Master

Every patient deserves the GOLD STANDARD ...

Chemistry and Toxicology Checklist

CAP Accreditation Program

College of American Pathologists
325 Waukegan Road
Northfield, IL 60093-2750
www.cap.org

07.11.2011

Disclaimer and Copyright Notice

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.

The checklists used in connection with the inspection of laboratories by the Laboratory Accreditation Program of the College of American Pathologists have been created by the College and are copyrighted works of the College. The College has authorized copying and use of the checklists by College inspectors in conducting laboratory inspections for the CLA and by laboratories that are preparing for such inspections. Except as permitted by section 107 of the Copyright Act, 17 U.S.C. sec. 107, any other use of the checklists constitutes infringement of the College's copyrights in the checklists. The College will take appropriate legal action to protect these copyrights.

Chemistry and Toxicology Checklist

TABLE OF CONTENTS

SUMMARY OF CHECKLIST EDITION CHANGES
Chemistry and Toxicology 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
CHM.13720	06/17/2010
CHM.16770	07/11/2011
CHM.17350	07/11/2011
CHM.17450	07/11/2011
CHM.30250	06/17/2010
CHM.31960	07/11/2011

REVISED Checklist Requirements

Requirement	Effective Date
CHM.10700	06/17/2010
CHM.10800	07/11/2011
CHM.11900	07/11/2011
CHM.12333	06/17/2010
CHM.12500	07/11/2011
CHM.12600	07/11/2011
CHM.12900	07/11/2011
CHM.13200	07/11/2011
CHM.13400	07/11/2011
CHM.13600	07/11/2011
CHM.13710	07/11/2011
CHM.13800	07/11/2011
CHM.13810	07/11/2011
CHM.13840	06/17/2010
CHM.13900	07/11/2011
CHM.14500	07/11/2011
CHM.14916	07/11/2011
CHM.16550	07/11/2011
CHM.16650	07/11/2011
CHM.16750	07/11/2011
CHM.16850	06/17/2010
CHM.17000	07/11/2011

CHM.17050	07/11/2011
CHM.17200	07/11/2011
CHM.17300	07/11/2011
CHM.18700	07/11/2011
CHM.18800	07/11/2011
CHM.22300	07/11/2011
CHM.22400	07/11/2011
CHM.23900	06/17/2010
CHM.24200	07/11/2011
CHM.24300	07/11/2011
CHM.24400	06/17/2010
CHM.24700	07/11/2011
CHM.29200	07/11/2011
CHM.30150	06/17/2010
CHM.30700	06/17/2010
CHM.31100	06/17/2010
CHM.31300	07/11/2011
CHM.31400	07/11/2011
CHM.31500	07/11/2011
CHM.31600	07/11/2011
CHM.31800	07/11/2011
CHM.31900	07/11/2011
CHM.31950	07/11/2011

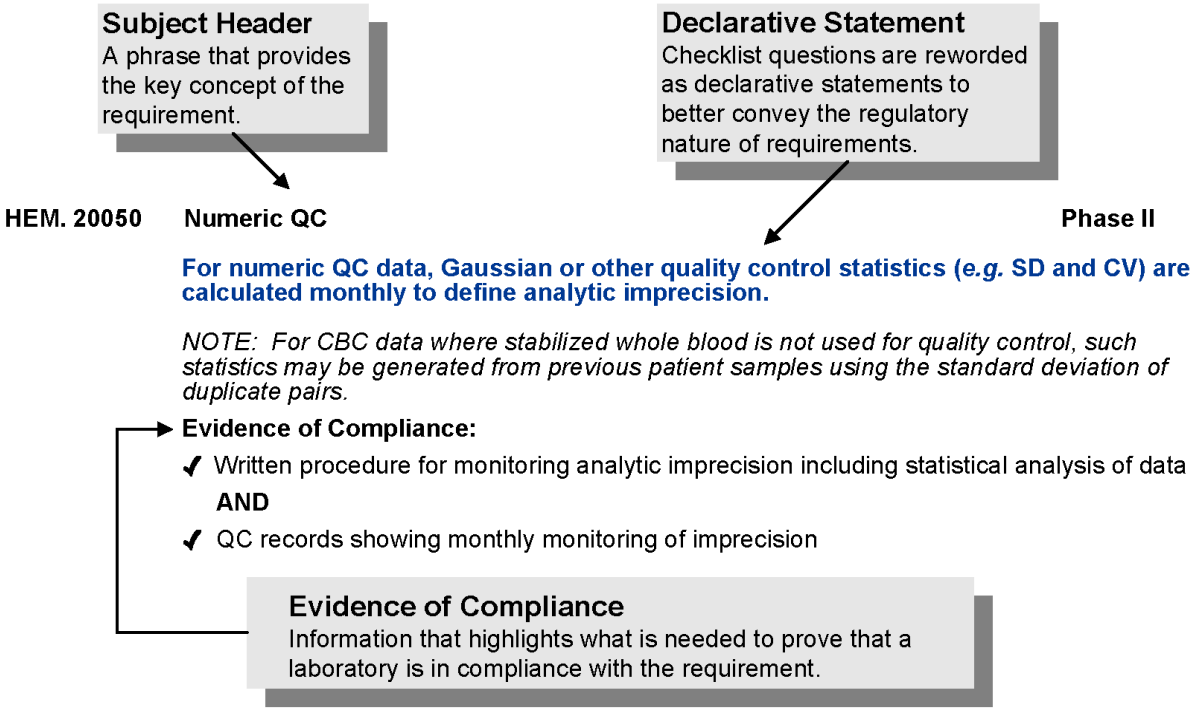
DELETED Checklist Requirements

Requirement	Effective Date
CHM.05000	07/10/2011
CHM.10000	07/10/2011
CHM.10100	07/10/2011
CHM.10200	07/10/2011
CHM.10300	07/10/2011
CHM.10433	07/10/2011
CHM.10466	07/10/2011
CHM.10950	06/16/2010
CHM.11000	07/10/2011
CHM.11100	07/10/2011
CHM.11200	07/10/2011
CHM.11300	07/10/2011
CHM.11400	07/10/2011
CHM.11500	07/10/2011
CHM.11550	07/10/2011
CHM.12000	07/10/2011
CHM.12266	07/10/2011
CHM.12700	07/10/2011
CHM.13300	07/10/2011
CHM.13700	07/10/2011
CHM.15100	07/10/2011
CHM.15200	07/10/2011
CHM.15400	06/16/2010
CHM.15500	06/16/2010
CHM.16100	07/10/2011
CHM.25000	07/10/2011
CHM.25100	07/10/2011
CHM.25200	07/10/2011

CHM.26000	07/10/2011
CHM.26100	07/10/2011
CHM.26200	07/10/2011
CHM.26300	07/10/2011
CHM.26400	07/10/2011
CHM.26500	07/10/2011
CHM.26600	07/10/2011
CHM.26700	07/10/2011
CHM.26800	07/10/2011
CHM.26900	07/10/2011
CHM.27000	07/10/2011
CHM.27200	07/10/2011
CHM.27300	07/10/2011
CHM.27400	07/10/2011
CHM.27500	07/10/2011
CHM.27600	07/10/2011
CHM.27700	07/10/2011
CHM.30650	07/10/2011
CHM.31000	07/10/2011
CHM.31850	07/10/2011
CHM.34100	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.

HOW TO INSPECT USING R.O.A.D INSPECTION TECHNIQUES (Read, Observe, Ask, Discover)

CAP has streamlined the inspection approach used during onsite inspections and is now offering guidance to inspectors by providing assessment techniques to facilitate a more efficient, consistent, and effective inspection process. Specific inspector instructions are listed at the beginning of a grouping of related requirements.

Rather than reviewing each individual requirement, CAP inspectors are encouraged to focus on the Inspector Instructions for a grouping of related requirements. Once an area of concern has been identified through "Read," "Observe," "Ask," "Discover," or a combination thereof, inspectors are encouraged to "drill down" to more specific requirements, when necessary and review more details outlined in the Evidence of Compliance statements. If a requirement is non-compliant, circle the requirement number to later list on the Inspector Summation Report. Inspectors may also make notes in the margins of the checklist document.

Inspector Instructions and Icons used to evaluate a laboratory's performance now appear in several areas throughout the Inspector Checklists. Please note that all four R.O.A.D elements are not always applicable for each grouping, or sections of related requirements.

Inspector Instructions:

	**READ**/review a sampling of laboratory documents. Information obtained from this review will be useful as you observe processes and engage in dialogue with the laboratory staff. *(Example of the complimentary inspector instructions for Quality Management/Quality Control General Issues section appearing across checklists):* • *Sampling of QM/QC policies and procedures* • *Incident/error log and corrective action*
	**OBSERVE** laboratory practices by looking at what the laboratory personnel are actually doing and note if practice deviates from the documented policies/procedures. *(Example)* • *Observe the settings/QC range limits established in the laboratory LIS/HIS to ensure that the laboratory's stated ranges are accurately reflected*
	**ASK** open-ended, probing questions that start with phrases such as "tell me about..." or "what would you do if..." This approach can be a means to corroborate inspection findings that were examined by other techniques, such as Read & Observe. Ask follow-up questions for clarification. Include a variety of staff levels in your communication process. *(Example)* • *As a staff member, what is your involvement with quality management?* • *How do you detect and correct laboratory errors?*
	**DISCOVER** is a technique that can be used to "drill down" or further evaluate areas of concern uncovered by the inspector. "Follow the specimen" and "teach me" are two examples of Discovery. Utilizing this technique will allow for the discovery of pre-analytic, analytic, and post-analytic processes while reviewing multiple requirements simultaneously. *(Example)* • *Select several occurrences in which QC is out of range and follow documentation to determine if the steps taken follow the laboratory policy for corrective action*

INTRODUCTION

This Checklist is intended for comprehensive clinical chemistry testing, including blood gas analysis and toxicology.

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

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

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 validation, 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; Reagents; 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.

CHEMISTRY & TOXICOLOGY GENERAL ISSUES

QUALITY MANAGEMENT AND QUALITY CONTROL

GENERAL ISSUES

Inspector Instructions:

	• Sampling of AM/QC policies and procedures • QM/QC program, including pre-analytic, analytic and post-analytic monitor records and corrective action when indicators do not meet threshold • Incident/error log and corrective action • Records of high school graduate high complexity test review by supervisor
	• How do you evaluate data on the incident/error log? How do you determine appropriate corrective action? • As a staff member, what is your involvement with quality management? • How do you detect and correct laboratory errors?
	• Follow an incident identified on the incident/error log and follow actions including notification

and resolution
- Select several problems identified by the QM plan and follow tracking and corrective action. Determine if the methods used led to discovery and effective correction of the problem.

CHM.10500 Documented QM/QC Plan **Phase II**

The chemistry 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 chemistry laboratory.

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 1992(Feb 28):7176 [42CFR493.1445(e)(5)

CHM.10600 Specimen Collection Manual **Phase II**

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

*****REVISED** 06/17/2010***
CHM.10700 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***
CHM.10800 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

REFERENCES
1) Dufour D, *et al*. The clinical significance of delta checks. *Am J Clin Pathol*. 1998;110:531

CHM.10900 Supervisory Result Review **Phase II**

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

NOTE: The CAP does NOT require supervisory review of all test results. This requirement addresses 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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 1992(Feb 28):7182 [42CFR493.1463(a)(3) and 42CFR493.1463(c)]: 7183 [42CFR493.1489(b)(1) and 42CFR493.1489(b)(5)]

SPECIMEN COLLECTION AND HANDLING

Specimen collection and handling are critical, even if the patient and the testing instrumentation are near one another. Specific instructions for the proper collection and handling of specimens must be made available to anyone collecting patient test materials that are sent to the laboratory.

Inspector Instructions:

	• Sampling of chemistry specimen and handling policy and procedure • Sampling of specimen rejection records/log
	• Sampling of chemistry specimens (labeling) • Aliquoting process. Determine if the procedure and process are adequate to prevent cross-contamination and specimen mix-ups.

- What is your course of action when you receive unacceptable chemistry specimens?
- What process does your laboratory follow when dilutions are made from the primary specimen?

CHM.11800 Specimen Identity/Integrity **Phase II**

Procedures are adequate to verify the identity and integrity of samples, including capillary specimens, aliquots and dilutions.

Evidence of Compliance:

✓ Patient collection and processing records

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

CHM.11900 Specimen Rejection Criteria **Phase II**

There are documented criteria for the rejection of unacceptable specimens, instructions for the special handling of sub-optimal specimens, and documentation of disposition of all unacceptable specimens in the patient/client report and/or quality management records.

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 the treating physician desires the result, then the laboratory must note the condition of the sample on the report. Some or all tests may not be analytically valid on such a specimen. The laboratory may wish to record that a dialogue was held with the physician, when such occurs.

Evidence of Compliance:

✓ Records of rejected specimens

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7183 [42CFR493.1249(a) and (b)]
2) Jones BA, *et al*. Chemistry specimen acceptability. A College of American Pathologists Q-Probes study of 453 laboratories. *Arch Pathol Lab Med.* 1997;121:19-26
3) Hill DM, *et al.* Effects of temperature on stability of blood homocysteine in collection tubes containing 3-deazaadenosine. *Clin Chem.* 2002;48:2017-2022
4) Kilinç AS, *et al.* Falsely increased free triiodothyronine in sera stored in serum separator tubes. *Clin Chem.* 2002;48:2296-2297

CHM.12133 Aliquoting **Phase II**

The documented aliquoting procedure prevents cross contamination of specimens and aliquots.

NOTE: Certain limited volume specimens may warrant the use of previously aliquotted specimens. In such cases, the laboratory must have a clearly defined, documented policy specifying such circumstances and a procedure describing how it is performed.

REAGENTS

Inspector Instructions:

- Sampling of test procedures for reagent handling

	• Sampling of new reagent/shipment validation records • Sampling of ambient temperature logs (if reagents stored at ambient temperature)
	• Sampling of reagents (expiration date, labeling, storage)
	• How do you store the reagents and controls used in test procedures? • How do you validate new reagent lots? • What are your laboratory's criteria for mixing components from one lot number of reagent kit with components from another lot number of kit? • How does your laboratory manage and control reagent inventory?

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

CHM.12333 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's instructions for each waived test

The remaining checklist requirements in the REAGENTS section do not apply to waived tests.

Verification of reagent performance is required. The intent of the requirements is that reagents are used according to manufacturer's instructions and new reagents are checked by an appropriate method before being placed in service. Results of reagent checks must be documented. Where individually packaged reagents/kits are used, criteria should be established for monitoring reagent quality and stability, based on volume of usage and storage requirements.

CHM.12400 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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7164 [42CFR493.1252(c)]
2) Gonzales Y, Kampa IS. The effect of various storage environments on reagent strips. *Lab Med.* 1997;28:135-137
3) Clinical and Laboratory Standards Institute (CLSI). *Laboratory Documents: Development and Control; Approved Guideline—Fifth Edition*. CLSI document GP2-A5 (ISBN 1-56238-600-X). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006

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

CHM.12500 Reagent Storage **Phase II**

All reagents are stored as recommended by the manufacturer.

NOTE: Reagents must be stored as recommended by the manufacturer to prevent environmentally-induced alterations that could affect test performance. If ambient storage temperature is indicated, there must be documentation that the defined ambient temperature is maintained and corrective action taken when tolerance limits are exceeded.

A frost-free freezer may not be used to store reagents and controls 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:

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

REFERENCES
1) Gonzales Y, Kampa IS. The effect of various storage environments on reagent strips. *Lab Med.* 1997;28:135-137

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

CHM.12600 Reagent Expiration Date **Phase II**

All reagents 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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7164 [42CFR493.1252(d)]

CHM.12800 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

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

CHM.12900 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 use for patient testing.

NOTE: For quantitative and qualitative tests, new reagent lots and/or shipments must be tested in parallel with old lots before or concurrently with being placed in service to ensure that the calibration with the new lot of reagent maintains consistent results for patient specimens.

For qualitative tests, minimum cross-checking includes retesting at least one known positive and one known negative sample from the old reagent lot against the new reagent lot. A weakly positive sample should also be used in systems where patient results are reported in that fashion.

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, 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.

The use of QC material alone is adequate to check a new shipment of a reagent lot currently in use, as there should be no change in potential matrix interactions between QC material and different shipments of the same lot number of reagent.

Evidence of Compliance:

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

REFERENCES
1) Clinical and Laboratory Standards Institute. *Evaluation of Matrix Effects; Approved Guideline—Second Edition*. CLSI document EP14-A2 (ISBN 1-56238-561-5). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 190871898, USA, 2005
2) Clinical and Laboratory Standards Institute. *Verification of comparability of patient results within one healthcare system: Approved Guideline.* CLSI document C54-A (ISBN 1-56238-671-9).Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 190871898, USA, 2008
3) Miller WG, Myers GL, Rej R. Why commutability matters. *Clin Chem.* 2006;52:553-554

CALIBRATION AND STANDARDS

Inspector Instructions:

	• Sampling of calibration and AMR policies and procedures • Sampling of calibration/calibration verification records • Sampling of AMR validation records • Sampling of patient reports/worksheets for verification of results outside of AMR • Current DEA license (for US laboratories that handle pure controlled substance(s)) • Biannual instrument correlation records
	• Sampling of calibration materials (labeling, storage, grade quality)
	• What is your course of action if calibration is unacceptable? • When was the last time you performed a calibration procedure and how did you verify the calibration? • What is your course of action when results fall outside the AMR? • What is your course of action when you receive calibration materials for non-FDA cleared assays? • How does your laboratory verify concentration methods?
	• Further evaluate the responses, corrective actions, and resolutions for unacceptable calibration, and unacceptable calibration verification

CHM.12950 Calibration, Calibration/Verification - Waived Tests **Phase II**

For waived tests, testing personnel follow manufacturer's 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 CALIBRATION, CALIBRATION VERIFICATION, ANALYTIC MEASUREMENT RANGE (AMR), and INTERINSTRUMENT COMPARISONS do not apply to waived tests.

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 or 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 µL 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, CLIA requires samples that 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, 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 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.

CHM.13000 Calibration Procedure — Phase II

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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 1992(Feb 28):7165 [42CFR493.1217]
2) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register*. 2003(Jan 24):3707 [42CFR493.1255]
3) Kroll MH, Emancipator K. A theoretical evaluation of linearity. *Clin Chem.* 1993;39:405-413
4) Clinical and Laboratory Standards Institute. *Evaluation of Matrix Effects; Approved Guideline—Second Edition*. CLSI document EP14-A2 (ISBN 1-56238-561-5). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 190871898, USA, 2005
5) Miller WG. Quality control. In: Henry's Clinical Diagnostic and Management by Laboratory Methods, 21st Edition, , ed McPherson RA, Pincus MR. Saunders Elsevier , 2007,p 99-111
6) Kroll MH, *et al*. Evaluation of the extent of nonlinearity in reportable range studies. *Arch Pathol Lab Med.* 2000;124:1331-1338

CHM.13100 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

REFERENCES
1) ISO 17511:2003 *In vitro diagnostic medical devices--Measurement of quantities in biological samples--Metrological traceability of values assigned to calibrators and control materials.*

CHM.13125 Calibration Materials - Non-FDA Cleared Assays — Phase II

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

NOTE: Standards used to prepare calibrators for non-FDA-cleared assays require certificates of purity from the vendor, or a check on purity as part of the initial assay validation process. The

laboratory should document the accuracy of a new lot of calibrators by checking the new lot against the current lot.

Evidence of Compliance:

✓ Records of accuracy checks with each new lot

CHM.13175 Pure Controlled Substances **Phase II**

If the laboratory handles pure controlled substances, there is a current Drug Enforcement Administration (DEA) license.

NOTE: A US laboratory handling pure controlled substance(s) must possess a current Drug Enforcement Administration (DEA) license. It is not necessary to have a DEA license if controlled substances are analyzed, only if they are possessed in pure form. Many manufacturers prepare commercial solutions of controlled substances. It may not be necessary to have a DEA license to purchase these. This checklist requirement is applicable only to US laboratories.

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

CHM.13200 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

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

CHM.13400 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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):3707[42CFR493.1255(b)(3)]
2) Miller WG. "Quality control." Professional Practice in Clinical Chemistry: A Companion Text, ed DR Dufour. Washington, DC: AACC Press, 1999:12-1 to 12-22

CHM.13500 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**** ***07/11/2011***

CHM.13600 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, 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).

AMR validation is not required for methods that measure an analyte quantitatively or semi-quantitatively, and report a qualitative value based on concentration threshold. For such methods, e.g. drugs of abuse, refer to checklist requirement CHM.13750.

Evidence of Compliance:

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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):3707 [42CFR493.1255]

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

CHM.13710 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. *For each analyte, the composition of the diluent solution and the appropriate volumes of sample and diluent must be specified in the procedure manual. Specifying acceptable volumes is intended to ensure that the volumes pipetted are large enough to be accurate without introducing errors in the dilution ratio.*
3. *All dilutions, whether automatic or manual, should be performed in a way that ensures that the diluted specimen reacts similarly to the original specimen in the assay system. For some analytes, demonstrating that more than one dilution ratio similarly recovers the elevated concentration may be helpful.*
4. *This checklist requirement does not apply if the concentration or activity of the analyte that is outside the AMR is reported as "greater than" or " less than" the limits of the AMR.*

**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:

✓ Patient reports or worksheets

*****NEW** 06/17/2010***

CHM.13720 Maximum Dilution **Phase II**

For analytes that may have results falling outside the limits of the AMR, the laboratory procedure specifies the maximum 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, 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.

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.*
4. *In previous versions of the Chemistry and Toxicology Checklist, the concept of establishing allowable dilutions for an analyte was termed establishing the Clinical Reportable Range. This terminology has been discontinued.*

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, 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 reports or worksheets

CHM.13730 Concentration Methods **Phase I**

Concentration methods for quantitative tests are verified.

NOTE: Methods 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 methods **AND**

✓ Records of concentration method verification documented at defined frequency

CHM.13750 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 every 6 months thereafter.

NOTE: This checklist requirement applies only to certain tests that report qualitative results based on a quantitative measurement using a threshold (cut-off value) to discriminate between a positive and negative clinical interpretation (primarily, urine drugs of abuse). 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 six 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 satisfies 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 (listed in the introduction to the Calibration and Standards section of the Chemistry and Toxicology checklist). Note that QC materials are acceptable if the material is specifically designated 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*

CHM.13800 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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register*. 2003(Jan 24):5236 [42CFR493.1281(a)]
2) Podczasy JJ, *et al*. Clinical evaluation of the Accu-Chek Advantage blood glucose monitoring system. *Lab Med.* 1997;28:462-466
3) Ross JW, *et al*. The accuracy of laboratory measurements in clinical chemistry: a study of eleven analytes in the College of American Pathologists Chemistry Survey with fresh frozen serum, definitive methods and reference methods. *Arch Pathol Lab Med.* 1998;122:587-608
4) Miller WG, Myers GL, Ashwood ER, *et al*. State of the Art in Trueness and Inter-Laboratory Harmonization for 10 Analytes in General Clinical Chemistry. *Arch Pathol Lab Med 2008;132:838-846*
5) CLSI. *Verification of comparability of patient results within one healthcare system: Approved Guideline.* CLSI document C54-A (ISBN 1-56238-671-9).CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2008
6) Miller WG, Erek A, Cunningham TD, *et al*. Commutability limitations influence quality control results with different reagent lots. *Clin Chem*. 2011;57:76-83

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

CHM.13810 Neonatal Bilirubin Testing **Phase II**

If the laboratory performs neonatal bilirubin testing, it must ensure that results in the range of 5 to 25 mg/dL are suitable for use with standardized clinical practice interpretive guidelines.

NOTE: The laboratory must standardize its method against reference materials in the stated range as assigned by a reference measurement procedure, so that its results can be interpreted according to clinical guidelines. Proficiency testing material may be used to verify

standardization, but such material must be accuracy-based. (Not all proficiency testing material is accuracy-based.)

Evidence of Compliance:

✓ Records of method standardization

REFERENCES
1) Lo SF, Doumas BT, Ashwood ER. Bilirubin proficiency testing using specimens containing unconjugated bilirubin and human serum: results of a College of American Pathologists study. *Arch Pathol Lab Med* 2004;128:1219-1223
2) American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics* 2004;114:297-316

CONTROLS

Controls are used to ensure that a test system is performing correctly. Traditionally, controls are samples that act as surrogates for patient/client specimens, periodically processed like a patient/client sample to monitor the ongoing performance of the entire analytic process. Under certain circumstances, other types of controls (electronic, procedural, built-in) may be used. (Details are in the checklist requirements in this section, below.)

Controls – Waived Tests

Inspector Instructions:

	• Sampling of quality control policies and procedures • Sampling of QC records
	• How do you determine when QC is unacceptable and when corrective actions are needed?
	• Select several occurrences in which QC is out of range and follow documentation to determine if the steps taken follow the laboratory policy for corrective action

CHM.13820 Manufacturer Instructions - Waived Tests **Phase II**

Testing personnel follow manufacturer's instructions for quality control of waived tests.

Evidence of Compliance:

✓ Written procedure consistent with manufacturer's instructions for each waived test

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

CHM.13840 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.**

CHM.13860 QC Corrective Action - Waived Tests Phase II

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

CHM.13880 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

Controls – Nonwaived Tests

Inspector Instructions:

	• Sampling of quality control policies and procedures • Sampling of QC records
	• How have you validated the adequacy of limiting daily QC to electronic/procedural/built-in QC? • How do you determine when quality control is unacceptable and when corrective actions are needed? • How does your laboratory verify or establish acceptable quality control ranges? • What is your course of action when monthly precision data changes significantly from the previous month's data? • What is your course of action when you perform test procedures that do not have commercially available calibration or control materials?
	• Select several occurrences in which QC is out of range and follow documentation to determine if the steps taken follow the laboratory policy for corrective action

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

CHM.13900 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:*

- *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 (e.g. for blood gas testing).*
- *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. *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 external controls 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. For example, traditional external liquid control materials are considered 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

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register.* 2003(Jan 24):5232 [42CFR493.1256(d)(3) (i, ii)]
2) Steindel SJ, Tetrault G. Quality control practices for calcium, cholesterol, digoxin, and hemoglobin. A College of American Pathologists Q-Probes study in 505 hospital laboratories. *Arch Pathol Lab Med.* 1998;122:401-408
3) Voss EM, *et al*. Determining acceptability of blood glucose meters. Statistical methods for determining error. *Lab Med.* 1996;27:601-606
4) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition*. CLSI document C24-A3 (ISBN 1-56238-613-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006
5) Ye JJ, *et al*. Performance evaluation and planning for patient/client-based quality control procedures. *Am J Clin Pathol.* 2000;113:240-248
6) LaBeau KM, *et al*. Quality control of test systems waived by the clinical laboratory improvement amendments of 1988. Perceptions and practices. *Arch Pathol Lab Med.* 2000;124:1122-1127

CHM.14000 QC Acceptable 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. For assayed controls, the laboratory must verify the acceptability 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

REFERENCES

1) NCCLS. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition*. NCCLS document EP5-A2 (ISBN 1-56238-542-9). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087- 1898 USA, 2004
2) Clinical and Laboratory Standards Institute (CLSI). *User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition*. CLSI document EP15-A2 (ISBN 1-56238-574-7). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2005
3) Ross JW, Lawson NS. Analytic goals, concentration relationships, and the state of the art of clinical laboratory precision. *Arch Pathol Lab Med* 1995;119:495-513
4) Steindel SJ, Tetrault G. Quality control practices for calcium, cholesterol, digoxin, and hemoglobin. A College of American Pathologists Q-Probes study in 505 hospital laboratories. *Arch Pathol Lab Med* 1998;122:401-408
5) CLSI. Risk Management Techniques to Identify Error Sources - Approved Guideline - Second Edition. CLSI Document EP18-A2 (ISBN 1-56238-712-X). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA , 19087-1898, USA, 2009

CHM.14125 Calibrator Preparation — 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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):3708 [42CFR493.1256(d)(9)]

CHM.14150 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, this 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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):3708 [42CFR493.1256(d)(9)]

CHM.14200 Verification of Accuracy **Phase II**

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

CHM.14300 QC Data **Phase II**

Quality control data are organized and presented so they can be evaluated daily by the technical staff to detect problems, trends, *etc.*

NOTE: Results of controls must be recorded or plotted to readily detect a malfunction in the instrument or in the analytic system. These control records must be readily available to the person performing the test.

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition*. CLSI document C24-A3 (ISBN 1-56238-613-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006

*****REVISED**** ***07/11/2011***
CHM.14500 Numeric QC Data **Phase II**

For numeric QC data, quality control statistics (*e.g.* SD and CV) are calculated monthly to define and monitor analytic imprecision.

NOTE: The laboratory must evaluate the imprecision statistics (e.g. SD and CV, or other appropriate statistics) monthly to confirm that the test system is performing within acceptable limits. For whole blood methods, where stabilized whole blood or other suitable material is not available for QC, such statistics may be generated from previous patient/client samples using the SD of duplicate pairs or other patient data based statistical procedures.

This checklist requirement does not apply to external controls run only to validate new lots/shipments of test materials. However the laboratory should have defined acceptable limits

for such controls (either from the manufacturer, or developed by the laboratory).

Evidence of Compliance:

✓ Written procedure for monitoring analytic imprecision including statistical analysis of data **AND**

✓ QC records showing monthly monitoring for imprecision

REFERENCES

1) Mukherjee KL. Introductory mathematics for the clinical laboratory. Chicago: American Society of Clinical Pathology, 1979:81-94
2) Barnett RN. Clinical laboratory statistics, 2nd ed. Boston, MA: Little, Brown, 1979
3) Weisbrodt IM. Statistics for the clinical laboratory. Philadelphia. PA: JB Lippincott, 1985
4) Matthews DF, Farewell VT. Understanding and using medical statistics. New York, NY: Karger, 1988
5) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7146 [42CFR493.1256(d)(10)(i)]
6) Ross JW, Lawson NS. Analytic goals, concentrations relationships, and the state of the art for clinical laboratory precision. *Arch Pathol Lab Med.* 1995;119:495-513
7) NCCLS. *Laboratory Statistics—Standard Deviation; A Report.* NCCLS document EP13-R (ISBN 1-56238-277-2). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087, 1995
8) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition*. CLSI document C24-A3 (ISBN 1-56238-613-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006
9) Brooks ZC, *et al*. Critical systematic error supports used of varied QC rules in routine chemistry. *Clin Chem.* 2000;46:A70

CHM.14600 QC Corrective Action — Phase II

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

NOTE: In the case of complex metabolic profiles as seen in clinical biochemical genetics laboratories, controls of analytes of clinical significance should meet the laboratory's overall criteria for clinical interpretation.

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. 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).

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Oct 1):1046[42CFR493.1282(b)(2)]

CHM.14800 QC Handling — Phase II

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

NOTE: QC specimens must be analyzed by personnel who routinely perform patient/client 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 pre-analytic and post-analytic variables may differ from those encountered with patient/clients.

Evidence of Compliance:

✓ Records reflecting that QC is run by the same personnel performing patient testing

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7166 [42CFR493.1256(d)(8)]
2) *ibid*, 2003(Jan 24):3708[42CFR493.1256(d)(7-8)

CHM.14900 QC Verification **Phase II**

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

NOTE: Control results must be reviewed before reporting patient/client results. It is implicit in quality control that patient/client test results will not be reported when controls do not yield acceptable results. Controls must be run prior to reporting patient results after a change of analytically critical reagents, major preventive maintenance, or change of a critical instrument component.

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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7166 [42CFR493.1256(f)]
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):3708 [42CFR493.1256(d)(6)]

*****REVISED**** ***07/11/2011***
CHM.14916 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

RESULTS REPORTING

Inspector Instructions:

- Sampling of reporting policies and procedures
- Sampling of patient reports (reference range included)
- Sampling of patient toxicology reports

CHM.15000 Reference Intervals **Phase II**

All patient/client results are reported with reference (normal) intervals or interpretations as appropriate.

NOTE: The laboratory must report reference (normal) intervals or interpretations with patient/client results, where such exist. This is important to allow proper interpretation of patient/client data. In addition, the use of high and low flags (generally available with a computerized laboratory information system) is recommended. For urine testing for drugs of abuse, the cut-off value for positive results should be listed, either in the report or in a separate

chart/memorandum that is available to clinicians.

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.

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7162 [42CFR493.1291(d)]

CHM.15250 Toxicology Results **Phase II**

There are documented protocols for the reporting of toxicology results.

NOTE: In addition to the requirements found in the Laboratory General Checklist, the following information must be included in toxicology reports:

1. *If appropriate, substances or classes of substances analyzed as part of the toxicology test*
2. *Specimen type*
3. *Report status for positive results (i.e., unconfirmed, confirmed or pending confirmation)*
4. *For immunoassays, the assay cut-off concentration for each drug or drug class**
5. *If the report includes unconfirmed screening results, a statement that such results are to be used only for medical (i.e., treatment) purposes. Unconfirmed screening results must not be used for non-medical purposes (e.g., employment testing, legal testing)*

**The cut-off concentrations may either be included in the report or in a separate chart/memorandum available to clinicians.*

ANALYTIC METHODS AND PROCESSES

Inspector Instructions:

	• Sampling of reference range studies
	• How does your laboratory establish or verify reference ranges?

CHM.15300 Reference Intervals Established **Phase II**

Reference intervals (normal ranges) 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-A3c, 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 retain documentation of this evaluation.

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

REFERENCES
1) Fulwood R, *et al*. Hematological and nutritional biochemistry reference data for persons 6 months-74 years of age: United States, 1976-80. Hyattsville, MD: Dept Health and Human Services pub no (PHS) 83-1682
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register.* 2003(Jan 24):5231 [42CFR493. 1253(b)(1)(ii) and (b)(2)(vi)]
3) Hicks JM, *et al.* Vitamin B12 and folate. Pediatric reference ranges. *Arch Pathol Lab Med.* 1993;117:704-706
4) Knight JA. Laboratory issues regarding geriatric patients. *Lab Med.* 1997;28:458-461
5) Slovacek KJ, *et al*. Use of age-specific normal ranges for serum prostate-specific antigen. *Arch Pathol Lab Med.* 1998;122:330-332
6) Soldin S, Hicks J (eds). Pediatric reference ranges, 3rd edition. Washington, DC: American Association of Clinical Chemistry Press, 1999
7) Clinical Laboratory and Standards Institute (CLSI). *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition* CLSI Document C28-A3c (ISBN 1-56238-682-4). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA , 2008
8) Katzmann JA, *et al*. Serum reference intervals and diagnostic ranges for free κ and free λ immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. *Clin Chem.* 2002;48:1437-1444
9) Horn PS, Pesce AJ. Effect of ethnicity on reference intervals. *Clin Chem.* 2002;48:1802-1804
10) Lahti A, *et al* Impact of subgroup prevalence on partitioning of Gaussian-distributed reference values. *Clin Chem.* 2002;48:1987-1999

METHODS AND INSTRUMENT SYSTEMS

Inspector Instructions:

- If problems are identified during the review of the methods and instrument systems, or when asking questions, further evaluate the laboratory's responses, corrective actions and resolutions
- Select a representative assay and follow the entire process from specimen receipt to final result reporting

Immunoassays/Immunoanalyzers

Inspector Instructions:

- Sampling of immunoassay policies and procedures
- Validation data for modifications, if applicable
- Sampling of calibration data

CHM.15600 Immunoassay Validation **Phase II**

If immunoassay reagents are NOT used according to instrument and/or kit manufacturer's instructions, there are validation data to support that modifications produce reliable results consistent with the original assay claims of the instrument or kit manufacturer.

NOTE: For modifications such as altering dilution ratios, there must be validation data to support that the modification produces results that meet the clinical requirements for test use.

CHM.15700 Calibration Materials **Phase II**

Appropriate calibrators are used.

NOTE: Specified appropriate calibrators must be used in each run or batch of samples. Appropriate calibrators for screening assays should consist of at least one positive calibrator. If only one calibrator is used, it must be at the declared cut-off value(s). Laboratories may use historical calibrations; however, controls must be run with each batch to verify the calibration.

Evidence of Compliance:

✓ Written procedure defining use of appropriate calibrators **AND**

✓ Records of calibration

Radioimmunoassays

Inspector Instructions:

- Sampling of radioimmunoassay policies and procedures
- Sampling of calibration records
- Sampling of background radioactivity records

CHM.15900 Gamma Counter Calibration **Phase II**

Gamma counters and/or scintillation counters are calibrated, results recorded and compared to previous values each day of use.

Evidence of Compliance:

✓ Written procedure for calibration

CHM.16000 Background Radioactivity **Phase II**

The background radioactivity is determined each day of use, including the background in each well of a multi-well counter, with defined upper limits of acceptability.

Evidence of Compliance:

✓ Records of background radioactivity determinations documented at defined frequency

CHM.16200 Counting Times **Phase II**

Counting times for quantitative procedures are sufficiently long for statistical accuracy and precision.

Evidence of Compliance:

✓ Written procedure defining counting times for each quantitative assay

REFERENCES
1) Klee G, Post G. Effect of counting errors on immunoassay precision. *Clin Chem.* 1989;35:1362-1366

Chromatography and Mass Spectrometry

Thin Layer Chromatography (TLC)

Inspector Instructions:

- Sampling of TLC policies and procedures
- Sampling of control, standards/calibrator records

CHM.16300 Standard/Calibration Materials **Phase II**

Appropriate standards or calibrators (as applicable) are included with each TLC plate.

NOTE: Appropriate standards must include drugs/compounds that test the chromatographic range of the TLC plate, and that test all phases of the staining/development system. This may consist of a standard solution or dot that contains multiple drug/compound standards.

Evidence of Compliance:

✓ Written procedure defining standards/calibrators for TLC **AND**
✓ Records showing use of appropriate standards/calibrators with each plate

CHM.16400 Daily QC - TLC **Phase II**

Negative and appropriate positive controls are extracted and run through the entire procedure.

NOTE: Appropriate positive controls must include drugs/compounds that test the chromatographic range of the TLC plate and the staining/development system. For FDA-cleared kits not classified as high complexity, negative controls and positive controls (when available) must be extracted and carried through the entire procedure at least weekly. For high complexity tests, including non-FDA-cleared TLC procedures, positive and negative controls must be extracted and carried through the entire procedure during each day of patient/client testing.

Evidence of Compliance:

✓ Written QC procedure defining QC requirements appropriate to the complexity of the test system **AND**
✓ QC records documented at defined frequency

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register.* 2003(Jan 24):5232 [42CFR493.1253(a)]

CHM.16500 Solvent Mixtures **Phase II**

Solvent mixtures are prepared fresh as needed.

NOTE: If a mixture of solvents is used, certain components will evaporate with time faster than others. This leads to poor extraction or reproducibility of migration rates. If a commercial kit is used, the manufacturer's instructions should be followed.

Evidence of Compliance:

✓ Written procedure defining the criteria for preparation of solvent mixture

Gas Chromatography (GC)

Inspector Instructions:

	• Sampling of GC policies and procedures • Sampling of control, calibration/standards records • Sampling of column verification records • Sampling of instrument maintenance records
	• How does your laboratory evaluate the effectiveness of hydrolysis? • How does your laboratory evaluate potential carryover? • How have you determined the limit of detection and the AMR?

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

CHM.16550 Calibrator/Standard Material **Phase II**

Appropriate calibration or calibration verification is performed with each analytic batch.

NOTE: For qualitative assays, an appropriate calibrator should be run at normal and abnormal levels. For quantitative assays, a multipoint calibration may be required if the measurement has a non-linear response. For some assays, a level near the assay's limit of detection (LOD) or at critical decision point(s) is needed. For measurement systems that have a linear response validated by periodic multipoint calibration verification and AMR validation protocols, a calibration procedure that uses a single calibrator at an appropriate concentration is acceptable. Analyses based on a single point calibration must be controlled by appropriate quality control samples. In addition, inclusion of a negative control (reagent blank) is good laboratory practice.

Evidence of Compliance:

✓ Written procedure defining calibrators/standards appropriate for the test system used **AND**

✓ Records of calibration/calibration verification with each batch

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

CHM.16650 Quality Control - GC **Phase II**

Appropriate controls are extracted and run through the entire procedure.

NOTE: Controls used in GC procedures must evaluate as much of the complete testing process as is technically feasible. The control process includes any pre-treatment, pre-purification or extraction steps, unless non-pretreated control material is inappropriate. For qualitative assays, the negative and positive controls should be at concentrations that meaningfully confirm performance below and above the decision threshold for the analyte. For quantitative assays, appropriate controls must include at least one normal sample, and at least one sample reflecting a disease range. For some assays, an additional control concentration may be useful to confirm performance near the assay's LOD, LOQ** or cut-off, if appropriate, or at a concentration consistent with highly abnormal levels that test the AMR.*

**LOD - limit of detection*

***LOQ - limit of quantitation*

Evidence of Compliance:

✓ Written procedure defining QC requirements for each test system **AND**

✓ QC records documenting controls at defined frequency

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register.* 2003(Jan 24):5232 [42CFR493.1256(d)(3)(ii)]

CHM.16700 Hydrolysis Step **Phase I**

If a hydrolysis step is required in the assay, the laboratory includes a control (when available) with each batch to evaluate the effectiveness of hydrolysis.

Evidence of Compliance:

✓ QC results documented for each batch

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

CHM.16750 GC Elements Recorded **Phase II**

A record of the order in which samples are run is maintained for review.

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

CHM.16770 Chromatographic Characteristics/Column Review **Phase II**

There is a procedure for review and approval of chromatographic characteristics and column performance of each run before results are released.

CHM.16800 Carryover Detection **Phase II**

There is a procedure for 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 in each analytical batch analysis.

Evidence of Compliance:

✓ Records of reassessment of samples with potential carryover

REFERENCES
1) CLSI. *Preliminary Evaluation of Quantitative Clinical Laboratory Methods – Approved Guideline- Third Edition.* CLSI Document EP10-A3 (ISBN 1-56238-622-0). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2006
2) Society of Forensic Toxicologists/American Academy of Forensic Sciences. *Forensic Toxicology Laboratory Guidelines.* 2002; 8.2.8:13

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

CHM.16850 Column Verification **Phase II**

New columns are verified for performance before use.

Evidence of Compliance:

✓ Written procedure for column verification **AND**

✓ Records of column verification

CHM.16900 Instrument Operation **Phase II**

Procedures are documented for operation, calibration and maintenance.

CHM.16950 Column/Detector Monitoring **Phase II**

The documented procedure requires monitoring the performance of the column and detector on each day of use.

NOTE: Unextracted standards, extracted calibrators or controls, typically containing the target compound(s), may be analyzed each day to monitor critical aspects of GC performance. Appropriate criteria for evaluating such parameters as retention time, relative retention time, separation of closely eluting compounds of interest, plates, chromatography quality, and detector response should be established and monitored.

Evidence of Compliance:

✓ Records for column and detector monitoring documented at defined frequency

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

CHM.17000 Routine Maintenance **Phase II**

There is evidence that the need for routine maintenance is assessed on each day of use.

NOTE: Typical items include, but are not limited to, flow rates, injector liner, and septum changes.

Evidence of Compliance:

✓ Records of instrument maintenance documented at defined frequency

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

CHM.17050 Gas Leakage **Phase II**

There is a procedure that requires gas lines are checked for leaks every time tubing or a connection has been manipulated.

Evidence of Compliance:

✓ Records of gas line checks

CHM.17100 Reagent Grade **Phase II**

Reagents, solvents and gases of appropriate grade are used.

Evidence of Compliance:

✓ Written procedure detailing appropriate grade for materials used

CHM.17150 Limit of Detection/AMR **Phase II**

There is evidence that the limit of detection (sensitivity) and the AMR for quantitative methods have been determined for each procedure.

High Performance Liquid Chromatography (HPLC)

Inspector Instructions:

- Sampling of HPLC policies and procedures
- Sampling of control, calibration/standards records
- Sampling of column verification records

READ	• Sampling of instrument maintenance records • Sampling of records of sample order
ASK	• How does your laboratory evaluate the effectiveness of hydrolysis? • How does your laboratory evaluate potential carryover? • How have you determined the limit of detection and the AMR?

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

CHM.17200 Calibrator/Standard Material **Phase II**

Appropriate calibration or calibration verification is performed with each analytic batch.

NOTE: For qualitative assays, an appropriate calibrator should be run at normal and abnormal levels. For quantitative assays, a multipoint calibration may be required if the measurement has a non-linear response. For some assays, a level near the assay's limit of detection (LOD) or at critical decision point(s) is needed. For measurement systems that have a linear response validated by periodic multipoint calibration verification and AMR validation protocols, a calibration procedure that uses a single calibrator at an appropriate concentration is acceptable. Analyses based on a single point calibration must be controlled by appropriate quality control samples.

Evidence of Compliance:

✓ Written procedure defining calibrators/standards appropriate for the test system used **AND**

✓ Records of calibration/calibration verification documented for each batch

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

CHM.17300 Quality Control - HPLC **Phase II**

Appropriate controls are extracted and run through the entire procedure.

NOTE: Controls used in HPLC procedures must evaluate as much of the complete testing process as is technically feasible. The control process includes any pre-treatment, pre-purification or extraction steps, unless non-pretreated control material is inappropriate. For qualitative assays, the negative and positive controls should be at concentrations that meaningfully confirm performance below and above the decision threshold for the analyte. For quantitative assays, appropriate controls must include at least one normal sample, and at least one sample reflecting a disease range. For some assays, an additional control concentration may be useful to confirm performance near the assay's LOD, LOQ** or cut-off, if appropriate, or at a concentration consistent with highly abnormal levels that test the AMR.*

**LOD - limit of detection*

***LOQ - limit of quantitation*

Evidence of Compliance:

✓ Written procedure defining QC requirements for test systems used **AND**

✓ QC records documented at defined frequency

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register.* 2003(Jan 24):5232 [42CFR493.1256]

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

CHM.17350 Sample Order **Phase II**

A record of sample run order is maintained for review.

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

CHM.17450 Chromatographic Characteristics/Column Review **Phase II**

There is a procedure for review and approval of chromatographic characteristics and column performance of each run before results are released.

CHM.17500 Hydrolysis Step **Phase I**

If a hydrolysis step is required in the assay, the laboratory includes a control (when available) with each batch to evaluate the effectiveness of hydrolysis.

Evidence of Compliance:

✓ QC results documented with each batch, as applicable

CHM.17700 Column Verification **Phase II**

New columns are verified for performance before use.

Evidence of Compliance:

✓ Written procedure for column verification **AND**

✓ Records of column verification

CHM.17800 Reagent Grade **Phase II**

Reagents and solvents are of appropriate grade.

Evidence of Compliance:

✓ Written procedure detailing appropriate grade for materials used

CHM.17900 Instrument Operation **Phase II**

Procedures are documented for operation, calibration and maintenance.

CHM.18000 Column/Detector Monitoring **Phase II**

The documented procedure requires monitoring the performance of the column and detector on each day of use.

NOTE: Unextracted standards, extracted calibrators or controls, typically containing the target compound(s), may be analyzed to monitor critical aspects of HPLC performance. Appropriate criteria for evaluating such parameters as retention time, relative retention compounds time, separation of closely eluting compounds of interest, plates, chromatography quality and detector response should be established and monitored.

Evidence of Compliance:

✓ Records of column and detector monitoring documented at defined frequency

CHM.18100 Carryover Detection **Phase II**

There is a procedure for the detection 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) to the following sample in each analytical batch analysis.

Evidence of Compliance:

✓ Records for reassessment of samples with potential carryover

REFERENCES
1) CLSI. *Preliminary Evaluation of Quantitative Clinical Laboratory Methods – Approved Guideline- Third Edition.* CLSI Document EP10-A3 (ISBN 1-56238-622-0). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2006
2) Society of Forensic Toxicologists/American Academy of Forensic Sciences. *Forensic Toxicology Laboratory Guidelines.* 2002; 8.2.8:13

CHM.18200 Instrument Performance **Phase II**

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

Evidence of Compliance:

✓ Instrument performance records

CHM.18300 Limit of Detection/AMR **Phase II**

There is evidence that the limit of detection (sensitivity) and the AMR for quantitative methods have been determined for each procedure.

Mass Spectrometry (MS)

Inspector Instructions:

	• Sampling of MS policies and procedures • Sampling of instrument maintenance records • Identification criteria compliance
	• How does your laboratory identify possible ion-suppression?

CHM.18400 Instrument Operation **Phase II**

Procedures are documented for operation, calibration and maintenance of the mass spectrometer.

CHM.18500 Instrument Maintenance **Phase II**

The documented procedure requires that the mass spectrometer be maintained at regular intervals as suggested by the manufacturer, or if different criteria or procedures from the manufacturer are used, these procedures have been validated and the records maintained on file.

CHM.18600 Mass Spectrophotometer Tuning **Phase II**

The mass spectrometers are tuned each day of patient/client testing, or according to manufacturer's recommendations and tune records are maintained.

NOTE: Acceptable tolerance limits for tune parameters must be defined, and tune records maintained.

***REVISED** 07/11/2011*
CHM.18700 Identification Criteria **Phase II**

The identification criteria for single stage mass spectrometry (*i.e.* GC/MS, LC/MS) are in compliance with recommendations.

NOTE: One acceptable criterion for compound identification by GC/MS using ion ratios is that the unknown result must have ion ratios within a predefined or tolerance limit. This limit should be supported by either literature references or through experimental means. Such ion ratio tolerance limits may differ based on the technique applied (e.g. GC/MS versus LC/MS) as well as the analyte(s) being determined (e.g. compounds with mainly ions of low abundance); thus, a defined limit to cover all methods and analytes cannot be given.

Identification using ion ratios typically requires the use of at least 2 ion ratios. However, one ion ratio of 2 characteristic ions may be acceptable if there are only a few characteristic ratios AND if there are other identifying characteristics, e.g. retention time. The internal standard's identification should be monitored with at least one ion ratio. An acceptable criterion for compound identification using total spectra is that the unknown result must have a "spectral match" quality or fit that is within the defined limits that the laboratory has set and validated. Ion ratios determined from total spectra analysis are an acceptable identification method, and should fulfill the same criteria as given above for ion ratio identification.

Laboratories using mass spectrometric methods for quantitative purposes based on total ion current measurements without ion ratios should have ancillary information and assay characteristics that validate this process, e.g. known compound of interest, retention times, potential interferences by endogenous compounds or other drugs/metabolites, etc.

Evidence of Compliance:

✓ QC and test records

REFERENCES
1) CLSI. *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline - Second Edition.* CLSI Document C43-A2. (ISBN 1-56238-720-0). CLSI, 940 West Valley Road, Wayne, PA 19087-1898, USA, 2010
2) Official Journal of the European Communities. Commission Decision implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (17.8.2002)

***REVISED** 07/11/2011*
CHM.18800 Identification Criteria **Phase II**

The identification criterion for tandem mass spectrometry (MS/MS) are validated and documented.

NOTE: In tandem mass spectrometry using multiple reaction monitoring (MRM) there is at least

one transition monitored for the internal standard and another for the analyte.

Evidence of Compliance:

✓ QC and test records

REFERENCES
1) CLSI. *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline - Second Edition.* CLSI Document C43-A2. (ISBN 1-56238-720-0). CLSI, 940 West Valley Road, Wayne, PA 19087-1898, USA, 2010
2) Official Journal of the European Communities. Commission Decision implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (17.8.2002)

CHM.18900 Ion-Suppression Evaluation **Phase II**

For LC/MS, the laboratory's assay procedure includes an evaluation for possible ion-suppression.

NOTE: Ion suppression is a recognized analytical anomaly in LC/MS experiments. Such suppression can lead to false negative results or poor quantitative analyses. While difficult to predict and observe from specimen to specimen, certain precautions should be used to try to recognize when ion suppression occurs. As an example, for isotopically-labeled internal standards, if there is poor recovery of the internal standard, a signal to noise ratio greater than 3:1 should still suffice for acceptance of the specimen in question. If recovery of the isotopically-labeled internal standard is considered poor, then an alternate analysis should be considered, e.g. the method of standard addition. For analogue-type internal standards, internal standard recovery may be used as a guide for identification of ion suppression, although another option, such as the method of standard addition, would be a reasonable alternative.

Evidence of Compliance:

✓ Records showing recovery of the internal standard **OR** records for alternative methods used

REFERENCES
1) Annesley, T.M. Ion Suppression in Mass Spectrometry. *Clin. Chem.*, 49, pp. 1041-1044 (2003)
2) Krull, I. and Swartz, M. Quantitation in Method Validation. LC-GC, 16, pp. 1084-1090 (1998)

Inductively Coupled Plasma – Mass Spectrometry (ICP/MS)

Inspector Instructions:

	• Sampling of ICP/MS policies and procedures • Sampling of ICP/MS routine function checks and maintenance logs • Sampling of calibration records
	• How does your laboratory verify assay performance each day of use?

CHM.20300 Function Checks **Phase II**

Routine function parameters (as specified by the instrument manufacturer) are verified and recorded on each day of use.

NOTE: Such parameters should include routine check of tubing of peristaltic pumps for wear and/or contamination.

CHM.20400 Tuning Solution **Phase II**

An appropriate tuning solution or autotune is used to verify assay performance each day of use.

NOTE: Tuning solutions may contain a single element or multiple elements. Use of such solutions and/or autotuning verifies general system performance, control for potential interferences and mass resolution, optimization of ion lens voltages and check signal stability. Failure to use a tuning solution or autotune may affect ICP/MS sensitivity and selectivity.

Evidence of Compliance:

✓ Records of instrument tuning documented at defined frequency

CHM.20500 Peak Width **Phase II**

The peak width is optimized.

NOTE: ICP/MS peak width must be optimized. In quadrupole ICP/MS experiments, there is generally mass unit resolution. If a mass spectral peak is too broad, a false positive finding may occur, since it may overlap with another analyte. If a mass spectral peak is too narrow, sensitivity is sacrificed. Most manufacturers of ICP/MS instrumentation designate an acceptable peak width range. The peak width range is generally determined using a tuning solution. Some software packages automatically check and alter peak width range. Peak width optimization is generally verified daily. There may be times when it may be desirable to go outside the manufacturers specified peak width range. For example, brass is an alloy of copper and zinc. In ICP/MS, copper peaks surround that of zinc. Therefore, the copper peaks may interfere with the ability to detect zinc. Hence, by narrowing the zinc peak width, the possible interference due to copper may be mitigated or eliminated. With high resolution ICP/MS, it may be acceptable to have designated acceptable peak width range levels for different analytes.

Evidence of Compliance:

✓ Records of verification of peak width optimization

CHM.20600 Common Interferences Minimized **Phase II**

When appropriate, oxides and doubly-charged species are minimized.

NOTE: Oxides and doubly-charged species are common interferences in ICP/MS. Oxides of various elements may have overlapping signals with elements of the same mass, thus leading to false-positive findings. Special techniques such as high resolution ICP/MS, dynamic-reaction cell and collision-reaction cell processes may eliminate the concern for oxide interference. Elements with a second ionization potential greater than or equal to 15.8 eV (the ionization potential of argon) may be doubly-charged. Such doubly-charged species may suggest the presence of an element that is not truly present. For example, gadolinium has an isotope at m/e154. It has a doubly-charged species at m/e 77, which is also the same mass as an isotope of selenium, as well as a mass used as a correction factor for arsenic interference by $ArCl^{37}$. Despite the potential for a doubly-charged species, if the analyte of interest cannot be interfered with by known doubly-charged species, then such concern is unwarranted.

Evidence of Compliance:

✓ Written procedure defining technique to minimize common interference

CHM.20700 Dual Detector Mode **Phase II**

If the dual detector mode is applied, the calibration is verified.

NOTE: In ICP/MS, calibration can be performed in two modes – pulse counting for lower concentrations and analog for higher concentrations. If a range is necessary that overlaps with both modes, then the laboratory should employ a cross-calibration. This is generally accomplished by use of a tuning solution whereby a full calibration is performed in both modes followed by software adjustment for a smooth transition. If a concentration range is needed that only encompasses one mode or the other, then a cross-calibration is unnecessary as long as the appropriate mode is employed.

Evidence of Compliance:

✓ Records of calibration verification and cross-calibration, if needed

CHM.20800 Reaction/Collision Cell **Phase II**

If a reaction/collision cell is utilized, the reaction/collision gases are optimized.

NOTE: Optimization of reaction/collision gases will allow for maximization of sensitivity and minimization of background counts. Such optimization is generally accomplished through use of a separate tuning solution and is controlled by a separate part of most software packages than that used for autotuning.

Evidence of Compliance:

✓ Records of optimization of reaction/collision gases

CHM.20900 Calibration Curve **Phase II**

An adequate and appropriate calibration curve is established for quantitative testing.

Evidence of Compliance:

✓ Written procedure defining criteria for establishing the calibration curve for quantitative testing

CHM.21000 Instrument Operation **Phase II**

Procedures are documented for operation, calibration, detection of drift in performance, and maintenance for ICP/MS equipment.

NOTE: Documented procedures for ICP/MS equipment must include criteria for performance and procedures to detect drift, which can occur rapidly. One way in which instrument drift can be detected is by evaluating control materials at defined intervals during a run. Procedures must also include thresholds for maintenance, instructions for maintenance, instructions for verification of instrument performance after maintenance, as well as instructions how to document the associated operations.

CHM.21100 Isotope/Standard Criteria **Phase II**

Appropriate criteria are defined for selection of both the isotope(s) and the associated internal standard(s) related to each quantified element.

NOTE: When isotopes and internal standards are measured by ICP/MS, interferences (isobaric and polyatomic species) and relative abundances must be considered and described in written procedures and/or assay validation materials.

CHM.21200 Contamination **Phase I**

Mechanisms are in place to minimize and detect contamination of results obtained by ICP/MS.

NOTE: Potential sources of contamination that should be evaluated and managed in an ICP/MS laboratory include specimen collection, reagent handling, carryover between samples, and engineering controls within the analytical environment.

Evidence of Compliance:

✓ Written procedure detailing methods to limit and detect contamination

CHM.21300 Gas/Reagent Purity **Phase I**

The purity of each gas and reagent used with ICP/MS is defined, documented and appropriate for the intended use.

NOTE: Purity of gasses and reagents (including water) used with ICP/MS should be defined and validated to identify and minimize interferences and sources of contamination.

CHM.21400 Controls/Calibrators/Blanks **Phase I**

Controls, calibrators and blanks are matrix-matched to the sample type.

NOTE: The matrices of controls, calibrators and blanks may affect the ions generated and should be considered in the design and validation of each ICP/MS assay. If matrices are not an issue, the laboratory should have documented that matrix-matching is not necessary.

Evidence of Compliance:

✓ Written procedure defining the use of matrix-matched controls/calibrators/blanks **OR** documentation that matrix-matching is unnecessary

Atomic Absorption Spectrophotometers

Inspector Instructions:

	• Sampling of AA Spectrophotometer policies and procedures • Calibration records • Sampling of required daily and periodic instrument function checks
	• How does your laboratory ensure optimal lamp performance?

CHM.21500 Function Checks **Phase II**

Routine function checks are defined, performed and recorded on each day of use.

CHM.21600 Burner Head/Aspirator **Phase II**

The burner head and aspirator are flushed thoroughly with water each day of use.

Evidence of Compliance:

✓ Record of burner head and aspirator maintenance

CHM.21700 Optical Beam Alignment **Phase II**

The optical beam alignment is checked regularly, and results are recorded.

NOTE: This should be done at least weekly, although daily checking is preferred.

CHM.21800 Atomizer **Phase II**

The atomizer is cleaned and flow rate optimized at regular, specified intervals, and the results recorded.

CHM.21900 Sampler System **Phase II**

Automatic sampler systems (*e.g.* on graphite furnace) are checked for precision at specified periodic intervals.

Evidence of Compliance:

✓ Records of sampler system checks documented at defined frequency

CHM.22000 Graphite Furnace **Phase II**

If a graphite furnace is used, the blank value of a graphite tube is verified for each element tested.

NOTE: Residue from assayed samples may accumulate on the graphite tube, thus potentially resulting in false positive findings should the residue contain the element of interest. In addition, checking for the response of a blank may also serve as one of the indicators that the graphite tube may need replacement.

Evidence of Compliance:

✓ Record of graphite tube checks documented at defined frequency

CHM.22100 Calibration Curve **Phase II**

An adequate and appropriate calibration curve is established for quantitative testing.

Evidence of Compliance:

✓ Written procedure for establishing the calibration curve for quantitative testing

CHM.22200 Lamp Energy **Phase II**

The lamp's energy is verified and recorded for each run.

NOTE: Atomic absorption spectrophotometric lamp energy must be verified and recorded for each run. Lamps lose performance characteristics over time. Decrements in lamp performance may be observed by a loss of sensitivity. Poor lamp performance may also serve as an indicator of another system failure, e.g. loose connections.

Colorimeters, Spectrophotometers, and Fluorimeters

The following requirements apply to stand-alone instruments; they are not applicable to instruments embedded in automated equipment for which the manufacturer's instructions should be followed.

Inspector Instructions:

	• Sampling of colorimeter/spectrophotometer policies and procedures • Sampling of manufacturer required system checks
	• How does your laboratory verify calibration curves?

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

CHM.22300 Absorbance/Linearity **Phase II**

Absorbance and/or fluorescence linearity is checked and documented at least annually or as often as specified by the manufacturer, with filters or standard solutions.

Evidence of Compliance:

✓ Records of absorbance and linearity checks, as applicable

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

CHM.22400 Wavelength Calibration **Phase II**

Spectrophotometer (including ELISA plate readers) wavelength calibration, absorbance and linearity are checked at least annually (or as often as specified by the manufacturer), with appropriate solutions, filters or emission line source lamps, and the results documented.

NOTE: Some spectrophotometer designs, e.g. diode array, have no moving parts that can alter wavelength accuracy and do not require routine verification. The manufacturer's instructions should be followed.

Evidence of Compliance:

✓ Records of wavelength calibration checks, as applicable

CHM.22500 Stray Light **Phase II**

Stray light is checked at least annually with extinction filters or appropriate solutions, if required by the instrument manufacturer.

Evidence of Compliance:

✓ Records of stray light checks, as applicable

CHM.22600 Calibration Curves **Phase II**

For procedures using calibration curves, all the curves are rerun regularly and/or verified after servicing or recalibration of instruments.

Evidence of Compliance:

✓ Records of calibration curve rerun and/or verification documented at defined frequency

Flame Photometers

Inspector Instructions:

	• Sampling of flame photometer policies and procedures • Sampling of required system checks
	• Filters (clean, not scratched, not deteriorated)

CHM.22700 Filter Photometers **Phase II**

Filters (filter photometers) are clean, free of scratches and in good condition (not deteriorated).

CHM.22800 Function Checks **Phase II**

Routine function checks are defined, performed and recorded on each day of use.

CHM.22900 Burner/Chimney **Phase II**

The burner, chimney and appropriate optical surfaces are checked for dirt and film and cleaned at regular intervals.

Evidence of Compliance:

✓ Record of maintenance documented at defined frequency

Equipment Maintenance

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.

Inspector Instructions:

	• Sampling of instrument policies and procedures • Sampling of instrument maintenance logs and repair records
	• Instrument records (promptly retrievable)

CHM.23000 Instrument Operation **Phase II**

There are documented standard procedures for start-up, operation and shutdown of instruments.

NOTE: These procedures must readily be available to the operator in the immediate vicinity of the instrument, and ideally should include a procedure for emergency shutdown.

CHM.23100 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.

CHM.23300 Function Checks **Phase II**

Instrument function checks are documented BY THE TECHNICAL OPERATOR and readily available to detect trends or malfunctions.

CHM.23400 Instrument Tolerance Limits **Phase II**

Tolerance limits for acceptable function are documented for specific instruments wherever appropriate.

CHM.23500 Instrument Troubleshooting **Phase II**

Instructions are provided for minor troubleshooting and repairs of instruments (such as manufacturer's service manual).

CHM.23600 Instrument Service Records **Phase II**

Records are maintained AT OR NEAR each instrument to document all repairs and service procedures.

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. Offsite 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.

Glassware

Inspector Instructions:

	• Pipette verification procedure • Sampling of pipette/dilutor checks
	• What is your laboratory's course of action prior to using non-certified thermometers and non-certified volumetric glassware?

CHM.23800 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:

✓ Pipettes marked Class A **OR** NIST certificate **OR** Validation study of accuracy for non-certified glassware

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

CHM.23900 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** Validation study of accuracy for non-certified pipettes

REFERENCES
1) Curtis RH. Performance verification of manual action pipets. Part I. *Am Clin Lab. 1994*;12(7):8-9
2) Curtis RH. Performance verification of manual action pipets. Part II. *Am Clin Lab. 1994*;12(9):16-17
3) Perrier S, *et al*. Micro-pipette calibration using a ratiometric photometer-reagent system as compared to the gravimetric method. *Clin Chem.* 1995;41:S183
4) American Society for Testing and Materials. Standard specification for glass volumetric (transfer) pipets, designation E 969-95. Philadelphia, PA: ASTM, 1995
5) Johnson B. Calibration to dye for: Artel's new pipette calibration system. *Scientist.* 1999;13(12):14
6) Connors M, Curtis R. Pipetting error: a real problem with a simple solution. Parts I and II. *Am Lab News.* 1999;31(13):20-22
7) Skeen GA, Ashwood ER. Using spectrophotometry to evaluate volumetric devices. *Lab Med.* 2000;31:478-479

CHM.24000 Pipette Accuracy - Non Class A — 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.

REFERENCES
1) Curtis RH. Performance verification of manual action pipets. Part I. *Am Clin Lab. 1994*;12(7):8-9
2) Curtis RH. Performance verification of manual action pipets. Part II. *Am Clin Lab. 1994*;12(9):16-17
3) Perrier S, *et al*. Micro-pipette calibration using a ratiometric photometer-reagent system as compared to the gravimetric method. *Clin Chem.* 1995;41:S183
4) Bray W. Software for the gravimetric calibration testing of pipets. *Am Clin Lab.* Oct 1995
5) CLSI. *Laboratory Instrument Implementation, Verification, and Maintenance; Approved Guideline.* CLSI Document GP31-A. (ISBN 1-56238-697-2). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2009
6) Johnson B. Calibration to dye for: Artel's new pipette calibration system. *Scientist.* 1999;13(12):14
7) Connors M, Curtis R. Pipetting error: a real problem with a simple solution. Parts I and II. *Am Lab News.* 1999;31(13):20-22
8) Skeen GA, Ashwood ER. Using spectrophotometry to evaluate volumetric devices. *Lab Med.* 2000;31:478-479

CHM.24100 Measuring Devices — Phase I

The use of less precise measuring devices such as serological plastic pipettes and graduated cylinders are limited to situations where the accuracy and precision of calibrated glass pipettes are not required.

NOTE: In contrast with the more stringent accuracy requirements of glass pipettes, ASTM requirements for plastic pipettes are ± 3% of the stated volume. The procedure manual should specify when the use of non-class A measuring devices is permissible.

REFERENCES

1) American Society for Testing and Materials. Standard specification for serological pipets, disposable plastic, designation E 934-88, In 1993 Annual Book of ASTM Standards, section 14 (general methods and instrumentation). Philadelphia, PA: ASTM, 1993:14.02:485-486

Automatic Pipettes - Fixed Volume Adjustable and/or Micropipettes

Automatic pipettes and diluting devices of all types must be checked for accuracy and reproducibility before being placed in service and at least annually thereafter.

Inspector Instructions:

- Automatic pipette calibration procedure
- Sampling of pipette/dilutor checks

- How are you assured your automatic pipetting systems exhibit no carryover effects?

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

CHM.24200 Automatic Pipettes **Phase II**

There is a documented procedure defining how pipettes used for quantitative dispensing are checked for accuracy of calibration and reproducibility (gravimetric, colorimetric, volumetric or other verification procedure) before being placed in service initially, and results documented.

NOTE: Automatic pipettes (fixed volume, adjustable volume, micropipettes, and analytic instruments with integral automatic pipettors) must be checked for calibration accuracy and imprecision either by volumetric, colorimetric, gravimetric, or other means before being placed in service initially, and results documented. The initial calibration may be performed by the manufacturer or other outside facility, but in such cases the laboratory must have documentation from the manufacturer or other facility that includes the technique used to check calibration, the method of shipment to prevent damage in transit, and the bias and imprecision of the pipette(s). The bias and imprecision must meet the specifications established by the laboratory.

REFERENCES
1) Curtis RH. Performance verification of manual action pipets. Part I. *Am Clin Lab. 1994*;12(7):8-9
2) Curtis RH. Performance verification of manual action pipets. Part II. *Am Clin Lab. 1994*;12(9):16-17
3) Perrier S, *et al*. Micro-pipette calibration using a ratiometric photometer-reagent system as compared to the gravimetric method. *Clin Chem.* 1995;41:S183
4) Bray W. Software for the gravimetric calibration testing of pipets. *Am Clin Lab.* Oct 1995
5) CLSI. *Laboratory Instrument Implementation, Verification, and Maintenance; Approved Guideline.* CLSI Document GP31-A. (ISBN 1-56238-697-2). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2009
6) Johnson B. Calibration to dye for: Artel's new pipette calibration system. *Scientist.* 1999;13(12):14
7)) Connors M, Curtis R. Pipetting error: a real problem with a simple solution. Parts I and II. *Am Lab News.* 1999;31(13):20-22
8) Skeen GA, Ashwood ER. Using spectrophotometry to evaluate volumetric devices. *Lab Med.* 2000;31:478-479

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

CHM.24300 Automatic Pipettes **Phase II**

Automatic pipettes used for quantitative dispensing are checked for accuracy and reproducibility (gravimetric, colorimetric, volumetric or other verification procedure) at

specified, periodic (at least annually) intervals, and the results recorded.

NOTE: For analytic instruments with integral automatic pipettors, the accuracy and precision of the pipetting system should be checked at least annually, unless that is not practical for the end-user laboratory. Manufacturers' recommendations should be followed.

REFERENCES
1) Curtis RH. Performance verification of manual action pipets. Part I. *Am Clin Lab. 1994*;12(7):8-9
2) Curtis RH. Performance verification of manual action pipets. Part II. *Am Clin Lab. 1994*;12(9):16-17
3) Perrier S, *et al*. Micro-pipette calibration using a ratiometric photometer-reagent system as compared to the gravimetric method. *Clin Chem.* 1995;41:S183
4) Bray W. Software for the gravimetric calibration testing of pipets. *Am Clin Lab.* Oct 1995
5) CLSI. *Laboratory Instrument Implementation, Verification, and Maintenance; Approved Guideline.* CLSI Document GP31-A. (ISBN 1-56238-697-2). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2009
6) Johnson B. Calibration to dye for: Artel's new pipette calibration system. *Scientist.* 1999;13(12):14
7) Connors M, Curtis R. Pipetting error: a real problem with a simple solution. Parts I and II. *Am Lab News.* 1999;31(13):20-22
8) Skeen GA, Ashwood ER. Using spectrophotometry to evaluate volumetric devices. *Lab Med.* 2000;31:478-479

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

CHM.24400 Pipette Carryover **Phase II**

The laboratory evaluates its automatic pipetting systems for carryover.

NOTE: The laboratory must 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 wide clinical range of analyte concentration, such that a minute degree of carry-over could have significant clinical implications. Examples include immunoassays such as β-HCG, certain enzymes (e.g. CK), and certain drugs of abuse (e.g. benzoylecgonine [cocaine metabolite], which may be present in high concentrations). 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 must determine the analyte concentration above which subsequent samples may be affected, and define this value in the procedure. Results of each analytical run must 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 must be defined (repeat analysis of subsequent samples, for example).

Carryover studies must 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

REFERENCES
1) CLSI *Laboratory Instrument Implementation, Verification, and Maintenance: Approved Guideline.* CLSI Document GP31-A, (ISBN 1-56238-697-2) CLSI. 940 West Valley Road, Wayne, PA 19087-1898. USA. 2009

Thermometers

Inspector Instructions:

- Records of traceability to NIST standards

CHM.24500 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

CHM.24600 Non-certified Thermometers **Phase II**

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

Evidence of Compliance:

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

Temperature-Dependent Equipment

Inspector Instructions:

- Sampling of temperature logs (refrigerator, freezer, water bath, heat block, ambient)

***REVISED** 07/11/2011*
CHM.24700 Temperature Checks **Phase II**

Temperatures are checked and recorded daily for the following types of equipment.

1. **Water baths**
2. **Dry baths (heating blocks)**
3. **Instrument components (water baths, dialyzers, heating baths)**
4. **Incubators and ovens (where temperature control is necessary for a procedure)**
5. **Refrigerators and freezers**

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

For reagents with ambient (room temperature) storage requirements, the temperature of the storage area must be recorded.

CHM.24800 Temperature Range **Phase II**

Acceptable ranges have been defined for all temperature-dependent equipment.

Evidence of Compliance:

✓ Temperature log or record with defined acceptable range

CHM.24900 Temperature Corrective Action **Phase II**

There is evidence of corrective action taken if acceptable temperature ranges for refrigerators and/or freezers are exceeded, including evaluation of contents for adverse effects.

NOTE: If acceptable temperature ranges for refrigerators and/or freezers are exceeded, reagents, controls, calibrators, etc. must be evaluated for possible adverse effects, with documentation of results.

Analytic Balances

Inspector Instructions:

	• Sampling of analytic balance service records • Sampling of balance accuracy check records
	• Analytic balances (mounting) • Standard weights (maintained, appropriate class)

CHM.25300 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

CHM.25400 Balance Mounting **Phase I**

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

CHM.25500 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 (at least annually) 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).w 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

REFERENCES
1) American Society for Testing and Materials. Standard specification for laboratory weights and precision mass standards, designation E 617-97 (Reapproved 2003). ASTM International, West Conshohocken, PA 19428-2959 (www.astm.org)
2) American Society for Testing and Materials. E 898-88 (Reapproved 2000). Standard method of testing top-loading, direct reading laboratory scales and balances. ASTM International, West Conshohocken, PA 19428-2959 (www.astm.org)

CHM.25600 Accuracy Check **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 should be specified.

CHM.25700 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.

PERSONNEL

Inspector Instructions:

- Documentation of person in charge of technical operations education and experience

CHM.25800 Personnel - Technical Operations **Phase II**

The person in charge of technical operations in chemistry has education equivalent to an MT(ASCP) and at least 4 years experience (one of which must be in clinical chemistry) 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

CHM.25900 Personnel - Technical Operations **Phase II**

The person in charge of technical operations in toxicology has education equivalent to an MT(ASCP) or a Bachelor's degree in chemistry/biology/toxicology, and at least 4 years experience (one of which must be in toxicology) 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

CHM.25950 Personnel - Technical Operations **Phase II**

The person in charge of technical operations in the blood gas section has education and experience equivalent to an MT(ASCP) (or certified or registered respiratory therapist) and at least 4 years experience (one of which must be in blood gas testing) 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

LABORATORY SAFETY

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

RADIATION SAFETY

Inspector Instructions:

	• Sampling of radiation safety policies and procedures • Sampling of radiation area surveys/wipe tests documentation • Sampling of radioactive waste disposal documentation • Sampling of personnel records of radionuclide training
	• Radionuclide storage areas (properly shielded) • Appropriate signage where radioactive materials are used/stored
	• Does your laboratory have representation at radiation safety committee meetings? • How does your laboratory check the effectiveness of workbench decontamination?

CHM.27900 Radiation Safety Manual **Phase II**

There is an up-to-date radiation safety manual that includes sections on decontamination and radioactive waste.

NOTE: For US laboratories, this is required by the Nuclear Regulatory Commission (NRC).

REFERENCES
1) U.S. Nuclear Regulatory Commission. Guide for the preparation of applications for medical use programs. Regulatory guide 10.8: Appendix H model procedure for area surveys. Washington, DC: USNRC, 1987

CHM.28000 Workspace Decontamination **Phase I**

Workbenches and sinks are decontaminated each day of use, and the effectiveness checked at least monthly.

NOTE: If the laboratory uses only ^{125}I, either a wipe test or a portable scintillation probe can be used.

Evidence of Compliance:

✓ Records of daily workbench/sink decontamination **AND**
✓ Records of monthly effectiveness checks

CHM.28100 Radionuclides Handling **Phase I**

There are specific policies regarding authorization or restriction of personnel handling

radionuclides.

NOTE: These policies should be incorporated into the department's radiation safety manual.

CHM.28200 Radionuclide Leak **Phase II**

Policies include procedures for notification if a damaged or leaking radionuclide shipment is received.

NOTE: Procedures must include inspection, monitoring of shipments, and instructions for notification, if leakage or damage is noted in a radionuclide shipment. For US laboratories, this is a Department of Transportation requirement.

Evidence of Compliance:

✓ Records of inspections and notifications

REFERENCES
1) NCCLS. *Clinical Laboratory Safety; Approved Guideline—Second Edition.* NCCLS document GP17-A2 [ISBN 1-56238-530-5]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004
2) Department of Transportation, Research and Special Programs Administration. Hazardous materials table, special provisions, hazardous materials communications, emergency response information and training requirements. *Fed Register*. 2003 (Oct 1) [49CFR172.403, 600, 602, 604]

CHM.28300 Radionuclide Storage **Phase II**

Radionuclide storage and decay areas are properly shielded, if required for specific isotopic materials.

NOTE: Radionuclide storage and decay areas must be properly shielded, if required for specific isotopic materials, to avoid excessive exposure to personnel and interference with counting procedures.

Evidence of Compliance:

✓ Written procedure defining shielding requirements for radionuclide storage and decay areas

CHM.28400 Radiation Surveys **Phase II**

There are regular radiation area surveys and wipe tests, with records maintained.

NOTE: Routine radiation surveys and wipe tests to determine exposure rates and detect contamination must be performed and documented regularly.

Evidence of Compliance:

✓ Written procedure defining frequency of radiation survey and wipe tests to determine exposure rated and detect contamination

CHM.28500 Radioactive Material Sign **Phase II**

All areas or rooms where radioactive materials are being used or stored are posted to indicate the presence of radioactive materials.

NOTE: For US laboratories, all areas or rooms where radioactive materials are being used or stored must be posted to indicate the presence of radioactive materials, consistent with 10CFR20, Appendix C.

REFERENCES
1) Nuclear Regulatory Commission; Standards for Protection Against Radiation. *Fed Register* 2004(Jan1):354-421[10CFR20.2402]

CHM.28600 Radionuclide Training **Phase II**

Personnel receive documented training in decontamination routines and in the safe handling and proper disposal of radionuclides (wastes, syringes, needles, and sponges).

Evidence of Compliance:

✓ Records of radionuclide training in personnel file

CHM.28700 Radioactive Waste **Phase II**

Radioactive waste kept separate from normal trash, is stored, and appropriately discarded with documentation.

NOTE: For US laboratories, NRC regulations specify that separate areas be established for the receipt of radioactive waste and that these areas be properly shielded to reduce radiation levels below those maximum permissible limits specified in 10CFR20. Documentation of the radioactive trash disposal must be maintained.

Evidence of Compliance:

✓ Written procedure defining criteria for proper storage and disposal of radioactive waste

CHM.28800 Safety Committee Representation **Phase II**

There is evidence that a laboratory representative is a member of and/or attends institutional radiation safety committee meetings regularly.

NOTE: Independent laboratories must have a radiation safety officer who fulfills the functions of an institutional radiation safety committee.

Evidence of Compliance:

✓ Records of laboratory participation in institutional Safety Committee meeting **OR** participation in other appropriate group responsible for radiation safety

GENERAL CHEMISTRY

CHEMISTRY

THERAPEUTIC DRUG MONITORING

Inspector Instructions:

	• Sampling of TDM policies and procedures • Sampling of TDM patient reports (dosage, time of drug administration)
	• How is the clinician able to link TDM laboratory results to the dosage and time the patient received the drug?

CHM.28900 Specimen Collection/Drug Dosing **Phase I**

Where such information is important, the laboratory has provided information to clinical personnel of the optimal specimen collection time in relation to drug dosing.

Evidence of Compliance:

✓ Written procedure defining criteria for specimen collection for TDM

REFERENCES
1) Nicholson PW, *et al.* Ideal sampling time for drug assays. *Br J Clin Pharm.* 1980;9:467470
2) Howanitz PJ, Steindel SJ. Digoxin therapeutic drug monitoring practices. A College of American Pathologists Q-Probes study of 666 institutions and 18679 toxic levels. *Arch Pathol Lab Med.* 1993;117:684-690
3) Schoenenberge RA, *et al.* Appropriateness of antiepileptic drug level monitoring. *JAMA.* 1995;274:1622-1626
4) Williamson KM, *et al.* Digoxin toxicity: an evaluation in current clinical practice. *Arch Intern Med.* 1998;158:2444-2499

CHM.29000 TDM Results **Phase II**

Where applicable, TDM results are reported in relation to patient dosing and/or timing information.

NOTE: The intent is to have a mechanism whereby the clinician can easily and accurately link TDM results from the laboratory to the dosage and time of drug administration. Ideally, the test result, dose and administration time would be reported in juxtaposition on the patient chart. This may be the responsibility of the laboratory, or an integrating function of reported laboratory analytic data with clinical information from other sources.

Evidence of Compliance:

✓ Written procedure defining criteria for reporting TDM results

REFERENCES
1) Elin RJ. Computer-assisted therapeutic drug monitoring. *Clin Lab Med.* 1987;7:485-492
2) Howanitz PJ, Steindel SJ. Digoxin therapeutic drug monitoring practices. A College of American Pathologists Q-Probes study of 666 institutions and 18679 toxic levels. *Arch Pathol Lab Med.* 1993;117:684-690
3) Schoenenberge RA, *et al.* Appropriateness of antiepileptic drug level monitoring. *JAMA.* 1995;274:16221626
4) Williamson KM, *et al.* Digoxin toxicity: an evaluation in current clinical practice. *Arch Intern Med.* 1998;158:2444-2499
5) Steele BW, *et al*. An evaluation of analytic goals for assays of drugs. A College of American Pathologists therapeutic drug monitoring survey study. *Arch Pathol Lab Med.* 2001;125:729-735

SWEAT TESTING FOR CYSTIC FIBROSIS

The laboratory diagnosis of cystic fibrosis includes SCREENING and CONFIRMATORY sweat testing. Screening tests include: sweat conductivity, cystic fibrosis indicator patch system, Orion skin measuring electrode, and sweat osmolality. Confirmatory tests include quantitative analysis of sweat chloride.

Specimen Collection and Handling

Inspector Instructions:

	• Sampling of sweat testing policies and procedures • Sampling of records/log of insufficient sweat samples • Sampling of iontophoresis unit maintenance records
	• An employee performing the sweat collection procedure, if possible

- How do you ensure effective disinfection of the sweat collection equipment?
- What is your course of action if the patient is receiving oxygen from an open delivery system?

CHM.29100 Sweat Test/Appropriate Age **Phase I**

The sweat test is offered only to patients at an appropriate age.

NOTE: Testing should be performed on patients who are at least 48 hours old. During the first 24 hours after birth, sweat electrolytes are transiently elevated, and rapidly decline on the second day. Thus, sweat testing should not be performed within 48 hours of birth; except for this limit, sweat testing should not be withheld from neonates. There is no reason to delay testing until after 6 weeks of age.

Evidence of Compliance:

✓ Written procedure defining criteria for sweat testing **OR** collection manual that includes parameters for patient age

REFERENCES
1) Hardy JD, *et al.* Sweat tests in the newborn period. *Arch Dis Child.* 1973;48:316-318

CHM.29150 Annual Review Equipment Disinfection **Phase I**

The laboratory reviews at least annually the procedures employed for disinfection of equipment and facilities used for sweat collection.

NOTE: The procedures for disinfection of sweat collection equipment and facilities should be reviewed annually to assure their continued effectiveness. One suggested approach is annual evaluation by the infection control department of the institution.

Evidence of Compliance:

✓ Written procedure defining criteria for disinfection of equipment and facility

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

CHM.29200 Sweat Collection and Analysis Procedure **Phase II**

The laboratory follows generally accepted procedures for sweat collection and analysis, including steps to minimize sample evaporation or contamination.

NOTE: Because sample evaporation and contamination can have significant impact on the validity of test results, laboratories must incorporate the following steps into their procedure and/or follow manufacturer's recommendations:

A. When using gauze or filter paper collection pads:

1. *Use gauze and/or filter paper that is low in electrolyte content*
2. *Wash the patient's skin thoroughly with distilled or deionized water, then dry before stimulation. Repeat after stimulation and before collection*

3. *Do not touch the weighing vial, wax film, collection site, or collection pad. Always use forceps or powder-free gloves*
4. *Use two pieces of waterproof adhesive tape on all sides of the paraffin wax film or wrap with a disposable stretch bandage to produce an airtight seal*
5. *Blot back into the collection pad any condensate that may have formed on the wax film during collection. Failure to collect the condensate can result in false positive test results*
6. *After collection, quickly transfer the specimen pad to the weighing vial and reweigh promptly*

B. When collecting sweat into macroduct coils:

1. *Wash the patient's skin thoroughly with distilled, deionized water. Leave the skin slightly wet after washing the area where the electrode will be attached or add a drop of distilled or deionized water to either the skin or the pilogel surface (after installation in the electrode). Repeat after stimulation and before collection*
2. *Avoid touching the collecting surface of the coil*
3. *Fasten the collector to the extremity with firm strap pressure. Test for proper attachment after sweat appears in the coils*
4. *Do not attempt to remove the entire collector assembly from the patient's extremity before separating the coil from the main body. Loss of specimen may occur*
5. *Do not contaminate the nippers or sweat dispensing needle with sweat sample*

C. When collecting and analyzing sweat using the Nanoduct system:

1. *Wash the patient's skin thoroughly with distilled, deionized water. Leave the skin slightly wet after washing the area where the electrode will be attached or add a drop of distilled or deionized water to either the skin or the pilogel surface (after installation in the electrode). Repeat after stimulation and before collection*
2. *Avoid touching the collecting surface of the device*

Evidence of Compliance:

✓ Written procedure defining criteria for sweat collection and analysis including required elements **OR** other procedure following manufacturer's recommendations

REFERENCES
1) Scott MG, *et al.* Electrolytes and blood gases, In Burtis C and Ashwood E (eds), Tietz textbook of clinical chemistry. Philadelphia: WB Saunders, 4th edition, 2006
2) CLSI. *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline-Third Edition.* CLSI document C34-A3 (ISBN 1-56238-713-8). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2009

CHM.29300 Sweat Stimulation — Phase II

The protocol requires sweat stimulation and collection from the patient's lower arm or upper leg, using a site that is free from diffuse inflammation or rash.

NOTE: The protocol should require that sweat is stimulated and collected from the patient's lower arm or upper leg. Sweat must not be stimulated or collected from the head or trunk. Sweat must not be stimulated or collected from an area of diffuse inflammation, such as a rash or eczematous lesion, because of the likelihood of contamination by serous fluid.

REFERENCES
1) Liebke C, *et al.* Sweat electrolyte concentrations in children with atopic dermatitis. *Lancet.* 1997;350:1678
2) CLSI. *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline-Third Edition.* CLSI document C34-A3 (ISBN 1-56238-713-8). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2009

CHM.29400 Pilocarpine Grade **Phase II**

If the laboratory prepares the pilocarpine solution for iontophoresis, the source of the pilocarpine is USP grade or equivalent.

CHM.29500 Electrode Placement **Phase II**

The protocol requires that the electrodes used for stimulation are placed such that iontophoretic current never crosses the patient's trunk.

NOTE: The protocol must specify that electrodes used for stimulation be placed so that current does not cross the trunk, to avoid the possibility of current crossing the heart, resulting in cardiac depolarization.

CHM.29600 Iontophoresis Conditions **Phase II**

The protocol specifies the conditions of iontophoresis.

NOTE: For safety reasons, the iontophoretic current source must be battery-powered, to avoid the possibility of patient exposure to line voltage. For manually controlled devices, iontophoresis must be performed for no more than 5 minutes at a current less than 4 mA, to prevent burns. The iontophoresis unit must undergo a documented, regular maintenance procedure by qualified personnel (such as engineering personnel) for current leakage and current control.

Evidence of Compliance:

✓ Records of instrument maintenance documented at defined frequency

CHM.29700 Iontophoresis Oxygen **Phase II**

The protocol specifies that iontophoresis is withheld from patients receiving oxygen by an open delivery system.

NOTE: While the possibility of an explosion due to the generation of an electrical spark is remote, it cannot be ignored. Often, these patients can temporarily receive oxygen via a facemask or nasal cannula, in which case sweat testing can be done.

CHM.29800 Iontophoretic Stimulation **Phase II**

The protocol specifies that the area of iontophoretic stimulation is equivalent to the area of sweat collection.

NOTE: The protocol must specify that the area of iontophoretic stimulation is equivalent to the area of sweat collection. Sweat electrolyte concentration is related to sweat rate. At low sweat rates, sweat electrolyte concentration decreases. The average sweat rate should exceed 1 g/m2/min.

To ensure adequate sweat stimulation and accurately reflect sweat electrolyte concentration, a minimum acceptable sweat volume or weight is required. This requirement is based on the size of the electrode and stimulation area, the type and size of collecting media, and the duration of sweat collection. To standardize the process, the stimulation and collection area should be equivalent, and the time of collection consistent. For example, for the positive electrode, use a 1 ½ inch x 1 ½ inch electrode over a 2 x 2 inch gauze pad saturated with pilocarpine for stimulation, then collect sweat onto a 2 x 2 inch pre-weighed gauze pad.

REFERENCES
1) CLSI. *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline-Third Edition.* CLSI document C34-A3 (ISBN

1-56238-713-8). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2009

CHM.29850 Appropriate Sweat Collection Device **Phase II**

The sweat collection device is appropriate for the iontophoresis system.

NOTE: The sweat collection device must be designed for use with the appropriate iontophoresis system so that the stimulation and collection area are equivalent and the appropriate minimum acceptable sweat volume or weight can be achieved. Examples of acceptable combinations include:

- *Stimulation with Wescor Pilogel iontophoresis and collection into Wescor Macroduct coils*
- *Stimulation with copper electrodes over gauze/filter paper pilocarpine pads and collection into gauze/filter paper*
- *Stimulation and collection into Wescor Nanoduct conductivity cell*

Examples of unacceptable combinations include:

- *Stimulation with Wescor Pilogel iontophoresis and collection into gauze/filter paper*
- *Stimulation with Polychrome iontophoresis and collection into Wescor Macroduct coils, gauze or filter paper*
- *Stimulation with copper electrodes over gauze/filter paper pilocarpine pads and collection into Wescor Macroduct coils*

REFERENCES
1) CLSI. *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline-Third Edition.* CLSI document C34-A3 (ISBN 1-56238-713-8). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2009
2) Cystic Fibrosis Foundation Center Accreditation Guidelines. Bethesda, MD, 2004

CHM.29900 Sweat Collection Time **Phase II**

The protocol specifies that sweat collection time may not exceed 30 minutes.

NOTE: Extending the collection time will not significantly increase the sweat yield and may lead to sample evaporation. Samples may be taken from less than maximally stimulated glands. This may lead to a false-negative result. In addition, altering the collection time will affect the minimum acceptable sweat weight or volume, because the time parameter of the rate equation has been changed.

Evidence of Compliance:

✓ Patient records or worksheets documenting collection time

CHM.30000 Sweat Collection Parameters **Phase II**

The protocol specifies the parameters of sweat collection.

NOTE: These must include an established minimum acceptable sweat volume or weight based on the area of stimulation, area of collection and standardized time for collection. The average sweat rate should exceed 1 g/m2/min, which in general corresponds to a minimum sample weight of about 75 mg of sweat collected on a 2x2 inch gauze or filter paper and about 15 µL of sweat collected in macroduct coil in 30 min. Samples less than the required volume or weight must not be analyzed..

REFERENCES
1) Hjelm M, *et al.* Sweat sodium related to amount of sweat after sweat test in children with and without cystic fibrosis. *Acta Paediatr Scand.* 1986;75:652-656

2) CLSI. *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline-Third Edition.* CLSI document C34-A3 (ISBN 1-56238-713-8). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2009

CHM.30100 Sweat Sample Rejection **Phase II**

The protocol specifies that multiple insufficient sweat samples are rejected and not pooled for analysis.

NOTE: The laboratory must reject individual samples that do not meet minimum sample size requirements. The average sweat rate of 1 g/m2/min is determined independently for each site. The requirement is a physiologic one, not an analytic one. Samples less than the required volume or weight must not be pooled to achieve the weight/volume requirement. Measurement on samples from less than maximally stimulated sweat glands may lead to false-negative results.

Evidence of Compliance:

✓ Records of specimen rejection

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

CHM.30150 Sweat Rejection Incidence Rate **Phase I**

The incidence of insufficient sweat samples is routinely monitored.

NOTE: Laboratories should collect data on the number of patients from whom a sufficient sweat sample has not been obtained. For patients older than 3 months of age, the annual insufficient rate should not exceed 5%. For patients 3 months of age or younger, the rate of insufficient samples should not exceed 10%. If these rates are exceeded, the collection procedure should be reevaluated for consistency with the CLSI document C34-A3. The most common cause of insufficient samples is the use of inappropriate collection devices (see CHM.29850).

Evidence of Compliance:

✓ insufficient collection **AND**

✓ Records of follow-up if rate exceeds the norm

REFERENCES

1) Cystic Fibrosis Foundation Center Accreditation Guidelines. Bethesda, MD, 2009

CHM.30200 Sweat Sample Labels **Phase II**

The sweat samples are labeled with appropriate patient identification.

NOTE: The label must be attached before determining the initial weight.

Evidence of Compliance:

✓ Written procedure for specimen labeling

*****NEW**** ***06/17/2010***

CHM.30250 Sweat Sample Storage **Phase I**

The protocol describes appropriate storage conditions for collected sweat samples.

NOTE: If there is a significant delay between collection and analysis, appropriate storage conditions must be followed: a. Sweat collected on gauze is stable at refrigerator temperatures for up to 72 hours once reweighed and secured in a vial with a tightly fitting cap; and b) Sweat collected in Macroduct is stable at refrigeration or room temperature for up to 72 hours in a 0.2 mL microcentrifuge tube with a tight fitting cap. Storage of sweat in microbore tubing is not recommended.

REFERENCES
1) Cystic Fibrosis Foundation Center Accreditation Guidelines. Bethesda, MD, 2009

CHM.30300 Sweat Collection Skin Reaction **Phase II**

The protocol describes the recognition of, and appropriate treatment for, patient skin reactions (allergic or burns) to pilocarpine and/or other reagents used in iontophoresis.

NOTE: Rarely, some patients may develop an area of, urticaria (hives) or small localized burns. In such cases, the procedure must be discontinued immediately and appropriate medical attention obtained. Sweat must not be collected over areas of urticaria or burns.

Evidence of Compliance:

✓ Records of follow-up treatment

Analytic Methods for Sweat Testing

Inspector Instructions:

- Sweat testing method validation records
- Sampling of QC logs

CHM.30400 Sweat Test Validation **Phase II**

For sweat testing, the analytical method is validated by the laboratory prior to patient testing using specimens equivalent to the volume and concentration of patient sweat samples.

NOTE: Validation procedures must include studies of accuracy, precision, and upper/lower limits of the analytic measurement range. The laboratory should be aware that some instruments designed for serum or urine electrolyte determination may lack the sensitivity required for sweat testing.

Evidence of Compliance:

✓ Written procedure defining criteria for validation of the analytical method **AND**

✓ Records of method validation study(ies)

REFERENCES
1) Pillion DJ, Meczan E. Chloride measurement by microelectrode in cystic fibrosis and normal sweat. *Miner Electrolyte Metab.* 1987;13:196-200
2) Barnes GL, *et al.* Sweat testing by capillary collection and osmometry: suitability of the Wescor macroduct system for screening suspected cystic fibrosis patients. *Aust Paediatr J.* 1988;24:191-193
3) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):3707 [42CFR493.1255]
4) CLSI. *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline-Third Edition.* CLSI document C34-A3 (ISBN 1-56238-713-8). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2009
5) Department of Health and Human Services, Centers for Medicare and Medicaid Services Center for Disease Control and Prevention. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register.* 2003 (Jan 24): Vol. 68, No. 16 [42CFR493]

CHM.30550 Sweat Chloride AMR **Phase II**

The lower limit of the sweat chloride analytical measurement range is less than or equal to 10 mmol/L.

NOTE: The lower limit of the sweat chloride analytical measurement range must be less than or equal to 10 mmol/L without any dilution, concentration or other pretreatment that is not part of the usual assay process.

REFERENCES
1) Cystic Fibrosis Foundation Center Accreditation Guidelines. Bethesda, MD, 2009
2) Farrell PM, *et al.* Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *J Pediatr* 2008;153:S4-S14

CHM.30575 Confirmatory Sweat Test Report **Phase II**

If the test performed is a confirmatory test (*i.e.* quantitative analysis of sweat chloride), the upper limit of AMR for sweat chloride results is less than or equal to 160 mmol/L.

NOTE: Even though the analytical instrument may have a higher upper limit of its AMR, sweat chloride concentrations > 160 mmol/L are not physiologically possible. Results of sweat chloride testing greater than 160 mmol/L must not be reported, and the patient must be retested.

Evidence of Compliance:

✓ Patient reports or worksheets

REFERENCES
1) Schultz IJ. Micropuncture studies of the sweat formation in cystic fibrosis patients. *J Clin Invest.* 1969;48:1470-1477

CHM.30600 Daily QC - Sweat Testing **Phase II**

The laboratory analyzes 2 levels of controls (one in the negative range and one in the positive range) at least once each day patient specimens are assayed.

NOTE: If sweat is collected from patients on gauze or filter paper, controls should be placed directly onto the same collection material, eluted, and treated in the same manner as a patient specimen.

For test systems with internal controls, the laboratory may limit daily quality control to the internal controls ONLY if all CAP requirements for internal controls are met, as listed in CHM.13900.

Evidence of Compliance:

✓ Written QC policy **AND**

✓ QC results documented at defined frequency

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7165 [42CFR493.1255]
2) CLSI. *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline-Third Edition.* CLSI document C34-A3 (ISBN 1-56238-713-8). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2009
3) Department of Health and Human Services, Centers for Medicare and Medicaid Services Center for Disease Control and Prevention. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register.* 2003 (Jan 24): Vol. 68, No. 16 [42CFR493]

Reporting of Results

Inspector Instructions:

- Sampling of sweat analysis reports (appropriate reference ranges and disclaimer if applicable)

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

CHM.30700 Sweat Test Reporting **Phase II**

The laboratory report indicates the specific analytes measured in the sweat analysis, and applies the appropriate reference ranges and/or decision levels to patient results.

NOTE: The laboratory report must clearly indicate the analytes measured in the sweat test, and apply the appropriate reference intervals and/or decision levels to patient results. Osmolality and conductivity are nonselective methods for sweat analysis; they are not equivalent to sweat chloride concentrations and therefore have their own unique set of reference ranges. When sweat conductivity is expressed as units of aqueous sodium chloride solution, the values are approximately 15 mmol/L higher than when chloride is measured directly.

The Cystic Fibrosis Foundation reference intervals for chloride and conductivity are:

SWEAT CHLORIDE: For infants up to and including 6 months of age: less than or equal to 29 mmol/L, CF unlikely; 30-59 mmol/L, intermediate; greater than or equal to 60 mmol/L, indicative of CF. For individuals older than 6 months of age: < 40 mmol/L, CF unlikely; 40-59 mmol/L, intermediate; > 60 mmol/L, indicative of CF. The result must be interpreted with regard to the patient's age and clinical presentation.

SWEAT CONDUCTIVITY: A patient having a sweat conductivity greater than or equal to 50 mmol/L should be referred to a specialized cystic fibrosis care center for a quantitative analysis of sweat chloride with or without sweat sodium.

SWEAT OSMOLALITY: 50-150 mmol/kg, negative; 151-200 mmol/kg, equivocal; > 200 mmol/kg, positive for cystic fibrosis.

REFERENCES
1) Farrell PM, *et al.* Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *J Pediatr* 2008;153:S4-S14
2) CLSI. *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline-Third Edition.* CLSI document C34-A3 (ISBN 1-56238-713-8). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2009

CHM.30800 Sweat Test Report Disclaimer **Phase II**

If the test performed is a screening test (*e.g.* sweat conductivity, CF Indicator patch system, Orion skin measuring electrode, *etc.*), the report includes a statement regarding the limits of clinical interpretation.

NOTE: Suggested wording for such a disclaimer might be: "This result represents a screening test for cystic fibrosis. Patients having borderline or positive results should be referred for a quantitative sweat chloride concentration."

REFERENCES
1) Rosenstein BJ, Langbaum TS. Misdiagnosis of cystic fibrosis. Need for continuing follow-up and reevaluation. *Clin Pediatr.* 1987;26:78-82
2) CLSI. *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline-Third Edition.* CLSI document C34-A3 (ISBN 1-56238-713-8). CLSI, 940 West Valley Road, Wayne, PA, 19087-1898, USA, 2009

PRENATAL SCREENING

Test panels include: 1) Second trimester quadruple panel: maternal serum alpha-fetoprotein (MSAFP), unconjugated estriol (uE3), and beta-human chorionic gonadotropin (beta-hCG) and dimeric inhibin-A (DIA); 2) First trimester panel: total or free beta-hCG, pregnancy associated placental protein A (PAPP-A), and nuchal translucency (NT); 3) Sequential and

integrated panels: various combinations of first and second trimester tests

Requisitions/Calculations/Reports

Requests for prenatal screening (neural tube defects, Down syndrome, etc.) must include specific information for meaningful interpretation of laboratory tests. For clinical screening purposes, analyte concentrations must be converted to multiple of the median (MoM) values, using gestational-age specific medians. The MoM value is used directly as the interpretative result for neural tube defect screening and for calculating risk for fetal trisomies. Gestational age-specific MoM values need to be adjusted for each patient, based on several variables. The laboratory must work cooperatively with the clinician to ensure that all necessary information is obtained.

Inspector Instructions:

	• Sampling of prenatal screen policies and procedures • Sampling of prenatal screening requisitions for required elements • Sampling of median value records • Sampling of records verifying calculated gestational age, maternal age and patient-specific risks
	• How does your laboratory verify or establish acceptable median values? • How does your laboratory monitor assay quality, appropriateness of medians and accuracy of gestational dating? • What is your course of action if the requisition does not include all necessary information?

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

CHM.31100 Prenatal Screen Requisitions **Phase II**

Prenatal screening requisitions solicit the first day of the last menstrual period (LMP), the estimated date of confinement (EDC), or estimated gestational age by ultrasound dating.

NOTE: Accurate screening requires that the laboratory know the clinician's best estimate of the gestational age at the time of the specimen collection. The gestational age allows determination of the best median values for a given screen. The optimum time for neural tube defect detection using MSAFP measurements is between 16-18 weeks gestation.

REFERENCES
1) Wald NJ, *et al*. Maternal serum-alphafetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on alpha-fetoprotein in relation to neural-tube defects. *Lancet.* 1977;i:1323-1332
2) Wald NJ, *et al.* Maternal serum screening for Down's syndrome in early pregnancy. *Brit Med J.* 1988;297:883-887
3) Bock JL. Current issues in maternal serum alphafetoprotein screening. *Am J Clin Pathol.* 1992;97:541-554

CHM.31200 Prenatal Screen Requisitions **Phase II**

Prenatal screening requisitions solicit maternal birth date.

NOTE: Maternal birth date must be included as part of the test requesting process. Maternal age is not necessary when screening for neural tube defects, but is needed to calculate the patient-specific risk for Down syndrome. The patient-specific risk, not the analyte concentration, is used as the screening variable to identify pregnancies at high-risk for Down syndrome.

REFERENCES
1) Cuckle HS, *et al*. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alphafetoprotein level. *Brit J Obstet Gynecol*. 1987;94:387-402
2) Wald NJ, *et al.* Maternal serum screening for Down's syndrome in early pregnancy. *Brit Med J.* 1988;297:883-887

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

CHM.31300 Prenatal Screen Requisitions **Phase II**

Prenatal screening requisitions solicit patient race.

NOTE: Patient race must be included as part of the test requesting process.

Both MSAFP and hCG values in black women are approximately 10% to 15% higher than in Caucasian women. Depending upon the racial distribution of the patient population, the laboratory should either have separate MSAFP medians for blacks if enough data is available and Caucasians, or apply an appropriate correction factor for the nonnumerically dominant race. Black women also have a lower birth prevalence of neural tube defects, and some screening programs raise the MoM cut-off to take this difference into account (after adjusting the AFP MoM value).

uE3 levels are similar in the two racial groups, uE3 medians need not be adjusted.

DIA medians in blacks are approximately 8% lower than those in Caucasians, and adjustment is recommended.

PAPP-A values are approximately 25% higher in black women, therefore medians should be adjusted.

Current data indicate that statistically significant differences in median values exist for other races and ethnic groups (e.g. Hispanic, Asian, Native American). Most differences tend to be small and do not materially affect the risk calculations for Down syndrome and neural tube defects. However, laboratories that screen large numbers of women may consider using median values specific to ethnic or racial groups if significant differences are identified in their screening population.

REFERENCES
1) Crandall BF, *et al*. Alphafetoprotein concentrations in maternal serum: relation to race and body weight. *Clin Chem.* 1983;29:531-533
2) Greenberg F, *et al.* Estimates of birth prevalence rates of spina bifida in the United States from computer generated maps. *Am J Obstet Gynecol.* 1983;145:570-573
3) Bock JL. Current issues in maternal serum alphafetoprotein screening. *Am J Clin Pathol.* 1992;97:541-554
4) Kulch P, *et al.* Racial differences in maternal serum human chorionic gonadotrophin and unconjugated oestriol levels. *Prenat Diag.* 1993;13:191-195
5) O/Brien JE, Dvorin E, Drugan A, Johnson MP, Yaron TY, Evans MI. Race-ethnicity -specific variation in multiple-marker biochemical screening: alpha-fetoprotein, hCG, and estriol. *Obstet Gynecol* 1997 89(3):355-8
6) Benn PA, Clive JM, Collins R. Medians for second-trimester maternal serum alpha-fetoprotein human chorionic gonadotrophin, and unconjugated estriol; differences between races or ethnic groups. *Clin Chem* 1997 43(2):333-7
7) Spencer K, *et al*. The influence of ethnic origin on first trimester biochemical markers of chromosomal abnormalities. *Prenat Diagn* 2000 June;20(6):491-4

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

CHM.31400 Prenatal Screen Requisitions **Phase II**

Prenatal screening requisitions solicit maternal weight.

NOTE: Maternal weight must be included as part of the test requesting process. The average concentration of all of the analytes used for second trimester screening decreases with increasing maternal weight. Heavier women have lower analyte values, and lighter women have higher levels. This is presumably due to a greater maternal blood volume in the former group. The influence of weight adjustment on neural tube defects screening is small, but adjustment aids in equalizing false positive and false negative rates in all weight categories. The concentration of first trimester markers also decrease with increasing maternal weight; the relationship between PAPP-A and maternal weight is greater than for any other marker. All first trimester markers should be adjusted for maternal weight. Laboratories can utilize published

weight correction equations until sufficient data are collected to establish correction equations using in-house patient data. The linear-reciprocal equation model allows the correction equation to apply to a broader range of maternal weights than the log-linear model and only requires approximately 1000 data points for calculation.

REFERENCES
1) Johnson AM, *et al.* The effect of adjusting maternal serum alphafetoprotein levels for maternal weight in pregnancies with open spina bifida: a United States collaborative study. *Am J Obstet Gynecol.* 1990;163:911
2) Bock JL. Current issues in maternal serum alphafetoprotein screening. *Am J Clin Pathol.* 1992;97:541-554
3) Neveux LM, *et al.* Refinements in managing maternal weight adjustment for interpreting prenatal screening results. *Prenat Diag.* 1996;16:1115-1119

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

CHM.31500 Prenatal Screen Requisitions **Phase II**

Prenatal screening requisitions solicit a history of the patient receiving medication (e.g. insulin) to control diabetes at the time of conception.

NOTE: A history of medication-dependent diabetes mellitus (IDDM) at the time of conception must be included as part of the test requesting process. Pregnant women who have diabetes prior to conception have 12% lower MSAFP levels than nondiabetics of the same gestational age, but have a higher birth prevalence of neural tube defects. If medians derived from nondiabetic women are used to screen women with IDDM, the percentage of women with values exceeding the laboratory's MoM screening cut-off will be inappropriately low, resulting in a lower detection rate. Conversely, if medians from nondiabetic women are used for screening pregnancies from women with IDDM for Down syndrome, the AFP MoM values calculated using non-diabetic medians will be inappropriately low, resulting in an increased number of patients in the screen positive group. Differences are slight for the other analytes. uE3 is 8% lower, second trimester hCG is 5% lower, and DIA is 9% lower in pregnant women with DM. Adjustment for these analytes is optional. PAPP-A and first trimester hCG are not significantly different in DM, and adjustment is not recommended for these analytes. There is no consensus whether correction for gestational diabetes is warranted.

REFERENCES
1) Wald NJ, *et al.* Maternal serum alphafetoprotein and diabetes mellitus. *Br J Obstet Gynecol.* 1979;86:101-105
2) Kucera J. Rate and type of congenital anomalies among offspring of diabetic women. *J Reprod Med.* 1979;7:61-70
3) Greene MF, *et al.* Maternal serum alphafetoprotein levels in diabetic pregnancies. *Lancet.* 1988;ii:345-346
4) Wald NJ, *et al.* Maternal serum unconjugated oestriol, human chorionic gonadotrophin, and alpha-fetoprotein levels in pregnancies with insulin-dependent diabetes: implications for Down's syndrome screening. *Brit J Obstet Gynaecol.* 1992;99:51-53
5) Hutley W, *et al.* Second-trimester prenatal screening markers for Down syndrome in women with insulin-dependent diabetes mellitus. *Prenat Diagn* 2004;24:804-807

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

CHM.31600 Prenatal Screen Requisitions **Phase II**

Prenatal screening requisitions solicit clinical evidence of multiple gestations (twins, *etc.*).

NOTE: Clinical evidence of multiple gestations (twins, etc.) must be included as part of the test requesting process. Women with ultrasonographically confirmed twin pregnancies have, on average, twice the level of MSAFP than that seen in women with singleton pregnancies at the same gestational age. Most laboratories use a screening cutoff level for twin pregnancies between 3.5 and 5.0 MoM, or divide the MoM by 2 and use an adjusted cutoff level as for singleton pregnancies. However, screening for neural tube defects in twin pregnancies is less efficient than singleton pregnancies. Approximately 50% of twin pregnancies with an open neural tube defect and 5% of unaffected twin pregnancies have a MoM of 4.5 or greater. If twin pregnancies are to be screened for Down syndrome, laboratories need to calculate risks using a method specifically designed for this application. hCG and DIA levels are also approximately twice as high in twin pregnancies, while uE3 levels in twin pregnancies are approximately 1.7 times as high as in singleton pregnancies. There are few data on analyte concentrations from

twin pregnancies with one or both fetuses affected with Down's syndrome; therefore, only "pseudo risks" can be calculated. Pseudo risks are less reliable than those calculated for singleton pregnancy and some laboratories choose not to report the actual risk, but only report specimens as screen negative or screen positive. Insufficient data are available to calculate Down syndrome risk in pregnancies with three or more fetuses.

REFERENCES
1) Cuckle HS, *et al.* Maternal serum alphafetoprotein screening for open neural tube defects in twin pregnancies. *Prenat Diagn.* 1990;10:71-77
2) Bock JL. Current issues in maternal serum alphafetoprotein screening. *Am J Clin Pathol.* 1992;97:541-554
3) Wald NJ, *et al.* Maternal serum unconjugated oestriol and human chorionic levels in twin pregnancies: implications for screening for Downs syndrome. *Brit J Obstet Gynaecol.* 1991;98:905-908
4) Wald NJ, Rish S. Prenatal screening for Down syndrome and neural tube defects in twin pregnancies. *Prenat Diagn 2005;25:740-745*

CHM.31700 Prenatal Screen Requisitions **Phase I**

The test requisitions ask if this is the initial screening sample or a repeat test for this pregnancy.

NOTE: The interpretation of a repeat maternal serum sample may be different from the interpretation of an initial serum specimen. In neural tube defect screening using AFP, a repeat sample can be interpreted as if it were an initial sample if fixed MoM cut-offs are used to identify high-risk women. If patient-specific risks are calculated for a repeat test result, one should combine the results of both the initial and repeat sample, using published algorithms. Repeat testing is not recommended for Down syndrome screening unless a sample is drawn too early for reliable interpretation. If a test on a second sample is performed, it is essential that the revised risk be calculated using the results from both samples. A method has been published for these calculations.

REFERENCES
1) Estimating an individuals risk of having a fetus with open spina bifida and the value of repeat alphafetoprotein testing. Fourth report of the U.K. collaborative study on alphafetoprotein in relation to neural tube defects. *J Epidemiol Commun Health.* 1982;36:87-95
2) Cuckle HS, *et al.* Repeat maternal serum testing in multiple marker downs syndrome screening programmes. *Prenat Diagn.* 1994;14:603-607
3) Hackshaw AK. *et al.* Repeat testing in antenatal screening for Down syndrome using dimeric inhibin-A in combination with other maternal serum markers. Prenat Diagn, 2001;21:58-61

*****REVISED**** ***07/11/2011***
CHM.31800 Median Values **Phase II**

There is documentation that the laboratory has established its own median values or verified that the medians from another source are appropriate for the population being screened.

NOTE: Systematic biases in maternal serum assay values of up to 30% can occur when kits from different manufacturers are used. In addition, between laboratory differences in equipment, reagents, and technique may introduce bias in assay results even when the same kit lot is used. These differences can be minimized by reporting results in multiples of the normal median (assuming that the medians are calculated using values measured on the population to be tested using the kit designated for screening). Ideally, week-specific medians would be established by testing approximately 100 patients per week of gestation. Because analytes are stable, it is possible to use stored frozen specimens collected over a period of years. A second approach is to perform a split specimen study with a reference laboratory and transfer the reference laboratory's medians using the comparison regression equation from the split specimen study. However, in practice the most practical method is to measure values on 300 consecutively collected specimens spread over the appropriate gestational age range, and perform weighted regression analysis using published models. It is not necessary to document that all specimens are collected from unaffected pregnancies. Smoothing data by weighted regression analysis allows median values to be calculated for weeks with limited data. Package insert medians may

be outdated or inappropriate and should not be used even for a short time. Incorrect reference data may lead to inappropriate recommendations in the laboratory report.

Evidence of Compliance:

✓ Records for median value determination **OR** records for verification of package inserts or other sources

REFERENCES
1) Report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHD), Bethesda, Maryland. The quality control of alphafetoprotein reagents and assay for the antenatal screening and diagnosis of open neuraltube defects. *Clin Chim Acta.* 1980;105:9-24
2) Knight GJ, *et al.* Assessing reliability of AFP test kits. *Contemp Obstet Gynecol.* 1987 (Technology issue);30:37-52
3) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening; Approved Guideline—Second Edition*. CLSI document I/LA25-A2 (ISBN 1-56238-749-9). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011
4) Knight GJ. Quality assessment of a prenatal screening program. *Early Human Development.* 1996;47(Supp):S49-S53
5) Haddow *et al*. Screening for maternal serum for fetal Down's syndrome in the first trimester. *N Eng J Med,* 1998;338:955-961

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

CHM.31900 Median Value Reverification **Phase II**

Medians are reverified at specified intervals and when new reagent lots are introduced, and the medians are recalculated if necessary.

NOTE: Changing reagent lots can introduce significant bias. One method for assessing a new lot is performing a split specimen comparison study between the new and old lot.

In addition, re-evaluation of medians at specified intervals is a valuable quality control mechanism to ensure validity of reported MoMs. Epidemiological monitoring of Down syndrome screening can be accomplished by determining the median MoMs at frequent intervals (e.g. every 500-1000 patients, weekly or monthly). If a persistent shift is noted, (less than 0.95 or greater than 1.05), new medians should be determined. Documentation of the median MoMs must be maintained.

Evidence of Compliance:

✓ Written procedure defining criteria and frequency for recalculation or reverification of median values **AND**

✓ Records of median value recalculation or reverification data documented at defined frequency

REFERENCES
1) Report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHD), Bethesda, Maryland. The quality control of alphafetoprotein reagents and assay for the antenatal screening and diagnosis of open neuraltube defects. *Clin Chim Acta.* 1980;105:9-24
2) Bock JL. Current issues in maternal serum alphafetoprotein screening. *Am J Clin Pathol.* 1992;97:541-554
3) Ashwood ER, Knight GJ. Clinical Chemistry of Pregnancy. *In*: Tietz Textbook of Clinical Chemistry and Molecular Pathology, 4th ed. Burtis CA, Ashwood ER, Bruns DE, Eds. Philadelphia, Elsevier Saunders, 2006, pp. 2153-206
4) Erickson JA, Ashwood ER, Gin CA. Evaluation of a dimeric inhibitin-A assay for assessing fetal Down syndrome: establishment, comparison, and monitoring of median concentrations for normal pregnancies. *Arch Pathol Lab Med,* 2004 Apr;128(4):415-20

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

CHM.31950 Nuchal Translucency (NT) Measurements Establishing **Phase I**

If screening panels are offered using nuchal translucency (NT) values, the laboratory should have a written plan for their establishment and use.

NOTE: Some potential content of these plans might include:

- *Requirements for verifying the credentialing of each sonographer*
- *Having sonographers provide a set number of NT/CRL measurements prior to initial interpretations*
- *Implementing sonographer-specific medians when the median NT MoM levels are outside of set limits*
- *Reviewing data for sonographers available from certifying organization (e.g. NT Quality*

Review Program - NTQR (www.ntgr.org) or the Fetal Medicine Foundation - FMF (www.fetalmedicineusa.com))

- *Establishing communication with sonographers to inform them of monitoring results*
- *Establishing communications with credentialing organizations to inform them of sonographer performance*

Evidence of Compliance:

✓ Written procedure defining criteria for establishing a new sonographer **AND**
✓ Records of sonographer establishment

REFERENCES
1) Palomaki GE, Neveux LM, Donnenfeld A, Lee JE, McDowell G, Canick JA, Summers A, Lambert-Messerlian G, Kellner LH, Zebelman A, Haddow JE. Quality assessment of routine nuchal translucency measurements: A North American laboratory perspective. *Genet Med*, 2008 Feb;10(2):131-8
2) Ashwood ER, Lambert-Messerlian G. Clinical Chemistry of Pregnancy. *In*: Tietz Textbook of Clinical Chemistry and Molecular Pathology, 5th ed. Burtis CA, Ashwood ER, Bruns DE, Eds. Philadelphia, Elsevier Saunders, 2011, in press

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

CHM.31960 Nuchal Translucency (NT) Measurements, Monitoring **Phase I**

If screening panels are offered using nuchal translucency (NT) values, the laboratory should routinely perform epidemiological monitoring of these measurements.

NOTE: An example of such a monitoring plan (with action limits) is provided below. For each sonographer with sufficient data (typically at least 30 to 50 measurements over six months), monitor and provide limits for three quality parameters.

- *Percent increase in NT measurements (in mm) by gestational age (e.g. 15% to 35%)*
- *The NT median MoM (e.g. 0.90 to 1.10)*
- *The distribution of NT MoMs after a logarithmic (log standard deviation), (e.g. 0.08 to 0.13)*

Evidence of Compliance:

✓ Records of NT median data study(ies) **AND**
✓ Records of review documented at defined frequency

REFERENCES
1) Palomaki GE, Neveux LM, Donnenfeld A, Lee JE, McDowell G, Canick JA, Summers A, Lambert-Messerlian G, Kellner LH, Zebelman A, Haddow JE. Quality assessment of routine nuchal translucency measurements: A North American laboratory perspective. *Genet Med*, 2008 Feb;10(2):131-8
2) Ashwood ER, Lambert-Messerlian G. Clinical Chemistry of Pregnancy. *In*: Tietz Textbook of Clinical Chemistry and Molecular Pathology, 5th ed. Burtis CA, Ashwood ER, Bruns DE, Eds. Philadelphia, Elsevier Saunders, 2011, in press
3) Palomaki GE, *et al*. Technical standards and guidelines: Prenatal screening for Down syndrome that includes first-trimester biochemistry and/or ultrasound measurements. *Genet Med,* 2009;11(9):669-681

CHM.32000 Screen Result Statistics **Phase II**

The percentages of women with screen-positive test results for neural tube defects, Down syndrome, and Trisomy 21 are calculated and reviewed at least quarterly.

NOTE: Data from large studies provide guidelines for the percentage of pregnancies that will fall above specified maternal serum AFP MoM levels (NTD screening) or with risks greater than specified risk cutoff levels (Down syndrome screening). Regular comparison of a laboratory's screen-positive rates with expected rates serves as a continuing measure of assay quality, appropriateness of medians, and accuracy of gestational dating. As noted above, it is recommended that checks be performed more frequently.

Evidence of Compliance:

✓ Written procedure defining criteria for comparison of screen-positive tests to expected rates to include frequency of analysis **AND**
✓ Records of statistical analysis and evaluation of screen-positive test results documented at least quarterly

REFERENCES
1) Report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHD), Bethesda Maryland. The quality control of alphafetoprotein reagents and assay for the antenatal screening and diagnosis of open neuraltube defects. *Clin Chim Acta.* 1980;105:9-24
2) Palomaki GJ, *et al*. Risk based screening for trisomy 18 using alphaphetoprotein, unconjugated estriol, and human chorionic. *Prenat Diagn* 1995;15:713-723
3) Knight GJ, *et al*. Epidemiologic monitoring of prenatal screening for neural tube defects and Down syndrome. *Clin Lab Med* 2003;22:531-551

CHM.32100 Dimeric Inhibin A **Phase I**

If the laboratory adds dimeric inhibin A (DIA) (or another marker) to its screening panel, it has followed the same requirements outlined for established markers.

NOTE: Many screening laboratories have added a fourth marker, dimeric inhibin A, to the triple test. If so, the laboratory should be able to document that it adheres to the checklist items outlined for established markers in the Prenatal Screening section. These include establishing median values appropriate for the population being screened, and adjusting where appropriate for variables that have been shown to influence analyte values, such as maternal weight, maternal race, insulin dependent diabetes, and twin pregnancy. Laboratories should also verify that the risks calculated using the additional marker are valid.

Evidence of Compliance:

✓ Records of validation studies for dimeric inhibin A or other markers added to the screening panel

REFERENCES
1) Haddow JE, Palomaki GE, Knight GJ, Foster DL, Neveux LM. Second trimester screening for Down's syndrome using maternal serum dimeric inhibin A. J Med Screen 1998;5(3):115-9
2) Cuckle HS, Holding S, Jones R, Groome NP, Wallace EM. Combining inhibin A with existing second-trimester markers in maternal serum screening for Down's syndrome. *Prenat Diagn* 1996 16(12):1095-1100

CHM.32200 Computer Calculations **Phase II**

There is documentation that neural tube defect and Down syndrome risk calculations were initially verified for accuracy and reverified with any software updates or changes.

NOTE: Verification can be accomplished by interlaboratory comparisons, by comparison with results calculated or reported by proficiency testing programs, or by use of risk tables available on the CAP website in the Special Chemistry Committee section under the heading "Prenatal Risk." At a minimum, the accuracy of calculated gestational age, maternal age, and patient-specific risks should be verified.

Evidence of Compliance:

✓ Written procedure for verifying accuracy of calculations initially and with changes **AND**
✓ Records of initial and subsequent calculation checks

Interpretive Reporting for Maternal Screening

Inspector Instructions:

- Sampling of maternal screen patient reports (demographics, clinical information, results reported in MoM, cut-off values)

CHM.32300 Maternal Screen Reports **Phase II**

All of the following demographic and clinical information is included in the report: date of birth; maternal weight; maternal race; first day of the last menstrual period or gestational age as determined by ultrasound examination; specimen draw date; initial or repeat specimen; presence of medication-dependent diabetes; family history of neural tube defect, and presence of multiple gestation if known.

NOTE: Reports must include data essential to the interpretation of the test results to enable both the laboratory and physician to ensure that the interpretations on the report are based on complete and correct information.

In addition to the above, smoking history is recommended.

CHM.32400 Multiple of Population Median **Phase II**

Test results are reported as multiples of the population median (MoM).

NOTE: Reporting of results in terms of multiple of the population median (MoM) simplifies interpretation at various gestational ages, reduces possible systematic between-laboratory and between-kit bias in assay results, and facilitates comparison among laboratories. Laboratories can also compare their experiences with large-scale published studies more readily by using MoM as the reportable interpretive unit. The initial MoM is calculated as the measured analyte value divided by the median value for the appropriate gestational age. The MoM should also be adjusted for the other clinical variables known to influence the concentration of each analyte, generally by dividing by a factor specific for each variable.

Evidence of Compliance:

✓ Written procedure defining criteria for reporting results as MoM

REFERENCES
1) Report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHD), Bethesda Maryland. The quality control of alphafetoprotein reagents and assay for the antenatal screening and diagnosis of open neuraltube defects. *Clin Chim Acta.* 1980;105:9-24
2) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening; Approved Guideline—Second Edition*. CLSI document I/LA25-A2 (ISBN 1-56238-749-9). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

CHM.32500 Result Cut-off Values **Phase I**

The report classifies a pregnancy as screen-positive or screen-negative for open neural tube defects, based on the MSAFP test results.

NOTE: Cut-off levels based on maternal serum AFP MoM values or risk have been established by large screening programs. Use of these cut-off values in the laboratory report can assist the physician in making clinical decisions about pregnancy management.

REFERENCES
1) Burton BK. Elevated maternal serum alphafetoprotein (MSAFP): interpretation and followup. *Clin Obstet Gynecol.* 1988;31:293-305
2) Milunsky A, *et al.* Predictive values, relative risks, and overall benefits of high and low maternal serum alphafetoprotein screening in singleton pregnancies: new epidemiologic data. *Am J Obstet Gynecol.* 1989;161:291-297
3) Crandall BF, *et al.* Risks associated with an elevated maternal serum alphafetoprotein level. *Am J Obstet Gynecol.* 1991;165:581-586
4) Bock JL. Current issues in maternal serum alphafetoprotein screening. *Am J Clin Pathol.* 1992;97:541-554

CHM.32600 Result Cut-off Values **Phase I**

The report classifies a pregnancy as screen-positive or screen-negative for fetal Down syndrome, based on the calculated risk.

NOTE: Cut-off levels based on risk for fetal Down syndrome have been established by large

screening programs. Use of the cut-off values in the laboratory report can assist the physician in making clinical decisions about pregnancy management.

REFERENCES
1) Wald NJ, *et al.* Maternal serum screening for Down's syndrome in early pregnancy. *Brit Med J.* 1988;425:883-887

Amniotic Fluid Alpha-fetoprotein (AFAFP)

Inspector Instructions:

	• Sampling of AFAFP policies and procedures • Sampling of AFAFP patient reports (results reported in MoM) • Sampling of QC logs
	• How does your laboratory verify or establish acceptable median AFAFP values? • What is your laboratory's course of action when you receive an amniotic fluid sample that is visibly contaminated with blood? • What is your laboratory's course of action when an amniotic fluid has an elevated AFP?
	• Select an abnormal AFAFP result and further investigate documentation of the confirmatory testing performed, including QC for AChE testing

CHM.32700 Median AFAFP Values **Phase II**

There is documentation that the laboratory has established its own median AFAFP values or verified that medians are provided from another source are appropriate for the population being screened.

NOTE: Systematic biases in AFAFP assay values of up to 30% can occur when kits from different manufacturers are used. In addition, between-laboratory differences in equipment, reagents, and technique may introduce bias in assay results even when the same kit is used. These differences can be minimized by reporting results in multiples of the median (assuming that the medians are calculated using values measured on the population to be tested using the kit designed for screening). Package insert medians may be outdated or inappropriate and should only be used as a general guide as to expected medians. If a large percentage of the laboratory's AFAFP assays are performed because of elevated MSAFP, the medians may be erroneously high. This can be determined by checking the distribution of the log MoMs, which should be approximately Gaussian with few values higher than expected. It may be desirable to delete very high values if these are numerous, then recalculating the medians.

Evidence of Compliance:

✓ Records for median value determination **OR** records for median values from package inserts or other sources

REFERENCES
1) Amniotic fluid alphafetoprotein measurement in antenatal diagnosis of anencephaly and open spina bifida in early pregnancy. Second report of the U.K. collaborative study on alphafetoprotein in relation to neural tube defects. *Lancet.* 1979;ii:651
2) Report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHD), Bethesda Maryland: the quality control of alphafetoprotein reagents and assay for the antenatal screening and diagnosis of open neuraltube defects. *Clin Chim Acta.* 1980;105:9-24

3) Knight GJ. Maternal serum alphafetoprotein screening. In: techniques in diagnostic human biochemical genetics. New York, NY: WileyLiss, 1991:503
4) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening; Approved Guideline—Second Edition*. CLSI document I/LA25-A2 (ISBN 1-56238-749-9). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011

CHM.32800 Median Value Reverification **Phase II**

AFAFP medians are recalculated or reverified at specified intervals.

Evidence of Compliance:

✓ Written procedure defining criteria and frequency for recalculation or reverification of the AFAFP **AND**

✓ Records of median values recalculation or reverification documented at defined frequency

CHM.32900 Multiple of Median **Phase II**

AFAFP results are reported in multiples of the median (MoM).

NOTE: Reporting of AFAFP results in terms of multiples of the median (MoM) simplifies interpretation at various gestational weeks, reduces the systematic between-laboratory and between-kit bias in results, and facilitates comparison of results between laboratories. Laboratories may compare their experiences with large-scale published studies much more readily when using MoM as the interpretive unit for AFP measurements. AFAFP concentrations are higher in black women than in whites and may be different in other racial groups as well. Although no simple correction factors are available at present, the use of race-specific medians is worth consideration.

Evidence of Compliance:

✓ Written procedure defining criteria for reporting results as MoM

REFERENCES
1) Report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHD), Bethesda, Maryland: the quality control of alphafetoprotein reagents and assay for the antenatal screening and diagnosis of open neuraltube defects. *Clin Chim Acta.* 1980;105:9-24
2) Wald NJ, *et al.* Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. *Prenat Diagn.* 1989;9:813-829
3) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening; Approved Guideline—Second Edition*. CLSI document I/LA25-A2 (ISBN 1-56238-749-9). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011

CHM.33000 Amniotic Fluid Contamination **Phase II**

If an amniotic fluid sample is visibly contaminated with blood when received at the laboratory, or is reported as contaminated with blood on the laboratory slip, the fluid is checked for fetal blood contamination if the AFP MoM is elevated (abnormal).

NOTE: Fetal blood contains a much higher concentration of AFP than amniotic fluid, and contamination of fluid with fetal blood is a common source of false-positive AFAFP results.

Evidence of Compliance:

✓ Written procedure for handling and reporting of amniotic fluid contaminated with blood **AND**

✓ Records reflecting the testing of contaminated specimens for fetal blood, when appropriate **AND**

✓ Patient reports with notation of contamination

REFERENCES
1) Wald N, *et al.* Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. *Prenat Diagn.* 1989;9:813-829
2) Knight GJ. Maternal serum alphafetoprotein screening. In: techniques in diagnostic human biochemical genetics. New York, NY: WileyLiss, 1991:500

CHM.33100 Dilution Control **Phase II**

At least one amniotic fluid dilution control is processed with each analytic run of amniotic fluids.

Evidence of Compliance:

✓ Records of dilution control with each run

CHM.33200 AChE Testing **Phase II**

Acetylcholinesterase (AChE) testing is performed on ALL amniotic fluids having elevated AFAFP concentrations.

NOTE: Acetylcholinesterase (AChE) testing is an essential confirmatory test for amniotic fluids with abnormal AFP results. The odds of having a fetus with a neural tube defect are considerably greater if both the AFAFP is elevated and the AChE is positive. The addition of AChE for the detection of neural tube defects will reduce the false positive rate while maintaining a high detection rate. This procedure may be performed in-house or referred to a reference laboratory. If fetal blood is present, acetylcholinesterase results must be interpreted with caution.

Evidence of Compliance:

✓ Written procedure defining criteria for AChE testing on abnormal AFAFP results **AND**

✓ Patient reports showing AChE results, as applicable

REFERENCES
1) Haddow JE, *et al*. Acetylcholinesterase and fetal malformations: modified qualitative technique for diagnosis of neural tube defects. *Clin Chem.* 1981;27:61-63
2) Report of the collaborative acetylcholinesterase study. Amniotic fluid acetylcholinesterase electrophoresis as a secondary test in the diagnosis of anencephaly and open spina bifida in early pregnancy. *Lancet.* 1981;ii:321324
3) Wald N, *et al.* Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. *Prenat Diagn.* 1989;9:813-829
4) Crandall BF *et al.* Risks for fetal abnormalities after very and moderately elevated AFAFPS. *Prenat Diagn* 1997;17:837-841.

CHM.33300 Daily QC - Acetylcholinesterase **Phase II**

If acetylcholinesterase is run in-house, both positive and negative controls are included with each analytic run.

Evidence of Compliance:

✓ QC records for appropriate controls with each run

REFERENCES
1) Haddow JE, *et al.* Acetylcholinesterase and fetal malformations: modified qualitative technique for diagnosis of neural tube defects. *Clin Chem.* 1981;27:61-63
2) Report of the collaborative acetylcholinesterase study. Amniotic fluid acetylcholinesterase electrophoresis as a secondary test in the diagnosis of anencephaly and open spina bifida in early pregnancy. *Lancet.* 1981;ii:321324
3) Wald NJ, *et al.* Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. *Prenat Diagn.* 1989;9:813-829

CHM.33400 Acetylcholinesterase Confirmation **Phase II**

If acetylcholinesterase is run in-house, acetylcholinesterase-positive results are confirmed by addition of a specific inhibitor.

NOTE: Positive acetylcholinesterase results must be confirmed by the addition of a specific inhibitor of acetylcholinesterase, such as BW284C51.

Evidence of Compliance:

✓ Written procedure defining criteria for AChE confirmation testing **AND**

✓ Records of inhibitor testing for positive acetylcholinesterase results prior to reporting results

REFERENCES
1) Barlow RD, *et al.* A single method for amniotic fluid gelacetylcholinesterase determination, suitable for routine use in antenatal diagnosis of open neuraltube defects. *Clin Chim Acta.* 1982;119:137-142

ELECTROPHORESIS

Inspector Instructions:

	• Sampling of electrophoresis policies and procedures • Sampling of electrophoresis QC logs
	• Electrophoretic patterns (appropriate separations)

CHM.33500 Daily QC - Electrophoresis **Phase II**

Suitable control samples are run and reviewed with each batch of patient samples for all electrophoresis procedures for which controls are available.

Evidence of Compliance:

✓ Records of electrophoresis QC

CHM.33600 Electrophoresis Separations **Phase II**

Electrophoretic separations are satisfactory.

CHM.33700 Acceptable Limits - Controls **Phase II**

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

Evidence of Compliance:

✓ Records of defined acceptable limits for control range of each lot

Hemoglobin Electrophoresis

Hemoglobin solubility testing alone is NOT sufficient for detecting or confirming the presence of sickling hemoglobins in all situations. For purposes of diagnosing hemoglobinopathies, additional tests are required.

Inspector Instructions:

	• Sampling of abnormal hemoglobin policies or procedures • Sampling of patient reports (confirmatory testing, comments) • Sampling of QC records

READ	
OBSERVE	• Hemoglobin electrophoretic patterns (appropriate separations and controls) • Examine a sampling of medium (media) used to identify hemoglobin variants including alkaline/acid electrophoresis, isoelectric focusing, HPLC or other method
ASK	• What is your course of action when the primary screening method appears to show Hb S? • What is your course of action when the primary Hb electrophoresis method shows Hb variants migrating in nonA/nonS positions?

CHM.33708 Hb S Primary Screen **Phase II**

For samples that appear to have Hb S in the primary screening (by any method), the laboratory either 1) performs a second procedure (solubility testing, or other acceptable method) to confirm the presence of Hb S, or 2) includes a comment in the patient report recommending that confirmatory testing be performed.

NOTE: For primary definitive diagnosis screening by electrophoresis or other separation methods, all samples with hemoglobins migrating in the "S" positions or peak must be tested for solubility or by other acceptable confirmatory testing for sickling hemoglobin(s). Known sickling and non-sickling controls both must be included with each run of patient specimens tested.

Evidence of Compliance:

✓ Written procedure defining criteria for follow-up when Hb S appears in the primary screen

CHM.33716 Daily QC - Hgb Electrophoresis **Phase II**

Controls containing at least three known major hemoglobins, including both a sickling and a nonsickling hemoglobin (*e.g.* A, F and S) are applied with the patient specimen(s) and separations are satisfactory.

Evidence of Compliance:

✓ Written procedure defining QC requirements for hemoglobin electrophoresis **AND**

✓ QC records reflecting the use of appropriate controls **AND**

✓ Electrophoresis media demonstrating appropriate controls and separation

REFERENCES

1) Fairbanks VF. Hemoglobinopathies and thalassemias. Laboratory methods and case studies. New York, NY: BC Decker, 1980
2) Beuzard Y, *et al*. Isoelectric focusing of human hemoglobins, In Hanash, Brewer, eds. Advances in hemoglobin analysis. New York, NY: Alan R. Liss, 1981:177-195
3) Cossu G, *et al*. Neonatal screening of betathalassemias by thin layer isoelectric focusing. *Am J Hematol*. 1982;13:149
4) Bunn HF, Forget BG. Hemoglobin: molecular, genetic and clinical aspects. Philadelphia, PA: WB Saunders, 1986
5) Honig GR, Adams JG III. Human hemoglobin genetics. Vienna, Austria: SpringerVerlag, 1986
6) Jacobs S, *et al*. Newborn screening for hemoglobin abnormalities. A comparison of methods. *Am J Clin Pathol*. 1986;85:713-715
7) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part I. The introduction and thalassemia syndromes. *Lab Med*. 1987;18:368-372
8) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part II. The sickle cell disorders. *Lab Med*. 1987;18:441-443
9) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part III. Nonsickling disorders and cord blood screening. *Lab Med*. 1987;18:513-518
10) Armbruster DA. Neonatal hemoglobinopathy screening. Lab Med. 1990;21:815-822

11) Adams JG III, Steinberg MH. Analysis of hemoglobins, In Hoffman R, *et al*, eds. Hematology: basic principles and practice. New York, NY: ChurchillLivingstone, 1991:1815-1827
12) Mallory PA, *et al*. Comparison of isoelectric focusing and cellulose acetate electrophoresis for hemoglobin separation. *Clin Lab Sci*. 1994;7:348- 352
13) Awalt E, *et al*. Tandem mass spectrometry (MS) – A screening tool for hemoglobinopathies. *Clin Chem*. 2001;47(suppl):A165
14) Bradley CA, Kelly A. Comparison of high performance liquid chromatography with electrophoresis for measurement of hemoglobins A, A2, S, F, and C. *Clin Chem*. 2001;47(suppl):A172
15) Bradley CA, Kelly A. Calibration verification of hemoglobins A, A2, S, and F with an automated chromatography system. *Clin Chem*. 2001;47(suppl):A17315)
16) Hoyer JD, *et al*. Flow cytometric measurement of hemoglobin F in RBCs: diagnostic usefulness in the distinction of hereditary persistence of fetal hemoglobin (HPFH) and hemoglobin S-HPFH from other conditions with elevated levels of hemoglobin F. *Am J Clin Pathol*. 2002;117:857-863

CHM.33732 Hemoglobin Variants — Phase II

All samples with hemoglobin variants migrating in "nonA, nonS" positions on alkaline electrophoresis, isoelectric focusing, or HPLC are further defined with electrophoresis at acid pH or other acceptable methods where clinically and technically appropriate.

NOTE: Electrophoresis at acid pH is useful to further characterize hemoglobin variants migrating in the Hb A2 position, if all variants are not clearly separated by the primary method. This method will differentiate the three major hemoglobins that migrate in this position, namely Hb C, Hb E and Hb O-Arab, as well as give information on rare variants such as Hb C-Harlem. However, for hemoglobin variants that migrate in other "nonA, nonS" positions, such as fast hemoglobin variants, electrophoresis at acid pH is generally not informative. Further workup of such variants, including referral to a reference laboratory, is dependent upon the patient's overall clinical situation, such as findings of erythrocytosis or a hemolytic anemia.

Evidence of Compliance:

✓ Written procedure defining criteria for further identification of hemoglobin variants **AND**
✓ Patient reports and records reflecting further work-up, when appropriate

REFERENCES
1) Glacteros F, *et al*. Cord blood screening for hemoglobin abnormalities by thin layer isoelectric focusing. Blood. 1980;56:1068
2) Beuzard Y, *et al*. Isoelectric focusing of human hemoglobins, In Hanash, Brewer, eds. Advances in hemoglobin analysis. New York, NY: Alan R. Liss, 1981:177-195
3) Black J. Isoelectric focusing in agarose gel for detection and identification of hemoglobin variants. *Hemoglobin*. 1984;8:117
4) Bunn HF, Forget BG. Hemoglobin: molecular, genetic and clinical aspects. Philadelphia, PA: WB Saunders, 1986
5) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part I. The introduction and thalassemia syndromes. *Lab Med*. 1987;18:368-372
6) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part II. The sickle cell disorders. *Lab Med*. 1987;18:441-443
7) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part III. Nonsickling disorders and cord blood screening. *Lab Med*. 1987;18:513-518
8) Adams JG III, Steinberg MH. Analysis of hemoglobins, In Hoffman R, *et al*, eds. Hematology: basic principles and practice. New York, NY: ChurchillLivingstone, 1991:1815-1827
9) Mallory PA, *et al*. Comparison of isoelectric focusing and cellulose acetate electrophoresis for hemoglobin separation. *Clin Lab Sci*. 1994;7:348-352
10) Ou CN, Rognerud CL. Rapid analysis of hemoglobin variants by cation exchange HPLC. *Clin Chem*. 1993;39:820-824
11) Awalt E, *et al*. Tandem mass spectrometry (MS) – A screening tool for hemoglobinopathies. *Clin Chem*. 2001;47(suppl):A165
12) Bradley CA, Kelly A. Comparison of high performance liquid chromatography with electrophoresis for measurement of hemoglobins A, A2, S, F, and C. *Clin Chem*. 2001;47(suppl):A172
13) Bradley CA, Kelly A. Calibration verification of hemoglobins A, A2, S, and F with an automated chromatography system. *Clin Chem*. 2001;47(suppl):A173
14) Hoyer JD, *et al*. Flow cytometric measurement of hemoglobin F in RBCs: diagnostic usefulness in the distinction of hereditary persistence of fetal hemoglobin (HPFH) and hemoglobin S-HPFH from other conditions with elevated levels of hemoglobin F. *Am J Clin Pathol*. 2002;117:857-863

CHM.33764 Hb S Predominant Band — Phase II

All samples that appear to have Hb S as the predominant band by the primary screening (by whatever method) and that are confirmed as sickling by appropriate methods are further examined to ascertain whether the "Hb S" band or peak contains solely Hb S or both Hb S and Hb D, Hb G or other variant hemoglobins.

NOTE: When the predominant hemoglobin component appears to be Hb S, it is necessary to determine whether this represents homozygous Hb S or a heterozygote for Hb S and another

variant such as Hb D, Hb G, Hb-Lepore, or other hemoglobin variant(s). Given the clinical implications of homozygous Hb S (or Hb S/ß zero thalassemia) it is imperative to exclude other hemoglobin variants, however rare. Referral of these specimens to a reference laboratory for further workup is acceptable.

Evidence of Compliance:

✓ Written procedure defining criteria for determination of homozygous versus heterozygous Hb S **AND**

✓ Patient records or worksheets showing the exclusion of hemoglobin variants **OR** documentation of referral for further work-up

REFERENCES
1) Black J. Isoelectric focusing in agarose gel for detection and identification of hemoglobin variants. *Hemoglobin*. 1984;8:117
2) Bunn HF, Forget BG. Hemoglobin: molecular, genetic and clinical aspects. Philadelphia, PA: WB Saunders, 1986
3) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part I. The introduction and thalassemia syndromes. *Lab Med*. 1987;18:368-372
4) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part II. The sickle cell disorders. *Lab Med*. 1987;18:441-443
5) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part III. Nonsickling disorders and cord blood screening. *Lab Med*. 1987;18:513-518
6) Adams JG III, Steinberg MH. Analysis of hemoglobins, In Hoffman R, *et al*, eds. Hematology: basic principles and practice. New York, NY: ChurchillLivingstone, 1991:18151-827
7) Mallory PA, *et al*. Comparison of isoelectric focusing and cellulose acetate electrophoresis for hemoglobin separation. *Clin Lab Sci*. 1994;7:348-352

BLOOD GAS ANALYSIS

SPECIMEN COLLECTION AND HANDLING

Inspector Instructions:

	• Blood gas collection policy or procedure • Sampling of records performance of collateral circulation tests
	• How are personnel that perform arterial punctures made aware of possible complications?

CHM.33800 Knowledgeable - Arterial Punctures Phase II

Personnel performing arterial punctures are knowledgeable about the more significant complications of this procedure compared with venipuncture.

Evidence of Compliance:

✓ Written documentation of training in personnel files

REFERENCES
1) NCCLS. *Procedures for the Collection of Arterial Blood Specimens; Approved Standard—Fourth Edition*. NCCLS document H11-A4 (ISBN 1-56238-545-3). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004

CHM.33900 Collateral Circulation Phase II

For radial artery sampling, a test for collateral circulation is performed and documented

before arterial puncture, as applicable.

NOTE: The various technologies available have been evaluated in the published literature. Consensus should be established between the laboratory and involved clinicians to identify the patient/clients in whom such a test is medically useful in averting potential patient/client injury. The site from where the sample was obtained should be documented.

Evidence of Compliance:

✓ Written collection procedure defining situations that require testing for collateral circulation to include preferred technique(s)

REFERENCES
1) Vaghadia H, *et al*. Evaluation of a postocclusive circulatory hyperaemia (PORCH) test for the assessment of ulnar collateral circulation. *Can J Anaesth.* 1988;35:591-598
2) Cheng EY, *et al*. Evaluation of the palmar circulation by pulse oximetry. *J Clin Monit.* 1989;5:1-3
3) Levinsohn DG, *et al*. The Allen's test: analysis of four methods. *J Hand Surg.* 1991;16:279-282
4) Fuhrman TM, *et al*. Evaluation of collateral circulation of the hand. *J Clin Monit.* 1992;8:28-32
5) Furhman TM, *et al*. Evaluation of digital blood pressure, plethysmography, and the modified Allen's test as a means of evaluating the collateral circulation to the hand. *Anaesthesia.* 1992;47:959-961
6) Fuhrman TM, McSweeney E. Noninvasive evaluation of the collateral circulation to the hand. *Acad Emerg Med.* 1995;2:195-199
7) O'Mara K, Sullivan B. A simple bedside test to identify ulnar collateral flow. *Ann Intern Med.* 1995;123:637
8) Starnes SL, *et al*. Noninvasive evaluation of hand circulation before radial artery harvest for coronary artery bypass grafting. *J Thorac Cardiovasc Surg.* 1999;117:261-266
9) Cable DG, *et al*. The Allen test. *Ann Thorac Surg.* 1999;67:876-877

CHM.34000 Ambient Air Contamination **Phase II**

There is a system to prevent ambient air contamination of blood gas samples before analysis.

Evidence of Compliance:

✓ Written procedure defining system for prevention of ambient air contamination

REFERENCES
1) Ishikawa S, *et al*. The effects of air bubbles and time delay on blood gas analysis. *Ann Allergy.* 1974;33:72-77
2) Mueller RG, *et al*. Bubbles in samples for blood gas determinations. *Am J Clin Pathol.* 1976;65:242-249
3) Madiedo G, *et al*. Air bubbles and temperature effect on blood gas analysis. *J Clin Pathol.* 1980;33:864-867
4) Biswas CK, *et al*. Blood gas analysis: effect of air bubbles in syringe and delay in estimation. *Brit Med J.* 1982;284:923-927
5) McKane MH, *et al*. Sending blood gas specimens through pressurized transport tube systems exaggerates the error in oxygen tension measurements created by the presence of air bubbles. *Anesth Analg.* 1995;81:179-182
6) Astles JR, *et al*. Pneumatic transport exacerbates interference of room air contamination in blood gas samples. *Arch Pathol Lab Med.* 1996;120:642-647

BLOOD GAS INSTRUMENTS

If the institution has blood gas testing sites that are medically and/or administratively separate from the main laboratory, and the blood gas testing medical director is different from the central laboratory medical director, then a separate Chemistry and Toxicology Checklist must be completed. If blood gas analyses are performed in a central laboratory with other chemistry assays, and/or testing occurs physically separate from the main laboratory, but the medical director is the same as the main laboratory, then complete this section.

Inspector Instructions:

- Blood Gas analysis policy or procedure
- Sampling of blood gas calibration records
- Sampling of blood gas QC records

CHM.34200 Calibration Materials **Phase II**

The materials used for calibration of the pH, CO_2, and O_2 sensors are either in conformance with the instrument manufacturer's specifications or traceable to NIST Standard Reference Materials.

CHM.34300 Calibration - Blood Gas Instruments **Phase II**

Blood gas instruments are calibrated according to manufacturer's specifications and at least as frequently as recommended by the manufacturer.

NOTE: Instruments used infrequently must be recalibrated each time of use. Some instruments have built in calibration that is performed automatically by the instrument; however, there must be some defined procedure for verifying the reliability of this process. If appropriate, the calibration must compensate for the influence of barometric pressure.

Evidence of Compliance:

✓ Written procedure defining frequency and criteria for performing calibration **AND**
✓ Records for calibration documented at defined frequency

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):3709 [42CFR493.1267(a)]

CHM.34400 Daily QC - Blood Gas Instruments **Phase II**

A minimum of 1 quality control specimen for pH, pCO_2 and pO_2 (tonometered sample or liquid control material) is analyzed at least every 8 hours of operation when patient specimens are tested.

Evidence of Compliance:

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

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register.* 2003(Jan 24) [42CFR493.1267(b)]
2) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition*. CLSI document C24-A3 (ISBN 1-56238-613-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006

CHM.34500 Daily QC - Blood Gas Instruments **Phase II**

The control materials for pH, pCO_2 and pO_2 represent both high and low values on each day of patient testing.

Evidence of Compliance:

✓ Written procedure defining QC requirements **AND**
✓ QC records reflecting the appropriate use of controls

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register.* 2003(Jan 24) [42CFR493.1267(b)]
2) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition*. CLSI document C24-A3 (ISBN 1-56238-613-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006
3) Ng VL, *et al*. The rise and fall of i-STAT point-of-care blood gas testing in an acute care hospital. *Am J Clin Pathol.* 2000;114:128-138

CHM.34600 QC - Blood Gas Instruments **Phase II**

At least one sample of control material for pH, pCO_2 and pO_2 is included each time patient specimens are tested, except for automated instruments that internally calibrate at least once every 30 minutes of use.

Evidence of Compliance:

✓ Written procedure defining QC requirements **AND**
✓ QC results **OR** documentation of internal calibrator

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register*. 2003(Jan 24): 3709 [42CFR493.1267(c)]
2) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition*. CLSI document C24-A3 (ISBN 1-56238-613-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006

LEGAL TESTING

Some laboratories may choose to perform certain tests exclusively for legal purposes (e.g. alcohol for traffic law enforcement, criminal justice and medical examiner systems). In this case, the performance of legal testing must meet forensic, not clinical laboratory, standards. These forensic standards include the requirements for chain-of-custody protocols for specimens and aliquots, specimen seals, increased specimen and record security, appropriate confirmation testing, and a certifying review process.

Certain clinical tests have a higher potential for being involved in a legal proceeding, e.g. blood alcohol tests for motor vehicle accident patients and drugs of abuse tests for patients undergoing drug treatment or neonates suspected of drug exposure, in utero. Therefore, a laboratory may choose to conduct these clinical tests using procedures and policies that meet both forensic and clinical laboratory standards. It is not a requirement, however, to conduct any clinical testing using the standards of legal testing; it is an administrative decision to do so. Toxicology testing for diagnosis, treatment or other clinical purposes must meet only clinical laboratory practice standards.

This section includes requirements that specifically relate to legal or "forensic" toxicology testing requirements. It is not intended for accreditation of forensic or workplace drug testing. The CAP Forensic Drug Testing (FDT) program is appropriate for workplace testing.

Inspector Instructions:

	• Sampling of legal testing policies and procedures (includes collection, accessioning, specimen retention/storage, record retention) • Copies of or documented evaluation of applicable laws and regulations • Sampling of external and internal chain-of-custody documents • Sampling of certifying review of analytical and forensic records
	• Locked limited-access secured area (contains original specimens/containers) • Locked limited-access secured area (contains forensic records)
	• How do you know your specimen collection containers maintain analyte stability? • How have you evaluated ethanol specificity in your method for testing legal alcohols?

- Who has access to the secure area where original specimens are stored?
- What is your course of action when unacceptable specimens are received?
- What is your course of action after obtaining an unconfirmed positive test result?
- How do you ensure the confidentiality of patient reports?

CHM.36700 Regulations/Laws **Phase II**

The laboratory is aware of rules and regulations that may affect any legal testing performed by the laboratory.

Evidence of Compliance:

✓ Copies of applicable regulations and laws **OR** documentation that applicable regulations and laws were evaluated to ensure compliance

REFERENCES
1) Urry FM, Wong Y-W. Current issues in alcohol testing. *Lab Med.* 1995;26:194-197
2) Williams RH, Erickson T. Evaluating toxic alcohol poisoning in the emergency setting. *Lab Med.* 1998;29:102-107

CHM.36800 Specimen Collection Manual - Legal Testing **Phase I**

Specific instructions are available for collecting blood samples for legal testing, including aspects of skin preparation and use of preservatives.

Evidence of Compliance:

✓ Written specimen collection procedure defining criteria for specimen collection for legal testing

REFERENCES
1) Dubowski KM, Essary NA. Contamination of blood specimens for alcohol analysis during collection. *Abst Rev Alcohol Driving.* 1983;4:3-7
2) Peek GJ, *et al.* The effects of swabbing the skin on apparent blood ethanol concentration. *Alcohol & Alcoholism.* 1990;25:639-640
3) Jones AW. Severe isopropanolemia without acetonemia: contamination of specimens during venipuncture? *Clin Chem.* 1995;41:123
4) NCCLS. Blood alcohol testing in the clinical laboratory; approved guideline T/DM6-A. Wayne, PA: NCCLS, 1997
5) Sylvester PA, *et al.* Unacceptably high site variability in postmortem blood alcohol analysis. *J Clin Pathol.* 1998;51:250-252
6) Williams RH, Leikin JB. Medicolegal issues and specimen collection for ethanol testing. *Lab Med.* 1999;30:530-537

CHM.36900 Specimen Collection Container Effectiveness **Phase I**

The laboratory has evaluated the effectiveness of its specimen collection containers in maintaining analyte stability.

NOTE: The laboratory should evaluate the effectiveness of its specimen collection containers in accurately maintaining analyte stability over time, as changes may occur that would alter the validity of reported measurements. For example, both metabolic consumption of alcohol and production of alcohol by microorganisms must be considered.

Evidence of Compliance:

✓ Written procedure defining criteria for evaluation of specimen collection containers **AND**

✓ Records of evaluation studies

REFERENCES
1) Brown GA, *et al.* The stability of ethanol in stored blood. Part 1. Important variables and interpretation of results. *Analyt Chim Acta.* 1973;66:271-283
2) Kaye S. The collection and handling of the blood alcohol specimen. *Am J Clin Pathol.* 1980;74:743-746
3) Winek CL, Paul LJ. Effect of short-term storage conditions on alcohol concentrations in blood from living human subjects. *Clin Chem.* 1983;29:1959-1960
4) Dubowski KM, *et al.* The stability of ethanol in human whole blood controls: an inter-laboratory study. *J Analyt Toxicol.* 1997;21:486-491

CHM.37000 Ethanol Specificity **Phase II**

If the laboratory tests for legal alcohols, the method has been evaluated for ethanol specificity.

NOTE: The laboratory director must ensure that the method is sufficiently specific for ethanol in the setting in which the test is used. The alcohol dehydrogenase-mediated enzymatic assays are variably susceptible to positive interference by high lactate dehydrogenase and/or high lactate levels, resulting in false positive or falsely elevated ethanol levels. The use of an enzymatic assay is acceptable if the director has determined the extent of LD and lactate interferences and that the specificity is adequate for the setting in which it is used. The laboratory should have a protocol to manage this specificity problem.

Evidence of Compliance:

✓ Written procedure defining actions to be taken for potential interferences in testing for legal alcohols **AND**

✓ Records of ethanol specificity evaluation studies **OR** director evaluation of manufacturer's documentation of specificity

REFERENCES
1) Dubowski KM. Alcohol determination in the clinical laboratory. *Am J Clin Pathol.* 1980;74:747-750
2) NCCLS. Blood alcohol testing in the clinical laboratory; approved guideline T/DM6A. Wayne, PA: NCCLS, 1997
3) Osterloh JD, *et al.* Discrepancies in osmolal gaps and calculated alcohol concentrations. *Arch Pathol Lab Med.* 1996;120:637-341
4) Williams RH, Leikin JB. Assessment of ethanol intoxication and regulatory issues. *Lab Med.* 1999;30:587-594
5) Nine JS *et al. J Anal Toxicol. 1995;19:192-196*

CHM.37100 Receiving/Accessioning **Phase II**

The specimen receiving/accessioning procedure requires documentation of type of specimen, verification of specimen identity, completeness of external chain-of-custody documents, and integrity (tamper-evident) of the transmittal or shipping container.

Evidence of Compliance:

✓ Written procedure defining criteria for receiving/accessioning of specimens for legal testing

CHM.37200 Chain-of-Custody Documents **Phase II**

The laboratory properly completes appropriate sections of external chain-of-custody documents.

Evidence of Compliance:

✓ Written procedure stating chain-of-custody requirements

CHM.37300 Chain-of-Custody Documents **Phase II**

There is a requirement for preparation of internal chain-of-custody documentation for specimens received.

Evidence of Compliance:

✓ Written procedure stating chain-of-custody requirements

CHM.37400 Chain-of Custody Documents **Phase II**

The laboratory generates and properly completes internal chain-of-custody documents to

account for the specimens and aliquots.

NOTE: The chain-of-custody procedure must account for all authorized individuals who handle the specimens/aliquots, or the storage location when not in the possession of an authorized individual. The reason for the transfer of custody should be recorded along with the date of the transfer.

Evidence of Compliance:

✓ Written procedure stating chain-of-custody requirements

CHM.37500 Secured Specimen Storage **Phase II**

The original specimens are always maintained in the original containers, and in a limited-access secured area, when not in the possession of an authorized individual.

NOTE: The original specimens must always be maintained either in the direct custody of an authorized individual, or be in a locked secured area accessible only to authorized individuals. This locked and limited-access area may be a refrigerator, freezer or storage room within the laboratory.

Evidence of Compliance:

✓ Written procedure defining criteria for storage of and access to specimens for legal testing **AND**

✓ Records for internal chain-of-custody reflecting limited-access storage **OR** record of direct custody of the specimen by an authorized person at all times

CHM.37600 Limited-Access Area **Phase II**

Access to the limited-access area is restricted to authorized laboratory personnel.

CHM.37700 Accessioning Procedure **Phase II**

The accessioning procedure for specimens defines criteria for determining the forensic acceptability of specimens for analysis, and there is a documented protocol for the course of action (*i.e.* reporting back to the client) that must be followed when unacceptable specimens are identified.

NOTE: Clients and laboratories may have different rules for evaluating a specimen for its forensic acceptability for analysis (chain-of-custody failures, missing information, specimen leakage, inadequate volume, wrong type of specimen submitted, etc.). These evaluation criteria must be documented in the accessioning procedure along with the actions that laboratory personnel are required to take in reporting these problems to the client. Unacceptable specimens must be monitored by the laboratory as part of its quality management program.

Evidence of Compliance:

✓ Specimen rejection records

CHM.37800 Specimen Retention and Storage **Phase II**

Appropriate specimen retention and storage conditions are defined for positive and negative specimens.

NOTE: The policies for specimen security, storage, location, retention, and final disposition must be appropriate for each type of specimen tested by the laboratory. The minimum specimen retention time and storage condition must comply with applicable laws and regulations.

CHM.37900 Positive Result Confirmation **Phase II**

The laboratory requires confirmation of all positive results before release.

NOTE: Some clients may request that unconfirmed positive results be reported, with the laboratory storing the specimen for potential confirmation at a later date. If it is locally acceptable to report unconfirmed positive results, a disclaimer must accompany those results to indicate that unconfirmed positive results may NOT meet forensic requirements.

Evidence of Compliance:

- ✓ Written procedure requiring confirmation of all positive results **OR** the procedure requiring the use of a disclaimer on report if unconfirmed results are reported, when applicable **AND**
- ✓ Records reflecting the confirmatory testing performed **OR** patient reports including the use of the disclaimer for unconfirmed results

CHM.38000 Second Method Confirmation **Phase II**

The laboratory confirms all positive results, using a second method that is scientifically valid and legally defensible, and which is analytically different from the initial testing method.

NOTE: An exception is that for legal alcohol confirmation testing, the standard of practice does not necessarily require the use of a confirmation method that is analytically different from that of the initial test. However, legal alcohol testing must use a methodology with high specificity for ethanol. The alcohol dehydrogenase-mediated enzymatic assays are variably susceptible to positive interference by high lactate dehydrogenase and/or high lactate levels resulting in false positive or falsely elevated ethanol level. The use of an enzymatic assay is acceptable if the director has determined the extent of LD and lactate interferences and that the specificity is adequate for the setting in which it is used. The laboratory should have a protocol to manage this specificity problem.

Evidence of Compliance:

- ✓ Written procedure defining the method for confirmatory testing of all positive results for tests other than legal alcohol **AND**
- ✓ Records reflecting the confirmatory testing performed on positive results

REFERENCES
1) Nine JS *et al. J Anal Toxicol. 1995;19:192-196*

CHM.38100 Review of Analytic Process **Phase II**

There is a documented procedure that requires that each step of the analytical process be reviewed and documented, and this procedure requires at least the review of the following information for both screening and confirmatory testing.

1. **Results of standards or calibrators**
2. **Results of quality controls**
3. **Laboratory identification of samples tested in each batch and the testing sequence of calibrators, controls, and unknowns**
4. **Identity of analyst(s) performing and reviewing the test results**

CHM.38200 Certifying Review **Phase II**

There is a written procedure that defines the requirement for documented certifying

review of the analytical and forensic records for all specimens (negative and positive) before results are released, and that procedure directs the certifying review to include the following.

1. **External chain-of-custody documents**
2. **Internal chain-of-custody documents**
3. **Review and comparison of both screening and confirmation results**
4. **Acceptability of quality control results**
5. **Review of critical analytical data for the identification, quantitation (if required) of each drug in confirmation analyses for calibrators/standards, controls, and unknown specimens**
6. **Final report for completeness, accuracy, and agreement with the analytical data**

CHM.38300 Certifying Review **Phase II**

The certifying review procedure requires documentation of the identity of the reviewer and the date review was completed.

CHM.38400 Report Confidentiality **Phase II**

Documented protocols for reporting results emphasize and ensure maintenance of confidentiality of reports.

NOTE: The reporting of legal testing results should be done in a confidential manner to ensure that only authorized client representatives or authorized laboratory personnel can receive, review, or print these results regardless of the methods used for reporting (e.g. telephone, FAX, remote printer, computer terminal).

CHM.38500 Record Retention **Phase II**

There is a documented procedure that defines the records that must be retained to meet client, legal, regulatory, and accreditation requirements, and the length of retention time.

NOTE: The CAP Laboratory Accreditation Program requires the following forensic records be retained for at least 2 years. However, the laboratory must be able to store forensic records as long as any legal action is pending.

- *Laboratory specimen security logs*
- *Laboratory accessioning logs*
- *Chain-of-custody documents and requisitions*
- *Analytical data from screening and confirmation analyses*
- *Specimen reports*
- *Quality control program records*
- *Instrument maintenance/service records*
- *Instrument calibration records*
- *Reagent/standard/calibrator/control preparation and verification records*
- *Method performance validation records*
- *Personnel files on all laboratory personnel who are involved with the forensic testing performed by the laboratory*
- *Proficiency testing survey results, reports, and corrective actions*
- *CAP accreditation reports and corrective actions*

CHM.38600 Secured Forensic Records **Phase II**

The forensic records are maintained in a limited-access secured (locked) area that is only accessible to authorized laboratory personnel.

Evidence of Compliance:

✓ Written policy addressing restricted access to forensic records