COMMISSION ON LABORATORY ACCREDITATION

Laboratory Accreditation Program

MOLECULAR PATHOLOGY CHECKLIST

Disclaimer and Copyright Notice

The College of American Pathologists (CAP) Checklists are posted on the CAP's Web site for information only. 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.

MOLECULAR PATHOLOGY

OUTLINE

SUMMARY OF CHANGES
MOLECULAR PATHOLOGY Checklist
9/27/2007 Edition

The following questions 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 questions included may not appear in the customized checklist. Such questions are not applicable to the testing performed by the laboratory.

Note: For revised checklist questions, 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 Questions

Question	Effective Date
MOL.05075	09/27/2007
MOL.39323	09/27/2007
MOL.39358	09/27/2007
MOL.39393	09/27/2007
MOL.13466	12/12/2006
MOL.16732	12/12/2006
MOL.34003	12/12/2006
MOL.34495	12/12/2006

REVISED Checklist Questions

Question	Effective Date
MOL.10200	09/27/2007
MOL.31590	09/27/2007
MOL.29750	12/12/2006
MOL.34900	12/12/2006
MOL.34930	12/12/2006

DELETED Checklist Questions

Question	Effective Date
MOL.29635	09/27/2007
MOL.32165	09/27/2007
MOL.34928	09/27/2007
MOL.34942	09/27/2007

MOL.38767	09/27/2007
MOL.38814	09/27/2007
MOL.20100	12/12/2006
MOL.35546	12/12/2006
MOL.35590	12/12/2006
MOL.35634	12/12/2006
MOL.35678	12/12/2006

CONTINUING EDUCATION INFORMATION

Beginning January 2008, you may earn continuing education credits (CME/CE) by completing an online Inspection Preparation activity that includes review of this checklist.

Prior to reviewing the checklist, log on to the CAP Web site at <www.cap.org <http://www.cap.org>>, click the Education Programs tab, then select Laboratory Accreditation Program (LAP) Education Activities, and Inspection Preparation for complete instructions and enrollment information.

IMPORTANT: The contents of the Laboratory General Checklist are applicable to the Molecular Pathology section of the laboratory.

****

INSPECTION TECHNIQUES – KEY POINTS

****

I. READ – OBSERVE – ASK – the three methods of eliciting information during the inspection process. These three methods may be used throughout the day in no particular order. Plan the inspection in a way that allows adequate time for all three components.

READ = Review of Records and Documents
Document review verifies that procedures and manuals are complete, current, available to staff, accurate and reviewed, and describe good laboratory practice. Make notes of any questions you may have, or processes you would like to observe as you read the documentation.

OBSERVE – ASK = Direct Observation and Asking Questions
Observing and asking questions accomplish the following:

1. Verifies that the actual practice matches the written policy or procedure
2. Ensures that the laboratory processes are appropriate for the testing performed
3. Ensures that outcomes for any problem areas, such as PT failures and issues/problems identified through the quality management process, have been adequately investigated and resolved
4. Ensures that previously cited deficiencies have been corrected

Use the following techniques:

- **Observe laboratory practices** – look at what the laboratory is actually doing. Compare the written policy/procedure to what you actually observe in the laboratory to ensure the written policy/procedure accurately reflects laboratory practice. Note if practice deviates from the documented policies/procedures.

- **Ask open ended, probing questions –** these are starting points that will allow you to obtain large amounts of information, and help you clarify your understanding of the documentation you've seen and observations you've made. This eliminates the need to ask every single checklist question, as the dialogue between you and the laboratory may address multiple checklist questions.

    - Ask open-ended questions that start with phrases such as "show me how…" or "tell me about …" or "what would you do if…". By asking questions that are open-ended, or by posing a hypothetical problem, you will avoid "cookbook" answers. For example, ask "Could you show me the specimen transport policy and show me how you ensure optimum specimen quality?" This will help you to determine how well the technical staff is trained, whether or not they are adhering to the lab's procedures and policies, and give you a feel for the general level of performance of the laboratory.

    - Ask follow-up questions for clarification. Generally, it is best not to ask the checklist questions verbatim. For example, instead of asking the checklist question "Is there documentation of corrective action when control results exceed defined tolerance limits?" ask, "What would you do if the SD or CV doubles one month?" A follow-up probing question could be, "What would you do if you could not identify an obvious cause for the change in SD or CV?"

II. Evaluate Selected Specimens and Tests in Detail

For the Laboratory General Checklist: Follow a specimen through the laboratory. By following a specimen from collection to test result, you can cover multiple checklist questions in the Laboratory General checklist: questions on the specimen collection manual; phlebotomy; verbal orders; identification of patients and specimens; accessioning; and result reporting, including appropriate reference ranges, retention of test records, maintaining confidentiality of patient data, and proper handling of critical results and revisions to reports.

For the individual laboratory sections: Consult the laboratory's activity menu and focus on tests that potentially have the greatest impact on patient care. Examples of such tests include HIV antibodies, hepatitis B surface antigen, urine drugs of abuse, quantitative beta-hCG, cultures of blood or CSF, acid-fast cultures, prothrombin time and INR reporting, and compatibility testing and unexpected antibody detection. Other potentially high-impact tests may be identified by looking at very high or low volume tests in the particular laboratory, or problems identified by reviewing the Variant Proficiency Testing Performance Report.

To evaluate preanalytic and postanalytic issues: Choose a representative specimen and "follow" the specimen through the laboratory or section of the laboratory, reviewing appropriate records in the preanalytic and postanalytic categories.

To evaluate analytic processes: Choose 2 or 3 analytes and perform a comprehensive review of records, including procedure manuals, quality control and proficiency testing records, instrument maintenance records and method performance validations for the last 2 years, selecting timeframes at the beginning, mid-point, and end of this timeframe. Compare instrument print-outs to patient reports and proficiency testing results to ensure accurate data entry. If problems are identified, choose additional tests or months to review.

III. Verify that proficiency testing problem have been resolved: From the inspector's packet, review the Variant PT Performance Report that identifies, by analyte, all of the PT scores below 100%. Correlate any PT problems to QC or maintenance records from the same time period. Be thorough when reviewing these representative records, selecting data from the beginning, middle and end of the period since the last on-site inspection.

IV. Review correction of previous deficiencies: Review the list of deficiencies from the previous on-site inspection provided in the inspector's packet. Ensure that they have been appropriately addressed.

****

APPLICABILITY

****

Testing that involves DNA/RNA probe hybridization or amplification constitutes molecular testing. The Molecular Pathology Checklist covers most aspects of clinical molecular testing including oncology, hematology, infectious disease, inherited disease, HLA typing, forensics and parentage applications. The inspection of laboratories performing molecular testing requires the Molecular Pathology checklist, except for the following:

1) The Cytogenetics or Anatomic Pathology checklist (as appropriate) may be used to inspect fluorescence in situ hybridization (FISH), for FISH testing performed in the cytogenetics or anatomic pathology section. Also, the Anatomic Pathology checklist may be used to inspect in situ hybridization (ISH), for ISH testing performed in the anatomic pathology section.

2) The Microbiology checklist may be used to inspect laboratories that limit molecular testing to infectious disease testing using unmodified, FDA-approved methods. Microbiology laboratories that use molecular methods that are not FDA-approved, or that modify an FDA-approved method, must be inspected with the Molecular Pathology checklist. (Note: This requirement applies to all CAP-accredited laboratories, including non-U.S. laboratories.) Laboratories should consult the list of currently FDA-approved tests published on-line by the Association for Molecular Pathology (AMP) at www.amp.org

***Inspector Requirements**: Inspection of a molecular pathology laboratory requires special knowledge of the science. Ideally, the inspector should be an actively practicing molecular scientist familiar with*

the Checklist and possessing the technical and interpretive skills necessary to evaluate the quality of the laboratory's performance. If the team leader's laboratory performs similar molecular pathology services as the inspected lab, the inspecting laboratory's molecular pathology section director or section supervisor is a qualified inspector. If the team leader has no such resource, the list of qualified regional inspectors included in the Inspector's Inspection Packet should be consulted.

****

PROFICIENCY TESTING

****

Definitions:

Proficiency testing (PT) is defined as determination of laboratory testing performance by means of interlaboratory comparisons, in which a PT program periodically sends multiple specimens to members of a group of laboratories for analysis and/or identification; the program then compares each laboratory's results with those of other laboratories in the group and/or with an assigned value…(adapted from Clinical Laboratory Standards Institute Harmonized Terminology Database; available at http://www.nccls.org/).

Alternative assessment is defined as determination of laboratory testing performance by means other than PT--for example, split-sample testing, testing by a different method, *etc.*

*****NEW**** ***09/27/2007***

MOL.05075 Phase I **N/A YES NO**

Does the laboratory's current CAP Activity Menu accurately reflect the testing performed?

NOTE: An accurate Activity Menu is required to properly assess a laboratory's compliance with proficiency testing requirements. The accuracy of the Activity Menu can be assessed by inquiry of responsible individuals, and by examination of the laboratory's test requisition(s), computer order screens, procedure manuals, or patient reports. All tests performed by the laboratory should be listed on the Activity Menu, and visa versa.

If tests are identified that are not included on the laboratory's test menu, the inspector should contact the CAP (800-323-4040) for instructions.

Please note that unusual or esoteric tests performed in the laboratory section may not be specifically listed on the laboratory's activity menu but may be identified on the activity menu as a miscellaneous code. Further information may be found with the laboratory's instrumentation list. Some activities are also included on the Master Activity Menu using more generic groupings or panels instead of listing the individual tests. The Master Activity Menu represents only those analytes that are directly measured. Calculations are not included.

COMMENTARY:

N/A

REFERENCE: Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2004(Oct 1): 985 [42CFR493.51].

MOL.10150 Phase II N/A YES NO

Does the laboratory participate in the appropriate required CAP Surveys or another proficiency testing (PT) program accepted by CAP for the patient testing performed?

NOTE 1: The list of analytes for which CAP requires proficiency testing is available on the CAP website [http://www.cap.org/] or by phoning 800-323-4040 (or 847-832-7000), option 1. A laboratory's participation in proficiency testing must include all analytes on this list for which it performs patient testing. Participation in proficiency testing may be through CAP Surveys or another proficiency testing provider accepted by CAP. Laboratories will not be penalized if they are unable to participate in an oversubscribed program. If unable to participate, however, the laboratory must implement an alternative assessment procedure for the affected analytes. For regulated analytes, if the CAP and CAP-accepted PT programs are oversubscribed, CMS requires the laboratory to attempt to enroll in another CMS-approved PT program.

NOTE 2: HER2 PT is method specific, and laboratories performing HER2 testing by multiple methods must participate in PT for each method. Details are available on the CAP website www.cap.org. Satisfactory performance requires correct responses on at least 90% of graded challenges in each testing event (mailing).

If the laboratory interprets HER2 test results from stains prepared at another facility, the laboratory must (1) enroll in an appropriate PT survey, (2) send PT materials to the staining facility for preparation, and (3) interpret the resulting stains using the same procedures that are used for patient specimens.

COMMENTARY:

N/A

REFERENCES: 1) Ehrmeyer SS, *et al.* Performance of external quality control systems. *Lab Med.* 1989;20:428-431; 2) 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):7146 [42CFR493.801]; 3) Walker, RH. Molecular pathology programs of the College of American Pathologists. *Lab Med.* 1994;25:654-657; 4) Westgard JO, *et al.* Laboratory precision performance. State of the art *versus* operating specifications that assure the analytical quality required by Clinical Laboratory Improvement Amendments proficiency testing. *Arch Pathol Lab Med.* 1996;120:621-625; 5) NCCLS. *Continuous Quality Improvement: Integrating Five Key Quality*

System Components; Approved Guideline—Second Edition. NCCLS document GP22-A2 (ISBN 1-56238-552-6). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004; 6) Rachel JM, *et al.* CAP survey results not indicative of PCR testing errors. *Clin Lab News.* 1997(Aug);23(8):4; 7) College of American Pathologists, Commission on Laboratory Accreditation. Standards for laboratory accreditation; Standard III. Northfield, IL: CAP, 1998.

MOL.10160 Phase II N/A YES NO

For tests for which CAP does not require PT, does the laboratory at least semiannually 1) participate in external PT, or 2) exercise an alternative performance assessment system for determining the reliability of analytic testing?

NOTE: Appropriate alternative performance assessment procedures may include: split sample analysis with reference or other laboratories, split samples with an established in-house method, assayed material, regional pools, clinical validation by chart review, or other suitable and documented means. For FISH testing, alternative assessment may be performed by method and specimen type, rather than for each tested abnormality (i.e., one program for all FISH cytogenetics tests performed on cell suspensions).

It is the responsibility of the laboratory director to define such alternative performance assessment procedures, as applicable, in accordance with good clinical and scientific laboratory practice. Participation in ungraded/educational proficiency testing programs also satisfies this checklist question.

Semiannual alternative assessment must be performed on tests for which PT is not available.

COMMENTARY:

N/A

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):7184 [42CFR493.1281(a) and 1236(c)(1)]; 2) Shahangian S, *et al.* A system to monitor a portion of the total testing process in medical clinics and laboratories. Feasibility of a split-specimen design. *Arch Pathol Lab Med.* 1998;122:503-511.

MOL.10170 Phase II N/A YES NO

Does the laboratory integrate all proficiency testing samples within the routine laboratory workload, and are those samples analyzed by personnel who routinely test patient samples, using the same primary methods as for patient samples?

NOTE: Replicate analysis of proficiency testing samples is acceptable only if patient specimens are routinely analyzed in the same manner. With respect to morphologic examinations (identification of

cell types and microorganisms; review of electrophoretic patterns, etc.), group review and consensus identifications are permitted only for unknown samples that would ordinarily be reviewed by more than one person in an actual patient sample.

If the laboratory uses multiple methods for an analyte, proficiency testing samples should be analyzed by the primary method. The educational purposes of proficiency testing are best served by a rotation that allows all technologists to be involved in the proficiency testing program. Proficiency testing records must be retained and can be an important part of the competency and continuing education documentation in the personnel files of the individuals. When external proficiency testing materials are not available, the semi-annual alternative performance assessment process should also be integrated within the routine workload, if practical.

COMMENTARY:

N/A

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):7146 [42CFR493.801(b)]; 2) Shahangian S, *et al.* Toward optimal PT use. *Med Lab Observ.* 2000;32(4):32-43.

*****REVISED** 09/27/2007***

MOL.10200 Phase II N/A YES NO

Is there ongoing evaluation of PT and alternative assessment results, with prompt corrective action taken for unacceptable results?

NOTE: Compliance with this item can be examined by selecting a sample of PT evaluation results and alternative assessment records. Special attention should be devoted to unacceptable results. Compliance requires that all of the following are true:

1. *There is documented evidence of ongoing review of all PT reports and alternative assessment results by the laboratory director or the director's designee. Reviews should be completed within one month of the date reports and results become available to the laboratory.*
2. *All "unacceptable" PT results and alternative assessment test result have been investigated.*
3. *Corrective action has been initiated for all unacceptable PT and alternative assessment results. Corrective action is appropriate to the nature and magnitude of the problem; it might consist of staff education, instrument recalibration, change in procedures, institution of new clerical checks, discontinuation of patient testing for the analyte or discipline in question, or other appropriate measures.*
4. *Primary records related to PT and alternative assessment testing are retained for two years (unless a longer retention period is required elsewhere in this checklist for*

specific analytes or disciplines). These include all instrument tapes, work cards, computer printouts, evaluation reports, evidence of review, and documentation of follow-up/corrective action.

COMMENTARY:

N/A

REFERENCES: 1) Clinical and Laboratory Standards Institute (CLSI). *Using Proficiency Testing to Improve the Clinical Laboratory; Approved Guideline—Second Edition.* CLSI document GP27-A2 (ISBN 1-56238-632-8). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 200; 2) Shahangian S, *et al.* Toward optimal PT use. *Med Lab Observ.* 2000;32(4):32-43; 3) Zaki Z, *et al*. Self-improvement by participant interpretation of proficiency testing data from events with 2 to 5 samples. *Clin Chem*. 2000;46:A70.

*****NEW***** ***12/12/2006***

MOL.13466 Phase II N/A YES NO

Is there a policy that prohibits interlaboratory communication about proficiency testing samples until after the deadline for submission of data to the proficiency testing provider?

COMMENTARY:

N/A

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):7146 [42CFR493.801(b)(3)]; 2) Bierig JR. Comparing PT results can put a lab's CLIA license on the line. Northfield, IL: College of American Pathologists *CAP Today*. 2002;16(2):84-87.

*****NEW***** ***12/12/2006***

MOL.16732 Phase II N/A YES NO

Is there a policy that prohibits referral of proficiency testing specimens to another laboratory?

NOTE: Under CLIA-88 regulations, there is a strict prohibition against referring proficiency testing specimens to another laboratory. In other words, the laboratory may not refer a proficiency testing specimen to a laboratory with a different CLIA number (even if the second laboratory is in the same health care system).

COMMENTARY:

N/A

REFERENCE: Department of Health and Human Services, Centers for Medicare & Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 1992(Feb 28): [42CFR493.801(b)(4)].

****

QUALITY MANAGEMENT AND QUALITY CONTROL

****

GENERAL ISSUES

MOL.20000 Phase II **N/A YES NO**

Does the molecular pathology laboratory have a written quality management/quality control (QM/QC) program?

NOTE: The QM/QC program in the molecular pathology laboratory must be clearly defined and documented. The program must ensure quality throughout the preanalytic, 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 quality management (QM) questions in the Laboratory General Checklist pertain to the molecular pathology laboratory.

COMMENTARY:

N/A

REFERENCE: 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):7170 [42CFR493.1265], 7176 [42CFR493.1445(e)(5)].

MOL.20050 Phase II N/A YES NO

Is there a documented system in operation to detect and correct significant clerical and analytical errors, and unusual laboratory results, in a timely manner?

NOTE: The laboratory must have a documented system in operation to detect and correct significant clerical and analytical errors, and unusual laboratory results. One common method is review of results by a qualified person (technologist, supervisor, pathologist) before release from the laboratory, but there is no requirement for supervisory review of all reported data. The selective use of delta checks also may be useful in detecting clerical errors in consecutive samples from the same patient/client. 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 suspected errors detected by the end user after reporting, corrections must be promptly made if such errors are confirmed by the laboratory.

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.

COMMENTARY:

N/A

MOL.20200 Phase II N/A YES NO

Is there a documented procedure describing methods for specimen preservation and storage before testing, consistent with good laboratory practice?

COMMENTARY:

N/A

REFERENCES: 1) Schultz CL, *et al*. A lysis, storage, and transportation buffer for long-term, room-temperature preservation of human clinical lymphoid tissue samples yielding high molecular weight genomic DNA suitable for molecular diagnosis. *Am J Clin Pathol.* 1999;111:748-752; 2) Makowski GS, *et al.* In situ PCR amplification of Guthrie card DNA to detect cystic fibrosis mutations. *Clin Chem.* 1996;41:471-479; 3) Farkas DH, *et al.* Specimen stability for DNA-based diagnostic testing. *Diagn Mol Pathol.* 1996;5:227-235; 4) Kaul K, *et al.* Amplification of residual DNA sequences in sterile bronchoscopes leading to false-positive PCR results. *J Clin Microbiol.* 1996;34:1949-1951; 5) Kessler HH, *et al.* Effects of storage and types of blood collection tubes on hepatitis C virus level in

whole blood samples. *J Clin Microbiol.* 2001;39:1788-1790; 6) Tsui NMY, *et al.* Stability of endogenous and added RNA in blood specimens, serum, and plasma. *Clin Chem.* 2002;48:1647-1653; 7) Rainen L, *et al.* Stabilization of mRNA expression in whole blood samples. *Clin Chem.* 2002;48:1883-1890; 8) Pahl A, Brune K. Stabilization of gene expression profiles in blood after phlebotomy. *Clin Chem.* 2002;48:2251-2253.

MOL.20300 Phase I N/A YES NO

Is there evidence that the laboratory monitors sample turnaround times and that they are appropriate for the intended purpose of the test?

NOTE: Appropriate turnaround times will vary by test type and clinical application. There are certain clinical situations in which rapid completion is essential. For example, inappropriate delays in completing a prenatal diagnosis test can cause unacceptable emotional stress for the parents, make ultimate pregnancy termination (if chosen) much more difficult, or even render the results of the test unusable.

COMMENTARY:

N/A

MOL.20550 Phase I N/A YES NO

When appropriate, are statistics on molecular pathology test results (*e.g.*, percentages of normal and abnormal findings, allele frequencies) maintained, and appropriate comparative studies performed?

NOTE: Periodic evaluation of test result statistics (rates of positives and negatives) can be used to identify changes in test performance or shifts in populations being tested.

COMMENTARY:

N/A

MOL.22458 Phase II N/A YES NO

Are procedures documented to prevent specimen loss, alteration, or contamination?

COMMENTARY:

N/A

MOL.29290 Phase II N/A YES NO

Is there documentation of relevant data for all DNA probes, primers, and other nucleic acid reagents?

NOTE: Important data on DNA probes and PCR primers that must be available in the laboratory include the size, the complete or partial nucleotide sequence, chromosomal map location of the target sequence, allele frequencies of the mutation in various ethnic groups, recombination frequencies (for linkage probes), cloning vector, relevant restriction endonuclease sites, method of preparation, and relevant literature citations. Sequence and size data may not be available for commercially-obtained tests when this information is considered proprietary.

COMMENTARY:

N/A

MOL.29405 Phase II N/A YES NO

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

NOTE: The CAP does NOT require supervisory review of all test results before or after reporting to patient records. Rather, this question is intended to address only that situation defined under CLIA-88 for "high complexity testing" performed by trained high school graduates qualified under 42CFR493.1489(b)(5) when a qualified general supervisor is not present.

COMMENTARY:

N/A

REFERENCE: 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)].

MOL.29520 Phase II N/A YES NO

Are critical results established when important for prompt patient management decisions?

NOTE: *As applicable, critical results must be established for appropriate tests, so that immediate notification of a physician or other clinical personnel responsible for patient care occurs. These may be indicated in the procedure manual and/or in a separate manual or policy. The bench technologists must be familiar with critical results for procedures that they perform.*

COMMENTARY:

N/A

PROCEDURE MANUAL

The procedure manual should be used by personnel at the workbench and should include: test principle, clinical significance, specimen type, required reagents, test calibration, quality control, procedural steps, calculations, reference intervals, and interpretation of results. The manual should address relevant pre-analytic and post-analytic considerations, as well as the analytic activities of the laboratory. The specific style and format of procedure manuals are at the discretion of the director.

The inspection team should review the procedure manual in detail to understand the laboratory's standard operating procedures, ensure that all significant information and instructions are included, and that actual practice matches the contents of the procedure manuals.

*****REVISED** 12/12/2006***

MOL.29750 Phase II N/A YES NO

Is a complete, current procedure manual available at the workbench or in the work area?

NOTE 1: The use of inserts provided by manufacturers is not acceptable in place of a procedure manual. However, such inserts may be used as part of a procedure description, if the insert accurately and precisely describes the procedure as performed in the laboratory. Any variation from this printed or electronic procedure must be detailed in the procedure manual. In all cases, appropriate reviews must occur.

NOTE 2: A manufacturer's procedure manual for an instrument/reagent system may be acceptable as a component of the overall departmental procedures. Any modification to or deviation from the procedure manual must be clearly documented.

NOTE 3: Card files or similar systems that summarize key information are acceptable for use as quick reference at the workbench provided that:

a. A complete manual is available for reference
b. The card file or similar system corresponds to the complete manual and is subject to document control

NOTE 4: Electronic (computerized) manuals are fully acceptable. There is no requirement for paper copies to be available for the routine operation of the laboratory, so long as the

electronic versions are readily available to all personnel. However, procedures must be available to laboratory personnel when the electronic versions are inaccessible (e.g., during laboratory information system or network downtime); thus, the laboratory must maintain either paper copies or electronic copies on CD or other media that can be accessed via designated computers. All procedures, in either electronic or paper form, must be readily available for review by the inspector at the time of the CAP inspection.

Electronic versions of procedures must be subjected to proper document control (i.e., only authorized persons may make changes, changes are dated/signed (manual or electronic), and there is documentation of annual review). Documentation of review of electronic procedures may be accomplished by including statements such as "reviewed by [name of reviewer] on [date of review]" in the electronic record. Alternatively, paper review sheets may be used to document review of electronic procedures. Documentation of review by a secure electronic signature is NOT required.

COMMENTARY:

N/A

REFERENCES: 1) College of American Pathologists, Commission on Laboratory Accreditation. Standards for laboratory accreditation; Standard III. Northfield, IL: CAP, 1998; 2) van Leeuwen AM. 6 Steps to building an efficiency tool. *Advance/Laboratory*. 1999:8(6):88-91; 3) Borkowski A, *et al.* Intranet-based quality improvement documentation at the Veterans Affairs Maryland health care system. *Mod. Pathol*. 2001;14:1-5; 4) 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.

MOL.29865 Phase II **N/A YES NO**

Is there documentation of at least annual review of all policies and procedures by the current director or designee?

NOTE: The director must ensure that the collection of policies and technical protocols is complete, current, and has been thoroughly reviewed by a knowledgeable person. Technical approaches must be scientifically valid and clinically relevant. To minimize the burden on the laboratory and reviewer(s), it is suggested that a schedule be developed whereby roughly 1/12 of all procedures are reviewed monthly. Paper/electronic signature review must be at the level of each procedure, or as multiple signatures on a listing of named procedures. A single signature on a Title Page or Index of all procedures is not sufficient documentation that each procedure has been carefully reviewed. Signature or initials on each page of a procedure is not required.

COMMENTARY:

N/A

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):7173 [42CFR493.1407(e)(13)]; 2) Borkowski A, *et al.* Intranet-based quality improvement documentation at the Veterans Affairs Maryland health care system. *Mod. Pathol*. 2001;14:1-5.

MOL.29980 Phase II N/A YES NO

Does the director (or a designee who meets CAP director qualifications) review and approve all new policies and procedures, as well as substantial changes to existing documents, before implementation?

NOTE: Current practice must match the written procedure and all procedure manual changes must be initialed and dated by the director or designee.

COMMENTARY:

N/A

REFERENCE: 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 [42CFR493.1251(d)].

MOL.30095 Phase II N/A YES NO

If there is a change in directorship, does the new director ensure (over a reasonable period of time) that laboratory procedures are well-documented and undergo at least annual review?

COMMENTARY:

N/A

REFERENCE: 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 [42CFR493.1251(d)].

MOL.30210 Phase II N/A YES NO

When a procedure is discontinued, is a paper or electronic copy maintained for at least 2 years, recording initial date of use, and retirement date?

NOTE: In general, it is recommended that to meet the requirements of some states relating to the testing of minors (under the age of 21), laboratories should maintain all versions of the procedure manuals for at least 23 years (to cover the interval from the fetal period to age 21).

COMMENTARY:

N/A

REFERENCE: 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 [42CFR493.1251(e)].

MOL.30325 Phase II N/A YES NO

Does the laboratory have a system documenting that all analysts are knowledgeable about the contents (including changes) of procedure manuals relevant to the scope of their testing activities?

NOTE: This does not specifically require annual procedure sign-off by testing personnel. The form of this system is at the discretion of the director.

COMMENTARY:

N/A

MOL.30440 Phase II N/A YES NO

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

NOTE: Quantitative testing requires that the dynamic range of the assay be defined and assay performance tested with controls in each run, including a negative, low positive, and high positive control. When melting curves are generated, there should be criteria for interpreting results.

COMMENTARY:

N/A

MOL.30555 Phase II N/A YES NO

Are there written guidelines for analytic interpretation of results?

NOTE: For a qualitative assay, the procedure manual should describe, for example, the expected band pattern, melting temperature, or numeric cutoff to distinguish a positive from a negative result. For a quantitative assay, the manual should describe, for example, the criteria for verifying test performance characteristics of the run (e.g., assay sensitivity and linearity are within pre-established range, there is no significant inhibitor of the patient reaction, the calculated value appears reasonable from visual inspection of raw data) prior to releasing the quantitative result.

COMMENTARY:

N/A

MOL.30670 Phase II N/A YES NO

Does the procedure manual describe clinical indications for ordering the tests and clinical utility of the test in patient management, with pertinent literature references?

COMMENTARY:

N/A

ASSAY VALIDATION

It is important to confirm the analytical performance characteristics of the assay and to confirm the clinical validity of the assay. Performance characteristics that should be determined prior to reporting patient test results include diagnostic and analytical sensitivity, diagnostic and analytical specificity, precision, linearity (for quantitative tests), the reportable range of patient test results; the reference range (normal values); and any other applicable performance characteristic. (Refer to Subpart K,CFR §493.1253)

Analytic sensitivity refers to the ability of an assay to detect a given analyte (i.e., the lower limit of detection; for example, the ability to detect a clonal immunoglobulin gene rearrangement comprising only 1-5% of the cell population). Analytic specificity refers to the degree to which interfering substances are not detected by an assay.

Precision refers to the reproducibility of a test result (e.g., within-technologist, between-technologist, within-run and between-run).
Clinical sensitivity (also called diagnostic sensitivity) refers to the ability of an assay to detect a disease or clinical condition, while clinical specificity refers to the degree to which an assay is negative when disease is absent.

Diagnostic sensitivity and specificity must be determined relative to some "gold standard" (e.g., biopsy findings, clinical findings, etc.). The sensitivity of an assay equals [TP/(TP+FN)] X 100 and the

specificity of an assay equals [TN/(TN+FP)] X 100. (TP=true positive, TN=true negative, FN=false negative, FP=false positive.) Determinations of sensitivity and specificity should be done in a "blinded" fashion (i.e., without prior knowledge of the patient's disease status). For some disorders, it may not be possible to identify large numbers of positives (i.e., patients with the disorder) to establish the diagnostic sensitivity of the assay. In such instances, the laboratory should procure as many positive cases as is reasonably possible for method validation and in addition cite any publications that have investigated the diagnostic sensitivity of the assay.

MOL.30785 Phase II N/A YES NO

Is there documentation that the laboratory has performed validation studies to establish the performance characteristics of laboratory-developed assays?

NOTE: Laboratory-developed assays are defined as tests developed in-house, and FDA-cleared tests that have been modified by the laboratory. A summary of the validation data for all laboratory-developed tests introduced since the last on-site inspection should be available to the inspector. The inspector should also check validation data for assays introduced prior to the last on-site inspection.

COMMENTARY:

N/A

REFERENCES: 1) Association for molecular pathology statement. Recommendations for in-house development and operation of molecular diagnostic tests. *Am J Clin Pathol.* 1999;111:449-463; 2) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1253(b)(2)].

MOL.30900 Phase II N/A YES NO

Do validation studies include specimens representing each of the possible reportable results (genotypes)?

NOTE: Assays for genetic disorders with a limited number of possible genotypes (e.g., hereditary hemochromatosis) should confirm the ability of the assay to detect these genotypes. Assays for genetic disorders with considerable allelic heterogeneity (e.g., cystic fibrosis or hereditary nonpolyposis colorectal cancer) should confirm the ability of the assay to detect a high percentage of the possible genotypes. However, it will not be possible to document that such assays can detect every possible genotype.

COMMENTARY:

N/A

MOL.30957 Phase II N/A YES NO

For FDA-cleared/approved assays, are performance criteria verified by the laboratory?

NOTE: For example, the laboratory must verify the established performance specifications of FDA-approved assays (accuracy, precision, analytic sensitivity, interferences, reference range, and reportable range, as applicable) Refer to the Test Method Validation *section of the Laboratory General checklist for additional details.*

COMMENTARY:

N/A

REFERENCE: Department of Health and Human Services, Centers for Medicare & Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1253(b)(2)].

MOL.31015 Phase II N/A YES NO

Were validation studies with an adequate number and representative (reasonable) distribution of samples performed for each type of specimen expected for the assay (*e.g.*, blood, fresh/frozen tissue, paraffin-embedded tissue, prenatal specimens?

COMMENTARY:

N/A

MOL.31130 Phase II N/A YES NO

Were the results of each validation study compared to another valid assay, such as comparison to another test method or specimen exchange with a laboratory performing the same type of test?

NOTE: There may not be a closely related assay to be used for comparison. In such cases, the assay performance (sensitivity and specificity) should be assessed in relation to the patient's clinical diagnosis and in addition assessed by exchanges of specimens with a laboratory that performs the assay in a similar fashion.

COMMENTARY:

N/A

MOL.31245 Phase II N/A YES NO

For qualitative assays, are the reference value and reportable range defined?

NOTE: For qualitative assays (e.g., assays for germline mutation), the laboratory must define the reference value (normal versus abnormal result) and reportable outcomes (e.g., homozygous wild type, heterozygous or homozygous mutant). If the reference value depends on the clinical situation, then a plan for interpreting the patient result must be defined.

COMMENTARY:

N/A

REFERENCES: 1) American College of Medical Genetics Laboratory. Standards and Guidelines for Clinical Genetics Laboratories, 3rd ed. Bethesda, MD: ACMG, 2003. Available at: http://www.acmg.net Accessed 2006; 2) NCCLS. *Fluorescence* In Situ *Hybridization (FISH) Methods for Medical Genetics; Approved Guideline*. NCCLS document MM7-A (ISBN 1-56238-524-0). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 1908-1898 USA, 2004.

MOL.31360 Phase II N/A YES NO

For QUANTITATIVE assays, are the reference and reportable ranges defined?

NOTE: Reference and reportable ranges are pertinent to quantitative assays (e.g. assays for tumor burden, chimerism or pathogen load). The "reference range" is the range of results expected in the "normal" population, while the "reportable range" encompasses the full range of reported values. The laboratory must define the analytic measurement range (AMR) as described in the "Quantitative Assays; Calibration and Standards" section of the checklist. The laboratory must also determine how to handle positive patient results below or above the AMR, since numerical values outside the AMR may be inaccurate. For example, these may be reported a <x or>y, or they may be reported as "low positive" or " high positive" along with an explanation that values outside the linear range cannot be quantitated, or the sample may be concentrated or diluted and rerun to calculate an accurate value within the reportable range.

COMMENTARY:

N/A

MOL.31475 Phase II N/A YES NO

Do validation studies document test accuracy, analytical sensitivity, analytical specificity and precision?

COMMENTARY:

N/A

***REVISED** 09/27/2007*

MOL.31590 Phase II N/A YES NO

Are the clinical performance characteristics of each assay documented, using either literature citations or a summary of internal study results?

NOTE: The clinical performance characteristics of a test relate to its diagnostic sensitivity and specificity, and its positive and negative predictive values in the (various) target population(s). Issues that affect the clinical interpretation of a test which should be considered include (1) the clinical setting in which the test is used, (2) genotype/phenotype associations when these vary with particular mutations or polymorphisms, and (3) genetic, environmental or other factors which modify the clinical expression of the genetic alteration detected.

Establishing clinical validity may require extended studies and monitoring that go beyond the purview or control of the individual laboratory. In such cases, it is acceptable to provide documentation in the form of peer-reviewed studies in the scientific literature. It is essential that directors use their professional judgment in evaluating the results of such studies and in monitoring the state-of-the-art worldwide as it applies to newly discovered gene targets and potential new tests, especially those of a predictive or incompletely penetrant nature.

COMMENTARY:

N/A

REFERENCES: 1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, Third Edition, 2002, Section C8; 2) Clinical and Laboratory Standards Institute (CLSI). *Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline—Second Edition*. CLSI document MM1-A2 (ISBN 1-56238-615-8). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006.

MOL.31705 Phase II N/A YES NO

Do reports for laboratory-developed assays contain a description of the method, a statement that the assay was developed by the laboratory, and appropriate performance characteristics?

NOTE: General guidelines for reports are given in the Results Reporting section of this checklist. Particularly with respect to newly implemented assays, careful attention should be given to including in the report analytical and clinical performance specifications.

COMMENTARY:

N/A

MOL.31935 Phase I N/A YES NO

If the laboratory modifies an FDA-approved assay, has the modified procedure been validated to yield equivalent or superior performance?

COMMENTARY:

N/A

REFERENCES: 1) American College of Medical Genetics Laboratory. Standards and Guidelines for Clinical Genetics Laboratories, 3rd ed. Bethesda, MD: ACMG, 2003. Available at: http://www.acmg.net Accessed 2006; 2) NCCLS. *Fluorescence* In Situ *Hybridization (FISH) Methods for Medical Genetics; Approved Guideline*. NCCLS document MM7-A (ISBN 1-56238-524-0). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 1908-1898 USA, 2004.

MOL.32050 Phase II N/A YES NO

Is there a summary statement, signed by the laboratory director or designee, documenting review of validation studies and approval of the test for clinical use?

COMMENTARY:

N/A

REQUISITIONS

Requisitions should be designed to allow for sufficient identification of the patient and physician and any clinically pertinent information. Routinely required information elements are identified in the Laboratory General Checklist, and are not repeated here. The following represent additional information for molecular pathology assays.

MOL.32300 Phase II N/A YES NO

Are racial/ethnicity data provided to the laboratory, when appropriate?

COMMENTARY:

N/A

MOL.32350 Phase II N/A YES NO

Are test requests accompanied with a pedigree, when appropriate (*e.g.*, for linkage analysis)?

COMMENTARY:

N/A

SPECIMEN HANDLING

MOL.33050 Phase II N/A YES NO

Do the instructions for specimen collection and handling include all of the following?

1. **Patient preparation**
2. **Proper labeling and identification of specimens**
3. **Proper collection of specimens from all relevant sources**
4. **Proper shipping and delivery of specimens**
5. **Proper specimen preservation if processing before testing**

COMMENTARY:

N/A

REFERENCES: 1) Makowski GS, *et al*. *In situ* PCR amplification of Guthrie card DNA to detect cystic fibrosis mutations. *Clin Chem.* 1996;41:471-479; 2) Farkas DH, *et al*. Specimen stability for DNA-based diagnostic testing. *Diagn Mol Pathol.* 1996;5:227-235; 3) Kaul K, *et al*. Amplification of residual DNA sequences in sterile bronchoscopes leading to false-positive PCR results. *J Clin Microbiol.* 1996;34:1949-1951; 4) Kessler HH, *et al*. Effects of storage and types of blood collection tubes on hepatitis C virus level in whole blood samples. *J Clin Microbiol.* 2001;39:1788-1790; 5) Tsui NMY, *et al*. Stability of endogenous and added RNA in blood specimens, serum, and plasma. *Clin Chem.* 2002;48:1647-1653; 6) Rainen L, *et al.* Stabilization of mRNA expression in whole blood samples. *Clin Chem.* 2002;48:1883-1890; 7) Pahl A, Brune K. Stabilization of gene expression profiles in blood after phlebotomy. *Clin Chem.* 2002;48:2251-2253; 8) Schultz CL, *et al*. A lysis, storage, and transportation buffer for long-term, room-temperature preservation of human clinical lymphoid tissue samples yielding high molecular weight genomic DNA suitable for molecular diagnosis. *Am J Clin Pathol.* 1999; 111:748-752..

MOL.33150 Phase II N/A YES NO

Are stored specimens maintained in a way to allow prompt retrieval for further testing?

COMMENTARY:

N/A

MOL.33250 Phase II N/A YES NO

Are specimens retained in compliance with applicable laws and regulations?

NOTE: CAP retention guidelines may be found in the Quality Management section of the Laboratory General checklist, and also at http://www.cap.org/. However, state or local laws and/or regulations may be more stringent than CAP guidelines.

Retention of fluorochrome-stained slides should be determined by the laboratory director.

COMMENTARY:

N/A

REFERENCE: http://www.ncsl.org/programs/health/Genetics/charts.htm.

MOL.33350 Phase II N/A YES NO

Are there written criteria for rejection of unacceptable or possibly commingled specimens?

NOTE: Unacceptable specimens include those that are mislabeled, submitted in the wrong anticoagulant, or are an inappropriate specimen type. In addition, because of the high sensitivity and potential for contamination in molecular testing involving amplification of DNA, the laboratory must be alert to the possibility of commingled specimens. An example of a potentially commingled specimen is one that is received after the specimen container was entered by a sampling device that enters multiple samples, albeit with rinses in between specimens. If such samples must be tested by molecular methods, the results should be interpreted with caution, considering the potential for contamination.

COMMENTARY:

N/A

MOL.33370 Phase II N/A YES NO

Is the disposition of all unacceptable specimens documented in the patient report and/or quality management records?

COMMENTARY:

N/A

MOL.33450 Phase II N/A YES NO

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

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

COMMENTARY:

N/A

SPECIMEN PROCESSING

MOL.33491 Phase II N/A YES NO

Is the submitting physician (or requester) promptly notified when a specimen is inadequate or if insufficient nucleic acid is isolated?

COMMENTARY:

N/A

MOL.33532 Phase I N/A YES NO

Are ribonuclease-free conditions maintained for all assays that detect RNA or use an RNA probe?

NOTE: RNA is extremely susceptible to degradation by ribonucleases that are ubiquitous in the environment. To ensure preservation of target RNA or RNA probes, special precautions are needed.

COMMENTARY:

N/A

REFERENCE: Gulley ML, *et al*. Guidelines for interpreting EBER *in situ* hybridization and LMPI immunohistochemical tests for detecting Epstein-Barr virus in Hodgkin lymphoma. *Am J Clin Pathol. 2002*;117:259-267.

MOL.33573 Phase II N/A YES NO

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

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

COMMENTARY:

N/A

MOL.33614 Phase II N/A YES NO

Are patient samples processed promptly or stored appropriately to minimize degradation of nucleic acids?

COMMENTARY:

N/A

REFERENCES: 1) Farkas DH, Kaul KL, Wiedbrauk DL, *et al*. Specimen Collection and Storage for Diagnostic Molecular Pathology Investigation. *Arch Pathol Lab Med*. 1996;120:591-596; 2) Kiechle FL, Kaul KL, Farkas DH. Mitochondrial Disorders: Methods and Specimen Selection for Diagnostic Molecular Pathology. *Arch Pathol Lab Med*. 1996;120:597-603; 3) Farkas DH, Drevon AM, Kiechle FL, *et al*. Specimen Stability for DNA-based Diagnostic Testing. *Diag Molec Pathol*. 1996;5(4):227-235.

QUANTITATIVE ASSAYS; CALIBRATION AND STANDARDS

This section of the checklist is similar to the section of the Chemistry checklist dealing with the processes of calibration, calibration verification, and analytic measurement range (AMR) validation for quantitative assays.

CALIBRATION is the set of operations that establish, under specified conditions, the relationship between reagent system/instrument response and the corresponding concentration/activity values of an analyte. Calibration procedures are typically specified by a method manufacturer, but may also be established by the laboratory. The term "calibration" has the same meaning in this checklist as in the US CLIA'88 regulations.

However, the term "calibration verification," as used in this checklist, carries a more restrictive meaning than in CLIA'88. As defined in the January, 2003 revision of CLIA'88, "calibration verification" refers to 2 distinct processes: 1) verification of correct method calibration and 2) validation of the reportable range. This checklist restricts the use of the term "calibration verification" to the first process. The checklist uses a different term, "analytic measurement range(AMR) validation" to refer to the second process.

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

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

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

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

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

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

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

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

RECALIBRATION/CALIBRATION VERIFICATION and AMR VALIDATION INTERVAL: Recalibration or calibration verification, and AMR validation must be performed at least once every 6 months, as specified under CLIA-88 regulations at 42CFR493.1255(b)(3). Successful calibration verification certifies that the calibration is still valid; unsuccessful calibration verification requires remedial action, which usually includes recalibration. The performance of recalibration or a calibration verification procedure resets the calendar to a new maximum 6-month interval before the next required reassessment. Methods that are recalibrated more frequently than every 6 months do not require a separate calibration verification procedure.

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

determine what constitutes a verification and revalidation of the AMR. Manufacturers' instructions should be followed.

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

MOL.33655 Phase II N/A YES NO

Are calibration procedures for each method adequate, and are the calibration results documented?

COMMENTARY:

N/A

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. Clinical and Laboratory Standards Institute document EP14-A2 (ISBN 1-56238-561-5). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, 2005; 5) 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; 6) Kroll MH, *et al*. Evaluation of the extent of non linearity in reportable range studies. *Arch Pathol Lab Med.* 2000;124:1331-1338.

MOL.33696 Phase II N/A YES NO

Are high quality materials with method- and matrix-appropriate target values used for calibration and calibration verification whenever possible?

COMMENTARY:

N/A

REFERENCE: Clinical and Laboratory Standards Institute. *Evaluation of Matrix Effects; Approved Guideline—Second Edition*. Clinical and Laboratory Standards Institute document EP14-A2 (ISBN 1-56238-561-5). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2005.

MOL.33737 Phase II N/A YES NO

Are all calibration materials used for non-FDA cleared assays documented as to quality?

NOTE: Commercial standards used to prepare calibrators require certificates of quality from the vendor, or a quality check 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.

COMMENTARY:

N/A

MOL.33778 Phase II N/A YES NO

Are all calibration materials properly labeled as to content and calibration values?

NOTE: Complete values need not be recorded directly on each vial of calibrator material, so long as there is a clear indication where specific values may be found.

COMMENTARY:

N/A

MOL.33819 Phase II N/A YES NO

Do calibration materials include dates placed in service and expiration dates?

NOTE: 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.

COMMENTARY:

N/A

MOL.33860 Phase II N/A YES NO

Are criteria established for frequency of calibration or calibration verification, and the acceptability of results?

NOTE: Criteria typically include:

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

COMMENTARY:

N/A

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.

MOL.33901 Phase II N/A YES NO

Is the system recalibrated when calibration verification fails to meet the established criteria of the laboratory?

COMMENTARY:

N/A

MOL.33942 Phase II N/A YES NO

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

NOTE: Calibration, calibration verification, and validation of the analytical measurement range (AMR) are required to substantiate the continued accuracy of a test method.

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 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. The 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 lots of analytically critical reagents or major system components.

COMMENTARY:

N/A

REFERENCE: 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].

MOL.33983 Phase II N/A YES NO

Are criteria established for validating the analytical measurement range, and is compliance documented?

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

COMMENTARY:

N/A

*****NEW**** ***12/12/2006***

MOL.34003 Phase II N/A YES NO

If the laboratory uses more than one instrument to measure a given analyte, are the instruments checked against each other at least twice a year for correlation of patient/client results?

NOTE: This question applies to quantitative tests performed on the same or different instrument makes/models.

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

COMMENTARY:

N/A

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.

MOL.34024 Phase II N/A YES NO

Are calibrators and controls 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. For example, when using commercial calibrators and controls, use a different lot number for calibration than is used for QC, whenever possible.

COMMENTARY:

N/A

REFERENCE: 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)].

REAGENTS

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

MOL.34065 Phase II N/A YES NO

Are new reagent lots and/or shipments checked against old reagent lots or with suitable reference material before or concurrently with being placed in service?

NOTE: For qualitative tests, minimum cross-checking includes retesting at least one known positive and one known negative patient sample from the old reagent lot or shipment against the new reagent lot, ensuring that the same results are obtained with the new lot. For quantitative assays, several patient samples should be run at different levels to check the calibration of the system. Good clinical laboratory science includes patient-based comparisons in many situations, since it is patient results that are "controlled". A weakly positive control should also be used in systems where patient results are reported in that fashion. The use of QC material to check new reagent lots/shipments is acceptable, but the laboratory should be aware that matrix interference may affect such material and mask a change in patient results.

COMMENTARY:

N/A

MOL.34106 Phase II N/A YES NO

Are reagents and solutions 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. The inspector will describe specific issues of non-compliance in the Inspector's Summation Report.

COMMENTARY:

N/A

MOL.34147 Phase II N/A YES NO

Are all reagents 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 contamination.

COMMENTARY:

N/A

REFERENCE: 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)].

MOL.34188 Phase II N/A YES NO

Is sufficient information documented regarding the nature of any probe or primer used in an assay to permit interpretation and troubleshooting of test results?

NOTE: Items of importance where appropriate include: the type (genomic, cDNA, oligonucleotide or riboprobe) and origin (human, viral, etc.) of the probe or sequence; the oligonucleotide sequence and complementary sequence or gene region recognized; an appropriate restriction enzyme map of the DNA; known polymorphisms, sites resistant to endonuclease digestion, and cross-hybridizing bands; the labeling methods used and standards for adequacy of hybridization or amplification. For linkage analysis, recombination frequencies and map positions must be documented. Loci should be designated as defined by the Human Gene Mapping Nomenclature Committee. For inherited disease tests, additional information such as chromosomal location of the target, allele frequencies of the mutation in various ethnic groups, and recombination frequencies (for linkage probes) may be required.

COMMENTARY:

N/A

REFERENCE: McAlpine PJ, et al. The Catalog of mapped genes and report of the nomenclature committee. Human gene mapping. Cytogenet Cell Genet. (most recent version).

CONTROLS

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

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

MOL.34229 Phase II N/A YES NO

For QUALITATIVE tests, are positive, negative and sensitivity controls run for each assay, when available, appropriate, and practical?

NOTE: Ideally, one should use a positive control for each analyte in each run. However, in some circumstances such as in a large mutation panel for cystic fibrosis, this procedure is not practical. One way to address this situation is to rotate positive controls in a systematic fashion and at a frequency determined by the director. The sensitivity control is used to verify detection of low-level target sequences, e.g., pathogens or tumor markers.

COMMENTARY:

N/A

REFERENCE: 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)(3)(II)].

MOL.34270 Phase II N/A YES NO

For QUANTITATIVE tests, are control materials at more than one concentration (level) used in every run?

NOTE: Controls should verify assay performance at relevant analytic and clinical decision points.

COMMENTARY:

N/A

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; 2) Ye JJ, *et al*. Performance evaluation and planning for patient/client-based quality control procedures. *Am J Clin Pathol.* 2000;113:240-248..

MOL.34311 Phase II N/A YES NO

Are tolerance and acceptability limits defined for all control procedures, control materials, and standards?

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

COMMENTARY:

N/A

MOL.34352 Phase II N/A YES NO

Are the results of controls verified for acceptability before reporting of results?

NOTE: Controls must be reviewed before reporting patient results. It is implicit in quality control that patient test results will not be reported when controls are unacceptable.

COMMENTARY:

N/A

REFERENCE: 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):7166 [42CFR493.1218(e)].

MOL.34393 Phase II N/A YES NO

Is there evidence of corrective action when control results exceed defined tolerance limits?

NOTE: Patient test results obtained in an analytically unacceptable test run or since the last acceptable test run must be evaluated to determine if there is a significant clinical difference in patient 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).

COMMENTARY:

N/A

REFERENCE: 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)].

MOL.34434 Phase II N/A YES NO

Are control specimens tested in the same manner and by the same personnel (including specimen preparation) as patient samples?

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

COMMENTARY:

N/A

REFERENCE: 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):7166 [42CFR493.118(c)].

MOL.34475 Phase II N/A YES NO

For QUANTITATIVE assays, are quality control statistics performed at specified internals to define analytic imprecision and to monitor trends over time?

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

COMMENTARY:

N/A

REFERENCES: 1) Mukherjee KL. Introductory mathematics for the clinical laboratory. Chicago: ASCP, 1979:81-94; 2) Barnett RN. Clinical laboratory statistics, 2nd ed. Boston, M; 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. NY, NY: Karger, 1988; 5) Department of Health and Human Services, CMS. 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 of clinical laboratory precision. *Arch Pathol Lab Med.* 1995;119:495-513; 7) 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; 8) Brooks ZC, *et al*. Critical systematic error used of varied QC rules in routine chemistry. *Clin Chem*. 2000;46:A70.

*****NEW***** ***12/12/2006***

MOL.34495 Phase II N/A YES NO

Are quality control data reviewed and assessed at least monthly by the laboratory director or designee?

COMMENTARY:

N/A

MOL.34516 Phase I N/A YES NO

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

NOTE: This checklist question applies only to tests that report qualitative results based on a quantitative measurement using a cut-off threshold to discriminate between a positive and negative analytic interpretation. The cut-off value that distinguishes a positive from a negative result should be established when the test is initially placed in service, and verified every six months thereafter. The process and materials for establishing and verifying the cut-off are identical to those recommended for calibration verification (listed in the introduction to the Quantitative Assays: Calibration and Standards section of the Molecular Pathology Checklist) except that instead of calibrating a full range of values as is done for a quantitative test, only the cut-off value is evaluated. If the value of a control material or calibration verification material is near that of the cut-off, then the process of quality control, calibration or calibration verification satisfies this checklist question.

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.

For commercial assays that have a cut-off established by the vendor, the laboratory should run external controls to ensure that the assays return expected values.

COMMENTARY:

N/A

MOL.34557 Phase I N/A YES NO

Are controls stored in a manner that maintains their integrity?

COMMENTARY:

N/A

ANALYSIS

.....

Extraction

.....

MOL.34600 Phase II N/A YES NO

Are nucleic acids extracted and purified by methods reported in the literature, by an established commercially available kit or instrument, or is there validation of a method developed in-house?

COMMENTARY:

N/A

MOL.34700 Phase II N/A YES NO

Is the quantity of nucleic acid measured, when appropriate?

NOTE: The quantity of nucleic acid must be measured prior to use in a procedure whose success generally depends on the availability of large amounts of DNA, e.g., Southern blot-based tests or isolation of DNA for deposit in a DNA bank.

COMMENTARY:

N/A

MOL.34750 Phase II N/A YES NO

Is the quality (intactness) of the high molecular weight DNA assessed by gel electrophoresis or comparable method, when appropriate?

NOTE: The quantity of nucleic acid must be measured prior to use in a procedure whose success generally depends on the availability of large amounts of DNA, e.g., Southern blot-based tests or isolation of DNA for deposit in a DNA bank.

COMMENTARY:

N/A

MOL.34850 Phase II N/A YES NO

When appropriate, is RNA assessed for those assays that require its presence?

NOTE: RNA in specimens is highly labile because RNAase is ubiquitous and difficult to inhibit. For human RNA targets, RNA quality must be assessed. An appropriate "housekeeping" mRNA should be assessed as an internal control for RNA integrity. However, depending on the target, it may not be necessary for all specimens to be assessed for RNA quality. If RNA quality is not assessed, for example, for many types of viral RNA targets, the laboratory must document its false negative rate.

COMMENTARY:

N/A

REFERENCES: 1) Tsui NBY, Ng EKO, Lo YMD. Stability of Endogenous and Added RNA in Blood Specimens, Serum and Plasma. *Clin Chem* 48:1647-1653,2002; 2) Farrell R. Gel electrophoresis based assessment of cellular RNA quality may also be used (RNA Isolation Strategies). In: RNA Methodologies: A Laboratory Guide for Isolation and Characterization. Academic Press, 1998.

.....

Amplification

.....

MOL.34875 Phase II N/A YES NO

Are nucleic acid amplification procedures (*e.g.*, PCR) designed to minimize carry over (false positive results) using appropriate physical containment and procedural controls?

NOTE: This item is primarily directed at ensuring adequate physical separation of pre- and post-amplification samples to avoid amplicon contamination. The extreme sensitivity of amplification

systems requires that the laboratory take special precautions. For example, pre- and post-amplification samples should be manipulated in physically separate areas; gloves must be worn and frequently changed during processing; dedicated pipettes (positive displacement type or with aerosol barrier tips) must be used; and manipulations must minimize aerosolization. In a given run, specimens should be ordered in the following sequence: patient samples, positive controls, negative controls (including "no template" controls in which target DNA is omitted and therefore no product is expected). Enzymatic destruction of amplification products is often helpful, as is real-time measurement of products to avoid manual manipulation of amplification products.

COMMENTARY:

N/A

REFERENCE: Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 1989;339:237-238.

*****REVISED** 12/12/2006***

MOL.34900 Phase II N/A YES NO

In all nucleic acid amplification procedures, are internal controls run to detect a false negative reaction secondary to extraction failure or the presence of an inhibitor, when appropriate?

NOTE: The laboratory should be able to distinguish a true negative result from a false negative due to failure of extraction or amplification. Demonstration that another sequence can be successfully amplified in the same specimen should be sufficient to resolve this issue. For quantitative amplification assays, the effect of partial inhibition must also be addressed.

The false negative rate can be determined during the analytical validation phase, and during continual monitoring of test result trends. If the test is shown to be extremely robust, then the need for an internal positive control may be relaxed somewhat after considering the clinical implications of a false negative test result.

COMMENTARY:

N/A

.....

Restriction Endonucleases

.....

MOL.34907 Phase II N/A YES NO

Is the completeness and accuracy of restriction endonuclease digestion confirmed, when appropriate?

NOTE: The treatment of DNA with restriction endonucleases (RE) must be performed for an appropriate amount of time and under appropriate reaction conditions, i.e., to guard against non-specific activity. The efficacy of RE digestion must be established for each new lot of enzyme and in each run. Buffers must be used before their expiration date and properly stored.

COMMENTARY:

N/A

.....

Sequencing

.....

MOL.34914 Phase II N/A YES NO

Is there adequate information about the gene being tested regarding its wild type sequence and reported mutations and polymorphisms?

NOTE: DNA sequencing assays should be reserved for those disease genes that have been adequately characterized in the literature and in genomic databases so that the complete wild type sequence of the target region is known, as well as the identity and location of both clinically silent and clinically important mutations and polymorphisms.

COMMENTARY:

N/A

MOL.34921 Phase II N/A YES NO

Have sequencing assays been optimized to ensure a readable signal throughout the length of the target region and ready detection of sequence variants, especially those in heterozygous state?

NOTE: Sequencing assays differ from most other molecular pathology assays in that many targets (individual nucleotides) are examined at once, rather than addressing a discrete nucleotide mutation site. Assay procedures must assure that each of these targets is visualized adequately to produce an unequivocal sequence readout, whether this is done by manual or automated methods. Heterozygous point mutations in particular may be overlooked if the signals are low or unequal. One approach to preventing this problem is to perform sequencing in both directions (opposite strands).

COMMENTARY:

N/A

MOL.34929 Phase I N/A YES NO

Are criteria established for the acceptance and interpretation of primary sequencing data?

NOTE: Criteria for acceptance and interpretation of sequencing data must include correct assignments for non polymorphic positions, definition of the sequencing region, criteria for peak intensity, baseline fluctuation, signal-to-noise ratio and peak shapes.

COMMENTARY:

N/A

*****REVISED** 12/12/2006***

MOL.34930 Phase II N/A YES NO

Are methods established to ensure that sequences contributed by amplification primers are not considered in the assignment of alleles?

COMMENTARY:

N/A

MOL.34931 Phase I N/A YES NO

Is the sequence of sense and antisense strands determined for heterozygous templates, rare alleles or rare combinations of alleles?

NOTE: The sequence of sense and antisense strands must be determined for rare alleles and rare combinations of alleles. The sequence of sense and antisense strands should be determined for heterozygous templates. If only one strand is sequenced for heterozygous templates, validation must show that sequencing of only one strand will consistently yield accurate sequence assignments. If assignments are routinely based on only one strand, periodic confirmation of complementary strands is recommended.

COMMENTARY:

N/A

.....

Detection Methods

.....

MOL.34935 Phase II N/A YES NO

Are the autoradiographs and gel photographs of sufficient resolution and quality (low background, clear signal, absence of bubbles, *etc*.) to permit the reported interpretation?

COMMENTARY:

N/A

^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^

Agarose and Polyacrylamide Gel Electrophoresis

^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^

MOL.34950 Phase II N/A YES NO

Are standard amounts of nucleic acid loaded on analytical gels, when possible?

COMMENTARY:

N/A

MOL.35050 Phase II N/A YES NO

Are known molecular weight markers that span the range of expected bands used for each electrophoretic run?

COMMENTARY:

N/A

MOL.35100 Phase II N/A YES NO

Are visual or fluorescent markers used to determine the end-point of gel electrophoresis?

COMMENTARY:

N/A

MOL.35150 Phase I N/A YES NO

Are autoradiographs or electrophoretic gels interpreted using objective criteria?

COMMENTARY:

N/A

REFERENCES: 1) American Association of Blood Banks. Standards for parentage testing laboratories. Bethesda, MD: AABB, 1998:7.100, 7.500; 2) Cossman J, *et al.* Gene rearrangements in the diagnosis of lymphoma/leukemia. Guidelines for use based on a multiinstitutional study. *Am J Clin Pathol.* 1991;95:347-354; 3) NCCLS. Immunoglobulin and T-cell receptor gene rearrangement assays; approved guideline – second edition. NCCLS document MM2-A2 (ISBN 1-56238-466-X). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2002.

^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^

Real-Time Polymerase Chain Reaction (PCR)

^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^

MOL.35370 Phase I N/A YES NO

For tests that generate a result based on a T_m, are appropriately narrow temperature ranges (≤ +/- 2.5^{o}C) defined and monitored?

COMMENTARY:

N/A

MOL.35414 Phase I N/A YES NO

For quantitative real time PCR tests, do calibrator results fall within a specified range for each run?

COMMENTARY:

N/A

MOL.35458 Phase I N/A YES NO

Are new lots of fluorescent oligonucleotide reagents tested against control targets concurrent with or before being placed into service?

COMMENTARY:

N/A

.....

Arrays

.....

Arrays include a variety of reverse and forward hybridization formats. Reverse hybridization arrays use multiple unlabeled probes on a solid support to interrogate a patient sample that carries a label, either direct (fluorescent or radioactive) or indirect (affinity labels such as biotin, digoxigenin, etc.). Another form of array involves multiple real-time amplification assays to measure multiple targets simultaneously. Controls for arrays monitor those steps carried out by the laboratory (sample preparation and labeling, hybridization and detection) and by the manufacturer (assay preparation, detection and hybridization reagents). Manufacturers also contribute to QC by producing products under good manufacturing procedures (GMP), providing control material for each analyte, and by providing sequence information or confirmatory tests to resolve ambiguous results.

MOL.35722 Phase I N/A YES NO

Has patient nucleic acid integrity and labeling been verified?

NOTE: In many current applications, the sample is labeled during a PCR or RT-PCR reaction. Some arrays include a control feature that targets an endogenous positive target. Other possible controls include visualizing the material on electrophoretic gels/capillaries or by detection of label. Addition of an exogenous spiked control during labeling will monitor efficiency of labeling but will not control for quality of sample nucleic acid.

COMMENTARY:

N/A

MOL.35766 Phase I N/A YES NO

Has the quality of the arrays been verified and lot to lot comparisons done?

NOTE: Manufacturer quality control specifications should be available as a reference. Additional verification by the laboratory should include:

a) Verification of each probe for each lot. This can be achieved in part by using labeled oligonucleotides that hybridize to all probes, a mixture of labeled oligonucleotides specific for each probe, or a mixture of control samples that hybridize to each probe.

b) For quantitative assays such as gene dosage, pathogen load, or expression levels, a positive control near limiting dilution (low positive) for one or more probes should be included in each run. These controls should be rotated to include all analytes. A separate array for template blanks (i.e., no RT control or water) should also be included to monitor for inadvertent contamination.

c) Function checks on software used to analyze array data points.

COMMENTARY:

N/A

POST ANALYSIS

.....

Results Reporting

.....

MOL.35810 Phase II **N/A YES NO**

Are preliminary reports promptly generated, when indicated?

COMMENTARY:

N/A

MOL.35854 Phase II **N/A YES NO**

Are discrepancies between preliminary and final reports investigated and documented?

COMMENTARY:

N/A

MOL.35898 Phase I N/A YES NO

Are discrepancies between the molecular pathology laboratory's final results, other laboratory findings, and the clinical presentation investigated and documented, along with any necessary corrective action?

NOTE: When molecular results are questioned because they are discrepant with other clinicopathologic findings, an investigation should be carried out and documented along with any corrective action.

COMMENTARY:

N/A

MOL.35942 Phase II N/A YES NO

Are there protocols for the reporting of results?

COMMENTARY:

N/A

MOL.36000 Phase II N/A YES NO

Does the final report include an appropriate summary of the methods, the loci or mutations tested, the analytic interpretation, and clinical interpretation if appropriate?

NOTE: Laboratory reports should be designed to convey patient results effectively to a non-expert physician. This includes documentation of the analytic procedure used or the commercial kit version accompanied by an interpretation of the findings.

"Analytic interpretation" means examining the raw data to reach a conclusion about the quality or quantity of the analyte. "Clinical interpretation" means reaching a conclusion about the implications of the result for the patient. The clinical interpretation may be stated in general terms, or may be based on specific knowledge of the patient's situation.

COMMENTARY:

N/A

MOL.36012 Phase I N/A YES NO

Is the database for known alleles documented and updated as needed?

NOTE: The database for assignment of alleles must be documented and updated in a timely fashion after new alleles have been reported or verified in the published literature.

COMMENTARY:

N/A

MOL.36025 Phase II N/A YES NO

If patient testing is performed using Class I analyte-specific reagents (ASR's) obtained or purchased from an outside vendor, does the patient report include the disclaimer required by federal regulations?

NOTE: ASR's are antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens.

An ASR is the active ingredient of an in-house-developed test system.

This checklist question concerns Class I ASR's. Class I ASR's are not subject to preclearance by the U.S. Food and Drug Administration (FDA) or to special controls by FDA. Most ASR's are Class I. Exceptions include those used by blood banks to screen for infectious diseases (Class II or III), or used to diagnose certain contagious diseases (e.g., HIV infection and tuberculosis) (class III).

If the laboratory performs patient testing using Class I ASR's, federal regulations require that the following disclaimer accompany the test result on the patient report: "This test was developed and its performance characteristics determined by (laboratory name). It has not been cleared or approved by the U.S. Food and Drug Administration." The CAP recommends additional language, such as "The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity clinical laboratory testing."

The disclaimer is not required for tests using reagents that are sold in kit form with other materials or an instrument, nor reagents sold with instructions for use, nor reagents labeled "for in vitro diagnostic use" (IVD) by the manufacturer.

The laboratory must establish the performance characteristics of tests using Class I ASR's in accordance with the Assay Validation section of this checklist.

Antibodies, nucleic acid sequences, etc., labeled "Research Use Only" (RUO) purchased from commercial sources may be used in laboratory-developed tests only if the laboratory has made a reasonable effort to search for FDA-approved/cleared kits, and ASR class reagents. The results of that failed search should be documented by the laboratory director.

COMMENTARY:

N/A

REFERENCES: 1) Department of Health and Human Services, Food and Drug Administration. Medical devices; classification/reclassification; restricted devices; analyte specific reagents. Final rule. *Fed Register*. 1997(Nov 21);62243-45 [21CFR809, 21CFR864]; 2) Caldwell CW. Analyte-specific reagents in the flow cytometry laboratory. *Arch Pathol Lab Med*. 1998;122:861-864; 3) Graziano. Disclaimer now needed for analyte-specific reagents. Northfield, IL: College of American Pathologists *CAP Today*. 1998;12(11):5-11; 4) U.S. Department of Health and Human Services, Food and Drug Administration. Analyte Specific Reagents; Small Entity Compliance Guidance. http://www.fda.gov/cdrh/oivd/guidance/1205.html, February 26, 2003; 5) Shapiro JD and Prebula RJ. FDA's Regulation of Analyte-Specific Reagents. Medical Devicelink, February 2003. http://www.devicelink.com/mddi/archive/03/02/018.html.

MOL.36050 Phase II N/A YES NO

Is the final report reviewed and signed by the director or a qualified designee if there is a subjective or an interpretive component to the test?

NOTE: When diagnostic reports are generated by computer or telecommunications equipment, the actual signature or initials of the director need not appear on the report. Nevertheless, the laboratory must have a procedure that ensures and documents that the report has been reviewed and approved before its release.

COMMENTARY:

N/A

REFERENCES: 1) Standards for parentage testing laboratories. Bethesda, MD: AABB, 2003:6.4; 2) College of American Pathologists, Commission on Laboratory Accreditation. Standards for laboratory accreditation; Standard I. Northfield, IL: CAP, 1998.

MOL.36090 Phase I N/A YES NO

Are molecular genetic test reports released and transmitted in a manner adequate to maintain patient confidentiality at a level appropriate for the particular test?

NOTE: *In view of the recognized risks of genetic discrimination and stigmatization, confidentiality of molecular test results is an important consideration. Results should be communicated only to the referring physician, genetic counselor, the medical record, or (in some cases) the patient. Potentially non-confidential media (e.g., FAX) should be used with caution. Some patients, aware of the insurability risks, will choose to pay for testing out-of-pocket and request that the results not be recorded in their medical record; such requests should be honored by the laboratory to the extent allowable under applicable laws. Under no circumstances should results be provided to outside parties such as employers, insurers or other family members, without the patient's express consent, despite the fact that there will be cases in which such action would appear to be in the best interest of the patient, family, or society. Laboratory workers must even use caution when publishing or publicly presenting the results of such studies, as some family members have recognized their own pedigrees in published material and thereby derived otherwise confidential information.*

COMMENTARY:

N/A

REFERENCE: Health Insurance Portability and Accountability Act, 1996.

MOL.36100 Phase II N/A YES NO

When linkage analysis is performed, does the molecular inherited disease testing report include an estimate of the risk of false negatives and false positives arising from recombination between the linked probe(s) and the disease allele or mutation?

COMMENTARY:

N/A

REFERENCE: Keats BJB, *et al*. Guidelines for human linkage maps. An international system for human linkage maps (ISLM, 1990). *Ann Hum Genet.* 1991;55:1-6.

MOL.36110 Phase II N/A YES NO

In genetic testing for complex disease genes with multiple possible mutations, does the report include (where appropriate) an estimate of mutation detection rate and the residual risk of being a carrier for one of the mutations not tested for?

NOTE: Many disease genes, such as those for cystic fibrosis and familial breast/ovarian cancer, are extremely heterogeneous at the molecular level, with hundreds of different mutations reported in different patients and families. Even with gene sequencing, mutation detection is not 100%. A negative test result, therefore, does not completely rule out the possibility that the patient is a mutation carrier. The test report should convey this information in a fashion understandable to the physician

and, when appropriate, the patient. A calculated value for residual risk, based on the known population allele frequencies in the patient's ethnic group, is recommended.

COMMENTARY:

N/A

MOL.36120 Phase II N/A YES NO

Does the report include a discussion of the limitations of the findings and the clinical implications of the detected mutation (or negative result) for complex disorders with regard to recessive or dominant inheritance, recurrence risk, penetrance, severity and other aspects of genotype-phenotype correlation?

NOTE: *Because of the complexity of genotype-phenotype correlations for many genetic diseases, simply reporting a molecular genetic test as positive for a mutation is not acceptable since it conveys no information to the referring physician and patient as to the clinical ramifications of the result. Since major and often irreversible surgical or obstetric interventions may be initiated based on the test result, it is essential that the report convey the most current and accurate understanding of penetrance and recurrence risks.*

COMMENTARY:

N/A

MOL.36130 Phase I N/A YES NO

Does the report include a recommendation that patients receive appropriate genetic counseling to explain the implications of the test result, its residual risks and uncertainties, and the reproductive or medical options it raises, to the patient, where appropriate?

NOTE: *Molecular genetic test results are often extremely complex since they impart a probabilistic risk of disease rather than an objective positive/negative or quantitative answer. Physicians and counselors may require guidance to convey such subtle and emotionally charged information to patients in an understandable manner. In order to derive the most meaningful benefit from this testing, it is recommended that the results and subsequent options from these complex genetic tests be discussed with patients by a trained genetics professional.*

COMMENTARY:

N/A

REFERENCES: 1) American Board of Medical Genetics http://www.abmg.org/; 2) National Society of Genetic Counselors http://www.nsgc.org/; 3) American Board of Genetic Counseling http://www.abgc.net/.

MOL.36486 Phase II N/A YES NO

For assays performed on histology/cytology samples, does the interpretive report include correlation with the morphologic findings?

NOTE: In situ hybridization requires evaluation of the histo- or cytopathology to ensure that the sample assayed is representative of the lesion and to ensure that the result is interpreted in the context of localization of the hybridization signal to the lesional cells.

COMMENTARY:

N/A

MOL.36842 Phase II N/A YES NO

Is standard nomenclature used to designate genes and mutations?

NOTE: Human genes and loci should be designated as defined by the HUGO Gene Nomenclature Committee (http://www.gene.ucl.ac.uk/nomenclature/). Standard nomenclature must be used that is universally understandable to the genetics community and to allow correlation of test results with those of other family members or other patients reported in the literature. For interpretation of novel missense variants, the laboratory must follow existing guidelines for assessing the likely effect, if any, on gene and protein function so that the clinical implication of the results can be conveyed as accurately as possible to the referring physician.

COMMENTARY:

N/A

REFERENCES: 1) Wain HM, *et al*. Guidelines for Human Gene Nomenclature. *Genomics*. 2002;7:464-470; 2) ACMG, *Genet. Med.* 2000;2:302-30; 3) den Dunnen JR, *et al*. Mutation Nomenclature Extensions and Suggestions to Describe Complex Mutations: A Discussion. *Human Mutation*. 1999;15:7-12; 4) Wain HM, *et al*. Guidelines for Human Gene Nomenclature. *Genomics.* 2002;79:464-470; 5) Nomenclature for Description of Sequence Variations (http://www.hgvs.org/mutnomen); 6) Gulley ML, *et al*. Clinical Laboratory Reports in Molecular Pathology. *Arch Pathol Lab Med*. 2007;131;852-863; 7) Ogino S, *et al*. Standard Mutation Nomenclature in Molecular Diagnostics: Practical and Educational Challenges. *J Mol Diagn.* 2007;9:1-6.

.....

Records

.....

MOL.37200 Phase II N/A YES NO

Does the laboratory record include sufficient information regarding the individual specimen and assay conditions?

NOTE: Appropriate information may include the quantity and quality of nucleic acid isolated and the amount used in the assay; the lot numbers of the restriction endonucleases, probes or primers used and any assay variables.

COMMENTARY:

N/A

MOL.37250 Phase II N/A YES NO

Is a copy of each final report, all records of results, membranes, autoradiographs, gel photographs, and *in situ* hybridization slides, retained in compliance with applicable laws and regulations?

NOTE: Electronic versions are acceptable.

COMMENTARY:

N/A

MOL.37300 Phase II N/A YES NO

Are all autoradiographs, gel photographs and *in situ* hybridization slides adequately labeled for identification and adequately cross-referenced in the case records?

COMMENTARY:

N/A

PARENTAGE AND FORENSIC IDENTITY TESTING

MOL.37442 Phase II N/A YES NO

For paternity/forensic identity testing, are the following data obtained?

1. **Place and date of specimen collection**
2. **Identity of person collecting the specimen**
3. **Photograph or photocopy of a picture identification card for each individual tested**
4. **Signed record of information (including name, race, relationship) for each individual tested**
5. **Date of birth of child**
6. **Synopsis of case history/investigation, sample source**
7. **Documentation of informed consent**

COMMENTARY:

N/A

REFERENCE: Standards for Parentage testing laboratories. American Association of Blood Banks. Standards for parentage testing laboratories. Bethesda, MD: 2003:5.2.4.

MOL.37584 Phase II N/A YES NO

For parentage/forensic identity testing, does the laboratory seek a history of transfusion in the preceding three months or a history of allogeneic hematopoietic progenitor cell transplantation at any time?

COMMENTARY:

N/A

REFERENCE: Standards for Parentage testing laboratories. American Association of Blood Banks. Standards for parentage testing laboratories. Bethesda, MD: 2003:5.2.4.6.

MOL.37726 Phase II N/A YES NO

Is the information about each individual and the accuracy of the sample label verified by that individual or the legal guardian?

COMMENTARY:

N/A

MOL.37868 Phase II N/A YES NO

Is the condition of the specimen documented upon receipt in the laboratory including any evidence of tampering, adequacy of volume, and a firmly attached label bearing a unique identification?

COMMENTARY:

N/A

MOL.38010 Phase II N/A YES NO

For legal parentage and forensic testing, are specimens maintained in a limited access, secured area and appropriate documentation of chain-of-custody maintained?

COMMENTARY:

N/A

MOL.38152 Phase II N/A YES NO

Does the report include the individual paternity index for each genetic system, the combined paternity index, the probability of paternity as a percentage, prior probability of paternity used in calculations and the population for used for comparison?

COMMENTARY:

N/A

REFERENCE: Standards for parentage testing laboratories. Bethesda, MD: AABB, 2003:6.4 (6R-B).

MOL.38294 Phase II N/A YES NO

For parentage testing are DNA results (RFLP, STR, SNP) interpreted twice, independently?

COMMENTARY:

N/A

REFERENCE: Standards for parentage testing laboratories. Bethesda, MD: AABB, 2003:5.4.2.1-3.

MOL.38436 Phase II N/A YES NO

For parentage testing, are exclusions based on closely spaced alleles (usually defined as less than one tandem repeat apart) evaluated by co-electrophoresis or other methods?

COMMENTARY:

N/A

REFERENCE: Standards for parentage testing laboratories. Bethesda, MD: AABB, 2003:5.4.2.1-2.

MOL.38578 Phase II N/A YES NO

For forensic identity testing do laboratory methods, test validation, and interpretation and reporting of results meet current DNA Advisory Board Standards and SWGDAM guidelines?

COMMENTARY:

N/A

REFERENCES: 1) DNA Advisory Board. Quality assurance standards for DNA testing laboratories. Forensic Science Communications 2000:2.2; 2) Short tandem repeat (STR) interpretation guidelines. Ibid; 3) Guidelines for mitochondrial DNA (mtDNA) nucleotide sequence interpretation. Ibid. 2003:5.

IN SITU HYBRIDIZATION (ISH)

MOL.38720 Phase II N/A YES NO

For in situ hybridization studies, is the morphologic interpretation and correlation of results performed by a qualified anatomic or clinical pathologist as appropriate?

NOTE: This question applies primarily to anatomic pathology and cytopathology specimens.

COMMENTARY:

N/A

.....

Fluorescence *In Situ* Hybridization (FISH)

.....

MOL.38862 Phase II N/A YES NO

Are there documented policies and procedures for validation of all FISH probes?

COMMENTARY:

N/A

REFERENCES: 1) American College of Medical Genetics Laboratory. Standards and Guidelines for Clinical Genetics Laboratories, 3rd ed. Bethesda, MD: ACMG, 2003. Available at: http://www.acmg.net Accessed 2006; 2) NCCLS. *Fluorescence* In Situ *Hybridization (FISH) Methods for Medical Genetics; Approved Guideline*. NCCLS document MM7-A (ISBN 1-56238-524-0). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 1908-1898 USA, 2004.

MOL.39004 Phase II N/A YES NO

Are there documented procedures for scoring FISH results and are all analyses scored according to these procedures?

COMMENTARY:

N/A

REFERENCES: 1) American College of Medical Genetics Laboratory. Standards and Guidelines for Clinical Genetics Laboratories, 3rd ed. Bethesda, MD: ACMG, 2003. Available at: http://www.acmg.net Accessed 2006; 2) NCCLS. *Fluorescence* In Situ *Hybridization (FISH) Methods for Medical Genetics; Approved Guideline*. NCCLS document MM7-A (ISBN 1-56238-524-0). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 1908-1898 USA, 2004.

MOL.39146 Phase II N/A YES NO

Are control loci (internal or external) used with and documented for each FISH analysis?

NOTE: When normal chromosome targets are expected to be present within a sample, an internal control for that target should be used during each hybridization (i.e., a locus specific probe at a different site on the same chromosome and/or a normal locus on the abnormal homolog). If a probe is used that does not produce an internal control signal (e.g., a Y chromosome probe in a female), another sample that is known to have the probe target should be run in parallel with the patient sample.

COMMENTARY:

N/A

REFERENCES: 1) American College of Medical Genetics Laboratory. Standards and Guidelines for Clinical Genetics Laboratories, 3rd ed. Bethesda, MD: ACMG, 2003. Available at: http://www.acmg.net Accessed 2006; 2) NCCLS. *Fluorescence* In Situ *Hybridization (FISH) Methods for Medical Genetics; Approved Guideline*. NCCLS document MM7-A (ISBN 1-56238-524-0). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 1908-1898 USA, 2004.

MOL.39288 Phase I N/A YES NO

Are photographic or digitized images retained for documentation of all FISH assays (at least one cell for assays with normal results and at least two cells for assays with abnormal results)?

NOTE: For assays where multiple chromosomal loci (>2) are targets in part of a single test, an image of at least one cell must be retained for documentation of each target. Images of at least two cells are required to document all abnormalities.

COMMENTARY:

N/A

REFERENCE: American College of Medical Genetics Laboratory. Standards and Guidelines for Clinical Genetics Laboratories, 3rd ed. Bethesda, MD: ACMG, 2003. Available at: http://www.acmg.net Accessed 2006.

*****NEW***** ***09/27/2007***

MOL.39323 Phase I N/A YES NO

If the laboratory assesses HER2 gene amplification by fluorescence in situ hybridization (FISH), has the laboratory documented appropriate validation for the assay(s)?

NOTE: Initial test validation must be performed on a minimum of 25 cases (recommended 25-100). Validation may be performed by comparing the results of testing with a validated alternative method (i.e., IHC vs. FISH) either in the same laboratory or another laboratory, or with the same validated method performed in another laboratory; validation testing must be done using the same set of cases in both labs.

If specimens are fixed in a medium other than 10% neutral buffered formalin, the validation study must show that results are concordant with results from formalin-fixed tissues.

If significant changes are made in testing methods (e.g., antibody clone, antigen retrieval protocol or detection system), revalidation is required.

COMMENTARY:

N/A

*****NEW**** ***09/27/2007***

MOL.39358 Phase I **N/A YES NO**

If the laboratory assesses HER2 gene amplification by fluorescence in situ hybridization (FISH), does the laboratory have a documented procedure for ensuring appropriate length of fixation of specimens tested?

NOTE: Specimens subject to HER2 testing should be fixed in 10% neutral buffered formalin for at least 6 hours and no longer than 48 hours. While core biopsies must not be fixed for less than 1 hour, it is recommended that such specimens have the same fixation as larger specimens (i.e., 6 hours minimum). It is recommended that time of fixation be recorded and included in the report if available. While the maximum fixation time of 48 hours is not an exclusion criterion for HER2 testing, laboratories should qualify any negative results for specimens fixed longer than 48 hours. For cases with negative results by immunohistochemistry, consideration should be given to performing confirmatory analysis by FISH.

Laboratories testing specimens obtained from another institution should have a policy that addresses time of fixation. Information on time of fixation may be obtained by appropriate questions on the laboratory's requisition form. The time of fixation should be recorded in the final report.

COMMENTARY:

N/A

***NEW** 09/27/2007*

MOL.39393 Phase I N/A YES NO

If the laboratory assesses HER2 gene amplification by fluorescence in situ hybridization (FISH), does the laboratory report the results using the ASCO/CAP scoring criteria?

NOTE: For HER2 gene status determined by FISH, positive (amplified) cases are those with ratios of HER2 to CEP17 of > 2.2. Negative cases are defined as those with FISH ratios of < 1.8. Equivocal cases are those with a FISH ratio of 1.8 – 2.2. For test systems without an internal control probe, positive (amplified) cases are those with an average HER2 gene copy number > six signals/nucleus, negative cases are those with < four signals/nucleus, and equivocal cases are those with an average HER2 gene copy number of four to six signals/nucleus. Careful attention should be paid to the recommended exclusion criteria for performing or interpreting FISH for HER2 (e.g., signal obscured by background; difficulty in defining areas of invasive carcinoma under UV light; see table 6 in reference 1, below).

COMMENTARY:

N/A

REFERENCE: Wolff AC, Hammond ME, Schwartz JN, *et al.* American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 2007;131:18-43..

.....

Brightfield *In Situ* Hybridization

.....

MOL.39430 Phase II N/A YES NO

Have assay conditions and conditions of tissue pretreatment been verified for each sample, using an appropriate positive control probe(s) against endogenous targets?

NOTE: The laboratory must document that assay conditions and tissue pretreatment allow for detection of the intended target sequence. Nucleic acid preservation and accessibility vary with fixation and processing. Adjusting assay conditions to demonstrate the signal for an endogenous target with a positive control probe allows negative results with the test probe to be interpreted. Negative results with the endogenous positive control probe allow inadequately preserved samples to be eliminated. The positive control probe may be directed to any target known to be in the sample.

COMMENTARY:

N/A

REFERENCE: Unger ER, Lee DR. *In situ* hybridization: principles and diagnostic applications in infection. *J Histotechnol.* 1995;18:203-209.

MOL.39572 Phase I N/A YES NO

Are ribonuclease-free conditions maintained for all assays that detect RNA in target tissues or use an RNA probe?

NOTE: RNA is extremely susceptible to degradation by ribonucleases that are ubiquitous in the environment. To ensure preservation of target RNA or RNA probes, special precautions are needed.

COMMENTARY:

N/A

REFERENCE: Gulley ML, *et al*. Guidelines for interpreting EBER in situ hybridization and LMPI immunohistochemical tests for detecting Epstein-Barr virus in Hodgkin lymphoma. *Am J Clin Pathol.* 2002;117:259-267.

MOL.39714 Phase II N/A YES NO

For assays performed on histology/cytology samples, does the interpretive report include correlation with the morphologic findings?

NOTE: In situ hybridization requires reevaluation of the histopathology or cytopathology in the hybridized slide to ensure that the sample assayed is representative of the lesion.

COMMENTARY:

N/A

REFERENCE: Unger ER, Lee DR. *In situ* hybridization: principles and diagnostic applications in infection. *J Histotechnol.* 1995;18:203-209.

MOL.39856 Phase I N/A YES NO

Is appropriate interpretation of ISH results provided in the report?

COMMENTARY:

N/A

****

PERSONNEL

****

As applicable, the personnel requirements in the Laboratory General Checklist should be consulted. For optimal patient care, only qualified personnel may be involved with molecular pathology testing.

MOL.40000 Phase II N/A YES NO

Is the director of the molecular pathology laboratory a pathologist, board-certified physician in a specialty other than pathology, or doctoral scientist in a biologic science, with specialized training and/or appropriate experience in molecular pathology?

NOTE: In the case of forensic identity testing, the above or appropriate degree, training, or experience in forensic science is required.

COMMENTARY:

N/A

MOL.40100 Phase II N/A YES NO

Is the person in charge of technical operations of the molecular pathology laboratory qualified as one of the following?

1. **Person who qualifies as a director**
2. **CLSp(MB), BS, BA or MT(ASCP) with at least 4 years of experience (at least 1 of which is in molecular pathology methods) under a qualified director**

COMMENTARY:

N/A

MOL.40150 Phase II N/A YES NO

Do persons performing the technical aspects of molecular pathology qualify as one of the following?

1. **Experienced in the field under the direct supervision of a qualified director or supervisor**

2. **MT(ASCP) certified or equivalent**
3. **BA or BS degree in biologic sciences with appropriate experience in molecular pathology methods**

COMMENTARY:

N/A

MOL.40200 Phase I N/A YES NO

Is there an adequate training program for new technologists, and is there a continuing medical laboratory education program?

COMMENTARY:

N/A

REFERENCES: 1) Von Needa P. Keep everyone keen on continuing education. *Med Lab Observ.* 1979(May):117-126; 2) Berry CD. The JCAH approach to continuing education in the clinical laboratory. *Lab Med.* 1981;12:10-11; 3) Umiker W. The role of the pathologist in continuing education programs for laboratory personnel. *Lab Med.* 1981;12:18-21; 4) Yapit MK. Resources and strategies for a successful CE program. *Med Lab Observ.* 1989(Apr):47-566; 5) Matta N. Training new technologists in the basics of molecular pathology. *Lab Med.* 1993;24:636-641.

****

PHYSICAL FACILITIES AND EQUIPMENT

****

INSTRUMENTS

MOL.40666 Phase II N/A YES NO

Is there evidence of ongoing evaluation of instrument maintenance and function, temperature, *etc.*, for all procedures as required?

COMMENTARY:

N/A

MOL.41132 Phase II N/A YES NO

Are there documented standard procedures for set-up and normal operation of instruments?

NOTE: *All laboratory instruments and equipment must be maintained in a manner consistent with safe and reliable testing. The laboratory should have an organized system for monitoring and maintaining all instruments. The procedures and schedules for instrument maintenance must be as thorough and as frequent as specified by the manufacturer. Since some equipment has no standard frequency or extent of maintenance, each laboratory should establish a maintenance schedule that reasonably reflects the workload and specifications of its equipment. All servicing and repairs must be documented. The Inspector should identify the specific instruments involved in any deficiency during the summation conference.*

COMMENTARY:

N/A

MOL.41598 Phase II N/A YES NO

Is there a regular schedule or system for checking the critical operating characteristics for all instruments in use?

NOTE: Function checks should be designed to evaluate the critical operating characteristics to detect drift, instability, or malfunction before the problem affects patient results.

COMMENTARY:

N/A

MOL.42064 Phase II N/A YES NO

Are function checks conveniently documented to detect trends or malfunctions?

COMMENTARY:

N/A

MOL.42530 Phase II N/A YES NO

Are tolerance limits for acceptable function documented for specific instruments, when appropriate?

COMMENTARY:

N/A

MOL.42996 Phase II N/A YES NO

Are instructions provided for minor troubleshooting and repairs of instruments (*e.g.*, manufacturer's service manual)?

COMMENTARY:

N/A

MOL.43462 Phase II N/A YES NO

Are records maintained for each instrument to document all repairs and service procedures?

COMMENTARY:

N/A

MOL.43928 Phase II N/A YES NO

Are RECENT instrument maintenance, service, and repair records (or copies) available to and usable by the technical staff operating the equipment?

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

COMMENTARY:

N/A

.....

Spectrophotometers

.....

MOL.44394 Phase II N/A YES NO

Are filters (filter photometers) checked periodically to ensure they are in good condition (*e.g.*, clean, free of scratches)?

COMMENTARY:

N/A

MOL.44860 Phase II N/A YES NO

Is spectrophotometer wavelength calibration checked regularly with appropriate solutions, filters, or emission line source lamps, if so required by the manufacturer?

COMMENTARY:

N/A

MOL.45326 Phase II N/A YES NO

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

COMMENTARY:

N/A

.....

Signal Detection Instruments

.....

The following questions apply to scintillation counters, luminometers, densitometers, etc.

MOL.45792 Phase II N/A YES NO

Are background levels taken and recorded daily or with each use of the instrument if used less than daily?

COMMENTARY:

N/A

MOL.46258 Phase II N/A YES NO

Are there documented criteria for acceptable background levels?

COMMENTARY:

N/A

MOL.46491 Phase I N/A YES NO

For test platforms measuring multiple fluorochromes, are precautions taken to identify and correct for bleed-through signal from one channel to another?

COMMENTARY:

N/A

.....

Film Processing/Photographic Equipment

.....

MOL.46724 Phase II N/A YES NO

Is film-processing (developing) equipment routinely serviced, repaired, and appropriately replenished with reagents, if maintained by the laboratory?

NOTE: If the laboratory uses another department's film processing equipment, the quality of the autoradiographs produced must be monitored and the appropriate personnel notified if corrective action is required.

COMMENTARY:

N/A

MOL.47190 Phase I N/A YES NO

Are fixed camera mountings secure and level?

COMMENTARY:

N/A

MOL.47656 Phase I N/A YES NO

If ultraviolet light sources are used, is proper protective shielding available to users?

COMMENTARY:

N/A

.....

Electrophoresis Equipment and Power Supplies

.....

MOL.48122 Phase II N/A YES NO

Are all electrophoretic apparatus in the laboratory clean and properly maintained?

COMMENTARY:

N/A

.....

Pipettes

.....

MOL.48588 Phase II N/A YES NO

Are automatic pipettors that are used for quantitative dispensing of material checked for accuracy and reproducibility before being placed in service and at specified intervals, and are these 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, 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 provide convenient documentation.

COMMENTARY:

N/A

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) 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; 5) Bray W. Software for the gravimetric calibration testing of pipets. *Am Clin Lab*. Oct 1995 (available on the Internet at http://www.labtronics.com/pt_art.htm); 6) Kroll MH, *et al* (eds). Laboratory instrument evaluation, verification & maintenance manual, 5th edition. Northfield, IL: College of American Pathologists, 1999:126-127; 7) Johnson B. Calibration to dye for: Artel's new pipette calibration system. *Scientist*. 1999;13(12):14; 8) 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; 9) Skeen GA, Ashwood ER. Using spectrophotometry to evaluate volumetric devices. *Lab Med*. 2000;31:478-479.

.....

Thermometers and Temperature-Dependent Equipment

.....

MOL.49054 Phase II **N/A YES NO**

Are temperatures checked and recorded appropriately for the following types of equipment?

1. **Water baths**
2. **Heating blocks**
3. **Incubators and ovens (where temperature control is necessary for a procedure)**
4. **Refrigerators and freezers**

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

COMMENTARY:

N/A

MOL.49520 Phase I **N/A YES NO**

Are individual wells (or a representative sample thereof) of thermocyclers checked for temperature accuracy before being placed in service and periodically thereafter?

NOTE: A downstream measure of well-temperature accuracy (such as productivity of amplification) may be substituted to functionally meet this requirement.

COMMENTARY:

N/A

REFERENCE: Saunders GC, *et al.* Interlaboratory study on thermal cycler performance in controlled PCR and random amplified polymorphic DNA analyses. *Clin Chem.* 2001;47:47-55.

.....

Space

.....

Deficiencies in space should be documented so there is incentive to improve. Deficiencies in space are regarded as minor unless they are so severe as to interfere with the quality of work, quality control activities, or personnel safety, in which case they become a Phase II deficiency. Recurrent space deficiencies over an extended period with no apparent effort or plans for correction may be a Phase II deficiency.

MOL.50000 Phase II N/A YES NO

Is sufficient space available so that there is no compromise of the quality of work, (including quality control activities) or safety of personnel?

COMMENTARY:

N/A

REFERENCE: College of American Pathologists, Commission on Laboratory Accreditation. Standards for laboratory accreditation; Standard II. Northfield, IL: CAP, 2000.

****

LABORATORY SAFETY

****

The inspector should review relevant questions from the Safety section of the Laboratory General checklist, to assure that the molecular pathology laboratory is in compliance. Please elaborate upon the location and the details of each deficiency in the Inspector's Summation Report. In particular, the Inspector should review the use of universal precautions and the handling and disposal of hazardous chemicals such as ethidium bromide, acrylamide, and organic reagents.

MOL.52760 Phase II N/A YES NO

Is a properly functioning fume hood (or chemical filtration unit) available for any procedures using volatile chemicals?

COMMENTARY:

N/A

MOL.54570 Phase II N/A YES NO

Is a biological safety cabinet (or hood) available, when appropriate?

COMMENTARY:

N/A

REFERENCE: Classification of etiologic agents on the basis of hazard; US Department of Health, Education, and Welfare, PHS, Centers for Disease Control, Office of Biosafety. Atlanta, GA. Reprinted September, 1976.

MOL.56380 Phase II N/A YES NO

Is the biological safety cabinet (or hood) certified at least annually to ensure that filters are functioning properly and that airflow rates meet specifications?

COMMENTARY:

N/A

MOL.58190 Phase II N/A YES NO

Are refrigerators free of improper materials (such as food, externally contaminated specimens or unsealed volatile materials)?

COMMENTARY:

N/A

RADIATION SAFETY

MOL.61050 Phase II N/A YES NO

Is there an up-to-date radiation safety manual that includes sections on decontamination and radioactive waste?

NOTE: *A radiation safety manual providing procedures for the safe handling of radioactive substances in both routine and emergency situations is required by the Nuclear Regulatory Commission (NRC). Requirements for laboratory safety in nuclear medicine can be found in several references. These include: Code of Federal Regulations, Title 10, parts 19, 20 and 30; NIST handbooks 48, 53, 58, 59, and 92; OSHA 1910.96.*

COMMENTARY:

N/A

MOL.61055 Phase I N/A YES NO

Are workbenches and sinks decontaminated each day of use, and the effectiveness checked at least monthly?

COMMENTARY:

N/A

REFERENCE: 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.

MOL.61060 Phase I N/A YES NO

Are there specific policies regarding authorization or restriction of personnel handling radionuclides?

COMMENTARY:

N/A

MOL.61065 Phase II N/A YES NO

Do 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.

COMMENTARY:

N/A

MOL.61070 Phase II N/A YES NO

Are radionuclide storage and decay areas properly shielded, if required for specific isotopic materials?

COMMENTARY:

N/A

MOL.61075 Phase II N/A YES NO

Are there regular radiation area surveys and wipe tests, with records maintained?

COMMENTARY:

N/A

MOL.61080 Phase II N/A YES NO

Are all areas or rooms where radioactive materials are being used or stored posted to indicate the presence of radioactive materials?

COMMENTARY:

N/A

MOL.61085 Phase II N/A YES NO

Are personnel instructed in decontamination routines and in the safe handling and proper disposal of radionuclides (wastes, syringes, needles, and sponges)?

NOTE: Instruction must include decontamination routines and proper disposal of radioactive waste material. If the laboratory does NOT operate under a specific license (usually required by the Nuclear Regulatory Commission when amounts stored or used exceed those in commercial ^{125}I RIA kits), mark the remainder of this section "N/A".

COMMENTARY:

N/A

MOL.61090 Phase II N/A YES NO

Is radioactive waste kept separate from normal trash, 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 10 CFR 20. Documentation of the radioactive trash disposal must be maintained.

COMMENTARY:

N/A