ✓ Records of thermocycler verification

LABORATORY SAFETY

The inspector should review relevant requirements from the Safety section of the Laboratory General checklist, to assure that the molecular testing section is in compliance. Please elaborate upon the location and the details of each deficiency in the Inspector's Summation Report.

The following requirement pertains specifically to the molecular microbiology section.

✓ **MIC.64620** **Specimen Handling/Processing** **Phase II**

There are documented policies for the safe handling and processing of samples from patients with suspected infections due to avian influenza, SARS, or similar emerging pathogens.

PERSONNEL

✓ **MIC.64631** **Personnel Training** **Phase I**

There is an adequate training program for supervisory personnel and technologists.

Evidence of Compliance:
- ✓ Documented training program **AND**
- ✓ Records of training by the institution or appropriate outside organization

✗ **MIC.64634** **Personnel - Technical Operations** **Phase II**

If the laboratory performs non-FDA cleared/approved tests, the person(s) in charge of technical operations of molecular microbiology has education in microbiology equivalent to an MT(ASCP) and at least 4 years experience (one of which is in molecular microbiology) under a qualified laboratory director.

Evidence of Compliance:
- ✓ Records of qualifications including degree or transcript, certification/registration, current license (if required) and work history in related field

FDA CLEARED/APPROVED NON-AMPLIFICATION METHODS

Examples of this type of testing include C. trachomatis/N. gonorrhoeae DNA probes, Group A Streptococcus DNA probes, PNA FISH testing, and Affirm T. vaginalis, C. albicans, and G. vaginalis testing.

This section is used for the direct detection of pathogens from clinical samples and not for culture confirmation.

QUALITY CONTROL

✗ **MIC.64710** **ISH QC** **Phase II**

Appropriate positive and negative controls are run in parallel and results documented for each microbial in situ hybridization (ISH) analysis.

NOTE: Laboratories should refer to the manufacturer's guidelines for the selection of appropriate controls. Quality control must be performed with every run, independent of the number of samples tested (i.e. 1 sample or batch of several samples)

Evidence of Compliance:

- ✓ Written procedure for ISH QC consistent with manufacturer's guidelines

MIC.64720 QC Corrective Action **Phase II**

Corrective action is documented when microbial ISH (in situ hybridization) results do not correlate with culture findings.

NOTE: Discordant findings should be promptly investigated for potential false positive or false negative results from reagent failure, technical error, interpretive error or cross-reactivity of probes.

*****REVISED** 07/11/2011***
MIC.64730 Slide Usage - Manufacturer Recommendations **Phase I**

For microbial fluorescence in situ hybridization (FISH) testing, the laboratory uses only the microscope slides and filters recommended by the manufacturer.

NOTE: Use of other microscope slides can result in inaccurate or inconclusive results from non-specific or interfering background fluorescence.

MIC.64750 Group B Screening **Phase II**

Negative results obtained for Group B streptococcus intrapartum screening by direct DNA probe are followed up with a selective broth culture method.

NOTE: This test is insufficiently sensitive to detect light colonization and is therefore not adequate to replace culture based prenatal screening or to use in place of risk based approaches when culture results are unknown at time the of labor. An adequate rapid intrapartum test must be as sensitive as culture of vaginal and rectal swabs inoculated into selective broth media.

Evidence of Compliance:

- ✓ Written procedure requiring follow-up testing for negative Group B performed by direct DNA probe

ASSAY VERIFICATION

MIC.64760 Verification Study **Phase II**

There is documentation that the laboratory has performed a verification study prior to reporting patient results.

NOTE: Laboratories must verify manufacturer data on analytic accuracy, precision and reportable range. Verification studies must include an adequate number of positive and negative samples representing the specimen types used in the assay (e.g. cervical swabs, urethral swabs and urine). Samples may include spiked specimens (suspensions of target added to appropriate matrix), if patient samples are not available or inadequate in number across the dynamic range of a quantitative assay.

For analytic sensitivity, interferences and reference ranges, laboratories may use data from the manufacturer or the literature. Refer to the section "Test Method Validation" in the Laboratory General checklist for additional details.

MIC.64770 Validation Studies - Sample Type/Collection **Phase II**

If the laboratory tests sample types or uses collection devices other than those listed in the package insert, the laboratory performs validation studies to document adequate performance of the test.

NOTE: Results from tests performed on sample types not listed in the package insert may be reported without complete validation under two circumstances only: 1) validation studies are on-going but have not been completed; 2) the sample type is encountered rarely, precluding an adequate number for validation studies. Under these circumstances, the test report must include a disclaimer stating that the sample type has not been validated

FDA CLEARED/APPROVED TARGET & SIGNAL AMPLIFICATION METHODS & SEQUENCING

Examples of this type of testing include both target and signal nucleic acid amplification tests for the detection of C. trachomatis, N. gonorrhoeae , methicillin resistant Staphyloccocus aureus, human papillomavirus, Mycobacterium tuberculosis, HIV-1 and Hepatitis C viral load testing, and HIV-1 genotypic testing.

QUALITY CONTROL

*****REVISED** 06/17/2010***
MIC.64810 Test Performance - Manufacturer Instructions **Phase II**

Tests are performed and results reported as specified in package inserts without substitution of reagents or modification of testing protocol.

NOTE: If any part of the test procedure is modified, the test is subject to the requirements of the subsection "Laboratory-Developed or Modified FDA-Cleared/Approved Tests (see below)." Use of this subsection is not required for specimen types or collection devices not listed in the package insert.

MIC.64815 Validation Studies - Sample Type/Collection **Phase II**

If the laboratory tests sample types or uses collection devices other than those listed in the package insert, the laboratory performs validation studies to document adequate performance of the test.

NOTE: Results from tests performed on sample types not listed in the package insert may be reported without complete validation under two circumstances only: 1) validation studies are on-going but have not been completed; 2) the sample type is encountered rarely, precluding an adequate number for validation studies. In these circumstances, the test report must include a disclaimer stating that the sample type has not been validated.

MIC.64820 M.tb Molecular Testing **Phase II**

When performing molecular testing for the detection of *M. tuberculosis* directly from clinical specimens, culture is performed on all samples regardless of the molecular test result.

Evidence of Compliance:
- ✓ Patient reports or worksheets

MIC.64825 Modified Cut-Off **Phase II**

If the laboratory has modified the manufacturer's cut off-value for a positive result, the new cut-off value has been validated.

Evidence of Compliance:
- ✓ Records of cut-off validation when different cut-off values are utilized

✓ **MIC.64830** **Test Calibration** **Phase II**

For quantitative tests, test calibration is performed according to the manufacturer's specifications.

NOTE: Calibrators should be run following the manufacturer's recommendations. Some systems may use electronic calibration data.

Evidence of Compliance:
- ✓ Records of calibration

SEQUENCING

✗ **MIC.64835** **Sequencing Data Criteria** **Phase I**

Criteria are established for the acceptability and interpretation of primary sequencing data.

NOTE: The laboratory should follow manufacturer guidelines for rejection and acceptance criteria for assessing acceptability of sequencing results.

✗ **MIC.64840** **Sequence Data Interpretation** **Phase I**

The laboratory has a process in place to assure that interpretation of sequence data is based on the latest version of the manufacturer's interpretive software.

✗ **MIC.64845** **Alternative Sequencing Interpretive DB** **Phase I**

If the laboratory uses alternative sequence interpretive databases, either alone or in conjunction with manufacturer's software, the alternative databases have been validated for the interpretation of the sequence data.

NOTE: This validation can be completed using published literature that documents the interpretation of the sequence data (for example the ISA-USA resistance interpretation guidelines). If the use of alternative data bases is done by the clinician after laboratory reporting of sequence interpretation, this validation is not necessary.

Evidence of Compliance:
- ✓ Records of validation study if alternative interpretive databases are utilized, if applicable

✗ **MIC.64850** **Sample/Amplicon Contamination** **Phase II**

There is a procedure to prevent or detect potential cross-contamination of samples and/or amplicons.

NOTE: Examples of procedures are the use of negative controls in each batch, the manufacturer's use of Uracil N-glycosylase (UNG), or the fingerprinting program provided by the manufacturer.

✗ **MIC.64855** **Sample/Amplicon Contamination** **Phase II**

If results of fingerprint analysis or negative control indicate a potential for sample and/or amplicon contamination, the laboratory has a written procedure in place to investigate and resolve the problem.

ASSAY VERIFICATION

MIC.64860 Verification Study **Phase II**

There is documentation that the laboratory has performed a verification study prior to reporting patient results.

NOTE: For FDA cleared/approved tests, the laboratory is required to verify certain performance characteristics of the test as outlined in the package insert for all testable specimen types. For qualitative tests this includes comparison of positive and negative test results to a comparable test method. Specimens for the verification can include external control material, cultured organisms and proficiency testing material, and must include positive and negative patient samples. For quantitative tests, the manufacturer's limit of detection, linearity, reportable range, and precision should be verified by the laboratory, as well as a comparison of patient test results across the reportable range of the test. Specimens for the verification can include quantitative external control material, cultured organisms (quantified) and proficiency testing material, and must include patient samples.

Refer to the section "Test Method Validation" in the Laboratory General checklist for additional details.

LABORATORY-DEVELOPED OR MODIFIED FDA CLEARED / APPROVED TESTS

This section must be used for all laboratory-developed tests (qualitative, quantitative, or sequencing) as well as FDA cleared/approved tests in which the test methodology has been altered (for example, use of an alternative extraction method).*

A laboratory-developed test (LDT) is defined as follows: A test used in patient management that has all of the following characteristics:

- The test is performed by the clinical laboratory in which the test was developed
- The test is neither FDA-cleared nor FDA-approved, or is an FDA-cleared/approved test modified by the laboratory (sample types or the use of collection devices not listed in manufacturer instructions constitute modifications, by this definition)
- The test was first used for clinical testing after April 23, 2003

A laboratory is considered to have developed a test if the test procedure was created by the laboratory performing the testing, irrespective of whether fundamental research underlying the test was developed elsewhere or reagents (including ASRs), equipment, or technology integral to the test were purchased, adopted, or licensed from another entity.

***i.e. laboratory-developed tests using ASRs or reagents developed solely by the laboratory.*

QUANTITATIVE ASSAYS: CALIBRATION & STANDARDS

This section of the checklist only applies to quantitative tests for which appropriate external materials exist.

This section deals with the processes of calibration, calibration verification, and analytic measurement range (AMR) validation for quantitative assays.

CALIBRATION is the set of operations that establish, under specified conditions, the relationship between reagent system/instrument response and the corresponding concentration/activity values of an analyte. Calibration procedures are typically specified by a method manufacturer, but may also be established by the laboratory. The term "calibration" has the same meaning in this checklist as in the US CLIA regulations.

However, the term "calibration verification," as used in this checklist, carries a more restrictive meaning than in CLIA. As defined in the January, 2003 revision of CLIA, "calibration verification" refers to 2 distinct processes: 1) verification of correct method calibration and 2) validation of the reportable range. This checklist restricts the use of the term "calibration verification" to the first process. The checklist uses a different term, "analytic measurement range(AMR) validation" to refer to the second process.

In this checklist, CALIBRATION VERIFICATION denotes the process of confirming that the current calibration settings remain valid for a method. If calibration verification confirms that the current calibration settings are valid, it is not necessary to perform a complete calibration or recalibration of the method. Calibration verification can be accomplished in several ways. If the method manufacturer provides a calibration validation or verification process, it should be followed. Other techniques include: 1) assay of the current method calibration materials as unknown specimens, and determination that the correct target values are recovered, and 2) assay of matrix-appropriate materials with target values that are specific for the method.

Each laboratory must define limits for accepting or rejecting tests of calibration verification.

Materials for calibration verification must have a matrix appropriate for the clinical specimens assayed by that method, and target values appropriate for the measurement system. Materials may include, but are not limited to:

1. *Calibrators used to calibrate the analytical measurement system*
2. *Materials provided by the analytical measurement system vendor for the purpose of calibration verification*
3. *Previously tested unaltered patient/client specimens*
4. *Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method*
5. *Proficiency testing material or proficiency testing validated material with matrix characteristics and target values appropriate for the method*

In general, routine control materials are not suitable for calibration verification, except in situations where the material is specifically designated by the method manufacturer as suitable for verification of the method's calibration process.

The ANALYTICAL MEASUREMENT RANGE is the range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment not part of the usual assay process. AMR VALIDATION is the process of confirming that the assay system will correctly recover the concentration or activity of the analyte over the AMR. The materials used for validation must be known to have matrix characteristics appropriate for the method. The matrix of the sample (i.e. the environment in which the analyte is suspended or dissolved) may influence the measurement of the analyte. The method manufacturer may recommend suitable materials. The test specimens must have analyte values that, at a minimum, are near the low, midpoint, and high values of the AMR. Specimen target values can be established by comparison with peer group values for reference materials, by assignment of reference or comparative method values, and by dilution or admixture ratios of one

or more specimens with known values. Each laboratory must define limits for accepting or rejecting validation tests of the AMR.

Materials for AMR validation should have a matrix appropriate for the clinical specimens assayed by that method, and target values appropriate for the measurement system. Materials may include, but are not limited to:

1. *Linearity material of appropriate matrix, e.g. CAP Survey-based or other suitable linearity verification material*
2. *Proficiency testing survey material or proficiency testing survey-validated material*
3. *Previously tested patient/client specimens, unaltered*
4. *Previously tested patient/client specimens, altered by admixture with other specimens, dilution, spiking in known amounts of an analyte, or other technique*
5. *Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method*
6. *Calibrators used to calibrate the analytic measurement system*
7. *Control materials, if they adequately span the AMR.*

RECALIBRATION/CALIBRATION VERIFICATION and AMR VALIDATION INTERVAL: Recalibration or calibration verification, and AMR validation must be performed at least once every 6 months, as specified under CLIA regulations at 42CFR493.1255(b)(3). Successful calibration verification certifies that the calibration is still valid; unsuccessful calibration verification requires remedial action, which usually includes recalibration. The performance of recalibration or a calibration verification procedure resets the calendar to a new maximum 6-month interval before the next required reassessment. Methods that are recalibrated more frequently than every 6 months do not require a separate calibration verification procedure.

In addition to the every 6 month requirement, laboratories must perform recalibration or calibration verification and AMR validation at changes in major system components, and at changes of lots of chemically or physically active reagents unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient/client test results. The director should determine what constitutes a verification and revalidation of the AMR. Manufacturers' instructions should be followed.

The laboratory should establish other criteria, as appropriate, for recalibration/calibration verification. These include but are not limited to failure of quality control to meet established criteria, and major maintenance or service to the instrument.

MIC.64868 Calibration Procedures **Phase II**

Calibration procedures for each test are adequate, and the calibration results are documented.

MIC.64870 Calibration Materials **Phase II**

High quality materials with test- and matrix-appropriate target values are used for calibration and calibration verification whenever possible.

NOTE: For example, if multiple specimen types are tested in a quantitative test, the test calibration must encompass the range for all expected values for each specimen type. If one calibration range is not sufficient, then more than one calibration range may be required.

Evidence of Compliance:

- ✓ Written procedure defining the use of appropriate calibration/calibration verification materials

MIC.64872 Calibration Materials **Phase II**

All calibration materials used for non-FDA cleared tests are documented as to quality.

NOTE: Commercial standards used to prepare calibrators require certificates of analysis. The laboratory should document the accuracy of a new lot of calibrators by checking the new lot against the current lot.

***REVISED** 07/11/2011*
MIC.64874 Calibration Material Labeling **Phase II**

All calibration materials are properly labeled as to content, calibration values, dates placed in service, and expiration dates.

NOTE: Complete values need not be recorded directly on each vial of calibrator material, so long as there is a clear indication where specific values may be found for each analyte tested and each analyzer used by the laboratory.

The dates may be recorded in a log (paper or electronic), rather than on the containers themselves, providing that all containers are identified so as to be traceable to the appropriate data in the log.

Evidence of Compliance:
- ✓ Written procedure defining elements required for labeling of calibration material

MIC.64880 Calibration Verification Criteria **Phase II**

Criteria are established for frequency of calibration or calibration verification, and the acceptability of results.

NOTE: Criteria may include:

1. *At changes of reagent lots, unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client test results and the range used to report patient/client test data*
2. *QC fails to meet established criteria*
3. *After major maintenance or service*
4. *When recommended by the manufacturer*
5. *At least every 6 months*

Evidence of Compliance:
- ✓ Written procedure defining the method, frequency and limits of acceptability of calibration verification for each instrument/test system **AND**
- ✓ Records of calibration verification documented at defined frequency

MIC.64882 Recalibration **Phase II**

The system is recalibrated when calibration verification fails to meet the established criteria of the laboratory.

NOTE: An indication of a potential calibration failure would be external or kit controls with values that repeatedly fall outside of the established control range.

Evidence of Compliance:
- ✓ Written procedure defining criteria for recalibration **AND**
- ✓ Records of recalibration, if calibration or calibration verification has failed

***REVISED** 06/17/2010*
MIC.64884 AMR Validation **Phase II**

Validation of the analytical measurement range (AMR) is performed with matrix-appropriate materials that include the low, mid and high range of the AMR, and the process is documented.

NOTE: If the materials used for calibration or for calibration verification include low, midpoint, and high values that are near the stated AMR, and if calibration verification data are within the laboratory's

acceptance criteria, the AMR has been validated; no additional procedures are required. If the calibration and/or calibration verification materials do not span the full AMR, or the laboratory extends the AMR beyond the manufacturer's stated range, the AMR must be validated by assaying materials reasonably near the lowest and highest values of the AMR.

Calibration, calibration verification, and validation of the analytical measurement range (AMR) are required to substantiate the continued accuracy of a test method. The CLIA regulations use the term "calibration verification" to refer to both verification of correct method calibration and validation of the analytical measurement range. This Checklist uses separate terms to identify two distinct processes that are both required for good laboratory practice.

The AMR is the range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment that is not part of the usual assay process. Validation of the AMR is the process of confirming that the assay system will correctly recover the concentration or activity of the analyte over the AMR.

The materials used for validation must be known to have matrix characteristics appropriate for the method. The test specimens must have analyte values that as a minimum are near the low, midpoint, and high values of the AMR. Guidelines for analyte levels near the low and high range of the AMR should be determined by the laboratory director. Factors to consider are the expected analytic imprecision near the limits, the clinical impact of errors near the limits, and the availability of test specimens near the limits. It may be difficult to obtain specimens with values near the limits for some analytes (e.g. T-uptake, free thyroxine, free phenytoin, prolactin, FSH, troponin, pO_2). In such cases, reasonable procedures should be adopted based on available specimen materials. The method manufacturer's instructions for validating the AMR should be followed, when available. Specimen target values can be established by comparison with peer group values for reference materials, by assignment of reference or comparison method values, and by dilution ratios of one or more specimens with known values. Each laboratory must define limits for accepting or rejecting validation tests of the AMR.

The AMR must be revalidated at least every 6 months, and following changes in major system components or lots of analytically critical reagents (unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results, and control values are not adversely affected).

Evidence of Compliance:

- ✓ Written procedure for AMR validation defining the types of materials used and acceptability criteria consistent with manufacturer's instructions

MIC.64886 AMR Validation Criteria **Phase II**

Criteria are established for validating the analytical measurement range and compliance is documented.

NOTE: If the materials used for calibration or for calibration verification include low, midpoint, and high values that are near the stated AMR, and if calibration verification data are within the laboratory's acceptance criteria, the AMR has been validated; no additional procedures are required. If the calibration and/or calibration verification materials do not include the full AMR, or the laboratory extends the AMR beyond the manufacturer's stated range, the AMR must be validated by assaying materials reasonably near the lowest and highest values of the AMR.

Evidence of Compliance:

- ✓ Written procedure defining the method, frequency and acceptability criteria for AMR validation

QUALITY CONTROL

MIC.64910 Probe Characteristics **Phase II**

Sufficient information is documented regarding the nature of any probe or primer used in an assay to permit interpretation and troubleshooting of test results.

Evidence of Compliance:
- ✓ Records of probe details including oligonucleotide sequence, target, concentration, or purity, as applicable

✓ **MIC.64915** **Qualitative Cut-Off** **Phase I**

For qualitative tests that use a cut-off value to distinguish positive from negative, the cut-off value is established initially, and verified with every change in lot or at least every 6 months.

NOTE: The limit of detection that distinguishes a positive from a negative result should be established or verified when the test is initially placed in service, and verified with every change in lot (e.g. new master mix), instrument maintenance, or at least every six months thereafter. Note that a low-positive control that is close to the limit of detection can satisfy this checklist requirement, but must be external to the kit (e.g. weak-positive patient sample or reference material prepared in appropriate matrix).

Evidence of Compliance:
- ✓ Written procedure for initial establishment and verification of the cut-off value **AND**
- ✓ Records of initial establishment and verification documented at defined frequency

SEQUENCING

X **MIC.64920** **Sequencing Data Criteria** **Phase I**

Criteria are established for the acceptability and interpretation of primary sequencing data.

NOTE: Test procedures must assure that each target is visualized adequately to produce an unequivocal sequence readout, whether by manual or automated methods. Point mutations in particular may be overlooked if the signals are low or unequal. One approach to preventing this problem is to perform sequencing in both directions (opposite strands).

X **MIC.64922** **Sequencing Data Interpretation** **Phase II**

The laboratory has a process in place to assure that appropriate databases are used for the interpretation of sequencing data.

NOTE: Data bases should be comprehensive and current.

X **MIC.64924** **Sequence Data Correlation** **Phase I**

The sequence data are correlated with available phenotypic data.

Evidence of Compliance:
- ✓ Records of result review including correlation with phenotypic data

X *****REVISED** 07/11/2011***
MIC.64926 **Sample/Amplicon Contamination** **Phase II**

Procedures are in place to prevent or detect potential cross-contamination of samples and/or amplicons and to resolve problems from contamination of sequencing reactions.

NOTE: Examples of procedures are the use of negative controls in each batch, the manufacturer's use of Uracil N-glycosylase (UNG), or the fingerprinting program provided by the manufacturer.

TEST PROCEDURES

✓ **MIC.64930** **Nucleic Acid Extraction/Purification** **Phase II**

Nucleic acids are extracted and purified by validated methods.

NOTE: These can include methods reported in the literature, an established commercially available kit or instrument, or a laboratory-developed method.

Evidence of Compliance:
- ✓ Records to support nucleic acid extraction/purification is performed by a validated method

X **MIC.64934** **Melting Temperature** **Phase I**

For tests that generate a result based on a melting temperature (Tm), appropriately narrow temperature ranges (+/- 2.5^{o} C) are defined and monitored.

X **MIC.64938** **Autoradiograph Resolution** **Phase II**

The autoradiographs and gel photographs are of sufficient resolution and quality (low background, clear signal, absence of bubbles, *etc*.) to permit the reported interpretation.

X **MIC.64940** **Molecular Weight Markers** **Phase II**

Known molecular weight markers that span the range of expected bands are used for each electrophoretic run.

Evidence of Compliance:
- ✓ Records of appropriate markers documented with each run

X **MIC.64944** **Visual/Fluorescent Markers** **Phase II**

Visual or fluorescent markers are used to determine the endpoint of gel electrophoresis.

X **MIC.64948** **Autoradiograph/Gel Criteria** **Phase I**

Autoradiographs or electrophoretic gels are interpreted using objective criteria.

Evidence of Compliance:
- ✓ Written procedure including interpretive criteria for autoradiographs or gels

ASSAY VALIDATION

*It is important to confirm the analytical performance characteristics of the assay and to confirm the clinical validity of the assay. Performance characteristics that should be determined prior to reporting patient test results include analytical and diagnostic sensitivity and analytical and diagnostic specificity, precision, linearity (for quantitative tests), the reportable range of patient test results; the reference range (normal values); performance with clinical specimens**;** and any other applicable performance characteristic. Refer to the section "Test Method Validation" in the Laboratory General checklist for additional details.*

Analytic sensitivity refers to the ability of a test to detect a given analyte (i.e. the lower limit of detection). Analytic specificity refers to the degree to which related organisms are not detected by a test.

Precision refers to the reproducibility of a test result (e.g. within-technologist, between-technologist, within-run, and between-run).

Clinical sensitivity refers to the ability of a test to detect a disease or clinical condition, while clinical specificity refers to the degree to which a test is negative when disease is absent.

Diagnostic sensitivity and specificity must be determined relative to some "gold standard" (e.g. biopsy findings, clinical findings, etc.). The sensitivity of an assay equals [TP/(TP+FN)] X 100 and the specificity of an assay equals [TN/(TN+FP)] X 100. (TP=true positive, TN=true negative, FN=false negative, FP=false positive.) Determinations of sensitivity and specificity should be done in a "blinded" fashion (i.e. without prior knowledge of the patient's disease status). For some infections, it may not be possible to identify large numbers of positives (i.e. patients with the infection) to establish the diagnostic sensitivity of the assay. In such instances, the laboratory should procure as many positive cases as is reasonably possible for method validation and in addition cite any publications that have investigated the diagnostic sensitivity of the assay.

✓ **MIC.64952** **Validation Study** **Phase II**

There is documentation that the laboratory has performed a validation study prior to reporting patient results.

✓ **MIC.64956** **Modified FDA-Approved Assay** **Phase I**

If the laboratory modifies an FDA-approved assay, the modified procedure has been validated to yield equivalent or superior performance.

Evidence of Compliance:
- ✓ Records of validation studies for modified FDA-approved assays

✓ **MIC.64960** **Validation Studies - Specimen Selection** **Phase II**

Validation studies were performed with an adequate number and representative (reasonable) distribution of samples for each type of specimen (*e.g.* blood, fresh/frozen tissue, paraffin-embedded tissue).

NOTE: Validation studies must include an adequate number of positive and negative samples representing the specimen types used in the assay (e.g. plasma, blood, CSF). Samples may include spiked specimens (suspensions of target added to appropriate matrix), if patient samples are not available or inadequate in number across the dynamic range of a quantitative assay.

Evidence of Compliance:
- ✓ Records of validation studies

✗ **MIC.64964** **Validation Studies - Specimen Selection** **Phase II**

Validation studies include specimens representing each strain or genotype, when appropriate.

Evidence of Compliance:
- ✓ Records of validation studies

✓ **MIC.64968** **Validation Study Comparison** **Phase II**

The results of each validation study were compared to another valid test, such as a comparison to another test method or specimen exchange with a laboratory performing the same type of test in a similar fashion.

NOTE: There may not be a closely related test to be used for comparison. In such cases, the test performance (sensitivity and specificity) should be assessed in relation to the patient's clinical

diagnosis and in addition assessed by exchanges of specimens with a laboratory that performs the test in a similar fashion.

Evidence of Compliance:

- ✓ Records of comparison and evaluation of each validation study to another test method **OR** records of comparison using specimen exchange with another laboratory

MIC.64972 **Reference/Reportable Range Qualitative** **Phase II**

For qualitative assays, the reference value and reportable range are defined.

Evidence of Compliance:

- ✓ Written procedure defining reference and reportable range for each test

MIC.64976 **Reference/Reportable Range - Quantitative** **Phase II**

For quantitative assays, the reference and reportable ranges are defined.

NOTE: Reference and reportable ranges are pertinent to quantitative assays (e.g. viral load). The "reference range" is the range of results expected in the "normal" population, while the "reportable range" encompasses the full range of reported values. The laboratory must define the analytic measurement range (AMR) as described in the "Quantitative Assays; Calibration and Standards" section of the checklist. The laboratory must also determine how to handle positive patient results below or above the AMR, since numerical values outside the AMR may be inaccurate. For example, these may be reported a <x or >y, or they may be reported as "low positive" or " high positive" along with an explanation that values outside the linear range cannot be quantitated, or the sample may be concentrated or diluted and rerun to calculate an accurate value within the reportable range.

Evidence of Compliance:

- ✓ Written procedure defining reference and reportable range for each test

MIC.64980 **Validation Study** **Phase II**

Validation studies document test accuracy, analytical sensitivity, analytical specificity, precision, and linear range (quantitative tests only).

NOTE: For analytes without an international quantitative standard, it may not be possible to define numerical accuracy.

MIC.64984 **LDT Report** **Phase II**

Reports for laboratory-developed assays contain a description of the method, a statement that the assay was developed by the laboratory, and appropriate performance characteristics.

MIC.64988 **ASR Report** **Phase II**

If patient testing is performed using analyte-specific reagents (ASRs) obtained or purchased from an outside vendor, the patient report includes the disclaimer required by federal regulations.

NOTE: ASRs are antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens.

An ASR is the active ingredient of a laboratory-developed test system.

This checklist requirement concerns Class I ASRs. Class I ASRs are not subject to preclearance by the Us Food and Drug Administration (FDA) or to special controls by FDA. Most ASRs are Class

I. Exceptions include those used by blood banks to screen for infectious diseases (Class II or III), or used to diagnose certain contagious diseases (e.g. HIV infection and tuberculosis) (class III).

If the laboratory performs patient testing using Class I ASRs, federal regulations require that the following disclaimer accompany the test result on the patient report: "This test was developed and its performance characteristics determined by (laboratory name). It has not been cleared or approved by the US Food and Drug Administration."

The CAP recommends additional language, such as "FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing."

The disclaimer is not required for tests using reagents that are sold in kit form with other materials or an instrument, nor reagents sold with instructions for use, nor reagents labeled “for in vitro diagnostic use” (IVD) by the manufacturer.

The laboratory must establish the performance characteristics of tests using Class I ASRs in accordance with the Assay Validation section of this checklist.