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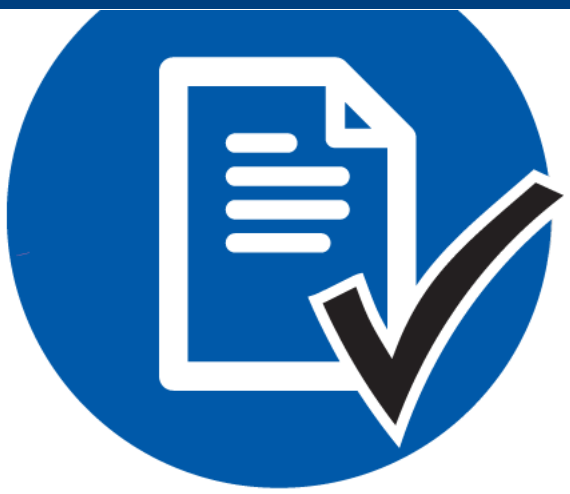
Yale-New Haven Hosp
Department of Laboratory Medicine

Immunology Checklist

CAP Accreditation Program



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Section/Department: Immuno, Chem, Flow Cyto, Mole Path

07.31.2012

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If you are enrolled in the CAP's Laboratory Accreditation Program and are preparing for an inspection, you must use the Checklists that were mailed in your application or reapplication packet, not those posted on the Web site. The Checklists undergo regular revision and Checklists may be revised after you receive your packet.

If a Checklist has been updated since receiving your packet, you will be inspected based upon the Checklists that were mailed. If you have any questions about the use of Checklists in the inspection process, please e-mail the CAP (accred@cap.org), or call (800) 323-4040, ext. 6065.

Immunology Checklist

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SUMMARY OF CHECKLIST EDITION CHANGES
Immunology Checklist
07/31/2012 Edition

The following lists of requirements provide information on what has changed in this edition of the checklist, or in the previous edition. This information is provided in three categories:

1. New — requirements that have been added
2. Revised — requirements listed in this section fall into two categories:
   - A major change to a requirement or a note that would necessitate a change in procedure for the laboratory
   - A change to the Phase
3. Deleted/Moved/Merged — requirements listed in this section fall into three categories:
   - Deleted — requirements that have been removed
   - Moved — requirements that have been relocated from this checklist into another checklist, or have been moved within this checklist and given a new checklist requirement number (resequenced)
   - Merged — requirements that have been combined with a similar requirement in the checklist

If this checklist was created for an 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 the detail of the changes, refer to the "Changes Only" document which may be found on the CAP website through e-LAB Solutions (Laboratory Accreditation Program Master and Custom Checklists). To access this document select "Changes Only" from the Checklist Type drop-down menu.

The "Changes Only" document contains the text of new and deleted checklist requirements, major and minor requirement revisions, and changes to explanatory text. These changes are presented, in order, as they appear in the checklist. Major requirement revisions will display a "Revised" flag. Minor revisions will not display a "Revised" flag and are defined as those editorial changes that are not likely to affect your laboratory operations, but are worded to better convey the intent of the requirement. Changes appear in redline/strikeout format that compares the previous checklist edition to this edition. Requirements that have been moved or merged will appear at the end of that file.

NEW Checklist Requirements

Requirement	Effective Date
IMM.33905	07/31/2012

REVISED Checklist Requirements

Requirement	Effective Date
IMM.33374	07/11/2011
IMM.33522	07/11/2011
IMM.33818	07/31/2012
IMM.33900	07/11/2011
IMM.33910	07/11/2011
IMM.34120	07/11/2011
IMM.34500	07/11/2011
IMM.41100	07/31/2012
IMM.50000	07/31/2012

DELETED/MOVED/MERGED Checklist Requirements

Requirement	Effective Date
IMM.05025	07/10/2011
IMM.10000	07/10/2011
IMM.10050	07/10/2011
IMM.10100	07/10/2011
IMM.10150	07/10/2011
IMM.16466	07/10/2011
IMM.22732	07/10/2011
IMM.29000	07/30/2012
IMM.29500	07/30/2012
IMM.30050	07/10/2011
IMM.30150	07/30/2012
IMM.30300	07/30/2012
IMM.31000	07/10/2011
IMM.31100	07/10/2011
IMM.31125	07/10/2011
IMM.31150	07/10/2011
IMM.31200	07/10/2011
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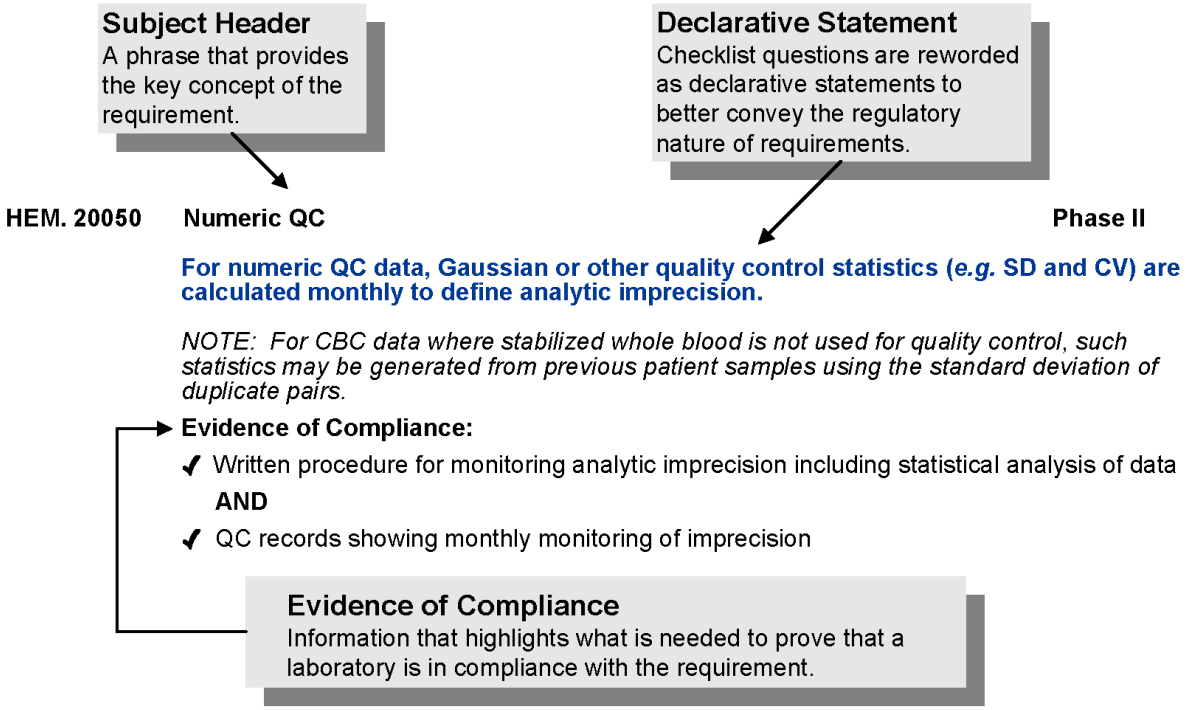
IMM.61200	07/10/2011
IMM.61300	07/10/2011
IMM.61400	07/10/2011
IMM.61500	07/10/2011
IMM.61600	07/10/2011

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.)

Evidence of Compliance suggests ways to document compliance with checklist requirements. 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.

Certain requirements in this checklist are now different for waived tests, versus nonwaived tests. Please refer to the checklist sections on Quality Management; Calibration and Standards; and Controls. The current list of tests waived under CLIA may be found at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfClia/analyteswaived.cfm.

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

DEFINITION OF TERMS

Analytical measurement range (AMR) validation - the process of confirming that the assay system will correctly recover the concentration or activity of the analyte over the AMR

Annual - every 12 calendar months

Biennial - every 24 calendar months

Calibrator, historical - the set of archived results of a single-point calibrator that demonstrates stability of the assay over time

Credentialing - the process of obtaining, verifying, and assessing the qualifications of a practitioner to provide care in a health care organization

Digital image analysis - the computer-assisted detection or quantification of specific features in an image following enhancement and processing of that image, including immunohistochemistry, DNA analysis, morphometric analysis, and *in situ* hybridization

Examination - in the context of checklist requirements, examination refers to the process of inspection of tissues and samples prior to analysis. An examination is not an analytical test.

FDA - in the context of checklist requirements, FDA should be taken to mean the national, state, or provincial authority having jurisdiction over *in vitro* diagnostic test systems

High complexity - rating given by the FDA to commercially marketed *in vitro* diagnostic tests based on their risks to public health. Tests in this category are seen to have the highest risks to public health.

Moderate complexity - rating given by the FDA to commercially marketed *in vitro* diagnostic tests based on their risks to public health

Nonwaived - tests categorized as either moderately complex (including provider-performed microscopy) or highly complex by the US Food and Drug Administration (FDA), according to a scoring system used by the FDA

Reagent - any substance in a test system other than a solvent or support material that is required for the target analyte to be detected and its value measured in a sample

Semiannual - every 6 calendar months

Telepathology - the practice in which the pathologist views digitized or analog video or still image(s), and renders an interpretation that is included in a formal diagnostic report or document in the patient record

Test system - the process that includes pre-analytic, analytic, and post-analytic steps used to produce a test result or set of results. A test system may be manual, automated, multi-channel or single-use and can include reagents components, equipment or instruments required to produce results. A test system may encompass multiple identical analyzers or devices. Different test systems may be used for the same analyte.

Waived - a category of tests defined as "simple laboratory examinations and procedures which have an insignificant risk of an erroneous result." Laboratories performing waived tests are subject to minimal regulatory requirements.

QUALITY MANAGEMENT AND QUALITY CONTROL

GENERAL ISSUES

IMM.30000 Instrument Maintenance Evaluation **Phase II**

There is documentation of monthly evaluation of the maintenance and function of all instruments, including documentation of corrective action taken when values for instrument function, temperature, etc. exceed defined tolerance limits.

IMM.30100 Numeric QC Data **Phase II**

For numeric QC data, statistics (such as S.D. and C.V.) are calculated monthly to define analytic precision.

Evidence of Compliance:
- ✓ Written procedure for monitoring analytic imprecision including statistical analysis of data **AND**
- ✓ QC records showing monthly monitoring for imprecision

IMM.30120 Precision Statistics Action **Phase II**

The laboratory has an action protocol when data from precision statistics change significantly from previous data.

Evidence of Compliance:
- ✓ Records of investigation and corrective actions taken

SPECIMEN COLLECTION AND HANDLING

IMM.31300 Specimen Identity/Integrity **Phase II**

Procedures are adequate to verify sample identity and integrity (includes capillary specimens, aliquots and dilutions).

Evidence of Compliance:
- ✓ Patient collection and processing records

IMM.31400 Specimen Rejection Criteria **Phase II**

There are documented criteria for the rejection of unacceptable specimens and the special handling of sub-optimal specimens.

NOTE: This requirement does not imply that all "unsuitable" specimens are discarded or not analyzed. If, for example, improper storage hemolyses a sample and hemolysis interferes with testing, there must be a mechanism to notify clinical personnel responsible for patient care. If a test result is still desired by the ordering physician, then the condition of the sample must be stated on the report, and a notation made of any limitation in test result interpretation. The laboratory may wish to record that a dialogue was held with the physician, when such occurs. If the specimen is to be disposed

of, all unacceptable specimens are documented in the patient report and/or quality management records.

Evidence of Compliance:
- ✓ Records of rejected specimens

RESULTS REPORTING

IMM.32000 Reference Intervals Established **Phase II**

Reference intervals (normal values) are established or verified by the laboratory for the population being tested.

NOTE: Age- and sex-specific reference intervals (normal values) must be verified or established by the laboratory. For example, a reference interval can be validated by testing samples from 20 healthy representative individuals; if no more than 2 results fall outside the proposed reference interval, that interval can be considered validated for the population studied (refer to CLSI guideline C28-A3, referenced below). If a formal reference interval study is not possible or practical, then the laboratory should carefully evaluate the use of published data for its own reference ranges, and document that review.

Evidence of Compliance:
- ✓ Record of reference range study **OR** records of verification of manufacturer's stated range when reference range study is not practical (*e.g.* unavailable normal population) **OR** other methods approved by the laboratory director

IMM.32050 Reference Intervals **Phase II**

As appropriate, all patient results are reported with accompanying reference (normal) intervals or interpretations.

NOTE: The use of high and low flags (generally available with a computerized laboratory information system) is recommended.

Under some circumstances it may be appropriate to distribute lists or tables of reference intervals to all users and sites where reports are received. This system is usually fraught with difficulties, but if in place and rigidly controlled, it is acceptable.

CALIBRATION AND STANDARDS

IMM.33337 Calibration, Calibration/Verification - Waived Tests **Phase II**

For waived tests, testing personnel follow manufacturer instructions for calibration, calibration verification, and related functions.

Evidence of Compliance:
- ✓ Written procedure consistent with the manufacturer's instructions for each waived test **AND**
- ✓ Records for calibration/calibration verification/related functions documented as required by the manufacturer

The remaining requirements in this checklist on CONTROLS, CALIBRATION, CALIBRATION VERIFICATION, ANALYTIC MEASUREMENT RANGE (AMR), and INTERINSTRUMENT COMPARISONS do not apply to waived tests.

The following requirements for calibration, calibration verification and analytic measurement range validation apply only to analyses that provide truly quantitative measurements expressed in mass units per unit volume (e.g. gm/L or mg/ml) OR in units traceable to a reference preparation or standard that is calibrated in mass units per unit volume. If these criteria are not met, the measurement is NOT quantitative and this section is not applicable.

This introduction discusses the processes of calibration, calibration verification, and analytical measurement range validation (AMR).

DEFINITIONS:

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.

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

REQUIRED FREQUENCY OF CALIBRATION VERIFICATION
Laboratories must calibrate a method when it is first placed in service and perform calibration verification at least every six months thereafter. However, a laboratory may opt to recalibrate a method (rather than perform calibration verification) at least every six months. If a method has been recalibrated then it is NOT necessary to also perform calibration verification sooner than six months following recalibration. In addition to this six-monthly schedule, calibration verification or recalibration is required (regardless of the length of time since last performed) immediately if any of the following occurs:

1. *A change of reagent lots for chemically or physically active or critical components, 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. *If QC fails to meet established criteria*
3. *After major maintenance or service. The Laboratory Director must determine what constitutes major maintenance or service.*
4. *When recommended by the manufacturer*

MATERIALS SUITABLE FOR 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. Suitable materials may include, but are not limited to:

1. *Calibrators used to calibrate the analytical 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. *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.*
6. *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.

ANALYTICAL MEASUREMENT RANGE

DEFINITIONS:

The ANALYTICAL MEASUREMENT RANGE (AMR) 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 sample is suspended or dissolved) may influence the measurement of the analyte. In many cases, the method manufacturer will recommend suitable materials. The test specimens must have analyte values, which 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.

USE OF THE AMR
It is important that the laboratory knows the AMRs of its methods. Patient samples that have measured values that fall within the AMR of a method can be reported by the laboratory without further analytical steps. If a patient sample has a measured value that is outside the AMR, then that value may be erroneous and the concentration of the analyte in the patient sample should be adjusted, usually by dilution, to bring it within the AMR.

In the case of samples with very high concentrations or activities of an analyte, very large dilutions may be required to bring the concentration of activity into the AMR. Making large dilutions of patient samples can introduce error, and the Laboratory Director should establish appropriate volumes of sample and diluent to be used to minimize dilution errors. For example, pipetting 1 uL of a sample is difficult to do accurately and larger sample and diluent volumes should be specified. Note that for some analytes, an acceptable dilution protocol may not exist because dilution would alter the analyte or the matrix causing erroneous results, e.g. free drugs or free hormones. Also note that for some analytes, there may be no clinical relevance to reporting a numeric result greater than a stated value. If it is not possible to achieve a measured value that is within the AMR by using allowable dilutions, then the result may be reported as "greater than" the value of the upper end of the AMR multiplied by the maximum allowable dilution.

LINEARITY AND THE AMR
Validation of the AMR is accomplished by demonstrating a linear relationship for an appropriate set of samples that cover the AMR. A plot of measured results for an analyte obtained across the AMR vs. expected concentrations or concentration relationships (or expected activity or activity relationships) in a set of samples should show a linear relationship. One can use matrix appropriate materials of known analyte concentration and demonstrate that measured values correspond with target values in a linear relationship. Note that for commercially available "linearity" sample sets, it is not expected that the measured values are the same as the target values because the "linearity" samples are not commutable with clinical samples. For commercially available "linearity" sample sets, it is expected that a plot of the measured values vs. the target values has a linear relationship because there is a known quantitative relationship between the concentrations or activities in the sample set. Alternatively, one can make admixtures of appropriate materials of high and low analyte concentrations and demonstrate that there is the expected linear relationship between measured values of these admixtures and the expected values based on the proportion of low and high concentration samples in each admixture. With either approach, the values should be suitably spaced across the AMR, preferably equidistant from each other.

CLOSENESS OF SAMPLE CONCENTRATIONS OR ACTIVITIES TO THE UPPER AND LOWER LIMITS OF THE AMR

When validating the AMR, it is required that samples are near the upper and lower limits of the AMR. Factors to consider in validating the AMR 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, p02). 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. The Laboratory Director must define limits for accepting or rejecting validation tests of the AMR.

REQUIRED FREQUENCY OF AMR VALIDATION
The AMR must be validated when a method is placed in service and at least every six months thereafter. The AMR must also be validated (regardless of the length of time since last performed) immediately if any of the following occur:

1. *A change of reagent lots for chemically or physically active or critical components, 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. *If QC fails to meet established criteria*
3. *After major preventive maintenance or change of a critical instrument component*
4. *When recommended by the manufacturer*

SUITABLE MATERIALS FOR AMR VALIDATION
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 and that represent the quantitative relationship among the specimens. 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 that are from a different lot than the one used for calibration*
7. *Control materials, if they adequately span the AMR and have method specific target values*

RECALIBRATION / CALIBRATION VERIFICATION and AMR VALIDATION INTERVALS: Recalibration or calibration verification, and AMR validation, must be performed at least once every 6 months. Successful calibration verification certifies that the calibration is still valid; unsuccessful calibration verification requires remedial action, which usually includes recalibration and AMR revalidation. 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 laboratory director should determine what constitutes a major system component change or a change in reagents that would require recalibration or calibration 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.

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

IMM.33374 Calibration and Controls **Phase II**

Calibration procedures for each method are adequate, and appropriate controls are used in each run or batch of samples.

NOTE: For immunoassays, appropriate controls must be used in each run or batch of samples. Appropriate controls for screening assays should consist of at least one positive control. If a single calibrator is used, the control must be at or near the declared cutoff value(s). Laboratories may use historical calibrations; however, controls must be run with each batch to verify the calibration.

The term "historical calibrator" refers to archived results for those assays that compile a ledger of previous results for a calibrator material (usually a so called "singer point" calibrator). The assays have demonstrated stability and do not require that the calibrator be run with each batch of samples. Several of the newer semiquantitative immunoassays for auto antibodies are of this type. The assays will state that calibrator values are stable for X number of days. This of course must be validated by the user. If the control(s) are acceptable on a given day it can be inferred that the calibration is also acceptable.

IMM.33448 Calibration Materials **Phase II**

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

NOTE: Calibration materials establish the relationship between method/instrument response and the corresponding concentration/activities of an analyte. They have defined analyte target values and appropriate matrix characteristics for the clinical specimens and specific assay method. Many instrument systems require calibration materials with system-specific target values to produce accurate results for clinical specimens.

Evidence of Compliance:

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

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

IMM.33522 Calibration Material Labeling **Phase II**

All calibration materials are properly labeled as to content, calibration values, date placed in service, and expiration date (if applicable).

NOTE: Complete values need not necessarily 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

IMM.33670 Calibration/Calibration Verification Criteria **Phase II**

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

NOTE: Criteria typically include:

1. *At changes of reagent lots for chemically or physically active or critical components, 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 preventive maintenance or change of a critical instrument component*
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

IMM.33744 Recalibration **Phase II**

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

Evidence of Compliance:

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

*****REVISED** 07/31/2012***

IMM.33818 AMR Validation **Phase II**

Validation of the analytical measurement range (AMR) is performed with matrix-appropriate materials which include the low, mid and high range of the AMR, appropriate acceptance criteria are defined, 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, prolactin, FSH). 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). AMR validation is not required for methods that measure an analyte quantitatively or semi-quantitatively, and report a qualitative value based on concentration threshold.

Evidence of Compliance:

✓ Written procedure for AMR validation/revalidation defining the types of materials used, frequency and acceptability criteria

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

IMM.33900 Diluted or Concentrated Samples **Phase II**

If a result is less than or greater than the AMR, a numeric result is not reported unless the sample is processed by dilution, a mixing procedure or concentration so that the processed result falls within the AMR.

NOTE:

1. *A measured value that is outside the AMR may be unreliable and should not be reported in routine practice. Dilution, a mixing procedure* or concentration of a sample may be required to achieve a measured analyte activity or concentration that falls within the AMR. The processed result must be within the AMR before it is mathematically corrected by the concentration or dilution factor to obtain a reportable numeric result.*
2. *If the concentration or activity of the analyte is determined to be outside the AMR and is reported as "greater than" or " less than" the limits of the AMR, then the checklist requirement is not applicable.*

**This procedure is termed the "method of standard additions." In this procedure, a known quantity (such as a control) is mixed with the unknown, and the concentration of the mixture is measured. If equal volumes of the two samples are used, then the result is multiplied by two, the concentration of the known subtracted, and the concentration of the unknown is the difference.*

Evidence of Compliance:

✓ Dilution protocol or patient results or worksheets

***NEW** 07/31/2012*

IMM.33905 Qualitative Cut-Off **Phase II**

For qualitative tests that use a cut-off value to distinguish positive from negative, the cut-off value is established initially, and verified every 6 months thereafter.

NOTE: This requirement does not apply to FDA-approved in vitro diagnostic assays that report the qualitative result based on a predefined cut-off value.

This requirement applies only to certain tests that report qualitative results based on a quantitative measurement using a laboratory established threshold (cut-off value) to discriminate between a positive and negative clinical interpretation. The cut-off value that distinguishes a positive from a negative result should be established when the test is initially placed in service, and verified every 6 months thereafter. If the value of a calibrator or calibration verification material is near that of the cut-off, then the process of calibration or calibration verification satisfied this checklist requirement.

Verification of the cut-off should also be performed at changes of lots of analytically critical reagents (unless the laboratory director has determined that such changes do not affect the cut-off); after replacement of major instrument components; after major service to the instrument; and failure of quality control to meet established criteria.

Appropriate materials for establishment and verification of the cut-off are identical to those recommended for calibration verification. Note that QC materials are acceptable if the material is specifically designed by the method manufacturer as suitable for verification of the method's calibration process.

Evidence of Compliance:

✓ Written procedure for initial establishment and verification of the cut-off value AND

✓ Records of initial establishment and verification of cut-off value documented at defined frequency

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

IMM.33910 Maximum Dilution/Concentration **Phase II**

For analytes that may have results falling outside the limits of the AMR, the laboratory procedure specifies the maximum concentration or dilution that may be performed to obtain a reportable numeric result.

NOTE:

1. *For each analyte, the laboratory protocol should define the maximum dilution that falls within the AMR and that can be subsequently corrected by the dilution factor to obtain a reportable numeric result. Note that for some analytes, an acceptable dilution protocol may not exist because dilution would alter the analyte or the matrix causing erroneous results. Also note that, for some analytes, there may be no clinical relevance to reporting a numeric result greater than a stated value.*
2. *Analytes for which a dilution protocol is unable to bring the activity or concentration into the AMR should be reported as "greater than" the highest estimated values.*
3. *Establishment of allowable dilutions is performed when a method is first placed into service and is reviewed biennially thereafter as part of the procedure manual review by the Laboratory Director or designee. The laboratory director is responsible for establishing the maximum allowable dilution of samples that will yield a credible laboratory result for clinical use.*

In a mixing procedure (also termed the "method of standard additions"), a known quantity (such as a control) is mixed with the unknown, and the concentration of the mixture is measured. If equal volumes of the two samples are used, then the result is multiplied by two, the concentration of the known subtracted, and the concentration of the unknown is the difference.

Evidence of Compliance:

✓ Patient results or worksheets

CONTROLS

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

WAIVED TESTS

IMM.33930 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, quantitative data should be plotted. 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.*

IMM.33940 QC Corrective Action - Waived Tests **Phase II**

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

IMM.33950 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

The remaining requirements in this checklist on QUALITY CONTROL and INTERINSTRUMENT COMPARISONS do not apply to waived tests.

NONWAIVED TESTS

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

IMM.34120 Daily QC - Nonwaived Tests **Phase II**

Controls are run daily for quantitative and qualitative tests.

NOTE 1: Controls should verify assay performance at relevant decision points. The selection of these points may be based on clinical or analytical criteria.

NOTE 2: Except for tests meeting the criteria in Note 3, below, daily external surrogate sample controls must be run as follows:*

1. *For quantitative tests, 2 controls at 2 different concentrations must be run daily or with each batch of samples/reagents, unless a different requirement is specifically required by this checklist*
2. *For qualitative tests, a negative control and a positive control (when available) must be run daily.*

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

NOTE 3: 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. *For laboratories subject to US regulations, 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 is in service, and for 2 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 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.*

**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

IMM.34140 QC Range Verification **Phase II**

For quantitative tests, a valid acceptable range has been established or verified for each lot of control material.

NOTE: For unassayed controls, the laboratory must establish a valid acceptable range by repetitive analysis in runs that include previously tested control material. This may be established through various mechanisms, such as multiple individual replicates or use of moving averages. For assayed controls, the laboratory must verify the recovery ranges supplied by the manufacturer.

Evidence of Compliance:

- ✓ Written procedure defining methods used to establish or verify control ranges **AND**
- ✓ Records for control range verification of each lot

IMM.34142 Calibrators and Controls **Phase II**

If the laboratory prepares calibrators and controls in-house, these materials are prepared separately.

NOTE: In general, calibrators should not be used as QC materials. If calibrators are used as controls, then different preparations should be used for these two functions.

Evidence of Compliance:

- ✓ Written procedure defining criteria for in-house preparation of calibrators and controls

IMM.34145 Calibrators as Controls **Phase II**

If a calibrator obtained from an outside supplier is used as a control, it is a different lot number from that used to calibrate the method.

NOTE: In general, calibrators should not be used as QC materials. However, the practice may be necessary for some methods when a separate control product is not available. In such cases, the calibrator used as a control must, whenever possible, be from a different lot number than that used to calibrate the method.

Evidence of Compliance:
- ✓ Written procedure defining the criteria for the use of calibrators as controls **AND**
- ✓ QC/calibrator records

IMM.34150 Control Labeling **Phase II**

Controls are properly labeled as to content, lot number, date of preparation and expiration date.

Evidence of Compliance:
- ✓ Written policy defining elements required for control labeling

IMM.34170 Weakly Reactive Controls **Phase II**

Reactive, weakly reactive and nonreactive controls are all used in test systems where results are reported in that fashion.

NOTE: Weakly reactive controls should be used when test results are reported in that fashion, unless such controls are not commercially available.

Evidence of Compliance:
- ✓ QC results

IMM.34190 Documented QC **Phase II**

There are records to reflect the results of all control procedures.

IMM.34250 QC Corrective Action **Phase II**

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

NOTE: When a QC result is unacceptable, patient/client test results obtained 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. 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).

IMM.34270 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. To the extent possible, all steps of the testing process must be controlled, recognizing that pre-analytic and post-analytic variables may differ from those encountered with patient samples.

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

IMM.34290 QC Verification **Phase II**

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

NOTE: It is implicit in quality control that patient test results will not be reported when controls yield unacceptable 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

IMM.34362 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

IMM.34450 Fluorescent/Enzyme Antibody Stain QC **Phase II**

Positive and negative controls are included with each patient run for all fluorescent or enzyme antibody stains.

NOTE: When examining tissue specimens, internal antigens, when present, may serve as positive controls (e.g. IgA in tubular casts, IgG in protein droplets, and C3 in blood vessels). Non-reactive elements in the tissue specimen may serve as a negative tissue control. A negative reagent control in which the patient tissue is processed in an identical manner to the test specimen but with the primary antibody omitted must be performed for each patient tissue specimen.

Evidence of Compliance:
- ✓ Written procedure for fluorescent/enzyme antibody stain QC **AND**
- ✓ Records of fluorescent/enzyme antibody stain QC documented at defined frequency

IMM.34475 Verification of Accuracy **Phase II**

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

NOTE: "Reliability" includes elements of accuracy, precision, and clinical discriminating power.

This checklist requirement does not apply to waived tests.

*****REVISED** 07/11/2011***
IMM.34500 Comparability of Instrument/Method **Phase II**

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

NOTE: This requirement applies to tests performed on the same or different instrument makes/models or by different methods. This comparison must include all nonwaived instruments/methods. The laboratory director must establish a protocol for this check.

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

Otherwise, the use of human samples, rather than stabilized commercial controls, is preferred to avoid potential matrix effects. The use of pooled patient samples is acceptable since there is no change in matrix. In cases when availability or pre-analytical stability of patient/client specimens is a limiting factor, alternative protocols based on QC or reference materials may be necessary but the materials used should be validated (when applicable) to have the same response as fresh human samples for the instruments/methods involved.

This checklist requirement applies only to instruments/methods accredited under a single CAP number.

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

INSTRUMENTS AND EQUIPMENT

A variety of instruments and equipment are used to support the performance of analytical procedures. 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. The procedures and schedules for instrument maintenance must be as thorough and as frequent as specified by the manufacturer.

IMM.34550 Thermometric Standard Device **Phase II**

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

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

IMM.35000 Non-Certified Thermometers **Phase II**

All non-certified thermometers in use in the laboratory 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

IMM.35050 Temperature Checks **Phase II**

The temperature of water baths and/or heat blocks, refrigerators and other temperature-dependent equipment is 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.

IMM.35075 Serologic Centrifuge Checks **Phase I**

Mechanical timers on serologic centrifuges, and the speed of the centrifuge, are checked for accuracy every 6 months.

NOTE: Most serologic centrifuges and timers do not require frequent recalibration. Accuracy of speed and timing must be checked initially, after adjustments/repairs or implementation of new techniques. The frequency of periodic checks should be based on the historical stability of the centrifuge, but at least every 6 months.

Evidence of Compliance:

- ✓ Records of serologic centrifuge checks documented at defined frequency

IMM.35100 Function Checks **Phase II**

Appropriate function checks are performed for all instruments prior to testing patient samples.

NOTE: There must be a schedule and procedure at the instrument for appropriate function checks. These may include (but are not limited to) electronic, mechanical and operational checks. The procedure and schedule must be as thorough and as frequent as specified by the manufacturer. Function checks should be designed to check the critical operating characteristics to detect drift, instability or malfunction before the problem is allowed to affect test results. All servicing and repairs must be documented.

IMM.35150 Routine Maintenance Schedule **Phase II**

All instruments are on a routine maintenance program.

IMM.35200 Instrument Repair 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.

IMM.35216 Pipette Accuracy **Phase II**

Glass volumetric pipettes are of certified accuracy (Class A); or they are checked by gravimetric, colorimetric, or some other verification procedure before initial use.

NOTE: The following Table shows the American Society for Testing and Materials' calibration (accuracy) specifications for Class A volumetric pipettes:

Reconstitution of lyophilized calibrators, controls, or proficiency testing materials, or any other tasks requiring accurate volumetric measurement, must be performed only with measuring devices of Class A accuracy, or those for which accuracy has been defined and deemed acceptable for the intended use.

If initial calibration is performed by the manufacturer or other outside facility, sufficient information must be provided to justify acceptance of the pipette's calibration based on the laboratory's documented specifications of acceptable bias and imprecision. The outside facility must also document the technique used to check calibration and ship the pipette in a manner that protects it from damage in transit.

Nominal Capacity (mL)	*Variation (± mL)*
0.5 - 2	*0.006*
3 - 7	*0.01*
8 - 10	*0.02*
15 - 30	*0.03*
40 - 50	*0.05*
100	*0.08*

Evidence of Compliance:

- ✓ Pipettes marked Class A **OR** NIST certificate **OR** etched markings indicating Class A **OR** validation study of accuracy for non-certified glassware

IMM.35232 Pipette Accuracy **Phase II**

Non-class A pipettes that are used for quantitative dispensing of material are checked for accuracy and reproducibility at specified intervals, and results documented.

NOTE: Such checks are most simply done gravimetrically. This consists of transferring a number of measured samples of water from the pipette to a balance. Each weight is recorded, the weights are converted to volumes, and then means (for accuracy), and SD/CV (for imprecision) are calculated. Alternative approaches include spectrophotometry or (less frequently) the use of radioactive isotopes, and commercial kits are available from a number of vendors. Computer software is useful where there are many pipettes, and provides convenient documentation.

This checklist requirement does not apply to applications not requiring the accuracy of class A pipettes (e.g. serologic pipettes). Refer to the next checklist requirement.

IMM.35250 Automatic Pipette Accuracy **Phase II**

Automatic and adjustable pipetting devices are checked at least annually for accuracy and reproducibility, and results recorded.

Evidence of Compliance:

- ✓ Written procedure detailing method for checking the accuracy and reproducibility of automatic pipettes **AND**
- ✓ Records of initial and periodic pipette verification documented at defined frequency

IMM.35275 Concentration Techniques **Phase I**

Concentration techniques are verified.

NOTE: Techniques used to concentrate specimens for analysis must be verified at specified, periodic intervals (not to exceed one year or manufacturer's recommendations.

Evidence of Compliance:
- ✓ Written procedure for verifying the accuracy of concentration techniques **AND**
- ✓ Records of concentration technique verification documented at defined frequency

IMM.35603 Pipette Carryover **Phase I**

The laboratory has evaluated its automatic pipetting systems for carryover.

NOTE: The laboratory should have procedures in place for evaluating whether carryover effects are present. This requirement applies to both stand-alone pipette systems and to sample pipettes integrated with analytic instruments.

In practice, carryover is a problem only for analytes with a very wide clinical range of analyte concentration, such that a minute degree of carryover could have significant clinical implications (for example, serologic tumor markers). The laboratory should select representative examples of such analytes for carryover studies.

Evaluation for carryover is not required for automatic pipettes that use disposable tips.

One suggested method to study carryover is to run known high patient samples, followed by known low samples to see if the results of the low-level material are affected. If carryover is detected, the laboratory should determine the analyte concentration above which subsequent samples may be affected, and define this value in the procedure. Results of each analytical run should be reviewed to ensure that no results exceed this level. If results that exceed the defined level are detected, then the appropriate course of action should be defined (repeat analysis of subsequent samples, for example).

Carryover studies should be performed, as applicable, as part of the initial evaluation of an instrument. (The laboratory may use the data from carryover studies performed by instrument manufacturers, as appropriate.) It is recommended that carryover studies be repeated after major maintenance or repair of the pipetting assembly of the instrument.

Evidence of Compliance:
- ✓ Record of carryover studies documented at defined frequency

IMM.35957 Glassware Accuracy **Phase II**

Glass volumetric flasks are of certified accuracy (Class A, National Institute of Standards and Technology (NIST) standard or equivalent), or if non-certified volumetric glassware is used, all items are checked for accuracy of calibration before initial use.

Evidence of Compliance:
- ✓ Glassware marked Class A **OR** NIST certificate **OR** Validation study of accuracy for non-certified glassware

ANALYTIC BALANCES

IMM.36664 Balance Maintenance **Phase I**

Balances are cleaned, serviced and checked at least annually only by qualified service personnel (*i.e.* service contract or as needed).

Evidence of Compliance:
- ✓ Records of balance maintenance

IMM.37371 Balance Mounting **Phase I**

Analytic balances are mounted such that vibrations do not interfere with readings.

IMM.38078 Standard Weights **Phase II**

Standard weights of the appropriate ANSI/ASTM Class are available and used for checking accuracy.

NOTE: The verification of accuracy of the analytical balance must be performed on a regular schedule to ensure accurate creation of analytical calibrators and/or weighed-in controls from standard materials, as well as when gravimetrically checking the accuracy of pipettes.

There are three general types of balances in use. First, many contemporary balance designs use force transducers of various designs to provide mass readings. These balances typically have built-in certified calibration weights that are utilized automatically each time of use. The second type of balance employs a force transducer design that uses external weights for calibration each time the balance is used. Typically a single mass at the maximum weighing range, in conjunction with a zero point for the pan, is used for calibration of a force transducer balance design. The third type of balance, an older design, is a mechanical balance beam with internal moveable or external calibration weights. This design may have an electronic read-out.

In all cases, verification of accuracy over the weighing range with external calibrated masses is required on a periodic schedule appropriate to the use of the balance. Balances must be checked at least every 6 months, if used for weighing out materials to make up standard solutions for method calibration. For other purposes, annual verification may be adequate. Accuracy must be verified when a new balance is installed and whenever a balance is moved.

External validation of accuracy requires the appropriate class of ASTM specification weights. ASTM Class 1 weights are appropriate for calibrating high precision analytical balances (0.01 to 0.1 mg limit of precision). ASTM Class 2 weights are appropriate for calibrating precision top-loading balances (0.001 to 0.01 g precision). ASTM Class 3 weights are appropriate for calibrating moderate precision balances, (0.01 to 0.1 g precision).

Periodic external validation of accuracy is required to ensure that internal weights have not deteriorated from adsorption of surface film or corrosion; and to ensure that electronics remain correctly calibrated.

Evidence of Compliance:

- ✓ Written procedure defining criteria for the use of standard weights for accuracy checks of analytical balances

IMM.38785 Balance Accuracy **Phase II**

Results of periodic accuracy checks are recorded.

NOTE: Mass readings should be recorded in a log book. The deviations in log book readings should be no more than the precision required in the applications for which the balance is used. Acceptable ranges for readings must be specified.

IMM.39492 Weight Maintenance **Phase II**

Weights are well-maintained (clean, in a covered container, not corroded) and appropriate lifting or handling devices are available.

NOTE: Weights must be well-maintained (covered when not in use, not corroded) and only be handled by devices that will not allow residual contaminants to remain on the masses. Certified masses will only meet their specifications if maintained in pristine condition.

PROCEDURES AND TEST SYSTEMS

SYPHILIS SEROLOGY

***REVISED** 07/31/2012*

IMM.41100 RPR Needles **Phase II**

If antigen is delivered by needles, the volume of delivery is checked under each of the following circumstances:

1. **Each time a new needle is used**
2. **When control patterns cannot be reproduced**
3. **When the antigen drop does not fall cleanly from the tip**

NOTE: The Centers for Medicare and Medicaid Services (CMS) has adopted the Centers for Disease Control (CDC) recommendations for checking needles used for the RPR and syphilis-related cardiolipin-based tests [e.g. toluidine red unheated serum test (TRUST)].

Evidence of Compliance:

- ✓ Written procedure defining process and criteria for RPR needle verification **AND**
- ✓ Records of needle verification

IMM.41300 Syphilis Serology Controls **Phase II**

A negative control plus positive serum controls of known titer or controls of graded reactivity are run each day of patient testing.

NOTE: A negative control plus positive serum controls of known titer must be run each day of patient testing. If the laboratory reports graded patient results, then graded controls must be run. However, serially diluted positive controls are not required.

Evidence of Compliance:

- ✓ QC results

IMM.41400 New Reagent Lot Verification **Phase II**

New reagent lots of antigen for VDRL, RPR, TRUST (toluidine red unheated serum test), and USR (unheated serum reagin) tests are checked in parallel with reference reagents to verify that they are of standard reactivity.

NOTE: New reagent lots of antigen for VDRL, RPR, TRUST, and USR tests must be checked in parallel with reference reagents to verify that they are of standard reactivity. Because the ability of a reagent to detect specimens with low-grade reactivity is necessary for the diagnosis of primary syphilis, serum samples of graded reactivity, including those with weak reactivity, must be used. Reactive serum diluted with nonreactive serum to produce various degrees of reactivity may also be used. Roughness of an antigen can best be detected using fresh serum samples obtained from persons without syphilis. Prior to testing patient samples with a new reagent lot, parallel testing must be performed by using specimens of graded reactivity, including minimum/weakly reactive samples (for example, a reactive, a weakly reactive and a negative specimen). If one set of parallel tests shows borderline results, a second set of parallel tests should be performed. The use of quality control materials to verify new reagent lots is acceptable, providing that the controls include materials with negative, low-grade positive, and high-grade positive reactivity.

Evidence of Compliance:
- ✓ Written procedure for verification of new antigen lots prior to use **AND**
- ✓ Records of verification data of new lots

WESTERN BLOT ASSAYS

IMM.41500 Molecular Weight Markers **Phase II**

Known molecular weight markers are included and reviewed with each Western blot assay of patient samples.

IMM.41600 Separations **Phase II**

Western blot separations are satisfactory with sufficient resolution (low background, clear signal, absence of bubbles, *etc.*) to interpret band size easily.

IMM.41700 Acceptable Limits - Controls **Phase II**

Acceptable limits are set for controls of procedures where the Western blot bands are quantified.

NOTE: The criterion to designate a Western blot test as positive is based on the detection of a certain combination of positive bands. The laboratory should define a minimum intensity that allows a band to be considered positive.

Evidence of Compliance:
- ✓ Records of defined acceptable limits for control range of each lot

IMM.41800 Interpretation **Phase II**

Objective criteria are defined for interpretation of Western blot.

PERSONNEL

*****REVISED** 07/31/2012***
IMM.50000 Personnel - Bench Testing **Phase II**

The person in charge of the bench testing in immunology and syphilis serology has education equivalent to an associate's degree (or beyond) in a chemical, physical or biological science or medical technology and at least 4 years experience (one of which is in immunology and syphilis serology) under a qualified director.

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