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**Hematology and Coagulation Checklist**

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ON-LINE CHECKLIST AVAILABILITY AND RESOURCES

Participants of the CAP accreditation programs may download the checklists from the CAP website (cap.org) by logging into e-LAB Solutions Suite. They are available in different checklist types and formatting options, including:

- Master — contains ALL of the requirements and instructions available in PDF, Word/XML or Excel formats
- Custom — customized based on the laboratory’s activity (test) menu; available in PDF, Word/XML or Excel formats
- Changes Only — contains only those requirements with significant changes since the previous checklist edition in a track changes format to show the differences; in PDF version only. Requirements that have been moved or merged appear in a table at the end of the file.

A repository of questions and answers and other resources is also available in e-LAB Solutions Suite under Accreditation Resources, Checklist Requirement Q & A.

SUMMARY OF CHECKLIST EDITION CHANGES
Hematology and Coagulation Checklist
09/22/2021 Edition

The information below includes a listing of checklist requirements with significant changes in the current edition and previous edition of this checklist. The list is separated into three categories:

1. New
2. Revised:
   - Modifications that may require a change in policy, procedure, or process for continued compliance; or
   - A change to the Phase
3. Deleted/Moved/Merged:
   - Deleted
   - Moved — Relocation of a requirement into a different checklist (requirements that have been resequenced within the same checklist are not listed)
   - Merged — The combining of similar requirements

NOTE: The requirements listed below are from the Master version of the checklist. The customized checklist version created for on-site inspections and self-evaluations may not list all of these requirements.

NEW Checklist Requirements

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REVISED Checklist Requirements

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**DELETED/MOVED/MERGED Checklist Requirements**

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INTRODUCTION

This checklist is used in conjunction with the All Common and Laboratory General Checklists to inspect a hematology laboratory section or department.

Certain requirements are different for waived versus nonwaived tests. Refer to the checklist headings and explanatory text to determine applicability based on test complexity. The current list of tests waived under CLIA may be found at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfClia/analyteswaived.cfm.

Laboratories not subject to US regulations: Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist. When the phrase "FDA-cleared/approved test (or assay)" is used within the checklist, it also applies to tests approved by an internationally recognized regulatory authority (eg, CE-marking).

QUALITY CONTROL

Inspector Instructions:

- Sampling of QC policies and procedures
- Sampling of QC records

- What do you do if controls are out of range?
- How does your laboratory verify or establish acceptable quality control ranges?
- What is your course of action when monthly precision data changes significantly from the previous month's data?
- How is quality control performed for test procedures that do not have commercially available calibration or control materials?

WAIVED TESTS - GENERAL

HEM.18038 QC - Waived Tests

The laboratory follows manufacturer's instructions for quality control and records and reviews results for acceptability prior to reporting patient results.
NOTE: Quality control must be performed according to manufacturer's instructions. To detect problems and evaluate trends, testing personnel or supervisory staff must review quality control data on days when controls are run prior to reporting patient results. The laboratory director or designee must review QC data at least monthly or more frequently if specified in the laboratory QC policy.

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

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

Evidence of Compliance:
✓ Written procedure consistent with manufacturer's instructions for each waived test AND
✓ Records showing confirmation of acceptable QC results

HEM.18691  QC Corrective Action - Waived Tests  Phase II
There is evidence of corrective action when control results exceed defined acceptability limits.

HEM.18705  Calibration, Calibration/Verification - Waived Tests  Phase II
For waived tests, the laboratory follows manufacturer’s instructions for calibration, calibration verification, and related functions.

Evidence of Compliance:
✓ Written procedure consistent with the manufacturer's instructions for each waived test AND
✓ Records for calibration/calibration verification/related functions as required by the manufacturer AND
✓ Records of recalibration or other appropriate corrective action when calibration verification is unacceptable

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

NONWAIVED TESTS - GENERAL

The following group of requirements is applicable to nonwaived manual, automated, and semi-automated testing, unless a separate checklist requirement exists in another checklist section that defines a specific QC frequency (eg, CBC instrument, coagulation testing, manual cell counts).

**REVISED** 09/22/2021
HEM.19360  Daily QC - Nonwaived Tests  Phase II
Controls are run at least each day testing is performed, or more frequently if specified in manufacturer’s instructions, laboratory procedure, or the CAP Checklist for quantitative and qualitative tests, and when changes occur that may impact patient results.

NOTE: The laboratory must define the number and type of quality control used and the frequency of testing in its quality control procedures. Control testing is not required on days when patient testing is not performed.

Controls must be run prior to resuming patient testing when changes occur that may impact patient results, including after a change of analytically critical reagents, major preventive
maintenance, change of a critical instrument component, or with software changes, as appropriate.

Daily quality control must be run as follows:

- **Quantitative tests** - two controls at different concentrations at least daily, except for coagulation tests (two controls every eight hours), or unless otherwise required elsewhere in this checklist
- **Qualitative tests** - a negative control and a positive control (when applicable) at least daily

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

If an internal quality control process (eg, electronic/procedural/built-in) is used instead of an external control material to meet daily quality control requirements, the laboratory must have an individualized quality control plan (IQCP) approved by the laboratory director defining the control process, including the frequency and use of external and internal controls. At a minimum, external control materials must be analyzed with new lots and shipments of reagents or more frequently if indicated in the manufacturer's instructions. Please refer to the IQCP section of the All Common Checklist for the eligibility of tests for IQCP and requirements for implementation and ongoing monitoring of an IQCP.

**Evidence of Compliance:**
✓ Records of QC results including external and internal control procedures
✓ Written quality control procedures
✓ Manufacturer product insert or manual

**REFERENCES**

---

Control Range Establishment or Verification

**Phase II**

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

**NOTE:** For unassayed control materials, an acceptable control range must be established by repetitive analysis in runs that include previously tested control material. For assayed control materials, control ranges supplied by the manufacturer must be verified.

Control ranges supplied by the manufacturer may be used without verification for qualitative (eg, positive or negative) testing.

**Evidence of Compliance:**
✓ Written procedure to establish or verify control ranges
✓ Records for control range establishment or verification of each lot

**REFERENCES**

---

Numeric QC Data

**Phase I**

For numeric QC data, quality control statistics (eg, SD and CV) are calculated monthly to define and monitor 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 records 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 commercially sponsored interlaboratory QC data for the same control lot and instrument model show SD/CV values outside those of the peer group, an explanation is required.

Evidence of Compliance:
✓ Written procedure for investigation and corrective action if a significant change in precision statistics occurs AND
✓ Records of investigation and corrective actions taken

HEM.20090 Alternative Control Procedures

If the laboratory performs test procedures for which control materials are not commercially available, there are written procedures for an alternative mechanism to detect immediate errors and monitor test system performance over time. The performance of alternative control procedures must be recorded.

NOTE: “Performance” includes elements of accuracy, precision, and clinical discriminating power. Examples of alternative procedures may include split sample testing with another method or with another laboratory, the testing of previously tested patient specimens in duplicate, testing of patient specimens in duplicate, or other defined processes approved by the laboratory director.

Evidence of Compliance:
✓ Written procedures for alternative quality control AND
✓ Records of alternative control procedures

REFERENCES
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.

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

REFERENCES

HEM.20140 QC Confirmation of Acceptability Phase II

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

Evidence of Compliance:
✓ Written policy stating that controls are reviewed and acceptable prior to reporting patient results AND
✓ Records of control result approval

REFERENCES

HEM.20143 QC Corrective Action Phase II

There are records 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.

The corrective action for tests that have an IQCP approved by the laboratory director must include an assessment of whether further evaluation of the risk assessment and quality control plan is needed based on the problems identified (eg, trending for repeat failures, etc.).

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 review of quality control data must be recorded and include follow-up for outliers, trends, or omissions that were not previously addressed.

The QC data for tests performed less frequently than once per month should be reviewed when the tests are performed.
The review of quality control data for tests that have an IQCP approved by the laboratory director must include an assessment of whether further evaluation of the risk assessment and quality control plan is needed based on problems identified (eg, trending for repeat failures, etc.).

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

HEMATOLOGY

SPECIMEN COLLECTION AND HANDLING

Inspector Instructions:

- Sampling of hematology specimen collection and handling policies and procedures
- 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.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 of evaluation of each specimen mixing method (eg, rotary mixer, rocker, automated sampler, or manual inversions) for reproducibility of results, as applicable

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

**NOTE:** Blood specimens for routine hematology tests (eg, CBC, leukocyte differential) must be collected in potassium EDTA to minimize changes in cell characteristics. Laboratories must follow manufacturer's recommendations for use of alternative anticoagulants.

**REFERENCES**

**HEM.22100** Capillary Tube Collection Criteria

Samples collected in capillary tubes for microhematocrits or capillary/dilution systems are obtained in duplicate whenever possible.

**NOTE:** Microspecimen containers such as those used for other capillary blood CBC parameter determinations need not be collected in duplicate. Because of the risk of injury, the use of glass capillary tubes is discouraged; if glass capillary tubes are used, measures have been implemented to reduce risk or injury.

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

**REFERENCES**

**HEM.22150** Specimen Quality Assessment - CBC

**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 testing personnel 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.

**REFERENCES**

**HEM.22200** Hemolyzed or Lipemic Specimens - CBC

**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**

*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**

**COMPLETE BLOOD COUNT (CBC) INSTRUMENTS**

**CALIBRATION**

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. Commercial control materials are not suitable for routine instrument calibration.

**Inspector Instructions:**

- Sampling of CBC calibration policies and procedures
- Sampling of CBC calibration records
Hematology and Coagulation Checklist

**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**

**HEM.25700 Calibration**  
Phase II

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

**REFERENCES**

**HEM.25760 Calibration Verification Criteria**  
Phase II

Criteria are established for calibration verification.

NOTE: The laboratory must have a written procedure for calibration verification. Criteria for calibration verification include:

1. At complete changes of reagents (ie, 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

For automated CBC cell counting instruments, requirements for calibration verification may be considered met if the laboratory follows the manufacturer's instructions for instrument operation and tests two levels of control materials each day of testing. The control results must meet the laboratory's criteria for acceptability. 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 at defined frequency
HEM.25780 Recalibration Phase II

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 for recalibration

CBC INSTRUMENT QUALITY CONTROL

Longitudinal process quality control (QC) procedures for individual instruments 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 two 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 from the previous two-year period
- Sampling of CBC error detection policies and procedures

- How do you determine when QC is unacceptable and when corrective actions are needed?
- How does your laboratory establish or verify acceptable QC ranges?
- How do you ensure results from CBC specimens with cold agglutinins, nucleated RBCs and lipemia are reported accurately?

- Review a sampling of QC data over the previous two-year period. Select several occurrences in which QC is out of range and follow records to determine if the steps taken follow the laboratory procedure for corrective action
- Select a spurious result example and follow the process used to ensure the correct results are reported

STABILIZED CONTROLS

HEM.25850 Stabilized Controls Phase II
Two different stabilized control specimens are analyzed and results recorded during each 24-hours of analyzer use.

NOTE: Stabilized control materials must be at two different analytic levels (ie, "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" (eg, 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 two control specimens per 24 hours, one or more of the additional approaches to QC must be employed to produce a total of at least two 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)

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.

Labs 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
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

If a "moving averages" system is combined with another control system (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

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

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

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

**ERROR DETECTION AND VERIFICATION**

**HEM.30070** Sampling Mode Comparison

There are records that at least annually compare all results obtained for patient specimens analyzed in the multiple sampling modes of the CBC analyzer (eg, "primary" and "secondary" 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 must be performed in the alternate mode(s), and results must 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 that 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

**REVISED** 06/04/2020

**HEM.30100** Detection/Correction Procedure - WBC

There is a written procedure 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 must 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 inspection of a stained blood film. 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 WBC concentration prior to reporting

REFERENCES

**REVISED** 06/04/2020

**HEM.30150** Spurious CBC Results

There is a written procedure to detect spurious CBC instrument results that may be clinically significant (eg, pseudomacrocytosis from rouleaux or agglutinates;
pseudoleukocytosis with erroneous hemoglobin, falsely low erythrocyte count and hematocrit; hyperlipemias) prior to reporting.

**NOTE:** Analytic sources of error with automated instruments depend on the type of instrument and reagents used by the laboratory.

**Evidence of Compliance:**
- ✓ Written procedure to detect spurious CBC results **AND**
- ✓ 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 (MCV, MCH, MCHC) must be monitored routinely to detect random errors, instrument malfunction, or spurious results. 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. 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, and results that fall outside these limits are reported properly.**

**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 policy 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 Platelet Abnormalities**

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 platelet satellitosis (satellitism), significant numbers of giant platelets and/or platelet clumps are suspected/detected by cyto/histographic abnormalities or instrument rejection of a platelet result, the platelet concentration must be independently verified. Correlation with a well-prepared blood film must be made. If platelets 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; platelets must be quantified from blood collected directly into a counting diluent, by use of a different anticoagulant per manufacturer's recommendations, 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**

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 the 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 and correction of falsely elevated WBC counts **AND**
- Record showing actions taken to verify WBC concentration prior to reporting

**REFERENCES**
HEM.30400  Platelet Count 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

MANUAL HEMATOCRIT

Inspector Instructions:

- Hematocrit procedure
- Sampling of annual centrifuge speed checks
- Sampling of timer checks

HEM.32050  Microhematocrit Centrifuge  Phase I

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 an RCF of 10,000 to 15,000 at the periphery for five 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 policy defining criteria for verification of centrifuge operating speeds AND
✓ Records of microhematocrit centrifuge speed checks

REFERENCES
HEM.32100  Mechanical Timer  Phase I

If a mechanical timer is used, its accuracy is checked at least annually.

**NOTE:** Not applicable to electronic timers.

**Evidence of Compliance:**
✓ Records of mechanical timer checks

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 policy defining criteria for establishing/reassessing constant packing time **AND**
✓ Records of initial and reassessment studies as appropriate

**REFERENCES**

**MANUAL BLOOD COUNT**

**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:**

- Manual cell counts procedure
- Sampling of QC logs
- 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

**REFERENCES**
HEM.33250 Manual Counts - PLT/WBC

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 written procedure must specify an increased number of cells counted (eg, 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

There is a written procedure 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:
✓ Written procedure for contamination checks on dilution fluids and reagents AND
✓ Records of contamination checks on dilution fluids/reagents

HEM.33330 Cell Count Controls

At least one cell count control specimen is analyzed in duplicate, or a procedural control employed for each eight 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 must 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 at defined frequency

REFERENCES

HEM.33350 PLT Estimate

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

Evidence of Compliance:
✓ Records of slide review/correlation

REFERENCES
AUTOMATED DIFFERENTIALS

Inspector Instructions:

- Automated differential procedure
- Sampling of QC records

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

HEM.34100 Limit of Agreement - WBC  Phase II

Acceptable limits for quality control procedures for WBC subclasses using manually counted blood films or commercial controls are defined.

NOTE: For automated analyzers, 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 pattern recognition microscopy systems, QC can be done by periodic processing of prepared control slides and maintenance/analysis of Levey-Jennings charts.

For commercial controls, mixed leukocyte subclasses (eg, "mononuclear" or "large unclassified cells") or "remainder" fractions do not need to be assessed with QC procedures. The commercial material must 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 (eg, 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) Etzell, JE. For WBC differentials reporting absolute numbers. CAP Today, March 2010
5) Ross DW, Bentley SA. Evaluation of an automated hematology system (Technicon H1). Arch Pathol Lab Med. 1986;110:803-808
HEM.34200  WBC Differential Verification    Phase II

The laboratory establishes criteria for checking and reviewing leukocyte differential counter data, histograms, and/or blood films for clinically important results flagged by the automated differential counter.

NOTE: Clinically important results include pathologic quantities of normal cell types and abnormal cells. Flagging mechanisms include those within the particular instrument, inspection of histographic/cytographic displays, laboratory criteria based on local experience, and awareness of published evaluations.

Evidence of Compliance:
✓ Written procedure defining criteria for review and evaluation of automated differential results prior to reporting AND
✓ Records of verification of flagged values

REFERENCES
4) Ross DW, Bentley SA. Evaluation of an automated hematology system (Technicon H-1). Arch Pathol Lab Med. 1986;110:803-808

MANUAL DIFFERENTIALS

This section applies to all manually interpreted differentials, including those performed using automated image analysis systems requiring manual verification or interpretation of cell classification or other morphologic findings.

Inspector Instructions:

- Manual differential policies and procedures
- 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?
- What criteria are used for referring a blood film for review by a pathologist, supervisor, or technologist with expertise in hematomorphology?
HEM.34300  Blood Film Quality  Phase I

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.34320  Stain Reactivity  Phase II

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

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

REFERENCES

HEM.34400  Morphologic Observation Evaluation - CBC  Phase II

The laboratory evaluates consistency of morphologic observation among personnel performing blood cell microscopy at least annually.

NOTE: The laboratory must ensure the identification and morphology of blood cells is reported consistently amongst all personnel performing the microscopic analysis.

Suggested methods to accomplish this include:

1. Circulation of a pre-graded set of blood films with defined leukocyte differential distributions, and RBC and platelet morphology.
2. Multi-headed microscopy
3. Use of blood or marrow photomicrographs with referee and consensus identifications (eg, former CAP surveys photomicrographs)
4. Use of digital images

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

Acceptability criteria for agreement must be determined by the laboratory director or designee. The laboratory must maintain records of performance and record corrective actions taken for personnel demonstrating significant discrepancies from the group consensus.

Evidence of Compliance:
✓ Written procedure defining the method and criteria used for evaluation of consistency AND
✓ Records of evaluation

REFERENCES
4) Brigden ML, Dalal BI. Morphologic abnormalities, pseudosyndromes, and spurious test results. Lab Med. 1999;30:397-405
HEM.34450  Slide Retention - CBC Differential  

**Phase I**

**Blood film slides (peripheral blood smears) 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 (eg, 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 written 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 MICROORGANISMS**

**Inspector Instructions:**

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

HEM.34655  Blood Film Microorganism Detection  

**Phase II**

Blood films submitted for microscopic examination allow for detection of microorganisms that may be present.
NOTE: Microorganisms that should be recognized, if present, include parasites, such as Plasmodium species, trypanosomes, and microfilaria. Occasionally, the morulae of Anaplasma and Ehrlichia, which are bacteria, may be seen. Spirochetes of the Borellia genus may be seen in patients with relapsing fever. Yeasts may sometimes be seen in patients with disseminated histoplasmosis or with fungemia caused by other yeast species (eg, Candida species or Malassezia species).

Evidence of Compliance:
✓ Written procedure defining microorganisms that may be present during blood film examination AND
✓ Blood parasitology atlas or reference materials

REFERENCES

**REVISED** 06/04/2020
HEM.34687 Parasite Load Reporting

When blood films are positive for malaria parasites (Plasmodium spp.), the parasite load (provided as percentage parasitemia or the number of parasites per µL of blood) is reported along with the organism identification.

NOTE: It is important to determine the parasite load when blood films are reviewed and found to be positive for malaria parasites because this information may be used to guide treatment decisions and monitor the response to therapy. Due to 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 parasite load reported exactly the same way on follow-up specimens as on the initial specimen. This allows the parasite load to be monitored after therapy has been initiated. The parasite load 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.

Although there are currently no requirements for reporting parasite load when blood films are positive for Babesia species, physicians may ask for these data to guide treatment decisions and monitor the response to therapy.

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

REFERENCES

HEM.34724 Thick and Thin Films

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
HEM.34798  Malaria Stain Procedure

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

REFERENCES

HEM.34872  Slide Review Procedure

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

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

REFERENCES

AUTOMATED RETICULOCYTES

Inspector Instructions:

- Automated reticulocyte procedure

HEM.35150  Spurious Reticulocyte Results

There is a written procedure to detect spurious automated reticulocyte results prior to reporting.

NOTE: Since all DNA- and RNA-containing cells will stain with DNA-RNA fluorescent dyes, the written procedure must 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:
✓ Written procedure to detect spurious automated reticulocyte results AND
✓ Records showing actions taken to verify reticulocyte count prior to reporting

REFERENCES

MANUAL RETICULOCYTES

Inspector Instructions:

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

HEM.35250  Reticulocyte Blood Film Quality  Phase I

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

REFERENCES

HEM.35300  Reticulocyte Concentration  Phase I

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:

READ

- Sampling of manual or automated body fluid policies and procedures
- Sampling of patient reports
- Sampling of QC records

OBSERVE

- 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

ASK

- 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?

MANUAL CELL COUNT - BODY FLUID

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

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 Manual Cell Count Controls Phase II
For manual body fluid cell counts, at least one cell count control specimen is analyzed in duplicate, or a procedural control used, for each eight 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 control materials must be tested in duplicate.

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

REFERENCES

HEM.35347 Counting Chamber and Optical Grid Quality Phase I
The lines in all counting or motility chambers, ocular micrometers, and optical grids are bright and free from scratches, dirt, or debris.

HEM.35357 Body Fluid Analysis Procedure Phase II
For manual body fluid cell counts, each sample is counted in duplicate.

NOTE: Testing records must reflect the performance of the counts in duplicate for all counting chambers. 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

REFERENCES

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.

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

AUTOMATED CELL COUNT - BODY FLUID

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 policy 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 II**

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 each day of testing with results recorded and reviewed for acceptability.

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

**NUCLEATED CELL DIFFERENTIALS - BODY FLUID**

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 supravitally-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 cytopsin 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

HEM.35547 Body Fluid Smear Quality Phase I

The quality of body fluid smears is satisfactory (uniform cell distribution, appropriate dilution so cells are not crowded, properly stained, adequate cell yield, ready recognition of cell types that are reported).

REFERENCES

HEM.35566 Morphologic Observation Evaluation - Body Fluid Phase II

The laboratory evaluates consistency of morphologic observation among personnel performing body fluid cell differentials at least annually.

NOTE: The laboratory must ensure the identification of body fluid cells is reported consistently amongst all personnel performing the microscopic analysis.

Suggested methods to accomplish this include:

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

Acceptability criteria for agreement must be determined by the laboratory director or designee. The laboratory must maintain records of performance and record corrective actions taken for personnel demonstrating significant discrepancies from the group consensus.

Evidence of Compliance:
✓ Written procedure defining the method and criteria used for evaluation of consistency AND
✓ Records of evaluation

REFERENCES

HEM.35585 Slide Review Phase II

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

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

HEM.35604 Microscopic Result Comparison Phase I

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

Evidence of Compliance:
✓ Written procedure for comparing microscopic results performed in multiple laboratory sections when malignancy is suspected AND
✓ Records of comparison

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

**REVISED** 09/22/2021

HEM.35642 Slide Retention - Body Fluid Differential Phase I
Body fluid slides (eg, pleural fluid, seminal fluid) are retained for at least one week for possible review and/or reference.

NOTE: The laboratory may choose to retain significantly abnormal smears (eg, those demonstrating microorganisms, cytologically suspicious or overtly malignant cells, etc.) for longer periods to allow for review as part of the laboratory’s correlative or quality assurance programs or delayed clinical queries, as defined in the laboratory’s slide retention policy. If a longer retention period is defined, it must be followed.

RESULT REPORTING - BODY FLUID

**NEW** 09/22/2021

HEM.35650 Body Fluid Result Reporting of Nucleated Cells

Phase I

When absolute total cell counting methods cannot reliably distinguish white blood cells from other nucleated cells, body fluid cell counts and differential results are reported with the total nucleated cell count and a differential with all nucleated cell types observed.

NOTE: If the absolute total cell counting method in the laboratory cannot reliably distinguish white blood cells from other nucleated cells (eg, unstained bright-field visualization of cells in a hemocytometer chamber and certain automated counting technologies), the laboratory must report the absolute total cell count (cells/µL) as TNC (total nucleated cells) not WBC (total white blood cells). The relative differential (% of total cells counted) performed on a stained cytocentrifuge slide, which can reliably distinguish white blood cells from other nucleated cells, must include the percentage of all nucleated cell types (eg, lymphocyte, neutrophil, monocyte/macrophage, basophils, eosinophil, plasma cell, mesothelial cell, bronchial lining cell, synovial lining cell, ventricular lining cell, endothelial cell, squamous epithelial, and other) when TNC is reported for the absolute total cell count.

REFERENCES


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:**

| READ | Sampling of manual and automated semen analysis policies and procedures  
Sampling of specimen collection and handling policies and procedures  
Sampling of patient records for all necessary collection information  
Patient instructions  
Sampling of stain QC records  
Sampling of patient reports  
Sampling of calibration/calibration verification records  
Sampling of QC records |
|---|---|
| OBSERVE | Stained smear (properly stained, free of precipitate, uniform cell distribution, recognition of reportable cell types)  
File of unusual slides |
| ASK | 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?  
What is your course of action when the concentration of the specimen is outside of the instrument measurement range? |
| DISCOVER | Follow a semen analysis from requisition, collection information, testing, reporting and recording of results. Determine if practice follows laboratory procedure. |

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**REQUISITIONS, SPECIMEN RECEIPT AND RESULTS REPORTING**

**HEM.35661 Azoospermic Specimen Result Reporting Phase I**

For azoospermic and post-vasectomy seminal fluid specimens, the laboratory clearly communicates the findings of the assay and either employs a concentrating technique on seminal fluid or includes a comment in the patient report indicating that a concentrating technique was not performed.

**NOTE:** Without a concentration technique, the presence of both motile and non-motile sperm may not be detected. The method for detection of motile and non-motile sperm and the laboratory findings must be clearly communicated on the patient report so that the clinician can interpret the results in context to the method performed. The decision on the method used and extent of testing to be performed should be made in consultation with the medical staff served.

The American Urological Association (AUA) Vasectomy Guideline recommends a careful evaluation of an uncentrifuged specimen, and does not recommend centrifugation of the specimen for further assessment. The AUA Guideline also recommends reporting both the presence and absence of sperm and presence or absence of sperm motility on the patient report. If no sperm are seen in the uncentrifuged specimen, the guideline recommends reporting that the presence of sperm is below the limit of detection.
Evidence of Compliance:

☑ Patient report with concentration findings or appropriate comment indicating that concentration was not performed

REFERENCES
1) Evaluation of the Azoospermic Male. Fertil Steril. 2008; 90 (S74-7)

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

HEM.35680 Specimen Collection/Handling

There are written 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.

REFERENCES

HEM.35699 Specimen Collection/Handling

Semen specimens are accompanied by the following collection information, and records are retained on the following.

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

REFERENCES

HEM.35718 Liquefaction

All semen specimens are given sufficient time for liquefaction before testing.

Evidence of Compliance:

☑ Written policy defining criteria for liquefaction

REFERENCES

HEM.35737 Specimen Handling - Pre-analytic

Semen specimens are mixed thoroughly before testing.

REFERENCES
HEM.3576  Specimen Characteristics - Analytic  

All characteristics of the semen specimens are noted and reported (eg, gelatinous clumps, viscosity, contaminants, erythrocytes, and abnormalities of liquefaction).

NOTE: Macroscopic and microscopic characteristics of the semen specimens must be noted and reported, in accordance with the WHO laboratory manual for the examination of human semen (ie, fourth or fifth edition).

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

REFERENCES

SPERM MOTILITY

HEM.35762 Motility Method Verification  

A procedure exists to verify the sperm motility method used (eg, 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  

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

NOTE: The laboratory must have a written 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 World Health Organization (WHO) Standards (ie, fourth or fifth edition).

REFERENCES

HEM.35768 Forward Progression  

Forward progression of sperm is evaluated.

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

REFERENCES

HEM.35775 Motility/Progression Evaluation  

Phase II

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

REFERENCES
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 appropriate

REFERENCES

HEM.35794  Standard Temperature Range  Phase II
The laboratory has established a standard temperature range for semen analysis assessment, and deviations from this temperature are noted on the report.

NOTE: Specimen motility is temperature-dependent. Temperature ranges must be defined.

Evidence of Compliance:
✓ Written procedure with acceptable temperature range defined
✓ Records showing monitoring of temperatures

REFERENCES

HEM.35813  Motility Microscopic Examination  Phase II
The laboratory has written instructions for evaluating a sufficient number of separate and randomly chosen microscopic fields and sperm cells.

REFERENCES

HEM.35822  Viability Testing Criteria  Phase I
The laboratory performs viability testing on specimens with low percent motility (eg, 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 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

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 WHO Standards (ie, fourth or fifth edition), and the Kruger classification system, and discontinuing the use of older classification systems.

**REFERENCES**

HEM.35851 Morphologic Observation Evaluation - Sperm

The laboratory evaluates consistency of morphologic observation among personnel performing microscopic morphologic classification of sperm and other cells at least annually.

**NOTE:** The laboratory must ensure the identification of sperm and other cells is reported consistently amongst all personnel performing the microscopic analysis.

Suggested methods to accomplish this include:

1. Circulation of a pre-graded set of stained semen smears with defined specific qualitative abnormalities of sperm
2. Multi-headed microscopy
3. Use of current published references
4. Digital images

Acceptability criteria for agreement must be determined by the laboratory director or designee. The laboratory must maintain records of performance and record corrective actions taken for personnel demonstrating significant discrepancies from the group consensus.

**Evidence of Compliance:**
✓ Written procedure defining the method and criteria used for evaluation of consistency AND
✓ Records of evaluation

**REFERENCES**

HEM.35870 Consultation

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

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

REFERENCES

HEM.35892 Stain Usage

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

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

REFERENCES

HEM.35893 Leukocyte Confirmation Techniques

There is an additional procedure beyond unstained bright-field microscopy to ensure the accurate distinction of leukocytes from other round cells (eg, Wright's, leukocyte alkaline phosphatase, or myeloperoxidase stains).

NOTE: This requirement only applies to laboratories that differentiate leukocytes from other round cells on the patient report.

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

REFERENCES

HEM.35895 Stain QC

Quality control of all stains is performed and recorded to check for contamination and intended reactivity each day of use.

Evidence of Compliance:
✓ Written procedures for stain QC AND
✓ Records of stain QC

REFERENCES

HEM.35902 Stain Quality

The stains used (Wright’s, Papanicolaou, eosin-nigrosin, peroxidase, etc.) and slide preparations are of sufficient quality to demonstrate the cellular characteristics for which they are designed.
**NOTE:** The stains used for semen analysis must be defined in the laboratory's procedure manual.

**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, with results recorded and reviewed for acceptability.*

**Evidence of Compliance:**
- Written procedure for QC  
- Records of 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, with results recorded and reviewed for acceptability.*

**Evidence of Compliance:**
- Written procedure for QC  
- QC records
REFERENCES
1) Keel BA, Webster BW. CRC handbook of the laboratory diagnosis and treatment of infertility. Boca Raton, FL: CRC Press, 19RLM.185

AUTOMATED SEMEN ANALYSIS INSTRUMENTS

Various systems are in use and some requirements may not apply to every system. The requirements 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 (eg, fixed or preserved)
3. Sperm surrogates (eg, latex particles)
4. Digital images/videotaped sperm specimens

NOTE: If stabilized control materials are used, they must represent different analytic levels (eg, normal and high). Similarly, retained patient specimens must 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 recorded.

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. Laboratories must verify at least every six months that instruments are functioning correctly and are in control.

REFERENCES

HEM.35915 Daily QC

The laboratory performs and records quality control for the automated instrument during each day of use, following the manufacturer instructions or using at least two levels of control at different concentrations.

REFERENCES

HEM.35916 Recalibration

The test system is recalibrated when calibration verification fails to meet the established criteria of the laboratory.
Evidence of Compliance:
✓ Written policy defining criteria for recalibration AND
✓ Records of recalibration, if calibration or calibration verification has failed

REFERENCES

HEM.35918 Calibration Material Validation Phase II
The material used for calibration is validated using primary reference procedures (eg, 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 certification/validation of commercial products OR in-house validation data

REFERENCES
2) Krause W. Value of computer-assisted sperm analysis (CASA), reproducibility--online documentation--prognostic value. [Article in German]. Fortschr Med. 1996;114:470-473

HEM.35919 System Control Phase II
If a manual method is used as the system control for automated sperm counts, its accuracy is verified and recorded 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 Range Phase II
For automated sperm counts and motility, there is a written procedure to confirm that the concentration of the specimen is within the range appropriate for automated analysis.

REFERENCES

HEM.35923 Reportable Range Phase II
Upper and lower limits of all reportable parameters on instruments are defined, and results that fall outside these limits are reported properly.

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 policy defining the upper and lower reporting limits and verification of results AND
✓ Patient test verification records

REFERENCES

HEM.35924 Calibration Verification Criteria

There are written criteria for method calibration verification.

NOTE: Laboratories must either recalibrate or perform calibration verification at least every six months and if any of the following occur:

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. If QC shows an unusual trend or shift or is outside acceptable limits, and the system cannot be corrected to bring control values into the acceptable range
3. After major preventive maintenance or change of a critical instrument component
4. When recommended by the manufacturer

For automated semen analysis instruments, requirements for calibration verification may be considered met if the laboratory follows the manufacturer’s instructions for instrument operation and tests two levels of control materials each day of testing. The control results must meet the laboratory’s criteria for acceptability.

Evidence of Compliance:
✓ Written policy 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

For purposes of diagnosing hemoglobinopathies, more than one test may be necessary. As an example, hemoglobin solubility testing alone is not sufficient for detecting or confirming the presence of sickling hemoglobins in all situations.
Inspector Instructions:

- Sampling of abnormal hemoglobin policies and 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?

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 policy defining criteria for follow-up when Hb S appears in the primary screen

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 (eg, A, F, and S) are performed with the patient specimen(s) and separations are satisfactory.

**NOTE:** There are written procedures for instruments with multiple electrophoretic chambers or capillaries to ensure that QC is performed on each individual chamber or capillary.

**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
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 must be performed to further characterize these hemoglobin variants. Examples include:

- Performance by a complementary, separate methodology
- Increasing the duration of the assay (for HPLC) where the hemoglobins migrate/elute at different configurations.

Further workup of such variants, including referral to another laboratory, is dependent upon the patient's overall clinical situation, such as findings of erythrocytosis or a hemolytic anemia.

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

REFERENCES
8) 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/ß-zero thalassemia) it is imperative to exclude other...
hemoglobin variants, however rare. Referral of these specimens to another laboratory for further workup is acceptable.

Evidence of Compliance:
✓ Written policy 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
1) Black J. Isoelectric focusing in agarose gel for detection and identification of hemoglobin variants. Hemoglobin. 1984;8:117

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HEM.35986 Calibration and Calibration Verification

Appropriate calibration or calibration verification is performed on each day of patient testing or more frequently if required by the manufacturer’s instructions.

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

Evidence of Compliance:
✓ Written procedure defining calibrators/standards appropriate for the test system used AND
✓ Records of calibration/calibration verification

HEM.35987 Quality Control

Appropriate controls are extracted and run through the entire procedure on each day of patient testing.

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

*LOD - limit of detection
**LOQ - limit of quantitation**

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

**Evidence of Compliance:**
- Written procedure defining QC requirements for each test system **AND**
- QC records at defined frequency

**REFERENCES**

**HEM.35988** Sample Run Order

*Phase II*

**A record of sample run order is maintained for review.**

*NOTE: The run list must include blanks, standards, controls, and patients included in each run and be stored with the results of each batch run.*

**HEM.35990** Chromatographic Characteristics/Column Review

*Phase II*

**Chromatographic characteristics and column performance are reviewed and approved for each run before results are released.**

*NOTE: Checks should record testing variables such as the amount of sample injected and indications of error, including split peaks, doublets, and tailing.*

**HEM.35992** Column Verification

*Phase II*

**New columns are verified for performance before use.**

**Evidence of Compliance:**
- Written procedure for column verification **AND**
- Records of column verification

**HEM.35998** Reagent Grade

*Phase II*

**Reagents, solvents and gases are of appropriate grade.**

**Evidence of Compliance:**
- Written procedure detailing appropriate grade for materials used

**HEM.36001** Limit of Detection/AMR

*Phase II*

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

**HEM.36005** Column/Detector Monitoring

*Phase II*

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

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

HEM.36010  Carryover Detection  Phase II

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

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

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

BONE MARROW PREPARATIONS

Inspector Instructions:

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

- Bone Marrow aspirate and/or biopsy slides (uniquely identified, with satisfactory staining and cell distribution)
- Sample report (with integration of ancillary testing, as indicated)

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

HEM.36030  Bone Marrow Procedures  Phase II

If bone marrow aspiration and/or biopsy procedures are performed, there is a written procedure to verify patient identification using at least two patient identifiers, the procedure site, and the procedure to be performed.

REFERENCES

**REVISED** 09/22/2021

HEM.36100  Bone Marrow Slide Quality  Phase I

The quality of bone marrow aspirate and touch slide preparations are satisfactory (properly stained, free of precipitate).

NOTE: The aspirate smears must be properly stained and free of artifacts (eg, excessive stain precipitate, cellular crushing, excessive blood) to allow for reliable differentiation of bone marrow elements and their stages of maturation.
HEM.36150  Fixed Sections  Phase I

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 records of aspirate and fixed section review, as applicable

REFERENCES
6) Foucar K. Bone marrow pathology. Chicago, IL: American Society of Clinical Pathology, 1995

**REVISED** 09/22/2021
HEM.36200  Fixed Tissue Quality  Phase II

The quality of fixed tissue sections of bone marrow is conducive to a reliable diagnosis (eg, properly stained, no distortion).

NOTE: The sections must be properly stained and free of distortions (eg, thick or wrinkled sections) to allow for reliable differentiation of bone marrow elements such as myeloid, erythroid, and lymphoid populations.

HEM.36250  Fixed Tissue Correlation  Phase I

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.

NOTE: Unified reporting of bone marrow aspirates and biopsies is strongly recommended. If aspirate smears and biopsy reports are released by different sections of the laboratory, or at different times, a mechanism must be in place to comment upon the existing report and interpretation when the subsequent report is released. Any conflicting data should be commented upon. Such data correlation is essential for diagnostic consistency and effective patient management.

Evidence of Compliance:
✓ Written procedure 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

**REVISED** 09/22/2021
HEM.36270  Slide and Report Retention - Bone Marrow Evaluation  Phase II

Bone marrow slides and reports are retained for at least 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

**REVISED** 06/04/2020
Correlation of Results

There is a mechanism to correlate the results of ancillary studies (immunohistochemistry, cytogenetics, molecular pathology, 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 records review of such studies not available at the time of initial request. Clinically significant discrepancies must be reconciled and recorded.

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

**REFERENCES**

Iron Stain

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

**NOTE:** The preferred specimen for the iron stain is an aspirate smear and/or clot section, not a decalcified core biopsy.

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

Stain Reactivity

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
✓ Records of stain QC at defined frequency

**REFERENCES**
How have you established or verified reference intervals?

HEM.36820  Reference Intervals

Phase II

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

NOTE: For WBC differential counts, the CAP recommends that laboratories report absolute cell counts, along with their corresponding reference intervals. The CAP discourages the reporting of percent cell counts without absolute counts on WBC differentials. Laboratories reporting only percent cell counts must provide laboratory established reference intervals.

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.

Reference interval citations from the manufacturer's insert or published literature citations may be used to determine the reference interval. However, reference intervals have not been published for many body fluid analytes and obtaining normal fluids to establish reference intervals may not be feasible. If reference intervals are not available, results must be accompanied by an appropriate comment such as, "The reference interval(s) and other method performance specifications are unavailable for this body fluid. Comparison of the result with concentration in the blood, serum, or plasma is recommended."

REFERENCES
3) Etzell, JE. For WBC differentials reporting absolute numbers. CAP Today. 2010; 3:12
COAGULATION

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?
- How do you ensure that platelet-poor plasma is used for testing?

HEM.36840  Specimen Collection - Intravenous Lines  Phase I

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 not used for coagulation testing. 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
3) Laxson CJ, Titler MG. Drawing coagulation studies from arterial lines; an integrative literature review. Am J Critical Care. 1994; 1:18-24
Routine coagulation specimens are 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 Na$_3$C$_6$H$_5$O$_7$·2H$_2$O) 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 (ie, PT and aPTT) performed on slightly underfilled samples and samples with high hematocrits. The prolonged results are also more pronounced when the clotting time is abnormal, such as in samples from patients on warfarin therapy. Both the World Health Organization and CLSI recommend utilizing 3.2% sodium citrate (105-109 nmol/L), as the thromboplastin International Sensitivity Index (ISI) values applied in the INR calculations are based on specimens collected in 3.2% sodium citrate. Coagulation testing cannot be performed in samples collected in EDTA due to the more potent calcium chelation. While certain assay systems, such as platelet mapping via thromboelastography require heparin, heparinized tubes are not appropriate for clot-based plasma assays due to the inhibitory effect of heparin on multiple coagulation proteins. Other testing for platelet function, such as light transmission platelet aggregation assay can be performed on 3.2% or 3.8% sodium citrate.

Evidence of Compliance:
✓ Written policy defining the use of 3.2% buffered sodium citrate for coagulation specimen collection AND/OR
✓ Written procedure for use of an alternative anticoagulant that follows manufacturer's instructions or has been validated by the laboratory

REFERENCES

HEM.36880  Fill Volume - Coagulation  Phase I

There are written guidelines for the acceptable fill volume of specimen collection tubes for coagulation testing.

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. It is unacceptable to combine the contents from separate, underfilled sodium citrate collection tubes.

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.36900 Elevated Hematocrits - Coagulation

There are written 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, a written procedure for the detection and special handling of polycytemic specimens is required. 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%).

Evidence of Compliance:
✓ Written procedure outlining the detection and handling of coagulation specimens with elevated hematocrits AND
✓ Written procedure for the adjustment of citrate concentration for coagulation specimens with a known hematocrit >55%

REFERENCES
5) Goodwin AJ. Q & A: Should a patient with a hematocrit greater than 55 percent be redrawn for correction always or only when prothrombin time and partial prothrombin time are elevated? CAP Today: August 2016.

**REVISED** 06/04/2020

HEM.36920 Specimen Quality Assessment - Coagulation

Coagulation specimens are checked for clots (eg, applicator sticks) or by analysis of testing results (eg, wave form analysis, delta checks) before reporting results.

NOTE: Specimens with grossly visible clots may have extremely low levels of fibrinogen and variably decreased levels of other coagulation proteins, causing PT, aPTT, fibrinogen and other coagulation assays results to be inaccurate or unobtainable. Checking for clots may be done:
- With applicator sticks
- By visual inspection of centrifuged plasma for small clots
- By analysis of results including waveform analysis or delta checks as applicable

Laboratories receiving centrifuged specimens (eg, frozen plasma) cannot rely on visual inspection alone to detect specimen quality issues. For example, if a clot is not detected during PT and aPTT testing and the fibrinogen level is <25 mg/dL, the sample may actually be serum instead of plasma.

The laboratory must have a written policy to identify these specimens appropriately and/or to reject specimens, as applicable. Laboratories must work with their clients that perform specimen processing to ensure that they practice appropriate specimen handling for coagulation specimens.

Evidence of Compliance:
✓ Written policy to assess quality of coagulation specimens AND
✓ Records of rejection for clotted specimens

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

HEM.36940 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, or as centrifuged plasma separated from the cells, should be kept at room temperature (18 to 24°C) and tested no longer than 24 hours from the time of specimen collection. PT specimens should not be refrigerated (during storage).
2. aPTT specimens that are uncentrifuged with plasma remaining in the capped tube above the packed cells should be kept at room temperature (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 refrigerated (2 to 8°C) or at room temperature (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 (eg, thrombin time, protein C, factor V, factor VIII) have variable stability and should be kept in the same manner as aPTT samples.

If PT or aPTT 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 12 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 policy 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

**REVISED** 09/22/2021

HEM.37150 DIC - Test Availability Phase II

Tests for defining or monitoring disseminated intravascular coagulation (DIC) are available, if applicable to the patient population served.

NOTE: At a minimum, the platelet count, aPTT, PT/INR, fibrinogen assay and D-dimer (or FDP) must be available. Laboratories may wish to refer to criteria published by the International Society on Thrombosis and Haemostasis (ISTH) and the Japanese Ministry of Health and Welfare for further information.
HEM.37165 Coagulation Testing and Therapeutic Anticoagulant Recommendations

Recommendations are available to clinicians on the following:

- Laboratory tests used for monitoring heparin, low molecular weight heparin, direct thrombin inhibitors (eg, lepirudin, bivalirudin, argatroban) and/or oral anticoagulant therapy
- The therapeutic range for the tests, if available
- Information about potential interferences of anticoagulant medications on coagulation testing.

NOTE: The coagulation tests available to clinicians should be applicable to the anticoagulant drugs in use, and information is available on the test values that indicate that the anticoagulant is present and/or is in a therapeutic range, when available.

For vitamin K antagonists (eg, warfarin), the prothrombin time (PT/INR) is recommended. Direct oral anticoagulant medications (non-vitamin K) should not be monitored with PT/INR or aPTT because the effect of these tests is not predictable. 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 parenteral thrombin inhibitors are often monitored using the aPTT. The thrombin time may be useful to qualitatively verify the presence of direct thrombin inhibitors.

Evidence of Compliance:

- Memorandums to physicians, test reference guide, interpretive comments in patient reports, or other mechanism for providing recommendations to physicians for ordering and interpreting coagulation tests used for diagnostic purposes and anticoagulant therapy monitoring

REFERENCES

At least annually and after major centrifuge maintenance or service, the laboratory measures 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 $10 \times 10^9$/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, XI, 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 $200 \times 10^9$/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 for measuring platelet concentration of platelet-poor plasma AND
✓ Records of platelet concentration checks on all centrifuges used to prepare platelet-poor plasma

REFERENCES
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
Hematology and Coagulation Checklist

**REVISED** 09/22/2021

HEM.37300 Coagulation Quality Control Phase II

Controls are run using two different levels of control material each eight hours of patient testing and each time there is a change in reagents, or more frequently if specified in manufacturer’s instructions, laboratory procedure, or the CAP Checklist.

**NOTE:** This includes photo-optical, electromechanical and manual methods.

For manual methods (ie, tilt tube method), controls must be performed by each individual who performs the tilt tube test in the same eight hour period.

If an internal quality control process (eg, electronic/procedural/built-in) is used instead of an external control material to meet daily quality control requirements, the laboratory must have an individualized quality control plan (IQCP) approved by the laboratory director defining the control process, including the frequency and use of external and internal controls. At a minimum, external control materials must be analyzed with new lots and shipments of reagents or more frequently if indicated in the manufacturer’s instructions. Please refer to the IQCP section of the All Common Checklist for the eligibility of tests for IQCP and requirements for implementation and ongoing monitoring of an IQCP.

**Evidence of Compliance:**
- Records of QC results including external and internal control processes AND
- Written quality control procedures AND
- Manufacturer product insert or manual

**REFERENCES**
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. Fed Register. 2003(Jan 24):5232 [42CFR493.1269(b)].
7) 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); [42CFR493.1269(b) & 42CFR493.1269(c)(2)]
COAGULATION TESTS BASED ON DIRECT MEASUREMENT OF ANALYTES

CAP accredited chemistry laboratories have been applying the concepts and procedures for calibration, calibration verification, and analytic measurement range (AMR) verification to calibrated analytical methods for many years. Section directors and technologists with chemistry backgrounds will be helpful consultants to their coagulation laboratory colleagues as calibration verification and AMR verification requirements evolve.

The 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), including ELISA and fluorescence immunoassay, 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 chromogenic 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 fall into the categories of methodologies described above.

Clot-based methods, (including PT, aPTT, thrombin time, factor assays and fibrinogen, lupus anticoagulant, activated protein C resistance, qualitative and semi-quantitative assays) and all platelet function assays, including ristocetin cofactor activity are exempt.

CALIBRATION: The process of adjusting an instrument or test system to establish a relationship between the measurement response and the concentration or amount of the analyte that is being measured by the test procedure.

CALIBRATION VERIFICATION: The process of confirming that the current calibration settings for each analyte remain valid for a test system.

Each laboratory must define limits for accepting or rejecting results of the calibration verification process. Calibration verification can be accomplished in several ways. If the manufacturer provides a calibration validation or verification process, it must be followed. Other techniques include (1) assay of the current calibration materials as unknown specimens and (2) assay of matrix-appropriate materials with target values that are specific for the test system.

ANALYTICAL MEASUREMENT RANGE (AMR): 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.

LINEARITY AND THE AMR
Linearity is a fundamental characteristic of many analytic measurement methods, whereby there is a straight-line relationship between "true" analyte concentrations and measured concentrations. In this context, linearity refers to the relationship between the predicted and observed measurement results and not to the relationship between instrument signal output and analyte concentration. For most assays, this relationship is linear within the AMR.

AMR VERIFICATION
Laboratories are required to verify that the appropriate relationship is maintained over the AMR. Laboratories may verify and use an AMR that is narrower than the range defined by the manufacturer. This may be appropriate when materials available for method validation and/or AMR verification are not available to verify the full range claimed by the manufacturer, or reporting values across the full range defined by the manufacturer is not clinically relevant. For many assays, results beyond the AMR can be reported through dilution studies (see HEM.37385).
Minimum requirements for AMR verification can be met by using matrix appropriate materials, which include low, mid and high concentration or activity range of the AMR with recovery of results that fall within a defined range of the target value. Records of the AMR verification process must be available.

CLOSENESS OF SAMPLE CONCENTRATIONS OR ACTIVITIES TO THE UPPER AND LOWER LIMITS OF THE AMR
When verifying the AMR, it is required that samples tested are near the upper and lower limits of the AMR. Factors to consider in verifying 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 closeness of sample concentrations or activities to the upper and lower limits of the AMR are defined at the laboratory director's discretion. The method manufacturer's instructions for verifying the AMR must be followed, when available. The laboratory director must define limits for accepting or rejecting tests of the AMR.

Inspector Instructions:

- Sampling of calibration and AMR policies and procedures
- Sampling of calibration/calibration verification records
- Sampling of AMR verification records

- What is your course of action if results fall outside the AMR?
- When was the last time you performed a calibration procedure for directly measured coagulation analytes? How did you verify the calibration?

HEM.37360 Calibration Procedures  Phase II
Calibration procedures for each test system are appropriate, and the calibration records are reviewed for acceptability.

NOTE: Calibration must be performed following manufacturer's instructions, at minimum, including the number, type, and concentration of calibration materials, frequency of calibration, and criteria for acceptable performance. Calibration procedures are typically specified in the manufacturer's instructions but may also be established by the laboratory.

REFERENCES
2) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. Fed Register. 2003(Jan 24);3707 [42CFR493.1255]

HEM.37363 Calibration and Calibration Verification Materials  Phase II
High quality materials with test system and matrix-appropriate target values are used for calibration and calibration verification whenever possible.

NOTE: Calibration and calibration verification must have defined analyte target values and appropriate matrix characteristics for the clinical specimens and specific assay method. Many...
instrument systems require calibration materials with system-specific target values to produce accurate results for clinical specimens.

Suitable materials for calibration verification include, but are not limited to:

1. Calibrators used to calibrate the analytical system
2. Materials provided by the manufacturer 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 that are suitable for verification

In general, routine control materials and proficiency testing materials are not suitable for calibration verification, except in situations where the material has been shown to be suitable (eg, specifically designated by the method manufacturer) or no other materials are available.

Evidence of Compliance
✓ Written policy defining appropriate calibration and calibration verification materials

REFERENCES

HEM.37365 Recalibration/Calibration Verification Criteria Phase II

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

NOTE: Laboratories must either recalibrate or perform calibration verification at least every six months and if any of the following occur:

1. At changes of reagent lots unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client results
2. If QC shows an unusual trend or shift or is outside acceptable limits, and the system cannot be corrected to bring control values into the acceptable range
3. After major preventive maintenance or change of critical instrument component
4. When recommended by the manufacturer

Single use devices, and other test devices that do not allow user calibration, do not require calibration verification.

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

REFERENCES

HEM.37370 Recalibration Phase II

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

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

REFERENCES
Verification of the analytical measurement range (AMR) is performed at least every six months and following the defined criteria. Records are retained.

NOTE: The AMR must be verified at least every six months after a method is placed in service and if any of the following occur:

1. A change of reagent lots unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client results, and the range used to report patient/client test data.
2. If QC shows an unusual trend or shift or is outside acceptable limits, and the system cannot be corrected to bring control values into the acceptable range.
3. After major preventive maintenance or change of a critical instrument component.
4. When recommended by the manufacturer.

It is not necessary to independently verify the AMR if the calibration of an assay includes calibrators that span the full range of the AMR, with low, midpoint and high values (ie, three points) and the system is calibrated at least every six months. A one-point or two-point calibration does not include all of the necessary points to validate the AMR.
**AMR verification is not required for clot-based coagulation tests, platelet function tests, and other tests where output is a unit of time or arbitrary reporting unit (rather than measured analyte concentration).**

**Evidence of Compliance:**

✓ Written policy for AMR verification defining the frequency performed AND
✓ Records of AMR verification at least every six months

**REFERENCES**


2) Anne Ford. As coag tests evolve, so do checklist requirements. Northfield, IL: College of American Pathologists. CAP Today November 2012

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**HEM.37380 Diluted or Concentrated Samples**

**Phase II**

If a result is greater than or less 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

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**HEM.37385 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.

Evidence of Compliance:
✓ Patient reports or worksheets

**NEW** 09/22/2021
HEM.37390 Quantitative Cut-Off Values

For qualitative tests that use a quantitative cut-off value to distinguish positive from negative results, the analytic performance around the cut-off value is verified or established initially, and reverified at least every six months thereafter.

NOTE: This requirement applies to tests that report qualitative results based on a quantitative measurement using a threshold (cut-off value) to discriminate between positive and negative results for clinical interpretation. It does not apply to methods where the laboratory is not able to access the actual numerical value from the instrument.

Appropriate materials for establishment and verification of the cut-off are identical to those recommended for calibration verification. The requirement can be satisfied by the process of calibration or calibration verification using calibrators or calibration verification materials with values near the cut-off. It may also be satisfied by the use of QC materials that are near the cut-off value if those materials are claimed by the method manufacturer to be suitable for verification of the method's calibration process.

Verification of the cut-off should also be performed at changes of lots of analytically critical reagents (unless the laboratory director has determined that such changes do not affect the cut-off); after replacement of major instrument components; after major service to the instrument; and when QC materials reflect an unusual trend or shift or are outside of the laboratory’s acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem.

For FDA-cleared or approved tests, the clinical appropriateness of the cut-off value is evaluated as part of the clinical validation performed by the manufacturer. For laboratory-developed tests and modified FDA-cleared or approved tests, refer to COM.40640 for validation of clinical claims.

Evidence of Compliance:
✓ Written procedure for initial establishment and verification of the cut-off value AND
✓ Records of initial establishment and verification of the cut-off value at defined frequency

COAGULATION STUDIES

PT/INR AND APTT

Inspector Instructions:

- Sampling of reporting policies and procedures
- Sampling of patient PT/INR and aPTT reports
How have you established or validated your PT and aPTT reference intervals 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 used to calculate the INR?

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 for the INR calculation, 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 interval correlate with the data obtained in the lot number conversion.

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, critical result notification (as applicable), reference intervals on patient report, and correct INR calculation and associated parameters.

HEM.37400 Alternative Method Criteria

For photo-optical coagulation systems, guidelines are established for determining when alternative procedures are performed (eg, 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 (eg, 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 policy defining criteria for when PT/aPTT alternative procedures should be performed AND

✓ Records showing results from alternative procedures, as applicable

REFERENCES


HEM.37600 Clot Detection

For electromechanical coagulation systems, if the system has reusable probes to detect a clot, written guidelines for cleaning the probes are available.

HEM.37800 Duplicate Testing - Manual Testing

For manual coagulation testing (eg, PT, aPTT, fibrinogen) 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
For PT, there is a record 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 must be appropriate for the particular reagent-instrument combination and method of clot detection. Acceptable records 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

The calculation of the INR is adjusted using the appropriate ISI value 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 must be able to provide records 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 records must be available whether the INR is calculated 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 ISI values used in the INR calculation were appropriate for new lots and types of PT reagent and for any other changes

REFERENCES
INR Geometric Mean

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.

\[ \text{GM} = \text{antilog} \left[ \frac{\log(X_1) + \log(X_2) + \log(X_3) + \ldots + \log(X_n)}{n} \right] \]

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


HEM.37870 Reference Intervals

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 and then verify the reference interval with each change of lot or change in reagent.

Evidence of Compliance:
✓ Written procedure 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

REFERENCES

HEM.37880 Heparin Therapeutic Range

There is a record that the aPTT-based heparin therapeutic range is established and subsequently verified using an appropriate technique.

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 verified with each new lot of a given aPTT reagent.

The aPTT is commonly used to monitor the anticoagulant effects of unfractionated heparin. The therapeutic range for heparin therapy should be initially validated for new reagents or instruments 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 by measuring the aPTT and heparin activity and then deriving the aPTT therapeutic range by comparison to heparin activity. For subsequent reagent lot changes, the therapeutic range can be verified by comparing the aPTT of patient samples using the new and the prior aPTT lots. 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.

Laboratories in a local care network or system using the same instrument and same lot of an APTT reagent, can share their nomogram of Heparin. However, a verification study using one laboratory as the reference laboratory to show that their results are comparable to each other must be performed.

Anti-Xa activity is the preferred alternate method to monitor heparin therapy.

Evidence of Compliance:
✓ Written procedure for establishing and verifying the aPTT heparin therapeutic range

REFERENCES
D-DIMER STUDIES

Inspector Instructions:

- Sampling of D-dimer policies and procedures
- Sampling of D-dimer patient reports

HEM.37924 D-dimer Unit Results

The unit type (e.g., FEU or D-DU) and unit of magnitude (e.g., ng/mL) reported with the patient results are the same units as generated directly by the D-dimer method (following manufacturer’s product insert); or if different units are reported, the laboratory verifies the correct conversion of the units on an annual basis.

NOTE: The CAP and Clinical Laboratory and Standards Institute (CLSI) recommend that units not be converted from those stated in the package insert. If units are converted, the laboratory must verify the conversion of the units in patient reports for patient values, cut-off values, and reference intervals with changes in reagents, instrument and at least once per year in the absence of a change, with records retained.

The units generated directly by the D-dimer method can be determined from the package insert. If units are not stated in the package insert, consult with the manufacturer of the D-dimer method.

The following chart demonstrates the correct conversion factor for the different reporting units:

<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:

✓ Patient reports with unit type (FEU vs. DDU) and unit of magnitude (ng/mL vs. µg/mL) that are the same as the units directly generated by the D-dimer method and in the manufacturer’s product insert OR

✓ Records of the annual verification to confirm correct conversion of the unit type (FEU vs. DDU) and unit of magnitude (ng/mL vs. µg/mL) if units are reported that are different than those directly generated by the D-dimer method

REFERENCES


2) Olson JD, Cunningham MT, Higgins RA, et. al. D-dimer: simple test, tough problems. 2013; 137:1030-1038
HEM.37925  D-dimer - Evaluation of VTE  Phase II

If a quantitative D-dimer method is used in the evaluation of venous thromboembolism (VTE), the method is valid for this purpose.

NOTE: D-Dimer methods intended for evaluation of VTE may be used, along with pretest probability, if a method specific cut-off value is available. Cut-off values are not universal, so method specific data regarding the negative predictive value and the sensitivity should be available. For cut-off data acquired from the literature, the CLSI (H59-A) recommends a negative predictive value of ≥98% (lower limit of CI ≥95%) and a sensitivity of ≥97% (lower limit of CI ≥90%) for non-high pretest probability of VTE.

For D-dimer methods that are FDA-cleared/approved for exclusion of VTE, the package insert includes the cut-off value and this value should be provided in the report. It is not feasible for most laboratories to perform a sufficient clinical validation of a D-dimer cut-off for use in the evaluation of VTE (ie, either exclusion or aid in diagnosis), including separate validation of the cut-off for deep vein thrombosis and pulmonary embolism. Therefore using the cutoff supplied from the manufacturer is strongly recommended.

If a laboratory or group of laboratories determine a cut-off (not published in literature or the package insert), a summary of data including the NPV, sensitivity, and power of determination must be available. The CLSI Guideline H59-A recommends correlation with imaging studies and follow-up after three months on a minimum of 200 cases to establish the threshold for VTE exclusion.

Evidence of Compliance:
✓ Package insert stating an Intended Use for the exclusion of VTE or aid in the diagnosis of VTE AND
✓ A method specific cut-off for the evaluation of VTE from the package insert, literature, or an extensive clinical validation study

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  D-dimer Reporting  Phase II

If a D-dimer test is used for evaluation of venous thromboembolism (VTE), the laboratory reports the VTE exclusion cut-off value as stated by the manufacturer. If the D-dimer test is intended for other purposes (eg, DIC evaluation) a reference interval is required.

NOTE: This requirement only applies to quantitative D-dimer tests.

The cut-off value and upper limit of the reference interval are not always identical. The upper limit of the reference interval may be used to evaluate disseminated intravascular coagulation (DIC), while the cut-off value is used for evaluation of VTE (see COM.29950 regarding reference interval reporting). The cut-off value and/or reference interval must be reported in units identical to the patient results, including both unit type (FEU or D-DU) and unit of magnitude (eg, ng/mL).
Evidence of Compliance:
✓ Patient reports including both the reference interval and/or the cut-off value for VTE evaluation

REFERENCES

HEM.37935 Sensitivity of D-dimer Test - Evaluation of VTE Phase I

If a D-dimer test is insufficiently sensitive to exclude venous thromboembolism, the laboratory informs clinicians that the test must not be used for this purpose.

NOTE: Manual agglutination D-dimer and FDP (fibrin degradation products) assays are not adequately sensitive for evaluation of deep vein thrombosis and/or pulmonary embolism.

MIXING STUDIES

Inspector Instructions:

- Sampling of mixing studies policies and procedures
- Sampling of mixing study testing records

Plasma-mixing studies (ie, 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.37937 Mixing Studies Procedure Phase II

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. Pooled plasma prepared in the laboratory or commercial products comprised of at least 20 apparently healthy donors are acceptable.

Evidence of Compliance:
✓ Written procedure for local preparation of pooled plasma for plasma-mixing studies using at least 20 healthy donors OR written procedure describing the use of a commercial product comprising at least 20 healthy donors

REFERENCES

HEM.37938 Mixing Studies Procedure Phase II

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 (eg, 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 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 FACTOR ASSAYS (EXCLUDING FIBRINOGEN BY IMMUNOLOGIC METHODS)

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.

Fibrinogen can be measured using different methodologies. The Clauss method is a functional assay based on the time to fibrin clot formation when excess thrombin is added to patient plasma. The PT-derived fibrinogen assay reports a fibrinogen based on the prothrombin time. Immunologic methods, which measure fibrinogen antigens, are covered in the Coagulation Tests Based on Direct Measurement of Analytes section.

Inspector Instructions:

- Sampling of factor assay policies and procedures
- Sampling of patient reports
- Sampling of records for standard curves and standard curve verification
- Sampling of calibration/calibration verification/recalibration records

How do you evaluate results for inhibitor effects?
NOTE: Plotting less than three points may generate an erroneous line.

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

REFERENCES

**REVISED** 06/04/2020
HEM.37960 Standard Curve Verification Phase II

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

NOTE: The Y intercept of the standard curve varies according to the reagent and environmental or instrument conditions. Verifying the curve (eg, two or more points with assayed reference plasma) each time ensures accuracy of the result. If more than two standard curves exist (ie, normal concentration and low concentration curves), the CAP recommends using at least one reference point on each curve.

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

REFERENCES

**REVISED** 09/22/2021
HEM.37980 Factor Assay Criteria Phase II

Three or more dilutions are plotted for each functional factor activity assay to detect non-parallelism and report non-parallelism if detected.

NOTE: This requirement does not apply to chromogenic factor assays or fibrinogen assays.

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.
Use of at least three 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.

The goal is to provide clinically useful data when a non-specific inhibitor activity is detected, (eg, a lupus anticoagulant or an anticoagulant drug like heparin). A comment like “inhibitor pattern detected” along with reporting the activity obtained at the highest dilution or over serial dilutions clarifies the result.

Functional fibrinogen assays may be subject to interference by certain anticoagulants; for guidelines on fibrinogen assays refer to COM.40500 and HEM.37165.

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

REFERENCES

HEM.37984 Inhibitor Interference

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

NOTE: This requirement is not applicable to fibrinogen assays.

REFERENCES

PLATELET FUNCTION STUDIES

Inspector Instructions:

• Sampling of platelet function study policies and procedures
• Sampling of patient reports

HEM.38300 Platelet Function Studies

Phase II
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 several 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. Manufacturer's instructions for specimen stability must be followed for FDA-cleared/approved platelet function study assays.
Evidence of Compliance:
✓ Written policy defining specimen stability for platelet function studies AND
✓ Records of testing completed within the defined time period

REFERENCES

HEM.38350 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 policy defining the specimen handling requirements prior to analysis

REFERENCES

HEM.38400 Platelet Aggregation Studies Phase II

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

HEM.38450 Platelet Aggregation Studies Phase II

Platelet aggregation studies are completed between 30 minutes and four 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 CO\textsubscript{2} 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. Specimens collected for whole blood aggregometry should be stored capped at room temperature and tested within four hours.

REFERENCES

HEM.38500 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 10⁹/L. Samples with platelet concentrations greater than optimal can be diluted into the optimal range with platelet-poor plasma (PPP) (<10 X 10⁹/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

ELECTROPHORESIS - COAGULATION

Inspector Instructions:

- Sampling of electrophoretic coagulation study policies and procedures
- Sampling of QC records
- Sampling of patient reports
- Electrophoretic patterns (appropriate separations)

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.38550  Daily QC - Electrophoresis  Phase II

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

Evidence of Compliance:
✓ Records of electrophoresis QC

HEM.38600  Electrophoretic Separation  Phase II

Electrophoretic separations are satisfactory.

NOTE: The laboratory should be able to provide instrument printouts, sample electrophoresis results and patient reports.