Every patient deserves the GOLD STANDARD ...
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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.

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SUMMARY OF CHECKLIST EDITION CHANGES
Hematology and Coagulation Checklist
09/25/2012 Edition

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

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

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

**Note:** For the detail of the changes, refer to the "Changes Only" document which may be found on the CAP website through e-LAB Solutions (Laboratory Accreditation Program Master and Custom Checklists). To access this document select “Changes Only” from the Checklist Type drop-down menu.

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

**NEW Checklist Requirements**

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**REVISED Checklist Requirements**

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HEM.35927  07/31/2012
HEM.35946  07/31/2012
HEM.37216  07/11/2011
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HEM.37980  07/11/2011
HEM.37982  07/11/2011
HEM.38002  07/11/2011
HEM.38004  07/11/2011
HEM.38028  07/31/2012
HEM.40000  07/31/2012

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<tr>
<td>HEM.50800</td>
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UNDERSTANDING THE CAP ACCREDITATION CHECKLIST COMPONENTS

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

Subject Header
A phrase that provides the key concept of the requirement.

Declarative Statement
Checklist questions are reworded as declarative statements to better convey the regulatory nature of requirements.

HEM. 20050 Numeric QC

Phase II

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

NOTE: For CBC data where stabilized whole blood is not used for quality control, such statistics may be generated from previous patient samples using the standard deviation of duplicate pairs.

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

Using Evidence of Compliance (EOC)

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

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

Evidence of Compliance suggests ways to document compliance with checklist requirements. Other types of documentation may be acceptable. Whenever a policy/procedure/process is referenced within a requirement, it is only repeated in the Evidence of Compliance if such statement adds clarity. All policies/procedures/processes covered in the CAP checklists must be documented. A separate policy is not needed for each item listed in EOC as it may be referenced in an overarching policy.
HOW TO INSPECT USING R.O.A.D INSPECTION TECHNIQUES
(Read, Observe, Ask, Discover)

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

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

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

**Inspector Instructions:**

| READ | review a sampling of laboratory documents. Information obtained from this review will be useful as you observe processes and engage in dialogue with the laboratory staff.  
(Example of the complimentary inspector instructions for Quality Management/Quality Control General Issues section appearing across checklists): |
<table>
<thead>
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<tbody>
<tr>
<td>● Sampling of QM/QC policies and procedures</td>
<td></td>
</tr>
<tr>
<td>● Incident/error log and corrective action</td>
<td></td>
</tr>
</tbody>
</table>

| OBSERVE | laboratory practices by looking at what the laboratory personnel are actually doing and note if practice deviates from the documented policies/procedures.  
(Example) |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>● Observe the settings/QC range limits established in the laboratory LIS/HIS to ensure that the laboratory's stated ranges are accurately reflected</td>
<td></td>
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</tbody>
</table>

| ASK | open-ended, probing questions that start with phrases such as "tell me about..." or "what would you do if..." This approach can be a means to corroborate inspection findings that were examined by other techniques, such as Read & Observe. Ask follow-up questions for clarification. Include a variety of staff levels in your communication process.  
(Example) |
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>● As a staff member, what is your involvement with quality management?</td>
<td></td>
</tr>
<tr>
<td>● How do you detect and correct laboratory errors?</td>
<td></td>
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</tbody>
</table>

| DISCOVER | is a technique that can be used to "drill down" or further evaluate areas of concern uncovered by the inspector. "Follow the specimen" and "teach me" are two examples of Discovery. Utilizing this technique will allow for the discovery of pre-analytic, analytic, and post-analytic processes while reviewing multiple requirements simultaneously.  
(Example) |
<table>
<thead>
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</thead>
<tbody>
<tr>
<td>● Select several occurrences in which QC is out of range and follow documentation to determine if the steps taken follow the laboratory policy for corrective action</td>
<td></td>
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</table>
INTRODUCTION

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

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

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

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

DEFINITION OF TERMS

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

Annual - every 12 calendar months

Biennial - every 24 calendar months

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

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

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

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

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

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

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

Nonwaived - tests categorized as either moderately complex (including provider-performed microscopy) or highly complex by the US Food and Drug Administration (FDA), according to a scoring system used by the FDA
Reagent - any substance in a test system other than a solvent or support material that is required for the target analyte to be detected and its value measured in a sample

Semiannual - every 6 calendar months

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

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

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

Inspector Instructions:

- Sampling of QC policies and procedures

- What do you do if controls are out of range?
- What is your course of action when monthly precision data changes significantly from the previous month’s data?

- Select several occurrences in which QC is out of range and follow documentation to determine if the steps taken follow the laboratory policy for corrective action

WAIVED TESTS

HEM.18038 Documented QC Results - Waived Tests Phase II

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

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

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

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

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

HEM.18691 QC Corrective Action - Waived Tests Phase II

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

HEM.19344 QC Verification - Waived Tests Phase II

The results of controls are verified for acceptability before reporting results.
Evidence of Compliance:
✓ Records showing verification of acceptability of QC

NOTE: The remaining requirements in this checklist on controls; calibration; reportable range; and interinstrument comparisons do not apply to waived tests. The checklist section “RESULTS REPORTING - COAGULATION” also is not applicable to waived tests.

GENERAL ISSUES

HEM.20035 Acceptable Limits - Controls
Acceptable limits (numeric and/or non-numeric) are fully defined and documented for all hematology and coagulation control procedures.

NOTE: The goal is to have scientifically valid, logical “action limits” for quality control procedures that promptly alert the technologist of the need for immediate evaluation of the particular assay, including initiation of corrective action, before release of patient results.

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

HEM.20050 Numeric QC Data
For numeric QC data, Gaussian or other quality control statistics (e.g. SD and CV) are calculated monthly to define analytic imprecision.

NOTE: For CBC data where stabilized whole blood is not used for quality control, such statistics may be generated from previous patient samples using the standard deviation of duplicate pairs.

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

REFERENCES
1) Mukherjee KL. Introductory mathematics for the clinical laboratory. Chicago, IL: American Society of Clinical Pathology, 1979:81-94

HEM.20070 Precision Statistics
The laboratory has an action protocol when data from precision statistics change significantly from previous data.

NOTE: As an example, if the laboratory’s normal-level commercial control usually yields a monthly CV of 2% for WBC, but the most recent month shows a 4% CV, then something has caused increased imprecision, and investigation with documentation is required. Similarly, if the
monthly SD for MCV by moving averages is typically around 1.8 fL, but now is at 3.1 fL, the laboratory must find a cause for this shift and take action, if indicated. Finally, if commercially sponsored interlaboratory QC data for the same control lot and instrument model show SD/CV values markedly smaller or larger than the peer group, an explanation is required.

Evidence of Compliance:
✓ Written protocol for investigation, documentation and corrective action should a significant change in precision statistics occur AND
✓ Records of investigation and corrective actions taken

HEM.20120 QC Handling

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

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

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

REFERENCES

HEM.20140 QC Verification

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

NOTE: It is implicit in QC logic that patient test results are not reported when controls do not yield acceptable results.

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

REFERENCES

HEM.20143 QC Corrective Action

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

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

Even if patient samples are no longer available, test results can be re-evaluated to search for evidence of an out-of-control condition that might have affected patient results. For example, evaluation could include comparison of patient means for the run in question to historical patient
means, and/or review of selected patient results against previous results to see if there are consistent biases (all results higher or lower currently than previously) for the test(s) in question.

REFERENCES

HEM.20146 Monthly QC Review Phase II

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

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

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

SPECIMEN COLLECTION AND HANDLING - HEMATOLOGY

Inspector Instructions:

- Sampling of hematology specimen collection and handling policies and procedures
- Sampling of specimen rejection records/log
- Sampling of patient CBC specimens (anticoagulant, labeling, storage)
- How do you know if the CBC specimen is clotted, lipemic, or hemolyzed?
- How do you ensure the CBC sample is thoroughly mixed before analysis?
- What is your course of action when you receive unacceptable hematology specimens?

HEM.21575 Bone Marrow Procedures Phase I

If bone marrow aspiration and/or biopsy procedures are performed, there is a documented procedure to verify patient identification, procedure site and the procedure to be performed.

REFERENCES
1) http://www.jointcommission.org/PatientSafety/UniversalProtocol/

HEM.22000 Collection in Anticoagulant Phase II

All blood specimens collected in anticoagulant for hematology testing are mixed thoroughly immediately before analysis.
NOTE: Some rocking platforms may be adequate to maintain even cellular distribution of previously well-mixed specimens, but are incapable of fully mixing a settled specimen. For instruments with automated samplers, the laboratory must ensure that the automated mixing time is sufficient to homogeneously disperse the cells in a settled specimen.

Evidence of Compliance:
✓ Records documenting evaluation of each specimen mixing method (e.g. rotary mixer, rocker, automated sampler, or manual inversions) for reproducibility of results, as applicable

REFERENCES

HEM.22050 CBC Anticoagulant

Phase II

Samples for complete blood counts and blood film morphology are collected in potassium EDTA.

NOTE: Blood specimens for routine hematology tests (e.g. CBC, leukocyte differential) must be collected in potassium EDTA to minimize changes in cell characteristics. Oxalate can cause unsuitable morphologic changes such as cytoplasmic vacuoles, cytoplasmic crystals, and irregular nuclear lobulation. Heparin can cause cellular clumping (especially of platelets), pseudoleukocytosis with pseudothrombocytopenia in some particle counters, and troublesome blue background in Wright-stained blood films. Citrate may be useful in some cases of platelet agglutination due to EDTA, but those CBC data will require adjustment for the effects of dilution.

REFERENCES

HEM.22070 Specimen Rejection Criteria

Phase II

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

NOTE: This requirement does not imply that all "unsuitable" specimens are discarded or not analyzed. If, for example, a CBC is ordered and there is visible hemolysis, the hemoglobin concentration may still be valid, but other parameters are not. There must be a mechanism to notify clinical personnel responsible for patient care, and to note the condition of the sample on the report if the analytically valid incomplete test results are desired by the ordering physician. The laboratory should record that a dialogue was held with the physician, when such occurs.

Evidence of Compliance:
✓ Records of rejected specimens

REFERENCES
HEM.22100  Capillary Tube Collection Criteria  Phase II

Samples are collected in capillary tubes for microhematocrits or capillary/dilution systems, obtained in duplicate whenever possible and adequately labeled with patient identification information throughout the analytic sequence.

NOTE: This applies to capillary tubes used for microhematocrit determinations as well as capillary/dilution systems used in proximity to the patient. Microspecimen containers such as those used for capillary blood CBC parameter determinations need not be collected in duplicate. Because of the risk of injury, the use of plain glass capillary tubes is strongly discouraged.

Evidence of Compliance:
✓ Written procedure for collection in capillary tubes

HEM.22150  Specimen Quality Assessment - CBC  Phase II

CBC specimens are checked for clots (visual, applicator sticks, or automated analyzer histogram inspection/flags) before reporting results.

NOTE: This may be done visually or with applicator sticks before testing. Additionally, microclots will often present themselves histographically on automated and semi-automated particle counters or by flagging, and the laboratory must become familiar with such patterns. Finally, platelet clumps or fibrin may be microscopically detected if a blood film is prepared on the same sample.

HEM.22200  Hemolyzed or Lipemic Specimens - CBC  Phase II

CBC specimens are checked for significant in vitro hemolysis and possible interfering lipemia before reporting results.

NOTE: Specimens for complete blood counts must be checked for in vitro hemolysis that may falsely lower the erythrocyte count and the hematocrit, as well as falsely increase the platelet concentration from erythrocyte stroma. Visibly red plasma in a tube of EDTA-anticoagulated settled or centrifuged blood should trigger an investigation of in vivo hemolysis (in which case the CBC data are valid) versus in vitro hemolysis (in which case some or all of the CBC data are not valid and should not be reported). Lipemia may adversely affect the hemoglobin concentration and the leukocyte count. This does not imply that every CBC specimen must be subjected to centrifugation with visual inspection of the plasma supernatant, particularly if this would significantly impair the laboratory’s turnaround time. An acceptable alternative for high volume laboratories with automated instrumentation is to examine the numeric data for anomalous results (especially indices), as well as particle histogram inspection.

Evidence of Compliance:
✓ Written procedure defining method for checking specimens for in vitro hemolysis and lipemia

REFERENCES

HEM.22625  Storage and Stability - Hematology  Phase I

The laboratory clearly defines sample storage conditions and stability for all hematology
parameters.

NOTE: The laboratory should define sample storage conditions and stability for all hematology parameters, as time- and temperature-dependent alterations can occur, creating spurious results.

Evidence of Compliance:
✓ Written policy defining specimen stability and storage requirements

REFERENCES

SPECIMEN COLLECTION AND HANDLING - COAGULATION

Inspector Instructions:

- Sampling of coagulation specimen collection and handling policies and procedures
- Sampling of specimen rejection records/log

- Sampling of patient coagulation specimens (anticoagulant, labeling)

- How do you know if the specimen is clotted?
- What further actions are necessary if the specimen has a hematocrit of 60%?
- What is your course of action when you receive unacceptable coagulation specimens?

HEM.22707 Specimen Collection - Intravenous Lines

There is a documented procedure regarding clearing (flushing) of the volume of intravenous lines before drawing samples for hemostasis testing.

NOTE: Collection of blood for coagulation testing through intravenous lines that have been previously flushed with heparin should be avoided, if possible. If the blood must be drawn through an indwelling catheter, possible heparin contamination and specimen dilution should be considered. When obtaining specimens from indwelling lines that may contain heparin, the line should be flushed with 5 mL of saline, and the first 5 mL of blood or 6-times the line volume (dead space volume of the catheter) be drawn off and discarded before the coagulation tube is filled. For those samples collected from a normal saline lock (capped off venous port) twice the dead space volume of the catheter and extension set should be discarded.

REFERENCES
Hematology and Coagulation Checklist

HEM.22748  Anticoagulant - Coagulation  Phase I

All coagulation specimens should be collected into 3.2% buffered sodium citrate.

NOTE: Sodium citrate is effective as an anticoagulant due to its mild calcium-chelating properties. Of the 2 commercially available forms of citrate, 3.2% buffered sodium citrate (105-109 mmol/L of the dihydrate form of trisodium citrate Na3C6H5O7·2H2O) is the recommended anticoagulant for coagulation testing. Reference intervals for clot-based assays should be determined using the same concentration of sodium citrate that the laboratory uses for patient testing. The higher citrate concentration in 3.8% sodium citrate, may result in falsely lengthened clotting times (more so than 3.2% sodium citrate) for calcium-dependent coagulation tests (i.e. PT and aPTT) performed on slightly underfilled samples and samples with high hematocrits. Coagulation testing cannot be performed in samples collected in EDTA due to the more potent calcium chelation. Heparinized tubes are not appropriate due to the inhibitory effect of heparin on multiple coagulation proteins. Testing for platelet function cannot be performed on 3.2% or 3.8% sodium citrate.

Evidence of Compliance:
✓ Written procedure defining the use of 3.2% buffered sodium citrate for coagulation specimen collection OR procedure with an alternative anticoagulant defined with validation data

REFERENCES
2) Reneke, J et al. Prolonged prothrombin time and activated partial thromboplastin time due to underfilled specimen tubes with 109 mmol/L (3.2%) citrate anticoagulant. Am J Clin Pathol. 1998;109:754-757

HEM.22789  Specimen Rejection Criteria - Coagulation  Phase I

There are documented guidelines for rejection of under- or overfilled collection tubes.

NOTE: The recommended proportion of blood to the sodium citrate anticoagulant volume is 9:1. Inadequate filling of the collection device will decrease this ratio, and may lead to inaccurate results for calcium-dependent clotting tests, such as the PT and aPTT. The effect on clotting time from under-filled tubes is more pronounced when samples are collected in 3.8% rather than 3.2% sodium citrate. The effect of fill volume on coagulation results also depends on the reagent used for testing, size of the evacuated collection tube, and citrate concentration. A minimum of 90% fill is recommended; testing on samples with less than 90% fill should be validated by the laboratory.

Evidence of Compliance:
✓ Records of rejected specimens

REFERENCES
1) Peterson P, Gottfried EL. The effects of inaccurate blood sample volume on prothrombin time (PT) and activated partial thromboplastin time. Thromb Haemost. 1982;47:101-103
HEM.22830  Hematocrit Criteria - Coagulation  Phase I

There are documented guidelines for detection and special handling of specimens with elevated hematocrits.

NOTE: A hematocrit value >55% may lead to spurious coagulation results. The citrate anticoagulant distributes only in the plasma and not into the blood cells. For this reason, plasma citrate concentration will be increased if the patient's hematocrit is greater than 55%, potentially leading to spuriously prolonged PT and aPTT results, as well as erroneous results for other calcium-dependent clotting tests such as clottable protein C/protein S and factor assays. Accordingly, there should be a documented procedure for detection and special handling of polycythemic specimens. If possible, a new phlebotomy should be performed, using a reduced volume of sodium citrate, adjusted for the elevated hematocrit. Conversely, there are no current data to support a recommendation for adjusting the citrate concentration in the presence of severe anemia (hematocrit <20%).

REFERENCES

HEM.22871  Specimen Quality Assessment - Coagulation  Phase II

Coagulation specimens are checked for clots (visual, applicator sticks, or by analysis of testing results) before testing or reporting results.

NOTE: Specimens with grossly visible clots may have extremely low levels of fibrinogen and variably decreased levels of other coagulation proteins, so that results of the PT, aPTT, fibrinogen and other coagulation assays will be inaccurate or unobtainable. Checking for clots may be done with applicator sticks or by visual inspection of centrifuged plasma for small clots. This may also be performed by analysis of results (waveform analysis or delta checks). Additionally, when a clot is not detected during PT and aPTT testing and, where the fibrinogen level is <25 mg/dL, it should be suspected that the sample is actually serum. This may be important when coagulation specimens are received as centrifuged, frozen “plasma”. Centrifuged plasma and serum cannot be distinguished by visual inspection alone. There should be a mechanism in place to identify these specimens appropriately and/or to reject the sample as a probable serum sample. Laboratories should be encouraged to work with their clients that perform sample processing to ensure that they practice appropriate specimen handling for coagulation specimens.

REFERENCES
2) Arkin CF. Collection, handling, storage of coagulation specimens. Advance/Lab. 2002;11(1);33-38

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HEM.22912  Specimen Handling - Coagulation  Phase II

Coagulation tests are promptly performed on fresh plasma, or the platelet-poor plasma is frozen until testing can be performed.

NOTE: After blood collection, there is progressive degradation of the labile coagulation factors V and VIII, leading to increasing prolongation of the aPTT and PT. The allowable time interval
between specimen collection and sample testing depends on the temperature encountered during transport and storage of the specimen. Allowable time intervals are as follows:

1. PT specimens, uncentrifuged or centrifuged with plasma remaining in the capped tube above the packed cells, should be kept at 18 to 24°C and tested no longer than 24 hours from the time of specimen collection.

2. aPTT specimens that are uncentrifuged with plasma remaining in the capped tube above the packed cells should be kept at 18 to 24°C and tested no longer than 4 hours after the time of specimen collection.

3. aPTT specimens that are centrifuged and plasma separated from cells can be kept for 4 hours at 2 to 8°C or 18 to 24°C. Samples for unfractionated heparin testing should be centrifuged within one hour from the time of specimen collection.

4. Samples for other coagulation factors (e.g. thrombin time, protein C, factor V, factor VIII) have variable stability and should be kept in the same manner as aPTT samples. If testing cannot be performed within these times, platelet-poor plasma should be removed from the cells and frozen at −20°C for up to 2 weeks or at −70°C for up to 6 months. If a laboratory has established an allowable time interval different than that detailed above, data must be available to verify that coagulation testing is valid in the time interval established.

Evidence of Compliance:
✓ Written procedure defining specimen stability requirements and sample preservation for delays in coagulation testing

REFERENCES
4) Davis KD, et al. Use of different thromboplastin reagents causes greater variability in international normalized ratio results than prolonged room temperature storage of specimens. Arch Pathol Lab Med. 1998;122:972-977

Platelet Function Studies
Platelet functional studies (platelet aggregation or initial platelet function test) are performed within an appropriate period after venipuncture.

NOTE: Following venipuncture, platelets continue to activate in vitro, so that platelet functionality becomes abnormal after a period of approximately 3-4 hours. The laboratory must ensure that platelet aggregation studies are completed within 3-4 hours from the time of phlebotomy, or erroneous results could be obtained.

Evidence of Compliance:
✓ Written procedure defining specimen stability for platelet function studies AND
✓ Records reflecting completion of testing within defined time period

REFERENCES

RESULTS REPORTING - GENERAL
Inspector Instructions:
- Sampling of reporting policies and procedures
**HEM.23000**  Reference Intervals Established

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

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

**Evidence of Compliance:**

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

**REFERENCES**


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**HEM.23050**  Reference Intervals

**Phase II**

Patient results are reported with accompanying reference intervals or interpretive ranges.

**NOTE:** The results of commercial quality control plasmas that may be used in coagulation assays are internal data for quality assurance purposes, and must NOT be externally reported; if reported with patient results, they may be confused as normal values.
If absolute cell counts are reported with their reference ranges, then percent cell count reference ranges should not be reported because they can lead to misinterpretation of CBC data.

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

REFERENCES

HEM.23150 Turnaround Time

Phase II

Routine and STAT results are available within a reasonable time.

NOTE: A reasonable time for routine daily service, assuming receipt or collection of specimen in the morning, is 4-8 hours. For common hematology and coagulation tests, emergency or STAT results that do not require special additional verification procedures should be reported within 1 hour after specimen receipt in the laboratory.

Evidence of Compliance:
✓ Written policy with established TAT for routine and STAT tests AND
✓ QM records

REFERENCES

RESULTS REPORTING - COAGULATION

ABBREVIATIONS: aPTT = activated partial thromboplastin time(s); INR = International Normalized Ratio(s); ISI = International Sensitivity Index; PT = prothrombin time(s)

Inspector Instructions:

- Sampling of reporting policies and procedures
- Sampling of patient PT/aPTT reports

- How have you established or validated your PT and aPTT reference ranges using the current lot numbers of PT and aPTT reagents?
- How have you established and validated your aPTT-based heparin therapeutic range?
- How do you establish the geometric mean?

- Examine the current PT reagent lot package insert for the ISI value, lot number and instrument model, and verify that the reagent lot number and ISI value is programmed for the correct instrument model
- Review the data used to establish the geometric mean, and compare to that entered into the instrument
- Verify calculations for the current reagent lot in use and examine the lot number validation records for the correct INR calculations
- Check patient reports to ensure INR and reference range correlate with the data obtained in the lot number conversion

**HEM.23220 ISI Phase II**

For PT, there is documentation that the ISI is appropriate to the particular PT reagent and instrumentation used.

**NOTE:** The laboratory must demonstrate appropriateness of its ISI, a measurement of the sensitivity with which thromboplastin reagents detect decreased levels of vitamin K-dependent coagulation factors. The ISI used should be appropriate for the particular reagent-instrument combination and method of clot detection. Acceptable documentation would include information from the instrument/reagent manufacturer or local calibration using an FDA-approved product. This is especially true for photo-optical vs. electromechanical instruments, but may also vary among different instruments within the same classification.

**Evidence of Compliance:**
✓ Record showing information from the instrument/reagent manufacturer OR use of an ISI calculated from laboratory specimens

**REFERENCES**
3) CLSI. Procedures for validation of INR and local calibration of PT/INR systems; Approved Guideline. CLSI document H54-A. CLSI, Wayne, PA, 2008

**HEM.23290 INR Calculation Phase II**

The calculation of the INR is appropriately adjusted for every new lot of PT reagent, changes in types of reagent or change in instrumentation.

**NOTE:** The ISI value usually changes with each new lot of PT reagent. The ISI reflects the sensitivity of the PT reagent to decreased levels of the vitamin K-dependent coagulation factors. This change in sensitivity will affect the calculation of the INR value.

The laboratory should be able to provide documentation that calculation of the INR is correct and that the ISI value is appropriate for the lot of thromboplastin reagent and for the method of clot detection. Such documentation should be available wherever the INR is calculated, whether by the coagulation instrument, laboratory information system, or manually.

It is critical to calculate and report appropriate INR values. Reporting erroneous INR values may lead to use of excessive or insufficient vitamin K antagonist medication, which may result in bleeding or thrombotic complications in patients.

**Evidence of Compliance:**
✓ Records showing that the values used in the INR calculation were determined and recalculated for new kits if PT reagent and for any other changes

**REFERENCES**
1) CLSI. One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (aPTT) Test; Approved Guideline-Second Edition. CLSI Document H47-A2. (ISBN 1-56238-672-7). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA,
HEM.23360 INR Geometric Mean

Phase II

The appropriate geometric mean of the PT reference interval is used in the INR calculation.

NOTE: The appropriate geometric mean of the PT reference interval must be used in the INR calculation, given by the formula:

\[ \text{INR} = \left( \frac{\text{PT of patient}}{\text{PT of geometric mean normal population}} \right)^{\text{ISI}} \]

The mean normal population value may change when the specimen collection process, instrument, reagent lot, or reagent changes.

When the distribution of values is distributed normally, the geometric mean, the arithmetic mean, the median and the mode of the population being studied are identical theoretically. These values diverge from each other, however, as the population distribution becomes more skewed. The geometric mean is a more appropriate estimate of the average value than the arithmetic mean when the population of interest is lognormally distributed because the geometric mean takes skewing into account.

Calculation of the geometric mean is indicated below; this calculation is available in many spreadsheet programs, such as Microsoft Excel.

\[ \text{GM} = \exp \left( \frac{\log(x_1) + \log(x_2) + \log(x_3) + \ldots + \log(x_n)}{n} \right) \]

Evidence of Compliance:

✓ Written procedure for determining the geometric mean and its use in the INR calculation

AND

✓ Records for geometric mean determinations and INR calculations for each instrument and PT reagent lots used

REFERENCES


3) CLSI. Procedures for validation of INR and local calibration of PT/INR systems; Approved Guideline. CLSI document H54-A. CLSI, Wayne, PA. 2008

HEM.23430 Report Verification Criteria

Phase II

There are checks of patient reports for correct INR calculations, patient values, and reference ranges under the following circumstances.

1. Change in lot or type of PT reagent
2. Change in instrument
3. Establishment of new PT reference range
4. Change in INR calculation
5. At defined intervals, in the absence of the above changes

NOTE: It is suggested that the calculations be checked at the following INR values: 2.0 and 3.0. Patient reports should be checked at least once per year even in the absence of changes to the test system and calculations. This requirement applies whether the INR is calculated by the coagulation analyzer or by the laboratory information system.
Evidence of Compliance:
✓ Records of patient report checks documented at defined frequency

REFERENCES
4) CLSI. Procedures for validation of INR and local calibration of PT/INR systems; Approved Guideline. CLSI document H54-A. CLSI, Wayne, PA, 2008

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HEM.23453 Heparin Therapeutic Range

Phase I

There is documentation that the aPTT-based heparin therapeutic range is established and subsequently validated using an appropriate technique, when appropriate.

NOTE: The heparin-responsiveness of aPTT reagents may change from lot to lot and among different reagents used on different instrument platforms. For this reason, it is necessary to establish the heparin therapeutic range for the aPTT assay with each change of coagulation instrument and/or reagent type. The therapeutic range must be validated with each new lot of a given aPTT reagent. Anti-Xa activity is an acceptable alternate method to monitor heparin therapy.

The aPTT is commonly used to monitor the anticoagulant effects of unfractionated heparin. The therapeutic range for heparin therapy should be established by using ex vivo plasma samples anticoagulated with 3.2% sodium citrate obtained from patients receiving therapeutic doses of unfractionated heparin. This can be accomplished one of two ways: 1) the aPTT and heparin activity is measured for each sample and the aPTT range is calculated by comparison to heparin activity, or 2) the aPTT of patient samples using the new lot or aPTT method is compared to the prior aPTT lot. It is recommended that the first method be used initially to establish the therapeutic range before starting patient testing with a new instrument or new reagent, while the second can be used for validation of the therapeutic range with subsequent reagent lot changes. It is not best practice to use plasma samples spiked with heparin in vitro to calculate the therapeutic range, as differences in heparin binding proteins in vitro may lead to overestimation of the therapeutic range.

Evidence of Compliance:
✓ Written procedure defining criteria for establishing and validating the aPTT heparin therapeutic range

REFERENCES

HEM.23500 Reference Intervals

Phase II

Reference intervals for PT and aPTT are current for the reagent or lot number, and are appropriately determined.
NOTE: Because of the variability between different types of PT and aPTT reagents, and even different lots of PT and aPTT reagents, there may be significant changes in the reference interval after a change of the type or lot of reagent. For this reason, the laboratory should establish or verify the reference interval with each change of lot or change in reagent.

Evidence of Compliance:
✓ Written procedure with defined criteria for determining reference intervals for PT and aPTT AND
✓ Reports showing verification of the reference interval with changes of lot or reagent AND
✓ Patient reports reflecting the use of the correct reference intervals

Coagulation Test Recommendations

Recommendations are available to clinicians concerning which laboratory tests to use for monitoring heparin, low molecular weight heparin, direct thrombin inhibitors (e.g. lepirudin, bivalirudin, argatroban) and/or oral anticoagulant therapy, and the therapeutic range for the tests.

NOTE: For vitamin K antagonists (e.g. warfarin), the prothrombin time (PT/INR) is recommended, although many other methods are still in use. In addition, more than a dozen methods are in use for monitoring heparin and low molecular weight heparin therapy. For unfractionated heparin the activated partial thromboplastin time (aPTT) and/or activated clotting time are commonly used, but the heparin assay (factor Xa inhibition) may also be employed. For low molecular weight heparin or danaparoid, monitoring is often not necessary, but the heparin assay (Xa inhibition assay) may be used in certain circumstances, as the aPTT is generally insensitive to the effect of these agents. Direct thrombin inhibitors are becoming more widely utilized and these drugs are often monitored using the aPTT. The tests available should be applicable to the anticoagulant drugs available on the pharmacy formulary at the medical institution. The laboratory should work closely with the pharmacy or therapeutics committee to ensure that appropriate assays are available for the drugs in use by physicians, and that information is available on the test values that indicate that the anticoagulant is in a therapeutic range.

Evidence of Compliance:
✓ Memorandums to physicians, test reference guide, interpretive comments in patient reports, or other documented mechanism for providing recommendations to physicians for ordering and interpreting coagulation tests used to monitor anticoagulant therapy

REFERENCES
9) Davis KD, et al. Use of different thromboplastin reagents causes greater variability in international normalized ratio results than prolonged room temperature storage of specimens. Arch Pathol Lab Med. 1998;122:972-977
Hematology and Coagulation Checklist


REAGENTS

Inspector Instructions:

- Sampling of new reagent/shipment verification records
- How do you verify new reagent lots?
- How does your laboratory manage and control inventory?

Additional requirements are in the REAGENTS section of the All Common Checklist.

HEM.24150 Reagent Handling - Coagulation

Procedures are in place to ensure that prepared reagents are maintained at proper temperature for testing, correctly stored, mixed when appropriate, and discarded when stability parameters are exceeded.

NOTE: Prepared reagents require varying conditions for optimal stability and performance. Manufacturer guidelines must be strictly followed or deviations from the package insert validated.

REFERENCES

INSTRUMENTS AND EQUIPMENT

A variety of instruments and equipment are used to support the performance of analytical procedures. All instruments and equipment should be properly operated, maintained, serviced, and monitored to ensure that malfunctions of these instruments and equipment do not adversely affect the analytical results. The procedures and schedules for instrument maintenance must be as thorough and as frequent as specified by the manufacturer.

Inspector Instructions:

- Sampling of instrument policies and procedures
- Sampling of temperature logs (refrigerator, freezer, water bath, heat block)
- Sampling of pipette/dilutor checks
- Sampling of instrument/microscope maintenance logs and repair records
Hematology and Coagulation Checklist

**HEM.25000 Temperature Checks**

**Phase II**

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

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

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

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

**HEM.25150 Pipettors and Dilutors**

**Phase II**

Pipettors and dilutors (fixed volume or adjustable) are checked at least annually for accuracy and reproducibility, (gravimetric, colorimetric or other verification procedure), and results recorded.

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

**Evidence of Compliance:**

✓ Written procedure detailing method for checking the accuracy and reproducibility of automatic pipettes

**REFERENCES**

HEM.25175 Pipette Carryover

The laboratory has evaluated its automatic pipetting systems for carryover.

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

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

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

This requirement is not applicable to coagulation.

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

REFERENCES

HEM.25250 Glassware Accuracy

Volumetric glassware is of certified accuracy (Class A), or checked by the laboratory to verify accuracy.

NOTE: Volumetric glassware must be certified for accuracy (class A) or checked for accuracy before being placed in service.

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

REFERENCES

HEM.25300 Microscope Maintenance

Microscopes are clean, adequate (i.e. low, high dry and oil immersion lenses), optically aligned, and properly maintained with documentation of preventive maintenance.

NOTE: Koehler illumination must be maintained for optimal resolution. For manual platelet counting, a phase contrast microscope is recommended.

REFERENCES
1) Vetter JP. Solving problems with illumination, focus, and detail in color photomicrography. Lab Med. 1997;28:719-723

HEM.25350 Instrument Service Records

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

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

COMPLETE BLOOD COUNT (CBC) INSTRUMENTS

CALIBRATION

Several different methods may be used for calibration of an automated Complete Blood Count (CBC) instrument. The laboratory should have a document that describes in detail the procedures for calibration and calibration verification.

Calibration techniques include: A) the use of multiple analyzed fresh whole blood specimens, and B) the use of manufactured, stabilized preparations of red cells, white cells (or white cell surrogates) and platelets (or platelet surrogates). Typically, a laboratory uses one of these two approaches as their primary calibration technique, with the other for backup, or for verification of the primary method, or on an emergency basis.

When stabilized whole blood or other commercial preparations are used for the periodic recalibration of automated instruments, the target values for the measured parameters must have been assigned by using primary reference procedures. The laboratory may assign such values or the manufacturer may certify that the target values were derived through primary reference procedures. All calibration techniques should include periodic verifications of analyzer hemoglobin measurements against a certified hemoglobin preparation (ICSH/WHO international haemoglobincyanide standard), or material that has been certified by its manufacturer as being derived from the certified international haemoglobincyanide standard using reference procedures. Ordinary commercial control materials are not suitable for instrument calibration.

Inspector Instructions:

- Sampling of CBC calibration policies or procedures
- Sampling of CBC calibration records
- What is your course of action if the CBC instrument fails to pass all calibration parameters?
- When was the last time you performed a calibration procedure and how did you verify the calibration?

HEM.25400 Precalibrated Instrument Verification Phase II

If precalibrated instruments are used, the manufacturer's calibrations are verified with appropriate control materials for the system.

NOTE: This requirement does not apply to CBC instruments that can be calibrated by the laboratory.

Evidence of Compliance:
✓ Records of calibration verification following manufacturer’s instructions

REFERENCES

Fresh Whole Blood

HEM.25500 Periodic Calibration - Whole Blood Phase II

There is a document defining the specific procedural steps for the periodic calibration of the analyzer with fresh whole blood specimens.

NOTE: The laboratory must have criteria that define when recalibration is necessary, based upon the data from the daily quality control system.

REFERENCES
1) NCCLS. Calibration and Quality Control of Automated Hematology Analyzers; Proposed Standard. NCCLS document H38-P [ISBN 1-56238-398-1]. NCCLS, 940 West Valley Road, Wayne, Pennsylvania 19087, U.S.A 1999

HEM.25570 Calibration Verification Criteria Phase II

Criteria are established for calibration verification.

NOTE: Criteria must be established for calibration verification. Criteria include:

1. At complete changes of reagents (i.e. change in type of reagent from same vendor, or change to a different vendor)
2. When indicated by quality control data
3. After major maintenance or service
4. When recommended by the manufacturer
5. At least every six months

One common method of calibration verification involves processing a commercial calibrator and comparing results to those published by the manufacturer. Linearity studies are not required.

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

REFERENCES

HEM.25600 Recalibration Phase II

The laboratory’s procedure for recalibration of CBC instrument parameter(s) requires one of the following approaches.

1. Comparison to at least 10 fresh whole blood samples whose values have been determined by duplicate analysis in another instrument known to be accurately calibrated, or
2. Duplicate analysis of at least 10 fresh whole blood specimens by reference methods

NOTE: The selection of 10 different blood samples (or alternate protocol recommended by the instrument manufacturer) is needed to accommodate a diversity of matrices, and to have the
absolute minimum sample size on which to perform statistical calculations. Such fresh samples must have values within the instrument's operating ranges, and must not generate flags indicative of possible abnormalities.

Evidence of Compliance:
✓ Written procedure defining the criteria for recalibration

REFERENCES
1) NCCLS. Calibration and Quality Control of Automated Hematology Analyzers; Proposed Standard. NCCLS document H38-P [ISBN 1-56238-398-1]. NCCLS, 940 West Valley Road, Wayne, Pennsylvania 19087, U.S.A 1999

HEM.25625 Verification Following Whole Blood Calibration Phase I

Following whole blood calibration, there is a documented procedure for calibration verification.

NOTE: Following whole blood calibration, the laboratory should have a documented procedure for verifying that whole blood calibration has been successful, i.e. that the accuracy of test results is established.

Evidence of Compliance:
✓ Records of acceptable calibration verification

REFERENCES
1) NCCLS. Calibration and Quality Control of Automated Hematology Analyzers; Proposed Standard. NCCLS document H38-P [ISBN 1-56238-398-1]. NCCLS, 940 West Valley Road, Wayne, Pennsylvania 19087, U.S.A 1999

Commercial Calibrators

Commercially available calibrator materials represent a convenient way to ensure that CBC instruments yield accurate results. Because of differences in technology, such calibrators are typically instrument-specific, and are cleared by the Food and Drug Administration for such use. These calibrators have more rigorous assignment of target values than ordinary commercial QC materials, and the latter must not be used for routine instrument calibration.

HEM.25700 Periodic Calibration - Stabilized Materials Phase II

There is a document defining the specific procedural steps for the periodic calibration of the analyzer with stabilized materials whose target values have been certified by the manufacturer using primary reference procedures.

NOTE: The laboratory must have criteria that define when recalibration is necessary, based upon the data from the daily quality control system.

REFERENCES
3) NCCLS. Calibration and Quality Control of Automated Hematology Analyzers; Proposed Standard. NCCLS document H38-P [ISBN 1-56238-398-1]. NCCLS, 940 West Valley Road, Wayne, Pennsylvania 19087, U.S.A 1999

HEM.25760 Calibration Verification Criteria Phase II

Criteria are established for calibration verification.

NOTE: Criteria must be established for calibration verification. Criteria include:

1. At complete changes of reagents (i.e. change in type of reagent from same vendor,
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or change to a different vendor)
2. When indicated by quality control data
3. After major maintenance or service
4. When recommended by the manufacturer
5. At least every six months

One common method of calibration verification involves processing a commercial calibrator and comparing results to those published by the manufacturer. Linearity studies are not required.

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

REFERENCES

HEM.25780 Recalibration

The laboratory’s procedure for recalibration of a parameter(s) requires analysis of stabilized whole blood or other commercial preparations, the parameters of which have been certified by the manufacturer.

Evidence of Compliance:
✓ Written procedure defining the criteria for recalibration

HEM.25785 Verification Following Commercial Calibrator Calibration

Following calibration with commercial calibrators, there is a documented procedure for calibration verification.

Evidence of Compliance:
✓ Records of acceptable calibration verification

**CBC INSTRUMENT QUALITY CONTROL**

Longitudinal process quality control (QC) procedures for individual instruments or interinstrument comparisons may include:

1. Use of preserved or stabilized whole blood controls
2. "Moving average" monitoring
3. Retained patient specimens, or
4. Some combination of the above

At least 2 different controls must be assayed and evaluated every 24 hours. For each QC procedure employed, the laboratory must have appropriate QC ranges. For example, expected recovery ranges for commercial control materials are **NOT** the same as between-run SD ranges, and are probably too wide for daily QC of a single instrument. The laboratory should calculate its own imprecision statistics for each instrument.

**Inspector Instructions:**
- Sampling of QC policies and procedures
- Sampling of QC records
**Stabilized Controls**

HEM.25850  Stabilized Controls  

**Two different stabilized control specimens are analyzed and results recorded during each 24-hours of analyzer use.**

**NOTE:** Stabilized control materials must be at 2 different analytic levels (i.e., "normal" and "high"). Three levels of control is a conceptual carryover from clinical chemistry, and does not apply to hematology particle counting. Dilute, "low-level" (e.g. leukopenic and thrombocytopenic) "oncology" controls are less informative indicators of calibration status, and are neither required nor recommended. For example, a 10% calibration bias will be numerically most apparent in a high-level control, less apparent in a normal-level control, and perhaps inapparent in a low-level control; it would be quite extraordinary for a low-level control to indicate a calibration problem that is not revealed by the other controls. There should be some relationship between the frequency of control runs and the numbers of patient specimens processed. If the frequency of commercial control use is less than 2 control specimens per 24 hours, one or more of the additional approaches to QC must be employed to produce a total of at least 2 different data points per 24 hours.

**REFERENCES**

3) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. Fed Register. 2003(Jan 24);7168 42CFR493.1256(d)

HEM.25870  Commercially Assayed Controls  

If commercially ASSAYED controls are used for CBC instruments, control values correspond to the methodology and target values (mean and QC ranges) are verified or established by the laboratory.

**NOTE:** Most commercial controls have expected recovery ranges for each parameter, provided by the manufacturer. The mean of such ranges may not be the exact target value in a given laboratory. Each laboratory must assign its own initial target value, based on initial analysis of the material; this target value should fall within the recovery range supplied by the manufacturer, but need not exactly match the package insert mean. The laboratory must establish specific recovery ranges that accommodate known changes in product attributes, assuming that calibration status has not changed.
Evidence of Compliance:
✓ Written procedure for establishing or verifying control ranges for assayed control materials
AND
✓ Records for control range determination

HEM.25880  Unassayed Controls  Phase II

If UNASSAYED controls are used, statistically valid target mean and range are established for each lot by repetitive analysis in runs that include previously tested control materials.

Evidence of Compliance:
✓ Written procedure for establishing or verifying control ranges for unassayed control materials
AND
✓ Records for control range determination

REFERENCES

Moving Averages

The technique of weighted moving averages (derived from multiple batch analysis of patient samples) is acceptably sensitive to drifts or shifts in analyzer calibration if a supplemental QC routine (stabilized control material or retained patient specimens) is employed. The latter is needed to detect random error and to avoid bias due to masking of drift by characteristics of the subpopulations within each individual batch.

Laboratories analyzing fewer than 100 CBC specimens daily (long term average) should not use moving averages as the primary method for process control, as this would not generate sufficient data within a day to be of value.

Depending on the particular instrument, there may be "on-board" moving average analyses for RBC indices only. In such cases, additional QC techniques are required for WBC, PLT and WBC differential parameters. However, some laboratories have found the mathematical logic of moving averages, modified average of normals, etc, applicable to other CBC parameters, and some instruments have these capabilities built into their software. Or, such calculations may be performed with an associated computer.

HEM.25920  QC - Moving Averages  Phase II

Control limits for moving averages are appropriately sensitive.

NOTE: Control limits for moving averages must be appropriately sensitive such that significant calibration alterations are always detected. Recalibration is not required for minor calibration variations of no clinical consequence. In other words, there should be a high probability for error detection and a low probability for false rejection.

Evidence of Compliance:
✓ Written procedure defining the:
  • method used to establish the moving average AND
  • frequency of calculation (batch size) AND
  • definition of the basis for selection of upper and lower limits

REFERENCES
**HEM.25990 QC Procedure**

**Phase II**

If a "moving averages" system is combined with another control system (e.g. commercial controls or retained patient specimens), the process is well-defined and appropriately sensitive to drift in analyzer calibration.

**Evidence of Compliance:**

- ✓ Written QC procedure defining criteria for the use of a moving average system in conjunction with another QC system

**REFERENCES**


9) NCCLS. Calibration and Quality Control of Automated Hematology Analyzers; Proposed Standard. NCCLS document H38-P [ISBN 1-56238-398-1]. NCCLS, 940 West Valley Road, Wayne, Pennsylvania 19087, U.S.A. 1999

### Retained Patient Specimens

Use of retained patient specimens alone is inadequate for routine QC of the primary CBC instrument, and must be considered as a supplemental procedure, in combination with another QC system. Retained patient specimens, while conveniently available, present some difficulties in mathematically defining "agreement" between CBC results separated in time, as these are not stabilized samples. This is in contrast to commercial control materials that have been treated to reduce time-dependent degradation.

**HEM.26660 QC - Retained Patient Specimens**

**Phase I**

When the laboratory uses retained patient samples, statistically defined limits are used to determine agreement of sequential assays of a given sample.

**NOTE:** Allowance should be made for time-dependent alterations in data from such labile samples.

**Evidence of Compliance:**

- ✓ Written QC procedure defining the control limits for repeat analysis of retained patient specimens AND
- ✓ QC records showing the use of the defined control limits
HEM.27330  QC - CBC Defined Range  Phase I

**HEMATOLOGY AND COAGULATION CHECKLIST**

**HEM.27330 QC - CBC Defined Range**

There is a defined range of CBC values for which these limits are applicable.

**NOTE:** Because imprecision (standard deviation, coefficient of variation) is dependent upon the hematologic target value, the laboratory should restrict the use of these limits to appropriate ranges of CBC values.

**Evidence of Compliance:**
✓ Written QC procedure for retained patient specimen controls defining the CBC target values for which the defined control limits are applicable.

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**Interinstrument Comparisons**

The laboratory may use fresh patient or donor specimens analyzed on a primary instrument for daily QC of a secondary instrument. The selection of these materials (rather than simply stabilized commercial controls) is important to directly address the issue of whether a patient sample yields the same results on all of the laboratory's instruments.

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**REVISED** 07/11/2011

HEM.28000  Comparability of Instruments/Methods  Phase II

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

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

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

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

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

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

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**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 [42CFR493.1249(a) and (b)]


HEM.28666 Defined Tolerance Limits

There are statistically defined tolerance limits for agreement of interinstrument assays.

NOTE: Statistically defined tolerance limits typically refer to standard deviations and confidence intervals (e.g. 2SDs for 95% limit, 3SDs for 99% limit) which are defined for agreement for any given assay when run on multiple instruments (i.e. cross checks or correlations). Laboratories may use different approaches for defining these tolerance limits, and these thresholds are often based on medical decision criteria. Some laboratories use regression analysis software in which 10-20 patient specimens are run on two instruments that cover the range of expected patient results. Results when plotted are expected to fall within a defined limit.

Evidence of Compliance:
✓ Written procedure defining limits of agreement for multiple instrument comparison

HEM.29332 QC - CBC Defined Range

There is a defined range of CBC values for which these limits are applicable.

NOTE: Because imprecision (standard deviation, coefficient of variation) is dependent upon the hematologic target value, the laboratory should restrict the use of these limits to appropriate ranges of CBC values when comparing results across multiple instruments.

Evidence of Compliance:
✓ Written QC procedure defining the CBC specimen target values for which the control limits used for multiple instrument comparison are appropriate

ERROR DETECTION AND VERIFICATION

Inspector Instructions:

- Sampling of CBC error detection policies and procedures
- How do you ensure results from CBC specimens with cold agglutinins, nRBCs and lipemia are reported accurately?
- Select a spurious result example and follow the process used to ensure the correct results are reported

HEM.30070 Sampling Mode Comparison

There are data that at least annually compare all results obtained for patient specimens analyzed in the multiple sampling modes of the CBC analyzer (e.g. "open" and "closed" modes) to ensure that they are in agreement.
NOTE: Different modes may involve a different sample path before analysis. When samples are analyzed in more than one mode, it is important to ensure that all modes function properly. Re-analysis of a previously analyzed sample should be performed in the alternate mode(s), and results should agree with the initial mode within the tolerance limits established for agreement by the hematology laboratory’s quality control program, and any recommendations by the instrument manufacturer. Mode-to-mode correlation is not necessary for those analyzers which use the same pathway for all modes.

Evidence of Compliance:
✓ Written procedure for sampling mode comparison with defined criteria for agreement AND
✓ Records of sampling mode comparison studies

HEM.30080 New Reagent Lot Verification - Inert Materials Phase II
Checks are performed on inert materials to ensure that new lots do not interfere with the method (e.g. performance of background counts on diluents).

Evidence of Compliance
✓ Written procedure for checking inert materials AND
✓ Records of material checks, as applicable

HEM.30100 Detection/Correction Procedure - WBC Phase II
There is a documented procedure available and in use for detecting and correcting automated WBC counts for the presence of nucleated red cells or megakaryocytes.

NOTE: The effect of nucleated erythrocytes and blood megakaryocytes on the apparent WBC count varies with the system used for analysis. Each laboratory should evaluate its system(s) and develop appropriate detection and correction procedures. This is important to prevent reporting a falsely high WBC concentration. With some automated CBC instruments, nucleated erythrocytes or megakaryocytes may present themselves histographically or cytographically, and this can serve as an indicator for careful stained blood film inspection. The laboratory must establish if its particular instrument(s) includes some or all nucleated non-leukocytes in its apparent WBC “count”.

Evidence of Compliance:
✓ Records showing actions taken to verify CBC concentration prior to reporting

REFERENCES

HEM.30150 Spurious Results Phase II
A documented procedure is in use to detect other spurious CBC instrument results that may be clinically significant (e.g. pseudomacrocytosis from rouleaux or agglutinates; pseudoleukocytosis with erroneous hemoglobin, falsely low erythrocyte count and hematocrit; hyperlipemias).

NOTE: Analytic sources of error with automated instruments depend on the type of instrument and reagents used by the laboratory. Common potential errors for the hemogram (without platelets) include pseudomacrocytosis (due to microclots, cold agglutinins, rouleaux, or osmotic matrix effects), pseudoleukocytosis (due to platelet agglutination, giant platelets, unlysed erythrocytes, nucleated erythrocytes, megakaryocytes, red cell inclusions, cryoproteins, circulating mucin), erroneous hemoglobin and indices (due to lipemia or leukocytosis), falsely low red cell concentration and hematocrit (due to in vitro hemolysis or extreme microcytosis), and
falsely depressed results for all parameters (due to clots).

**Evidence of Compliance:**
✓ Record of action taken when spurious CBC instrument results are detected

**REFERENCES**

**HEM.30200 Red Cell Indices**

**Phase I**

**Red cell indices (MCV, MCH, MCHC) are monitored routinely to detect random errors.**

**NOTE:** Patient sample red cell indices (Wintrobe indices or MCV, MCH, MCHC) should be monitored routinely to detect random errors, instrument malfunction, or spurious results. If semiautomated methods are used, indices should be calculated. On many automated instruments, the MCHC is the most useful parameter to ensure accuracy of the red cell parameters in individual patient samples. Since MCHC varies over a narrow range, an abnormal MCHC will often flag potentially spurious red cell parameters. Truly elevated MCHCs may be seen with spherocytosis, while decreased MCHCs can accompany a low MCV in severe iron deficiency anemia. If such RBC abnormalities are not present on the blood film, one or more of the measured RBC parameters is likely erroneous. Incorrect data may be due to instrument malfunction or to problems with the blood sample itself. Some examples include: spuriously elevated MCVs and MCHCs with cold agglutinins, falsely elevated MCHCs with lipemia and plasma paraproteins, spuriously low MCHCs with leukocytosis and osmotic effects such as hyperglycemia altering MCV. MCV and MCH are fairly constant for each patient, and monitoring these indices in a delta check error detection program may provide rapid patient-based detection of instrument malfunction or specimen misidentification.

**Evidence of Compliance:**
✓ Written procedure defining the criteria used to monitor the red cell indices to detect random errors **AND**
✓ Record of action taken when RBC indices are in question, including the reporting of results

**REFERENCES**

**HEM.30250 Reportable Range**

**Phase II**

**Upper and lower limits of all reportable parameters on the CBC instrument are defined, so results that fall outside these limits are verified before reporting.**

**NOTE:** The laboratory must initially establish or verify the reportable range for each parameter of its automated or semi-automated CBC instrument. In particular, the laboratory must have data on its instrument’s accuracy with thrombocytopenic and leukopenic samples. Platelet concentrations below the established lower limits must be reanalyzed by another method (e.g. manual hemocytometry, or semiquantitative blood film estimates, or fluorescence flow cytometry using specific platelet monoclonal antibodies). Particle (WBC, RBC, PLT) concentrations above
the established upper limits must, as clinically needed, be reanalyzed by doing the minimum dilution necessary to bring the counts into the instrument's analytic range. When clinically appropriate, apparent analyte concentrations that are lower or higher than the reportable range may be reported as "less than" the lower limit or "greater than" the higher limit.

Evidence of Compliance:
✓ Written procedure defining the upper and lower instrument reporting limits AND
✓ Record of action taken when limits are exceeded, including the reporting of results

REFERENCES

HEM.30300 PLT Abnormalities Phase II

There is an adequate system (such as microscopic correlation with the blood film) to prevent reporting of spurious thrombocytopenia when platelet clumps, giant platelets, or platelet satellitism are present.

NOTE: When PLT satellitosis (satellitism), significant numbers of giant PLT and/or PLT clumps are suspected/detected by cyto/histographic abnormalities or instrument rejection of a PLT result, the PLT concentration must be independently verified. Correlation with a well-prepared blood film must be made. If PLT are clumped after collection in an EDTA-anticoagulated tube that was well-mixed at the time of collection, this may represent in vitro EDTA-induced changes; PLT should be quantified from blood collected directly into a counting diluent, or by use of a different anticoagulant (e.g. liquid sodium citrate with subsequent adjustment for dilution) or by estimation from a non-anticoagulated blood film.

Evidence of Compliance:
✓ Written procedure defining the methods used to detect spurious thrombocytopenia or platelet abnormalities and to correct results AND
✓ Record showing actions taken to verify platelet concentration prior to reporting

REFERENCES

HEM.30350 Spuriously High WBC Concentration Phase II

If significant numbers of unlysed RBC, giant platelets and/or platelet clumps are suspected/detected, the WBC concentration is rechecked by another method or blood films are examined to prevent reporting spuriously high WBC concentrations.

NOTE: When unlysed RBC, PLT satellitosis, significant numbers of giant PLT and/or PLT clumps are suspected/detected by histographic abnormalities or instrument rejection of a PLT result, the WBC count must be verified manually, by automated counting after collection into a different anticoagulant, by automated counting in a lyse-resistant mode, or by semiquantitative blood film evaluation to prevent reporting spuriously high WBC concentrations.

Evidence of Compliance:
✓ Written procedure defining the criteria for the detection of falsely elevated WBC counts and to correct results AND
✓ Record showing actions taken to verify WBC concentration prior to reporting

REFERENCES

HEM.30400 Platelet Concentration Verification Phase II

If significant numbers of microcytic erythrocytes and/or small cell fragments are detected/suspected, the platelet count is determined or verified using an alternate method.

NOTE: When a significant number of interfering particles are identified at the upper or lower PLT counting threshold (by inspection of the PLT histogram or instrument flag), the PLT concentration must be determined or verified by an alternate method. Such methods could include alternate instrumentation, hemocytometry, or blood film estimate, depending upon the PLT concentration and the degree of clinical accuracy required.

Evidence of Compliance:
✓ Written procedure defining the criteria for detection of microcytic RBC and cell fragments that interfere with platelet counts AND
✓ Records showing action taken to verify platelet concentration prior to reporting

REFERENCES

GENERAL INSTRUMENT ISSUES

Inspector Instructions:

- Sampling of instrument(s) policies and procedures
- Sampling of instrument function logs

HEM.30550 Function Checks Phase II

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

NOTE: There must be a schedule and procedure at the instrument for appropriate function checks. These may include (but are not limited to) electronic, mechanical and operational checks. The procedure and schedule must be as thorough and as frequent as specified by the manufacturer. Function checks should be designed to check the critical operating characteristics to detect drift, instability, or malfunction, before the problem is allowed to affect test results. All servicing and repairs must be documented.
**REVISED** 07/31/2012
HEM.30600 Function Checks Phase II
Function checks are documented and readily available to detect instrument trends or malfunction.

HEM.30650 Instrument Tolerance Limits Phase II
Performance or tolerance limits are defined for each instrument, component, or procedure in the system.

Evidence of Compliance:
✓ Written procedure defining tolerance limits for each instrument, component or procedure

MANUAL HEMATOCRIT (MICROHEMATOCRIT, PACKED CELL VOLUME)

Inspector Instructions:
- Hematocrit policy or procedure
- Sampling of semi-annual centrifuge speed checks
- Sampling of timer checks

HEM.32050 Microhematocrit Centrifuge Phase II
The speed of the microhematocrit centrifuge is checked at least annually.

NOTE: Relative centrifugal field (rcf) must be sufficient to achieve maximum packing of cells. The centrifuge must be capable of sustaining a relative centrifugal field (rcf) of 10,000 to 15,000 at the periphery for 5 minutes.

If the centrifuge speed cannot be checked by the user, the laboratory must annually compare centrifuge test results against another centrifuge with known speed and constant packing time. If the laboratory does not have such an instrument, another laboratory or an outside vendor may be used for this comparison.

Evidence of Compliance:
✓ Written procedure defining criteria for verification of centrifuge operating speeds AND
✓ Records of microhematocrit centrifuge speed checks

REFERENCES

**REVISED** 07/11/2011
HEM.32100 Mechanical Timer Phase II
Mechanical timers and the speed of the centrifuge are checked for accuracy every six months.

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

Evidence of Compliance:
✓ Records of centrifuge checks documented at defined frequency

HEM.32150  Constant Packing Time  Phase II

The constant packing time (minimum spin to reach maximum packing of cells) is established before initial use and reassessed when there has been a change in either the speed or time.

Evidence of Compliance:
✓ Written procedure defining criteria for establishing/reassessing constant packing time AND
✓ Records of initial and reassessment studies as appropriate

REFERENCES

MANUAL (COUNTING CHAMBER) LEUKOCYTE (WBC) AND PLATELET (PLT) COUNTS (BLOOD)

NOTE: Counting chamber RBC counts are not recommended because of the level of imprecision and inability to verify results against a stained blood film.

Inspector Instructions:

READ

• Manual cell counts policy or procedure
• Sampling of QC logs

ASK

• How do you correlate counting chamber platelet counts?
• How do you ensure that your diluting fluids are free from contamination?

HEM.33200  Manual Counts - PLT/WBC  Phase II

If WBC or PLT counts are performed manually by pipette dilution and hemocytometer chamber count, each sample is counted in duplicate, plating both chambers of the hemocytometer.

NOTE: Performance of the counts in duplicate is required for all hemocytometers, whether glass or disposable.

Evidence of Compliance:
✓ Written procedure for manual PLT/WBC count requiring duplicate counts and defined limits of agreement AND
✓ Records or worksheets reflecting duplicate counts and corrective action when limits of agreement are exceeded
HEM.33250 Manual Counts - PLT/WBC Phase II

When there is leukopenia or thrombocytopenia, the manual hemocytometer procedure requires a technique to offset the increased error associated with counting smaller numbers of cells in the hemocytometer.

NOTE: The laboratory protocol must specify an increased number of cells counted (e.g. increased number of hemocytometer squares enumerated or a lesser specimen dilution) when there is leukopenia or thrombocytopenia, in order to avoid increasing the imprecision of particle counting, which is governed by binomial distributions and Poisson statistics.

Evidence of Compliance:
✓ Records or worksheets for manual counts on leukopenic or thrombocytopenic specimens

REFERENCES
3) Savage RA. Evaluate your practice for platelet counts. Northfield, IL: College of American Pathologists Summing Up, Fall 1987

HEM.33300 Contamination Checks Phase II

A system is documented for assuring that dilution fluids and reagents are free of contaminants that may spuriously change the true cell counts.

NOTE: Suggested checks include pH, osmolality and background counts.

Evidence of Compliance:
✓ Records of contamination checks on dilution fluids/reagents

HEM.33330 Cell Count Controls Phase I

At least one cell count control specimen is analyzed, or a procedural control employed for each 8 hours of patient testing.

NOTE: For WBC and PLT, this requirement can be met with assayed liquid control material, a previously assayed patient sample, or comparison with a visual blood film concentration estimate. Visual estimates are not appropriate for RBC hemocytometry. Liquid controls performed in a hemocytometer should be run in duplicate.

Evidence of Compliance:
✓ Written procedure defining quality control requirements for manual cell counts AND ✓ Records of cell count or procedural controls documented at defined frequency

REFERENCES

HEM.33350 PLT Estimate Phase II

For hemocytometry platelets, the manual count is correlated with a platelet estimate from a properly prepared blood film.

Evidence of Compliance:
✓ Records of slide review/correlation

REFERENCES
2) Nosanchuk JS, et al. The analytic basis for the use of platelet estimates from peripheral blood smears. Laboratory and clinical
AUTOMATED DIFFERENTIAL COUNTERS

Inspector Instructions:

- Automated differential policy or procedure
- Sampling of QC records
- Evaluation of the automated differential method

- What action would you take when there is a flagged result?

HEM.34050 Method Validation - WBC Phase II

There is documentation to indicate that the automated method was carefully evaluated in the laboratory against a previously validated automated method or a manual method before it was placed into routine use.

NOTE: The laboratory is not required to verify the manufacturer’s studies on flagging of abnormal cells, although studies of the local patient population are recommended. The references that accompany this checklist requirement may be consulted for examples of specific evaluation protocols.

Evidence of Compliance:
✓ Records of new methods evaluation study(ies), either in summary form or actual data

REFERENCES
2) Ross DW, Bentley SA. Evaluation of an automated hematology system (Technicon H1). Arch Pathol Lab Med. 1986;110:803-808

HEM.34100 Limits of Agreement - WBC Phase II

The quality control procedure defines limits of agreement with WBC subclasses from manually counted blood films or commercially available material containing at least two classes of white cells and/or surrogate particles.

NOTE: For pattern recognition microscopy systems, QC can be done by periodic processing of prepared control slides and maintenance/analysis of Levey-Jennings charts. For flow-through
systems, at least two approaches are reasonable: 1) comparison of instrument differentials on fresh blood samples with a conventional manual differential count, and/or 2) use of commercially available stabilized leukocytes and/or particle surrogate control material. The automated instrument and reference determinations should be treated as replicate manual differentials and evaluated using the ± 2 or 3 SD agreement limits of Rümke.

For commercial controls, mixed leukocyte subclasses (e.g. "mononuclear" or "large unclassified cells") or "remainder" fractions do not need to be assessed with QC procedures. The commercial material should contain surrogate particles to measure total neutrophils, total granulocytes, total lymphoid cells, monocytes, eosinophils, and basophils, if these subtypes are enumerated by the instrument and reported by the laboratory. If discrete populations of abnormal cells are identified and enumerated by the instrument (e.g. nucleated RBC, blasts), then the QC material must contain surrogate particles to evaluate accuracy.

Evidence of Compliance:

✓ Written procedure defining quality control requirements for automated WBC differentials

REFERENCES

3) Ross DW, Bentley SA. Evaluation of an automated hematology system (Technicon H1). Arch Pathol Lab Med. 1986;110:803-808
MANUAL BLOOD FILM EXAMINATION (DIFFERENTIAL COUNT)

Inspector Instructions:

- Manual differential policy or procedure
- Sampling of patient peripheral blood smears (uniquely identified, properly stained, free of precipitate, good cell distribution)
- File of unusual slides
- How do you ensure consistency among personnel performing blood cell microscopy?

HEM.34300 Blood Film Quality

The quality of blood films is satisfactory (properly stained, free of precipitate, good cell distribution).

REFERENCES
5) Turgeon ML. Clinical hematology, theories and procedures, 2nd ed. Boston, MA: Little, Brown, 1993;16-25

HEM.34350 Unique Identifiers - Slides

Slides are uniquely identified.

**NOTE:** Slides or coverslips must be uniquely identified by element(s) such as specimen or accession number, or patient name and/or number.

**Evidence of Compliance:**

- ✓ Written procedure for slide labeling
HEM.34400  Morphologic Observation Assessment - CBC  Phase II

The hematology laboratory at least annually assesses morphologic observations among personnel performing blood cell microscopy, to ensure consistency.

NOTE: Suggested methods to accomplish this include:

1. Circulation of blood films with defined leukocyte differential distributions and specific qualitative abnormalities of each class of cells (WBC, RBC, PLT), and/or
2. Multi-headed microscopy, and/or
3. Use of blood or marrow photomicrographs with referee and consensus identifications (e.g. former CAP surveys photomicrographs)
4. Use of digital images

The procedure manual should include definitions of semiquantitative measurements such as 1+, 2+, 3+, etc.

In the case of comparative blood film WBC differentials, the method of Rümke is recommended to define statistical agreement between observers.

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

REFERENCES
4) Brigden ML, Dalal BI. Morphologic abnormalities, pseudosyndromes, and spurious test results. Lab Med. 1999;30:514-520

HEM.34450  Slide Retention  Phase II

Blood films are retained for at least one week for possible review and/or reference.

NOTE: It may be desirable to retain outpatient films for a longer period and significantly abnormal films indefinitely for teaching purposes.

HEM.34500  Morphology Assessment - PLT/RBC  Phase II

The laboratory staff fully assesses, and accurately reports, RBC and PLT morphology as part of a manual WBC differential and/or blood film review.

NOTE: The laboratory must have a system to ensure that technical personnel have fully assessed all morphologic findings in each patient film. Each laboratory director should, in consultation with the medical staff, determine which morphologic findings are reportable. For example, minor degrees of anisocytosis and poikilocytosis without specific types of RBC abnormalities may be considered within the normal spectrum and not reportable to the chart. For RBC abnormalities that are reported, the laboratory must define a qualitative or semiquantitative grading system. When defined abnormalities (e.g. spherocytes, target cells, fragments, etc.) are present, non-specific listings of "anisocytosis" and/or "poikilocytosis" may not provide additional clinically useful information.

Evidence of Compliance:
✓ Written procedure defining the criteria for microscopic assessment of RBC and platelet morphology
✓ Patient reports that show assessment and reporting of RBC and PLT morphology
REFERENCES

HEM.34600 Criteria for Blood Film Review
Phase II
There are documented criteria with specified findings for blood films that are reviewed by the pathologist, supervisor or other technologist qualified in hematomorphology, and there is evidence of such review.

REFERENCES

Blood Films for Malaria and Other Parasites

Inspector Instructions:

- Sampling of blood parasite policies and procedures
- Sampling of patient reports
- Buffer pH documentation

- Ocular micrometer

HEM.34655 Blood Parasite Detection
Phase I
The microscopic examination of blood films submitted for detection of blood parasites allows for detection of parasites responsible for malaria, babesiosis, trypanosomiasis and filariasis.

REFERENCES
1) CLSI. Laboratory diagnosis of blood-borne parasitic diseases; approved guideline M15-A. Wayne, PA: CLSI, 2000

HEM.34660 Ocular Micrometer
Phase II
An ocular micrometer is available for determining the size of bloodborne parasites.

HEM.34665 Calibration/Recalibration - Ocular Micrometer
Phase II
The ocular micrometer is calibrated for the microscope(s) in which it is used and it is recalibrated each time the eyepieces or objectives are changed.

NOTE: Calibrations can be checked against a micrometer or other objects of known dimensions. If there are no changes to a particular microscope’s optical components, there is no need to
Evidence of Compliance:
✓ Records of initial calibration and recalibration, if applicable

HEM.34687  Percentage Parasitemia Reporting  Phase I

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

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

Evidence of Compliance:
✓ Written procedure for performing and reporting parasitemia percentage with identification AND
✓ Patient reports

REFERENCES
1) NCCLS. Laboratory diagnosis of blood-borne parasitic diseases; approved guideline M15-A. Wayne, PA: NCCLS, 2000
3) http://www.dpd.cdc.gov/DPDx/HTML/DiagnosticProcedures.htm

HEM.34724  Thick and Thin Films  Phase I

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

Evidence of Compliance:
✓ Written procedure with instructions for preparing thin and thick films

REFERENCES
1) NCCLS. Laboratory diagnosis of blood-borne parasitic diseases; approved guideline M15-A. Wayne, PA: NCCLS, 2000

HEM.34798  Malaria Stain Procedure  Phase I

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

REFERENCES
1) NCCLS. Laboratory diagnosis of blood-borne parasitic diseases; approved guideline M15-A. Wayne, PA: NCCLS, 2000

HEM.34872  Slide Review Procedure  Phase I

An adequate number of fields is examined under a 100 X oil-immersion objective (e.g. 300 fields).

Evidence of Compliance:
✓ Written procedure defining criteria for assessment of malaria slides including objective and number of fields examined
REFERENCES
1) NCCLS. Laboratory diagnosis of blood-borne parasitic diseases; approved guideline M15-A. Wayne, PA: NCCLS, 2000

AUTOMATED RETICULOCYTES

Inspector Instructions:
- Automated reticulocyte policy or procedure
- Sampling of QC records
- Evaluation of the automated reticulocyte method

- How was your automated reticulocyte method validated?

HEM.35020 Method Validation - Retic Counts Phase I

There is documentation to indicate that the automated method was carefully evaluated in the laboratory against previous manual or automated reticulocyte methods before use for patient reporting.

Evidence of Compliance:
✓ Records of new method evaluation study(ies)

REFERENCES

HEM.35100 Reticulocyte QC Phase I

There is a quality control program in place to determine the reproducibility (precision) of reticulocyte quantification.

NOTE: It is preferable that QC material include both normal and abnormal values.

Evidence of Compliance:
✓ Written procedure for reticulocyte quality control AND
✓ Records of reticulocyte QC
Spurious Results - Retics

There are documented criteria for identifying samples that may give spurious reticulocyte results by the automated method.

**NOTE:** Since all DNA- and RNA-containing cells will stain with DNA-RNA fluorescent dyes, a procedure must be in place to identify when the instrument cannot discriminate such stained particles from true reticulocytes. Potential interferences include Howell-Jolly bodies, nucleated erythrocytes, Heinz bodies, basophilic stippling of red cells, macrothrombocytes, megakaryocyte fragments, platelet clumps, and malaria or other intracellular organisms. Erythrocyte agglutination also may give spuriously high results, as may very high leukocytosis or thrombocytosis. Interfering particles may vary, depending on instrumentation, dye, and reaction conditions. Based upon initial evaluation of the instrument by the laboratory, criteria must be developed to detect samples with potentially erroneous results. This may be accomplished through flagging algorithms incorporated in the instrument and by examination of a blood film from every sample to ensure absence of relevant interferences.

**Evidence of Compliance:**
✓ Records showing actions taken to verify reticulocyte count prior to reporting

**REFERENCES**

**MANUAL RETICULOCYTES**

**Inspector Instructions:**
- Manual reticulocyte policy or procedure
Hematology and Coagulation Checklist

- Reticulocyte blood smear (uniquely identified, properly stained, free of precipitate, good cell distribution)

HEM.35250  Blood Film Examination  
Examine a blood film stained for reticulocytes. It is satisfactory.

REFERENCES

HEM.35300  Reporting - Retics  
The reported reticulocyte concentration is based on a minimum sample size of 1,000 RBC.

NOTE: Commercial controls are not necessary for manual reticulocyte counts.

Evidence of Compliance:
✓ Written procedure for manual reticulocyte counts including the method, number of cells counted and calculations used

REFERENCES

BODY FLUIDS

Inspector Instructions:
- Sampling of manual or automated body fluid policies and procedures
- Sampling of patient reports
- Sampling of QC records
- Counting chamber condition
- Body fluid smear (uniquely identified, uniform cell distribution, appropriate dilution so cells are not crowded, properly stained, adequate cell yield, ready recognition of cell types that are reported)
- File of unusual slides
- How do you ensure that morphologic observations are consistent among all personnel who report body fluid cell differential results?
- What do you do if you suspect malignant or unusual cells on the body fluid smear?
Body Fluid Cell Counting - Manual

HEM.35319  Diluting Equipment  Phase II
Certified pipettes or commercial dilution systems are used when diluting body fluid samples.

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

HEM.35338  Background Checks - Manual Counts  Phase II
The diluting fluid is checked for non-specimen background particulates and changed when indicated.

NOTE: Checking can be done by examining samples of these fluids under the microscope. The check must be performed each day of use for manual diluting methods. If commercial microdilution systems are used, daily checks are not required but each lot must be examined visually for uniformity of filling and clarity. If diluting fluids are prepared by the laboratory, they must be prepared aseptically; refrigeration is recommended to prevent contamination with microorganisms.

Evidence of Compliance:
✓ Written procedure defining frequency and method for performing background checks AND
✓ Records of background checks

HEM.35340  Controls  Phase I
At least one cell count control specimen is analyzed, or a procedural control used, for each 8 hours of patient testing.

NOTE: This requirement can be met with assayed liquid control material, a previously assayed patient sample, or a procedural control. (An example of a procedural control is correlation of the cell count with the cellularity of a stained slide prepared by a standard, validated method.) Liquid controls performed by a hemocytometer should be run in duplicate.

Evidence of Compliance:
✓ Records of cell count or procedural controls documented at defined frequency

REFERENCES

HEM.35347  Counting Chamber Quality  Phase I
The lines in the counting or motility chambers are bright, and the chambers are clean and free of scratches.

HEM.35357  Body Fluid Analysis Procedure  Phase II
For samples counted using a standard hemocytometer, each body fluid sample is counted in duplicate.
NOTE: Performance of the counts in duplicate is required for all hemocytometers, whether glass or disposable. Limits of agreement between replicate counts must be defined.

Evidence of Compliance:
✓ Written procedure requiring duplicate counts to include limits of agreement AND
✓ Records or worksheets reflecting duplicate counts and corrective action when limits of agreement are exceeded

HEM.35376 Cell Clumps/Debris - Manual Methods Phase II

The laboratory indicates (as part of the report) that results may be inaccurate if the fluid specimen is partially clotted or has cell clumps or debris on the counting chamber.

**REVISED** 07/11/2011
HEM.35395 Red Cell Confirmation Techniques Phase I

There is an additional procedure beyond unstained bright-field microscopic visualization of cells on the hemocytometer used when necessary to ensure the accurate distinction of erythrocytes from other cell types.

NOTE: Suggested techniques include acid rinsing of the fluid sample to lyse erythrocytes after initially counting all cells, the addition of a stain such as methylene blue to improve recognition of non-erythrocytes, correlation with the number and proportion of cells on the cytospin preparation or phase microscopy.

Evidence of Compliance:
✓ Written procedure defining laboratory's confirmation method when leukocyte results are reported AND
✓ Records of confirmation testing

Body Fluid Cell Counting - Instrumental

HEM.35414 Background Checks - Automated Counts Phase II

Instrument background counts are performed each day of testing on the diluent fluid and lysing agent to check for contamination that might affect cell counts.

NOTE: This can be done by processing these fluids on the instrument used for cell counting and checking for the presence of significant background in the diluting fluids and lysing agents.

Evidence of Compliance:
✓ Written procedure defining frequency and method for performing background checks AND
✓ Records of background checks

HEM.35452 Acceptable Limits Phase II

The laboratory defines the upper and lower limits for counting body fluid cells (erythrocytes, nucleated cells) outside of which the use of automated or semi-automated cell counters is not reliable.

NOTE: The laboratory must have an appropriate protocol that limits the use of automated or semi-automated instruments for cell counting in the very low concentration ranges often seen with body fluids. The lower limit selected must reflect the particular instrument's background
count and sensitivity.

Evidence of Compliance:

✓ Written procedure defining the upper and lower reporting limits for automated and/or semi-automated cell counters and actions to be taken if the limits are exceeded AND
✓ Records of study to validate reportable range

REFERENCES

HEM.35471 Cell Clumps/Debris - Automated Counts Phase I

The laboratory has a procedure to detect clumps of cells or debris that may give spurious cell counts.

NOTE: The procedure should include performing macroscopic assessment of body fluid samples processed on cell counting instruments. Instrument generated flags and findings on microscopic examination that suggest the presence of debris are important observations and may require the performance of a wet mount. Marked clumping or clots precludes reporting an automated count. The laboratory report should note the limited accuracy of cell counts in these situations, and include a description of the specimen problem.

HEM.35490 Stabilized Controls Phase II

Two different stabilized control specimens are analyzed and results recorded at least daily.

NOTE: Manufacturers recommendations for control material selection should be followed, and the selected control should be compatible with the methodology used by the instrument.

Body Fluid Nucleated Cell Differentials

HEM.35528 Quantitative Differentials Phase I

The method for differentiating body fluid cells is appropriate for the intended clinical use.

NOTE: The laboratory should use stained cytocentrifuge preparations to facilitate quantitative differentials and complete classification of nucleated cell types in body fluids, as opposed to performing differentials of unstained hemocytometer preparations. Differentials based on supravital-stained hemocytometer preparations, wedge smears and drop preparations are considered suboptimal; their use should be limited to clinical circumstances requiring differentiation of polymorphonuclear from mononuclear cells (e.g. bacterial meningitis). Further sub-classification of nucleated cells, particularly the detection of malignant cells, should be performed using slide preparation methods that provide optimal cell recovery and morphologic detail, such as cytocentrifugation. Cytocentrifuge preparations provide excellent morphologic detail, deliver a high yield of cells even when the concentration is low, and have a high rate of detection for malignant cells. In cases of leukemia or lymphoma, Romanowsky-stained cytospin slides show excellent morphologic correlation with blood and bone marrow smears. If the laboratory uses an alternate slide preparation method or stain for sub-classification of body fluid mononuclear cells and/or detection of malignant cells, it must demonstrate from literature or in-house studies that this technique is equivalent in cell yield/recovery and morphologic detail to Romanowsky-stained cytocentrifuge preparations.
Evidence of Compliance:
✓ Written procedure defining method for performing cell differentiation on body fluid AND
✓ Records showing in-house or literature validation of techniques other than Romanowsky-stained cytocentrifuge preparations

REFERENCES
4) Clare N, Rone R. Detection of malignancy in body fluids. Lab Med. 1986;17:147-150

**REVISED** 07/11/2011

HEM.35566 Morphologic Observation Assessment - Body Fluid

The laboratory at least annually assesses morphologic observations among personnel performing body fluid cell differentials, to ensure consistency.

NOTE: Suggested methods to accomplish this include:

1. Circulation of body fluid smears with defined nucleated cell differential distributions, and/or
2. Multi-headed microscopy, and/or
3. Use of body fluid photomicrographs with referee and consensus identifications (e.g. former CAP Surveys photomicrographs)
4. Use of digital images

The procedure manual should include definitions of semiquantitative measurements such as 1+, 2+, 3+, etc.

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

HEM.35585 Slide Review

Slides with suspected malignant cells are reviewed by a pathologist or other qualified physician before final results reporting.

Evidence of Compliance:
✓ Written procedure defining criteria for slide review by pathologist/physician AND
✓ Records of slide review

HEM.35604  Result Review/Correlation

Phase II

If a body fluid specimen is examined in more than one area of the laboratory, there is a mechanism to compare the data and interpretations from these different areas, particularly when a diagnosis of malignancy is rendered.

Evidence of Compliance:
✓ Written procedure defining the process for review/correlation of body fluid results performed in multiple laboratory sections AND
✓ Records of review/correlation

REFERENCES
1) Clare N, Rone R. Detection of malignancy in body fluids. Lab Med. 1986;17:147-150

HEM.35623  Cytomorphology Reference Library

Phase I

There is a file of unusual slides and/or an atlas of body fluid cytomorphology readily available to the technologist evaluating the slides, to assist in the identification of cell types.

REFERENCES
8) Kjeldsberg CR, Knight JA. Body fluids, 3rd ed. Chicago, IL: American Society of Clinical Pathology, 1993

HEM.35642  Slide Retention

Phase I

Slides are retained for future reference.

NOTE: All body fluid smears should be retained for at least one week for possible review/reference. Significantly abnormal smears (e.g. those demonstrating microorganisms, cytologically suspicious or overtly malignant cells, etc.) should be retained for longer periods, subject to space availability.

Semen Analysis

The preceding items in the Body Fluid Cell Counting and Body Fluid Nucleated Cell Differentials are generally applicable to semen analysis. Additional items of importance to this specialized area are identified in this section.

Inspector Instructions:

- Sampling of manual and automated semen analysis policies and procedures
- Sampling of patient records for all necessary collection information
- Patient instructions
- Sampling of patient reports
- Sampling of QC/calibration records

- Stained smear (uniform cell distribution, recognition of reportable cell types)
- File of unusual slides

- What do you do if there is difficulty distinguishing leukocytes from other round cells when performing sperm counts using bright field microscopy?
- How is the sperm motility method in use verified?
- How do you ensure that morphologic observations are consistent among all personnel who report sperm differential results?

Requisitions, Specimen Receipt and Results Reporting

HEM.35661  Concentration Technique  Phase II

For azoospermic and post-vasectomy specimens, a concentration technique is employed on seminal fluid.

NOTE: Without a concentration technique, the presence of both motile and non-motile sperm may not be detected.

Evidence of Compliance:
✓ Written procedure for concentration technique AND
✓ Patient record or worksheet indicating use of concentration technique

REFERENCES
2) Jaffe TM, et al. Sperm pellet analysis: a technique to detect the presence of sperm in men considered to have azoospermia by routine semen analysis. J Urol. 1998;159:1548-1550

NOTE: If the laboratory only performs post-vasectomy checks, the remaining semen analysis requirements are not applicable.

**REVISED**  07/31/2012
HEM.35680  Specimen Collection/Handling  Phase II

There are specific patient instructions for collection and prompt delivery of a semen sample to the laboratory.

NOTE: This should be written in simple terms in a language readily understood by the patient. Elements should include the need to abstain from ejaculation for 2-7 days before collection of the specimen, avoidance of lubricants and other contamination, completeness of collection, use of the supplied container, maintenance of sample temperature, and prompt delivery. Instructions must be readily available and distributed to patients and to off-site physician offices that refer specimens.
Specimen Collection/Handling

**REVISED** 07/31/2012

**HEM.35699**  
Semen specimens are accompanied by the following collection information, and records are maintained on the following.

1. Method of collection  
2. Type of specimen container  
3. Days of abstinence  
4. Collection or transport problems (e.g. incomplete specimen, exposure to temperature extremes)  
5. Time of specimen receipt and analysis

REFERENCES

Liquefaction

**HEM.35718**  
All semen specimens are given sufficient time for adequate liquefaction before testing.

Evidence of Compliance:
✓ Written procedure defining criteria for liquefaction

REFERENCES

Specimen Handling - Pre-analytic

**HEM.35737**  
Semen specimens are mixed thoroughly before testing.

REFERENCES

Specimen Characteristics - Analytic

**HEM.35756**  
All characteristics of the semen specimens are noted and reported (e.g. gelatinous clumps, viscosity, contaminants, erythrocytes).

NOTE: Macroscopic and microscopic characteristics of the semen specimens must be noted and reported, in accordance with the most recent edition of the World Health Organization (WHO) laboratory manual for the examination of human semen and sperm-cervical mucus interaction.

Evidence of Compliance:
✓ Written procedure defining characteristics to be included in the report

REFERENCES
Sperm Motility

HEM.35762 Motility Method Verification Phase I

A procedure exists to verify the sperm motility method used (e.g. video tapes/digital images of specimens with known percent motility and/or specific motion quality) and it is exercised at least semi-annually.

Evidence of Compliance:
✓ Records of method verification

REFERENCES

HEM.35765 Motility Quantification Phase II

Manual measures of percent sperm motility are quantified in a standardized manner.

NOTE: The laboratory must have a documented method for determining and reporting sperm motility in their procedure manual that describes how sperm are assessed and counted (percent motility) and is based on a reference method, such as the current World Health Organization (WHO) Standards.

REFERENCES

HEM.35768 Forward Progression Phase II

Forward progression of sperm is evaluated.

Evidence of Compliance:
✓ Written procedure with defined criteria for evaluation of forward progression AND
✓ Patient reports or worksheets with results of forward progression

REFERENCES

HEM.35775 Motility/Progression Evaluation Phase II

Sperm motility percent and progression are routinely evaluated within one hour of collection.

Evidence of Compliance:
✓ Written procedure with requirement for motility evaluation AND
✓ Records indicating time of collection and evaluation AND
✓ Patient reports noting exceptions, when applicable

REFERENCES
HEM.35794  Acceptable Temperature Range  Phase II

The laboratory establishes a standard specimen temperature range for semen analysis assessment, and deviations from this temperature are noted on the report.

NOTE: Specimen motility is temperature-dependent.

Evidence of Compliance:
✓ Written procedure with acceptable temperature range defined

REFERENCES

HEM.35813  Motility Microscopic Examination  Phase II

The laboratory has an appropriate procedure for evaluating a sufficient number of separate and randomly chosen microscopic fields and motile sperm cells.

REFERENCES

HEM.35822  Viability Testing Criteria  Phase I

The laboratory performs either viability testing on specimens with low percent motility (e.g. less than 30%), or includes a comment that the decreased motility may be the result of non-viable or non-motile sperm.

NOTE: Non-motile sperm may represent forms that were originally non-viable in the ejaculate, or previously motile forms that have subsequently lost motility. Thus, viability assessment is useful in making the distinction, and is commonly performed with a dye-exclusion method such as eosin-nigrosin.

Evidence of Compliance:
✓ Written procedure with defined criteria and protocol for viability testing AND
✓ Patient records or worksheet with results of viability testing OR patient report with cautionary verbiage

REFERENCES

Stained Smear - Sperm Differential

HEM.35832  Morphology Classification  Phase II

The sperm morphology classification method used is indicated on the report.

NOTE: Different classification systems have different reference intervals for normality. To improve the consistency and usefulness of reporting, CAP recommends the use of the most current edition of the WHO Standards and the Kruger classification system, and discontinuing the use of older classification systems.

REFERENCES
Morphologic Observation Assessment - Sperm

The laboratory at least annually assesses morphologic observations among personnel performing microscopic morphologic classification of sperm and other cells, to ensure consistency.

NOTE: Suggested elements of such a system may include:

1. Circulation of stained semen smears with defined specific qualitative abnormalities of sperm
2. Multi-headed microscopy
3. Use of current published references
4. Digital images (e.g. from a CD-rom)

Evidence of Compliance:
✓ Employee records documenting annual assessment

REFERENCES

Consultation

An individual with expertise in sperm morphology (the pathologist, laboratory director, supervisor, or other technologist) is available for consultation, when needed.

REFERENCES

Sperm Morphology Reference

There is a file of unusual slides or current atlas of sperm morphology, available for training and reference.

REFERENCES

Stain Usage

Stains are used to facilitate classification of cell types (as opposed to performing differentials of unstained preparations).

Evidence of Compliance:
✓ Written procedure defining the use of stains for cell classification

REFERENCES
HEM.35893  Secondary Procedure  Phase I

There is an additional procedure besides unstained brightfield microscopy to ensure the accurate distinction of leukocytes from other round cells (e.g. Wright’s or PAP stain, leukocyte alkaline phosphatase, myeloperoxidase).

Evidence of Compliance:
✓ Patient records or worksheets indicating use of additional procedure

REFERENCES

HEM.35895  Stain QC  Phase II

All stains are checked for contamination and reactivity each day of use.

Evidence of Compliance:
✓ Records of contamination checks on all stains

REFERENCES

HEM.35901  Slide Identification  Phase II

Slides are adequately identified.

NOTE: Slides must be identified in a permanent fashion with 2 specimen identifiers.

REFERENCES

HEM.35908  Stain Quality  Phase II

The stains used (Wright’s, Papanicolaou, eosin-nigrosin, peroxidase, etc.) are of sufficient quality to demonstrate the cellular characteristics for which they are designed.

NOTE: The selection of stains used for semen analysis is at the discretion of the director.

Evidence of Compliance:
✓ Examples of each type of stained slide available for microscopic review by inspector, as applicable

Biochemical Tests

HEM.35909  Biochemical Tests - Daily QC  Phase II

For biochemical tests such as fructose, positive and negative controls are run with each assay.
Evidence of Compliance:
✓ Written procedure documenting QC requirements AND
✓ QC results

REFERENCES

Anti-sperm Antibody (ASA) Tests

HEM.35910 Heat Inactivation
Phase II

Serum and follicular fluid specimens used for indirect ASA testing are heat-inactivated before use.

NOTE: Serum and follicular fluid specimens used for indirect ASA testing must be treated to inactivate complement.

Evidence of Compliance:
✓ Written procedure defining pre-analytic treatment of specimens

REFERENCES
1) Keel BA, Webster BW. CRC handbook of the laboratory diagnosis and treatment of infertility. Boca Raton, FL: CRC Press, 1994

HEM.35911 Motility Testing
Phase I

If the testing for ASA requires motile sperm, specimens are assayed with minimal delay and the motility is assessed and recorded.

Evidence of Compliance:
✓ Patient records and worksheets showing time of collection and evaluation of motility

REFERENCES

HEM.35912 ASA Controls
Phase II

For indirect antibody testing, positive and negative controls are run with each assay.

Evidence of Compliance:
✓ Written procedure defining QC requirements AND
✓ QC results

REFERENCES
1) Keel BA, Webster BW. CRC handbook of the laboratory diagnosis and treatment of infertility. Boca Raton, FL: CRC Press, 1994

Automated Semen Analysis Instruments

Various systems are in use and some checklist items may not apply to every system. The checklist items are intended to check factors common to automated systems. Inspectors should use individual judgment in applying the requirements to the particular type of system being used.
Calibration and Quality Control

Several different methods may be used for calibration and quality control in the automated analysis of semen characteristics. “Calibration” techniques include use of:

1. Multiple analyzed sperm specimens
2. Stabilized preparations of sperm cells (e.g. fixed or preserved)
3. Sperm surrogates (e.g. latex particles)
4. Videotaped sperm specimens

NOTE: If stabilized control materials are used, they should represent different analytic levels (e.g. normal and high). Similarly, retained patient specimens should be of differing counts and/or motility, as applicable.

HEM.35914  Calibration Materials

Calibration is verified with materials appropriate to the reportable range of the instrument, and verification is documented.

NOTE: The quality control procedure for the automated instrument must include calibration and evaluation using defined limits of agreement with manually counted semen smears or stored digital images, as appropriate for the particular system. Automated semen analysis laboratories must at least annually establish that their analysis equipment is functioning correctly and there is a protocol to determine if the analysis is in control.

REFERENCES

HEM.35915  Daily QC

The laboratory performs and documents calibration and quality control methods for the analyzer during each day of use, using an appropriate number of controls with varying ranges of values.

REFERENCES

HEM.35916  Recalibration

The laboratory has a procedure for recalibration of instrument parameter(s) when problems are encountered.

REFERENCES

HEM.35918  Calibration Material Validation

The material used for calibration is validated using primary reference procedures (e.g. manual counts).

Evidence of Compliance:
✓ Written procedure identifying calibration materials and validation of materials used AND
✓ Records showing accuracy of calibration materials used to include manufacturer's documentation of certification/validation of commercial products OR in-house validation data

REFERENCES

HEM.35919 System Control Phase II

If a manual method is used as the system control for automated or semi-automated sperm counts, its accuracy is verified and documented at intervals appropriate for laboratory volume.

REFERENCES

HEM.35920 Acceptable Limits - Controls Phase II

Acceptable limits are established for the value of each quality control sample.

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

HEM.35921 Sperm Concentration - Automated Methods Phase II

If sperm motility and/or count are assessed by automated analysis, a procedure is established to determine that the concentration of the specimen is within the range appropriate for automated counting.

REFERENCES

HEM.35923 Reportable Range Phase II

Upper and lower limits of all reportable parameters on instruments are defined so that results that fall outside these limits are verified before reporting.

NOTE: Results that fall outside of these limits may be verified by repeating the test, using an alternative method or diluting/concentrating the specimen, as appropriate.

Evidence of Compliance:
✓ Written procedure defining the upper and lower reporting limits and verification of results AND
✓ Patient test verification records

REFERENCES

HEM.35924 Calibration Verification Criteria

Criteria are established for method calibration verification.

NOTE: Criteria must be established for method calibration verification. Criteria for determining the need for calibration verification typically include:

1. At complete changes of reagents, unless the laboratory can demonstrate that changing reagent lots does not affect either the range used to report patient test results or the control values
2. When indicated by quality control data
3. After major maintenance or service
4. When recommended by the manufacturer
5. At least every six months

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

REFERENCES

ABNORMAL HEMOGLOBIN DETECTION

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

Inspector Instructions:
- Sampling of abnormal hemoglobin policies or procedures
- Sampling of patient reports (confirmatory testing, comments)
- Sampling of QC records
- Hemoglobin separation patterns (appropriate separations and controls)
- Examine a sampling of medium (media) used to identify hemoglobin variants including alkaline/acid electrophoresis, isoelectric focusing, HPLC, or other methods
- What is your course of action when the primary screening method appears to show Hb S?
- What is your course of action when the primary Hb electrophoresis method shows Hb variants migrating in nonA/nonS positions?
Hematology and Coagulation Checklist

HEM.35925  Hb S Primary Screen  Phase II

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

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

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

**REVISED**  07/31/2012

HEM.35927  Daily QC - Hgb Separation  Phase II

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

Evidence of Compliance:
✓ Written procedure defining QC requirements for hemoglobin separation AND
✓ QC records reflecting the use of appropriate controls AND
✓ Electrophoresis media/separation tracings demonstrating appropriate controls and separation

REFERENCES
5) Honig GR. Adams JG III. Human hemoglobin genetics. Vienna, Austria: Springer-Verlag, 1986
16) Hoyer JD, et al. Flow cytometric measurement of hemoglobin F in RBCs: diagnostic usefulness in the distinction of hereditary persistence of fetal hemoglobin (HPFH) and hemoglobin S-HPFH from other conditions with elevated levels of hemoglobin F. Am J Clin Pathol. 2002;117:857-863

**REVISED**  07/31/2012

HEM.35946  Hemoglobin Variants  Phase II

All samples with hemoglobin variants migrating in "non-A, non-S" positions on alkaline electrophoresis or other low resolution procedure are further defined with other acceptable methods where clinically and technically appropriate.

NOTE: If all clinically significant variants are not clearly separated by the primary method,
additional testing is performed to further characterize these hemoglobin variants. This may include, but is not limited to: performance by a complementary, separate methodology or increasing the duration of the assay (for HPLC) where the hemoglobins migrate/elute at different configurations. Further workup of such variants, including referral to a reference laboratory, is dependent upon the patient's overall clinical situation, such as findings of erythrocytosis or a hemolytic anemia.

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

**REFERENCES**
14. Hoyer JD, et al. Flow cytometric measurement of hemoglobin F in RBCs: diagnostic usefulness in the distinction of hereditary persistence of fetal hemoglobin (HPFH) and hemoglobin S-HPFH from other conditions with elevated levels of hemoglobin F. Am J Clin Pathol. 2002;117:857-863

**HEM.35984  Hb S Predominant Band  Phase II**

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

**NOTE:** When the predominant hemoglobin component appears to be Hb S, it is necessary to determine whether this represents homozygous Hb S or a heterozygote for Hb S and another variant such as Hb D, Hb G, Hb Lepore, or other hemoglobin variant(s). Given the clinical implications of homozygous Hb S (or Hb S/β-zero thalassemia) it is imperative to exclude other hemoglobin variants, however rare. **Referral of these specimens to a reference laboratory for further workup is acceptable.**

**Evidence of Compliance:**
- Written procedure defining criteria for determination of homozygous versus heterozygous Hb S **AND**
- Patient records or worksheets showing exclusion of hemoglobin variants **OR** documentation of referral for further work-up

**REFERENCES**
Hematology and Coagulation Checklist


BONE MARROW PREPARATIONS

Inspector Instructions:

- Bone marrow policy and procedure
- Sampling of stain QC records

- Bone Marrow slide (uniquely identified, satisfactory staining and cell distribution)

- How do you reconcile clinically significant discrepancies between the bone marrow morphologic diagnosis and the results of ancillary studies?

HEM.36050 Slide Labeling

Bone marrow slides are uniquely identified.

NOTE: Slide or coverslip identification must include a unique identifier, such as specimen or accession number. The addition of the patient name and/or number, and date is common. The ability to identify the patient as well as the date the specimen was obtained applies to all parts of the bone marrow case, which may include blood films, bone marrow aspirate, marrow clot and core biopsy specimens.

Evidence of Compliance:
✓ Written procedure for slide labeling

HEM.36100 Slide Review

Examine a slide prepared by the laboratory. The preparation and staining are satisfactory for interpretation.

HEM.36150 Fixed Sections

Fixed sections (marrow biopsy or particle sections) are used as a diagnostic aid to the smear aspirate, as appropriate for the clinical situation.

Evidence of Compliance:
✓ Patient reports with documentation of aspirate and fixed section review, as applicable

REFERENCES
HEM.36200  Fixed Tissue Quality  Phase II
The quality of fixed tissue sections of bone marrow is conducive to a reliable diagnosis.

HEM.36250  Fixed Tissue Correlation  Phase II
If fixed tissue sections and bone marrow aspirate smears are evaluated in different sections of the laboratory, or if separate reports are released at different times, there is a mechanism to compare the data and interpretations from these different sections.

Evidence of Compliance:
✓ Written procedure defining process for review/correlation of fixed tissue sections and bone marrow aspiration smear results/interpretations AND
✓ Records of review/correlation with follow-up reporting if a discrepancy is identified

HEM.36270  Record Retention  Phase II
Bone marrow reports and smears are retained for 10 years.

HEM.36300  Bone Marrow Evaluation  Phase II
Bone marrow specimens are evaluated by a pathologist or qualified hematologist and formal reports prepared.

REFERENCES

HEM.36325  Correlation of Results  Phase I
There is a mechanism to correlate the results of ancillary studies (immunohistochemistry, cytogenetics, flow cytometry, etc.) with the morphologic diagnosis.

NOTE: The pathologist or qualified hematologist should correlate all of the special studies, reconcile conflicting data, and render a final interpretation of all correlated studies where appropriate. A mechanism should exist in the laboratory that documents review of such studies not available at the time of initial request. Clinically significant discrepancies must be reconciled and documented.

Evidence of Compliance:
✓ Written procedure for correlation of specialized studies with morphologic diagnoses

REFERENCES

HEM.36350 Iron Stain Phase II

An iron stain is prepared for bone marrow evaluations of anemia where indicated.

Evidence of Compliance:
✓ Written procedure defining criteria for performing the iron stain

HEM.36800 Stain Reactivity Phase II

All stains are checked for intended reactivity each day of use.

NOTE: Stains should be assessed using both a normal blood film and an evaluation of the staining of residual apparently normal blood cells on the smears being tested. Rarely, the normal control may react, but the expected staining of normal cells on the test smear may be absent for technical reasons. Failure to evaluate the expected reactions of normal cells may cause diagnostic errors.

Evidence of Compliance:
✓ Written procedures for stain QC AND
✓ Records of stain QC documented at defined frequency

BLOOD COAGULATION STUDIES

Inspector Instructions:

- Sampling of coagulation/D-dimer/platelet function policies and procedures
- Sampling of QC records
- Sampling of patient reports
- Biannual instrument correlation records
- Sampling of calibration/calibration verification/recalibration records
- How do you know the PPP is really platelet-poor?
- How do you know if an increased aPTT is not due to heparin?
- How do you compare instruments and define agreement?
- What is your course of action if results fall outside the AMR?
- Track a PT and aPTT specimen from testing in the laboratory to results reporting. Assess the following: proper ID on tube, proper anticoagulant, acceptable QC for that run, if critical result is called, reference ranges on patient report, recommendation for laboratory tests to monitor coumadin and heparin, INR calculation and all associated parameters are correct.
- When was the last time you performed a calibration procedure for directly measured coagulation analytes? How did you verify the calibration?
Tests for defining or monitoring disseminated intravascular coagulation (DIC) problems are available, if applicable to the patient population served.

REFERENCES
1) Bovill EG. Laboratory diagnosis of disseminated intravascular coagulation. Sem Hematol. 1994;31(2;suppl)35-29

Platelet-poor Plasma

There is a system to at least annually measure the actual platelet count of the "platelet-poor" plasma used for many coagulation tests.

NOTE: Platelet-poor plasma is particularly important when testing for the presence of a lupus anticoagulant, when measuring the level of unfractionated heparin, and in plasma samples to be frozen for later testing. Platelet-poor plasma should have a residual platelet concentration of less than 10 X 10^3/L. This is important because platelet membranes form a procoagulant surface that can accelerate coagulation and spuriously shorten clotting times. It is particularly important when testing for the presence of a lupus anticoagulant; due to the high content of lipid in the platelet plasma membrane, increased platelets in samples with the lupus anticoagulant can cause the antiphospholipid antibody to bind to the platelet membrane, thus effectively removing it from plasma. In this circumstance, the presence of lupus anticoagulant may not be detected during diagnostic testing. Use of a 0.2-μm filter to achieve platelet-poor plasma samples is not appropriate for all plasma-based coagulation studies. Filtration of plasma can result in selective removal of factors V, VIII, IX, XII, and vWF; thus filtration of plasma to achieve a platelet-poor specimen is discouraged. aPTT, prothrombin time/international normalized ratio (PT/NR) and thrombin clotting time (TT) performed on fresh plasma samples are not affected by platelet counts of at least up to 200 x 10^3/L (200,000/μL).

Samples to be frozen should be "platelet-poor" because plasma contaminated with significant numbers of platelets may yield different analytic results after thawing, due to lysis of platelets.

Evidence of Compliance:
✓ Written procedure defining method for measuring platelet concentration of platelet-poor plasma AND
✓ Records of platelet concentration checks on all centrifuges used to prepare platelet-poor plasma

REFERENCES
2) Middleton AL, Oakley E. Activated partial thromboplastin time (aPTT): Review of Methods. Chicago, IL: American Society of Clinical Pathology Check Sample PTS 91-6, 1991
5) Barnes PW, Eby CS, Lukoszyk M. Residual platelet counts in plasma prepared for routine coagulation testing with the Beckman Coulter power processor. Lab Hematol. 2002;8:205-209

Coagulation Tests - 37°C

Coagulation tests (e.g. PT, aPTT, fibrinogen, and factor assays) are performed at 37 °C.
Evidence of Compliance:
✓ Records of temperature checks or automated internal instrument temperature monitoring

REFERENCES

Interinstrument Comparisons (Coagulation)

The laboratory may use fresh patient or donor specimens analyzed on a primary instrument for daily QC of a secondary instrument. The selection of these materials (rather than commercial controls) is important to directly address the issue of whether a patient sample yields the same results on all of the laboratory’s instruments. If the laboratory has only one instrument for each type of coagulation assay for patient testing, this section does not apply.

**REVISED** 07/11/2011
HEM.37216 Comparability of Instrument/Method  Phase II

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

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

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

This requirement would only apply when the different instruments/reagents are producing the same reportable result. For example, some laboratories may use multiple aPTT reagents with variable sensitivity to the lupus anticoagulant. If these are defined as separate tests, then this requirement does not apply unless each type of aPTT test is performed on more than one analyzer.

Otherwise, the use of fresh human samples (whole blood, serum, plasma, urine, etc.), rather than stabilized commercial controls, is preferred to avoid potential matrix effects. In cases when availability or pre-analytic stability of patient/client specimens is a limiting factor, alternative protocols based on QC or reference materials may be necessary but the materials used should be validated (when applicable) to have the same response as fresh human samples for the instruments/methods involved.

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

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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. Fed Register, 2003(Jan 24):5236 [42CFR493.1281(a)]
HEM.37232 Defined Tolerance Limits

There are defined tolerance limits for results agreement of interinstrument assays.

NOTE: The laboratory should clearly define numeric ranges of agreement between multiple coagulation instruments used by the laboratory for a given assay. These ranges must be statistically defined, and not simply qualitative.

Evidence of Compliance:
✓ Written procedure defining limits of agreement for multiple instrument comparison

Photo-Optical Coagulation Systems

**REVISED** 07/11/2011
HEM.37300 Quality Control

The instrument is checked with two different levels of control material and during each 8 hours of patient testing.

NOTE: This includes photo-optical, electromechanical and manual methods. Manual coagulation systems are defined as PT, aPTT or fibrinogen testing performed using a method whereby patient plasma and reagents are pipetted into a test tube, the tube is rocked back and forth in a water bath until a clot forms. This is often referred to as a "tilt-tube" method. For manual methods, if two individuals both performed a tilt tube test in the same 8 hour period, the controls would have to be assayed twice (i.e. once for each person).

NOTE 1: Except for tests meeting the criteria in Note 2, below, two different levels of external control material must be run during each 8 hours of patient testing.

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

1. For quantitative tests, the test system includes two levels of electronic/procedural/build-in internal controls that are run every 8 hours of patient testing
2. For qualitative tests, the test system includes an electronic/procedural/build-in internal control run daily
3. The system is FDA-cleared or approved, and not modified by the laboratory*
4. The laboratory has performed and documented studies to validate the adequacy of limiting daily QC to the electronic/procedural/build-in controls. Initial validation studies must include comparison of external and built-in controls for at least 20 consecutive days when patient samples are tested. For validation of multiple mechanical devices, the minimum of 20 consecutive daily comparisons applies to the initial device; the laboratory director is responsible for determining the sample size for the other devices. The laboratory director is responsible for determining criteria for acceptability, and other details of the validation. Validation records must be retained while an instrument is in service, and for 2 years afterwards. The requirement for 20 consecutive daily comparisons is effective for validation studies performed after 1/31/2012. Corrective action must be taken if either the internal or external control is out of acceptable range during or after the evaluation process. Repeating controls or re-evaluation of the internal control system may be necessary to achieve acceptable results.
5. External controls are run for each new lot number or shipment of test materials; after major system maintenance; and after software upgrades. ** Regarding external
controls for qualitative tests, best practice is to run a weak positive control.

6. External controls are run as frequently as recommended by the test manufacturer, or every 30 days, whichever is more frequent.

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

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

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

REFERENCES

HEM.37350 Acceptable Limits Phase II
Acceptable limits are defined for each instrument, component or procedure in the system.

HEM.37400 Alternative Method Criteria Phase I
Guidelines are established for determining when alternative procedures are performed (e.g. lipemia, hyperbilirubinemia, turbidity, etc.).

NOTE: Very long clotting times may not be reproducible on an automated coagulation instrument. Criteria should be established by each laboratory for performance of the PT or aPTT by an alternate technique (e.g. manual method) when the readable range of the instrument is exceeded. In addition, criteria should be provided for performance of alternate procedures in the presence of significant hyperbilirubinemia or lipemia, paradoxically short aPTTs and nonduplicating aPTTs.

Evidence of Compliance:
✓ Written procedure defining criteria for when PT/aPTT alternative procedures should be performed AND
✓ Records showing results from alternative procedures, as applicable

REFERENCES

Electromechanical Coagulation Systems
HEM.37500 QC - Electromechanical Systems  Phase II

The electromechanical coagulation system (for PT, aPTT, fibrinogen, and other coagulation assays) is checked with 2 different levels of control material during each 8 hours of patient testing, and each time there is a change in reagents.

Evidence of Compliance:
✓ Written QC policy AND
✓ QC records documented at defined frequency

HEM.37550 Acceptable Limits  Phase II

Acceptable limits are defined for each instrument, component, or procedure in the system.

HEM.37600 Clot Detection  Phase II

If the electromechanical system has reusable probes to detect a clot, documented guidelines for cleaning the probes are available.

Manual Coagulation Systems

DEFINITION: Manual coagulation systems are defined as PT, aPTT or fibrinogen testing performed using a method whereby patient plasma and reagents are pipetted into a test tube, the tube is rocked back and forth in a water bath until a clot forms. This is often referred to as a “tilt-tube” method. The following requirements only apply to such methods.

HEM.37700 QC - Manual Coagulation Systems  Phase II

The manual coagulation system is checked by each testing person with 2 different levels of control material in duplicate during each 8 hours of patient testing, and each time there is a change of reagents.

NOTE: The term “each testing person” applies to all individuals performing a manual tilt tube test during each 8 hour period. Thus, if two individuals both performed a tilt tube test in the same 8 hour period, the controls would have to be assayed twice (i.e. once for each person).

Evidence of Compliance:
✓ Written QC policy AND
✓ QC records documented at defined frequency

REFERENCES

HEM.37750 Acceptable Limits  Phase II

Acceptable limits are defined for each manual procedure.

Evidence of Compliance:
✓ Written procedure defining acceptable limits for each manual procedure
**REVISED** 07/11/2011  
**HEM.37800** Duplicate Testing  
**Phase II**

**Determinations are performed in duplicate and criteria for agreement are defined.**

**Evidence of Compliance:**
✓ Records or worksheets reflecting duplicate testing of each sample including corrective action when limits of agreement are exceeded

**REFERENCES**

**REVISED** 07/11/2011  
**HEM.37900** Temperature-Dependent Equipment  
**Phase II**

**The temperature of the water bath or incubator is verified with a certified thermometer or equivalent technique.**

**NOTE:** The thermometer must be certified or guaranteed by the manufacturer to meet NIST standards, or checked against an appropriate thermometric standard device. Coagulation assays must be performed at 37°C.

**Evidence of Compliance:**
✓ Etched markings indicating Class A OR NIST certificate OR validation study of accuracy for non-certified thermometer

**REFERENCES**

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**Coagulation Studies by Electrophoresis**

**NOTE:** These requirements apply to electrophoresis procedures performed for studies for von Willebrand multimers and Protein C antigen, or other factor antigens by Laurel Rocket technique.

**HEM.37905** Daily QC - Electrophoresis  
**Phase I**

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

**Evidence of Compliance:**
✓ Records of electrophoresis QC

**HEM.37910** Electrophoretic Separation  
**Phase I**

**Electrophoretic separations are satisfactory.**

**NOTE:** The laboratory should be able to document instrument printouts, sample electrophoresis results and patient reports.
D-Dimer Studies

If the laboratory reports D-dimer assay results in units other than those recommended by the assay manufacturer, the type of unit and magnitude of the D-dimer result is reported correctly.

NOTE: The units generated directly by the D-dimer method can be determined from the package insert for the method being used. D-dimer is a molecular species produced from two molecules of fibrin that were crosslinked by factor XIIIa through the adjacent D domains and then degraded by plasmin. It is a marker of ongoing in vivo fibrin formation and fibrinolysis. The reportable units of the D-dimer may vary by manufacturer, but may include both Fibrinogen Equivalent Units (FEU) and D-dimer Units (D-DU).

Some laboratories may convert the units stated in the package insert into different units for the patient report. If such is the case, the laboratory should verify that the calculations are correct. For example, a method may generate data as μg/mL D-dimer Units (D-DU), and the laboratory may choose to convert this to ng/mL Fibrinogen Equivalent Units (FEU). A critical concept for this calculation is that the mass of one unit of FEU is twice that of the mass of one unit of D-DU. (1 D-DU = 2 FEU). In this example, the correct conversion factor is 2000, which is calculated as follows: 1 μg/mL D-DU x (1000 ng/1 μg) x (2 FEU/1 D-DU) = 2000 ng/mL FEU. Please see the table below for other example conversion factors.

<table>
<thead>
<tr>
<th>Manufacturer Units</th>
<th>Final Units</th>
<th>Correct Conversion Factor</th>
<th>Equivalency Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEU ng/mL</td>
<td>D-DU ng/mL</td>
<td>0.5</td>
<td>1 FEU ng/mL = 0.5 D-DU ng/mL</td>
</tr>
<tr>
<td>FEU ng/mL</td>
<td>D-DU μg/mL</td>
<td>0.0005</td>
<td>1 FEU ng/mL = 0.0005 D-DU μg/mL</td>
</tr>
<tr>
<td>FEU μg/mL</td>
<td>FEU ng/mL</td>
<td>1000</td>
<td>1 FEU μg/mL = 1000 FEU ng/mL</td>
</tr>
<tr>
<td>D-DU ng/mL</td>
<td>FEU ng/mL</td>
<td>2</td>
<td>1 D-DU ng/mL = 2 FEU ng/mL</td>
</tr>
<tr>
<td>D-DU μg/mL</td>
<td>FEU ng/mL</td>
<td>2000</td>
<td>1 D-DU μg/mL = 2000 FEU ng/mL</td>
</tr>
<tr>
<td>D-DU μg/mL</td>
<td>D-DU ng/mL</td>
<td>1000</td>
<td>1 D-DU μg/mL = 1000 D-DU ng/mL</td>
</tr>
</tbody>
</table>

Evidence of Compliance:

✓ Written procedure describing the reporting of D-dimer results consistent with manufacturer's instructions AND
✓ Patient reports reflecting the reporting of results in units defined in the procedure

REFERENCES
5) Perrier A. Review: several factors are associated with the performance of D-dimer assays for detecting deep venous thrombosis. ACP J Club. 2004 Nov-Dec;141(3):76

The results in laboratory reports are specified as D-dimer Units (D-DU) or Fibrinogen Equivalent Units (FEU).
D-Dimer Calculated Values

If calculations (including conversion of units) are used to obtain D-dimer results, the laboratory documents checks at least annually to ensure that the calculations are correct.

NOTE: The D-dimer may be a calculated value. In some institutions, the D-dimer result may be calculated by the coagulation analyzer, while in others it may be calculated by the laboratory information system. The accuracy of calculations should be verified at least annually.

Calculations should be checked for all instruments or methodologies that are used to generate reportable D-dimer values. It is suggested that the calculations be checked at both a normal and elevated D-dimer value. It is generally recommended that the laboratory report D-dimer in the units used by the reagent manufacturer rather than converting to different units.

Evidence of Compliance:
✓ Written procedure defining the process for verifying D-dimer calculations and the criteria for performing the check

Method Validation - D-dimer

If a D-dimer method is used in the exclusion of venous thromboembolism, the method is validated for this purpose.

NOTE: If a D-dimer method is used in the exclusion of deep vein thrombosis and/or pulmonary embolism, the cut-off value for exclusion of these conditions should be validated, either in the peer-reviewed published literature, by the laboratory, or, for FDA-cleared/approved methods only, by the manufacturer of the method. The validation study(ies) should establish whether the cut-off is applicable to all patients, or only those with certain pre-test probabilities of having venous thromboembolism. If the laboratory modifies the manufacturer's recommendations for an FDA-approved method, then the laboratory should perform the appropriate validation studies.

Typically, manual latex agglutination D-dimer or FDP (fibrin degradation products) assays are not adequately sensitive to be used for evaluation of deep vein thrombosis and/or pulmonary embolism.

Evidence of Compliance:
✓ Records of the validation study including cut-off values for excluding DVT and PE appropriate to the use of the assay

REFERENCES
5) Perrier A. Review: several factors are associated with the performance of D-dimer assays for detecting deep venous thrombosis. ACP J Club. 2004 Nov-Dec;141(3):76

REFERENCES
7) Gould MK. Review: of the various D-dimer assays, negative ELISA results are most useful for excluding a diagnosis of deep venous thrombosis or pulmonary embolism. ACP J Club. 2004 Nov-Dec;141(3):77
HEM.37930  Reporting  Phase I

If a D-dimer test is used for evaluation or exclusion of deep vein thrombosis and/or pulmonary embolism, the laboratory reports the cut-off value for exclusion of venous thromboembolism, as well as the reference range.

NOTE: The cut-off can be indicated within the patient report, or by a written memorandum to clinicians. The former is preferable. For laboratories subject to US regulations, to use D-dimer as exclusion criteria, the kit must be FDA-cleared/approved.

Evidence of Compliance:
✓ Patient reports including reference range AND
✓ Patient reports including cut-off value for exclusion of DVT OR other documented communication of cut-off value to physician

HEM.37935  Utilization of D-Dimer Test  Phase I

If a D-dimer test is not used for exclusion of deep vein thrombosis and/or pulmonary embolism, the laboratory informs clinicians that the test should be used as an aid in diagnosis and not be used to exclude deep vein thrombosis or pulmonary embolism.

NOTE: This disclaimer may be included in the laboratory report, or in a written memorandum to clinicians. The former is preferable.

Coagulation Factor Assays (including fibrinogen)

The factor activity of a plasma sample is measured by its ability to correct the prolonged clotting time of factor-deficient plasma. The aPTT or PT of mixtures of diluted test plasma and factor-deficient plasma are inversely proportional to the concentration of the factor in the test plasma mixtures. Mixtures of diluted reference plasma of known factor activity and factor-deficient plasma are used to construct a reference curve that can be used to convert aPTT or PT values of the test plasma mixtures to units of activity.

HEM.37940  Standard Curve  Phase II

For coagulation end point-based factor assays, three or more points are plotted for the standard curve.

NOTE: Plotting less than 3 points may generate an erroneous line.

Evidence of Compliance:
✓ Written procedure for establishing standard curves AND
✓ Records of standard curves for factor assays

REFERENCES
2) NCCLS. Determination of factor coagulant activities; approved guideline H48-A. Wayne, PA: NCCLS, 1997

HEM.37960  Standard Curve Validation  Phase I

The standard curves are validated with at least two reference points for each factor assay determination each 8 hours of patient testing, or each time a factor assay is performed if
factor assays are performed less frequently than one per 8 hours.

**NOTE:** The Y intercept of the standard curve varies according to the reagent and environmental or instrument conditions. Validating the curve (e.g. 2 or more points with assayed reference plasma) each time ensures accuracy of the result.

**Evidence of Compliance:**
- ✓ Written procedure describing the validation of standard curves with two reference points AND
- ✓ Records of QC documented at defined frequency

REFERENCES
1) NCCLS. Determination of factor coagulant activities; approved guideline H48-A. Wayne, PA: NCCLS, 1997

**REVISED** 07/11/2011
HEM.37980 Factor Assay Criteria

**Phase II**

Three or more dilutions are plotted for each factor assay.

**NOTE:** When performing factor assays, at least three dilutions of patient plasma in buffer are prepared either by the instrument or off the instrument. Multiple dilutions of test plasma are required to evaluate the extent of parallelism between test results and those of the reference plasma. This is necessary to be able to detect whether a factor inhibitor is present. Criteria for demonstration of non-parallelism (or non-specific inhibitor effect) may vary between laboratories and instrument types. For example, in some laboratories, individual results of each dilution are reviewed and should agree within 20% of each other to be considered linear or parallel. In this instance, the average of all three results may be reported. Some coagulation instruments perform this determination automatically based on criteria programmed into the instrument. Non-specific inhibitors often demonstrate a “dilution effect” characterized by non-parallelism of results with increasing dilutions. An example of non-parallel results is as follows: the 1:10 dilution yields 30% activity, the 1:20 dilution 50%, and the 1:40 dilution 75% activity. Further dilutions should be performed as needed and in accordance with the laboratory’s practice and instrument capability, at least until the factor activity falls within the reference interval. In situations of non-parallelism, the highest value obtained with dilution should be recorded with a comment about dilution effect made in the laboratory report. In this instance, the mean result should not be reported nor should the value of the least dilute result. At least 3 patient dilutions enhances accuracy by minimizing dilutor error, and allows for detection of inhibitors or anticoagulants. To be valid, at least one value must fall within the upper and lower limits of the standard curve used for the calculation of the result.

This requirement does not apply to fibrinogen assays.

**Evidence of Compliance:**
- ✓ Records or worksheets showing patient data analyzed at three or more dilutions

REFERENCES
1) NCCLS. Determination of factor coagulant activities; approved guideline H48-A. Wayne, PA: NCCLS, 1997
"inhibitor pattern detected" along with reporting the activity obtained at the highest dilution or over serial dilutions clarifies the result. Inhibitor effect is not applicable to fibrinogen assays.

HEM.37984  Inhibitor Interference

If non-specific inhibitor interference is apparent in a factor activity assay, the laboratory reports the highest factor activity apparent with dilution.

NOTE: Inhibitor interference is not applicable to fibrinogen assays.

REFERENCES

Mixing Studies

Plasma-mixing studies (i.e. mixing patient plasma with normal plasma) may be performed to distinguish whether an abnormal screening coagulation test result (PT or aPTT) is caused by a factor deficiency or an inhibitor.

HEM.37991  Mixing Studies Procedure

When plasma-mixing studies are performed, an appropriate pooled plasma is utilized.

NOTE: It is not appropriate to use single patient plasma samples with normal PT/aPTT values as the "normal" plasma reagent, as factor levels may vary over a wide range without affecting PT/aPTT results. In general, pooled plasma prepared in the laboratory from at least 20 apparently healthy donors or commercial products (including control materials) are acceptable.

Evidence of Compliance:
✓ Written procedure for preparation of pooled plasma for plasma-mixing studies OR written procedure describing the use of a commercial product with documented normal factor levels

REFERENCES

**REVISED** 07/11/2011

HEM.38002  Mixing Studies Procedure

For samples with positive mixing study results (suggestive of an inhibitor), there is either a procedure to detect heparin or other antithrombotic drugs that inhibit coagulation, or the result is reported with a comment that the effect of inhibitor drugs cannot be excluded.

NOTE: Anticoagulant drugs that act as coagulation inhibitors (e.g. heparin, factor Xa inhibitors or direct thrombin inhibitors) may give positive results in mixing study assays. Laboratories must have procedures established to screen mixing study samples with elevated PT and/or aPTT results for these anticoagulant drugs. For heparin, performing a thrombin time assay, heparin Xa inhibition assay, repeating the aPTT with polybrene, or treating the sample with heparinase may be acceptable. For direct thrombin inhibitors, performing a thrombin time should detect the presence of the inhibitor. A thrombin time should be greatly prolonged (or even give a "clot undetected" result) in the presence of a direct thrombin inhibitor. A thrombin time has the advantage of detecting not only heparin, but also the presence of direct thrombin inhibitors such as lepirudin, bivalirudin and argatroban. Alternately, the test result from a positive mixing study
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should include a comment that “the presence of anticoagulant inhibitor drugs such as heparin or direct thrombin inhibitors cannot be excluded.”

REFERENCES
1) Jim RTS. A study of the plasma thrombin time. J Lab Clin Med. 1957; 50:45-60

Coagulation Tests Based on Direct Measurement of Analytes

This introduction reviews two new coagulation checklist requirements: calibration verification and analytical measurable range (AMR) validation (chemistry equivalents). CAP accredited chemistry laboratories have been applying these concepts and procedures to calibrated analytical methods for many years. Medical directors and technologists with chemistry backgrounds will be helpful consultants to their coagulation laboratory colleagues as calibration verification and AMR validation requirements evolve.

The new checklist requirements apply to hemostasis test methods that are calibrated and directly measure the concentration or activity of an analyte by employing enzyme immunoassay (EIA), immunoturbidity and chromogenic methods. Examples of commonly performed hemostasis tests affected by these checklist requirements include: calibrated EIA or immunoturbidity methods for coagulation factors, protein C antigen, free and total protein S antigens, von Willebrand factor antigen, von Willebrand collagen binding activity, and quantitative D-dimer, and calibrated chromagenic assays for antithrombin activity, protein C activity, and heparin or low molecular weight heparin. This list is not exhaustive, and laboratory directors should review their laboratory’s test menu to identify additional tests which meet the definition highlighted above.

Clot-based methods, (including PT, aPTT, thrombin time, factor assays and fibrinogen) and all platelet function assays, including ristocetin cofactor activity are exempt.

DEFINITIONS:

CALIBRATION is the set of operations that establish, under specified conditions, the relationship between reagent system/instrument response and the corresponding concentration/activity values of an analyte. Calibration procedures are typically specified by a method manufacturer, but may also be established by the laboratory.

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

REQUIRED FREQUENCY OF CALIBRATION VERIFICATION

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

1. A change of reagent lots for chemically or physically active or critical components, unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client test results, and the range used to report patient/client test data
2. If QC fails to meet criteria established by the laboratory director or designee
3. After major maintenance or service. The Laboratory Director or designee must determine what constitutes major maintenance or service.
4. When recommended by the manufacturer

MATERIALS SUITABLE FOR CALIBRATION VERIFICATION

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

1. Calibrators used to calibrate the analytical system
2. Materials provided by the analytical measurement system vendor for the purpose of calibration verification
3. Previously tested unaltered patient/client specimens
4. Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method,
5. Third party general purpose reference materials may be suitable for validation of calibration following reagent lot changes if the material is documented in the package insert or by the method manufacturer to be commutable with patient specimens for the method. A commutable reference material is one that gives the same numeric result as would a patient specimen containing the same quantity of analyte in the analytic method under discussion; e.g. matrix effects are absent. Commutability between a reference material and patient specimens can be demonstrated using the protocol in CLSI EP14-A2,
6. Proficiency testing material or proficiency testing validated material with matrix characteristics and target values appropriate for the method

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

ANALYTICAL MEASUREMENT RANGE

DEFINITIONS:

The ANALYTICAL MEASUREMENT RANGE (AMR) is the range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment not part of the usual assay process.

AMR VALIDATION is the process of confirming that the assay system will correctly recover the concentration or activity of the analyte over the AMR. The materials used for validation must be known to have matrix characteristics appropriate for the method. The matrix of the sample (i.e. the environment in which the sample is suspended or dissolved) may influence the measurement of the analyte. In many cases, the method manufacturer will recommend suitable materials. At a minimum, three test specimens must have analyte values, which 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.

LINEARITY AND THE AMR

Calibration equations describing the relationship between analyte concentrations and instrument readings may be linear or more complex (non linear). Regardless of the calibration curve, there should be a linear relationship
between expected and measured analyte values when specimens of known value are mixed with other specimens of known value in different ratios: i.e. linear dilution recovery. The AMR is the range of analyte values for which this linear relationship has been established.

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

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

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

CLOSENESS OF SAMPLE CONCENTRATIONS OR ACTIVITIES TO THE UPPER AND LOWER LIMITS OF THE AMR
When validating the AMR, it is required that samples tested are near the upper and lower limits of the AMR. Factors to consider in validating the AMR are the expected analytic imprecision near the limits, the clinical impact of errors near the limits, and the availability of test specimens near the limits. It may be difficult to obtain specimens with values near the limits for some analytes. In such cases, reasonable procedures should be adopted based on available specimen materials. The method manufacturer’s instructions for validating the AMR should be followed; when available. Specimen target values can be established by comparison with peer group values for reference materials, by assignment of reference or comparison method values, and by dilution ratios of one or more specimens with known values. The Laboratory Director must define limits for accepting or rejecting validation tests of the AMR. One option is to establish imprecision and percent recovery limits for an assay and then determine at what point at the top and bottom of the AMR these limits fail.

REQUIRED FREQUENCY OF AMR VALIDATION
The AMR must be validated when a method is placed in service and at least every six months thereafter. The AMR must also be validated (regardless of the length of time since last performed) immediately if any of the following occur:
1. A change of reagent lots for chemically or physically active or critical components, unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client test results, and the range used to report patient/client test data.

2. If QC fails to meet established criteria by the laboratory director or designee.

3. After major preventive maintenance or change of a critical instrument component. The laboratory director or designee must determine what constitutes major maintenance or service.

4. When recommended by the manufacturer.

SUITABLE MATERIALS FOR AMR VALIDATION

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

1. Linearity material of appropriate matrix, e.g. CAP Survey-based or other suitable linearity verification material

2. Proficiency testing survey material or proficiency testing survey-validated material

3. Previously tested patient/client specimens, unaltered

4. Previously tested patient/client specimens, altered by admixture with other specimens, dilution, spiking in known amounts of an analyte, or other technique

5. Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method

6. Calibrators used to calibrate the analytic measurement system that are from a different lot than the one used for calibration

7. Control materials, if they adequately span the AMR and have method specific target values

RECALIBRATION / CALIBRATION VERIFICATION and AMR VALIDATION INTERVALS: Recalibration or calibration verification, and AMR validation, must be performed at least once every 6 months, unless the assay is calibrated at a more frequent basis. Successful calibration verification certifies that the calibration is still valid; unsuccessful calibration verification requires remedial action, which usually includes recalibration and AMR revalidation. The performance of recalibration or a calibration verification procedure resets the calendar to a new maximum 6-month interval before the next required reassessment. Methods that are recalibrated more frequently than every 6 months do not require a separate calibration verification procedure, and may not require separate AMR validation if the recalibration procedure also fulfills the AMR validation criteria. If the revalidation procedure does not fulfill AMR validation criteria, then an independent AMR validation must be performed at least every six months.

**REVISED** 07/11/2011
HEM.38004  Calibration Materials Labeling  Phase II

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

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

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

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

HEM.38006  Calibration/Verification Criteria  Phase II

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

NOTE: Criteria typically include:

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

Materials that may be used for calibration verification 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

Evidence of Compliance:
✓ Written procedure defining the method, frequency and limits of acceptability of calibration verification
✓ Records of calibration verification and/or recalibration documented at defined frequency

REFERENCES

HEM.38007  Recalibration  Phase II

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

Evidence of Compliance:
Validation of the analytical measurement range (AMR) is performed with matrix-appropriate materials, which include the low, mid and high range of the AMR, appropriate acceptance criteria are defined, and the process is documented.

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

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

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

The materials used for validation must be known to have matrix characteristics appropriate for the method. The test specimens must have analyte values that as a minimum are near the low, midpoint, and high values of the AMR. Guidelines for analyte levels near the low and high range of the AMR should be determined by the laboratory director. Factors to consider are the expected analytic imprecision near the limits, the clinical impact of errors near the limits, and the availability of test specimens near the limits. It may be difficult to obtain specimens with values near the limits for some analytes. In such cases, reasonable procedures should be adopted based on available specimen materials. The method manufacturer's instructions for validating the AMR should be followed, when available. Specimen target values can be established by comparison with peer group values for reference materials, by assignment of reference or comparison method values, and by dilution ratios of one or more specimens with known values. Each laboratory must define limits for accepting or rejecting validation tests of the AMR.

The AMR must be revalidated at least every 6 months, and following changes in major system components or lots of analytically critical reagents (unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results, and control values are not adversely affected). AMR validation is not required for methods that measure an analyte quantitatively or semi-quantitatively, and report a qualitative value based on concentration threshold.

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

REFERENCES
**HEM.38010**  Diluted or Concentrated Samples  Phase II

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

**NOTE:**

1. A measured value that is outside the AMR may be unreliable and should not be reported in routine practice. Dilution, a mixing procedure* or concentration of a sample may be required to achieve a measured analyte activity or concentration that falls within the AMR. The processed result must be within the AMR before it is mathematically corrected by the concentration or dilution factor to obtain a reportable numeric result.
2. For each analyte, the composition of the diluent solution and the appropriate volumes of sample and diluent must be specified in the procedure manual. Specifying acceptable volumes is intended to ensure that the volumes pipetted are large enough to be accurate without introducing errors in the dilution ratio.
3. All dilutions, whether automatic or manual, should be performed in a way that ensures that the diluted specimen reacts similarly to the original specimen in the assay system. For some analytes, demonstrating that more than one dilution ratio similarly recovers the elevated concentration may be helpful.
4. This checklist requirement does not apply if the concentration or activity of the analyte that is outside the AMR is reported as “greater than” or “less than” the limits of the AMR.

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

**Evidence of Compliance:**
✓ Patient reports or worksheets

**HEM.38011**  Maximum Dilution  Phase II

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

**NOTE:**

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

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

Evidence of Compliance:
✓ Patient reports or worksheets

HEM.38012  QC - Controls  Phase II

An acceptable range is established or verified for each lot of control material.

NOTE: For unassayed controls, the laboratory must establish an acceptable range by repetitive analysis in runs that include previously tested control material. For assayed controls, the laboratory must verify recovery ranges supplied by the manufacturer.

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

REFERENCES

Platelet Function Studies

HEM.38013  Specimen Handling - Platelets  Phase II

Blood specimens for platelet aggregation and platelet function studies are handled at room temperature before testing.

NOTE: Platelets develop a cold-induced conformational change and dysfunction when handled at temperatures <20°C. Even when re-warmed, platelets may not regain normal function. Therefore, platelet specimens should always be handled at "room temperature," which is generally defined as 20 to 25°C (68 to 77°F) before testing and should never be refrigerated, chilled on ice or frozen.

Evidence of Compliance:
✓ Written procedure defining the specimen handling requirements prior to analysis

REFERENCES

HEM.38024  Platelet Aggregation Studies  Phase I

Platelet aggregation studies are performed at the temperature recommended by the manufacturer.
Evidence of Compliance:
✓ Records of temperature checks OR automated internal instrument temperature monitoring

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HEM.38028 Platelet Aggregation Studies Phase I

Platelet aggregation studies are completed between 30 minutes and 3-4 hours of blood collection.

NOTE: PRP (platelet rich plasma) should be used within three to four hours of platelet donation. The effects of time are related to changes in pH, which are directly related to the escape of CO2 from the PRP sample tube. Platelets may be refractory to epinephrine when using PRP samples tested within 30 minutes of venipuncture; this is cited as the rationale for not testing PRP until at least 30 minutes after phlebotomy. There is evidence to suggest that this initial platelet refractoriness and subsequent gain of function occurs because centrifugation releases ADP from red blood cells and platelets.

REFERENCES

HEM.38035 Optical Aggregation Method Phase I

If platelet aggregation studies are performed by an optical aggregation methodology using platelet rich plasma, there is a procedure to define optimal platelet concentration range.

NOTE: Optical platelet aggregation studies measure the change in percent of light transmittance as platelets aggregate. These techniques typically use platelet rich plasma (PRP). If the platelet count in the PRP is too high or too low, erroneous results may occur. The laboratory must have a procedure for ensuring that the platelet count in the PRP is optimal for study. The optimal platelet concentration may vary from laboratory to laboratory but a commonly defined range is 200-300 x 10^9/L. Samples with platelet concentrations greater than optimal can be diluted into the optimal range with platelet-poor plasma (PPP) (<10 x 10^9/L). There is evidence that PPP can inhibit platelet aggregation, but also evidence that adjustment of PRP with PPP does not adversely affect interpretation of aggregation responses to platelet agonists in patients with abnormal bleeding histories. Therefore, the decision to adjust or not adjust PRP with PPP is at the discretion of the laboratory. Platelet agonist reference intervals derived from control subjects should be established with the same method used to evaluate patients. Samples with less than or greater than the defined optimal platelet concentration can be analyzed, but a disclaimer should be added when abnormal results are obtained, as the decreased platelet concentration alone may adversely affect the results.

Evidence of Compliance:
✓ Written procedure defining the optimal platelet concentration and special handling for samples outside of the optimal range AND
✓ Patient reports with disclaimer if concentration is less than or greater than the optimal concentration

REFERENCES

PERSONNEL

Inspector Instructions:

- Documentation of education and experience

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HEM.40000 Personnel - Bench Testing  Phase II

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

Evidence of Compliance:

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