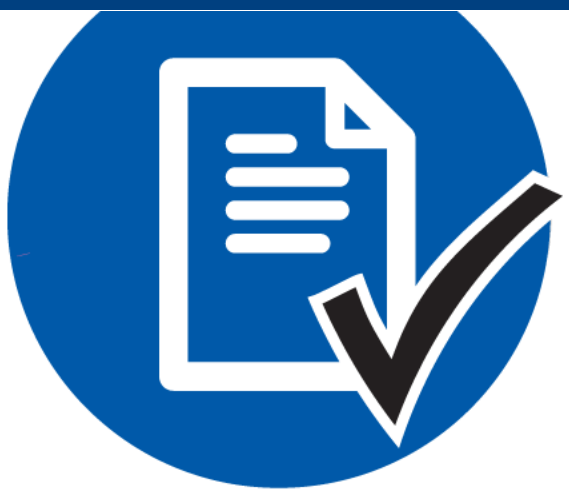
COLLEGE of AMERICAN PATHOLOGISTS

Master

Hematology and Coagulation Checklist

CAP Accreditation Program



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

08.21.2017

Disclaimer and Copyright Notice

On-site inspections are performed with the edition of the Checklists mailed to a facility at the completion of the application or reapplication process, not necessarily those currently posted on the website. The checklists undergo regular revision and a new edition may be published after the inspection materials are sent.

For questions about the use of the Checklists or Checklist interpretation, email accred@cap.org or call 800-323-4040 or 847-832-7000 (international customers, use country code 001).

Hematology and Coagulation Checklist

TABLE OF CONTENTS

ON-LINE CHECKLIST AVAILABILITY

Participants of the CAP accreditation programs may download the checklists from the CAP website (www.cap.org) by logging into e-*LAB* Solutions. 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.

SUMMARY OF CHECKLIST EDITION CHANGES
Hematology and Coagulation Checklist
08/21/2017 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 listing of requirements below is 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

Requirement	Effective Date
HEM.20090	08/17/2016
HEM.34320	08/21/2017
HEM.35905	08/21/2017

REVISED Checklist Requirements

Requirement	Effective Date
HEM.19360	08/17/2016
HEM.21575	08/17/2016
HEM.22748	08/21/2017
HEM.22789	08/21/2017
HEM.22830	08/21/2017
HEM.23050	08/21/2017
HEM.25570	08/21/2017
HEM.25760	08/21/2017
HEM.33330	08/17/2016

HEM.34655	08/21/2017
HEM.35150	08/21/2017
HEM.35340	08/21/2017
HEM.35347	08/21/2017
HEM.35357	08/21/2017
HEM.35642	08/17/2016
HEM.35895	08/21/2017
HEM.35916	08/17/2016
HEM.35924	08/21/2017
HEM.36350	08/21/2017
HEM.37924	08/17/2016

DELETED/MOVED/MERGED Checklist Requirements

Requirement	Effective Date
HEM.25300	08/20/2017
HEM.40000	08/20/2017

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.

Note for non-US laboratories: Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist.

QUALITY MANAGEMENT AND QUALITY CONTROL

Inspector Instructions:

	• Sampling of QC policies and procedures
	• What do you do if controls are out of range? • What is your course of action when monthly precision data changes significantly from the previous month's data?
	• Select several occurrences in which QC is out of range and follow records to determine if the steps taken follow the laboratory procedure for corrective action

WAIVED TESTS

HEM.18038 QC - Waived Tests **Phase II**

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. The checklist section "RESULTS REPORTING-COAGULATION" also is not applicable to waived tests.

NONWAIVED TESTS

The following group of requirements is applicable to nonwaived kit and slide tests, and automated and semi-automated testing, unless a separate checklist requirement exists in another checklist section that defines a specific QC frequency, e.g. coagulation testing, body fluid cell counts.

Inspector Instructions:

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

- Use QC data to identify tests that utilize internal quality control processes to confirm that any individualized quality control plan (IQCP) is used as approved by the laboratory director

***REVISED** 08/17/2016*

HEM.19360 Daily QC - Nonwaived Tests **Phase II**

Controls are run at least daily, 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 (e.g. 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 to address the use of the alternative control system. Please refer to the Individualized Quality Control Plan 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 **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. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):3708 [42CFR493.1256(d)(3)(ii)]
2) Clinical and Laboratory Standards Institute (CLSI). *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition*. CLSI document EP12-A2 (ISBN 1-56238-654-9). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2008.
3) Koepke JA. Update on reticulocyte counting. *Lab Med.* 1999;30:339-343

HEM.19380 Target Range Verification **Phase II**

For quantitative tests, a statistically valid target range (*e.g.* mean, SD, CV) is verified or established for each lot of control material by repetitive analysis in runs that include previously tested control materials.

Evidence of Compliance:

- ✓ Written procedure to establish target range **AND**
- ✓ Records of target range determination or verification, as applicable

HEM.20050 Numeric QC Data **Phase I**

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

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

Evidence of Compliance:

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

REFERENCES

1) Mukherjee KL. Introductory mathematics for the clinical laboratory. Chicago, IL: American Society of Clinical Pathology, 1979:81-94
2) Barnett RN. Clinical laboratory statistics, 2nd ed. Boston, MA: Little, Brown, 1979
3) Weisbrodt IM. Statistics for the clinical laboratory. Philadelphia. PA: JB Lippincott, 1985
4) Matthews DF, Farewell VT. Understanding and using medical statistics. New York, NY: Karger, 1988
5) Cembrowski GS, *et al*. An optimized quality control procedure for hematology analyzers with the use of retained patient specimens. *Am J Clin Pathol.* 1988;89:203-210
6) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7146 [42CFR493. 1256(d)(10)(i)]
7) Ross JW, Lawson NS. Analytic goals, concentrations relationships, and the state of the art for clinical laboratory precision. *Arch Pathol Lab Med.* 1995;119:495-513
8) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline.* 4th ed. CLSI document C24-ED4. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.

HEM.20070 Precision Statistics **Phase I**

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 indicated. Finally, if commercially sponsored interlaboratory QC data for the same control lot and instrument model show SD/CV values markedly smaller or larger than the peer group, an explanation is required.

Evidence of Compliance:

- ✓ Written procedure for investigation and corrective action should a significant change in precision statistics occur **AND**
- ✓ Records of investigation and corrective actions taken

*****NEW** 08/17/2016***

HEM.20090 Alternative Control Procedures **Phase II**

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

HEM.20120 QC Handling — Phase II

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

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

Evidence of Compliance:

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

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

HEM.20140 QC Confirmation of Acceptability — Phase II

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

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

Evidence of Compliance:

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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7166 [42CFR493.1256(f)], and 2003(Oct 1):1046[42CFR493.1282(b)(2)]

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 (e.g. trending for repeat failures, etc.).

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

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 (e.g. trending for repeat failures, etc.).

Evidence of Compliance:

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

SPECIMEN COLLECTION AND HANDLING - HEMATOLOGY

Inspector Instructions:

	• Sampling of hematology specimen collection and handling policies and procedures
	• Sampling of 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?

*****REVISED** 08/17/2016***

HEM.21575 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

1) Clinical and Laboratory Standards Institute. *Accuracy in Patient and Sample Identification; Approved Guideline.* CLSI Document GP33-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.

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 (*e.g.* rotary mixer, rocker, automated sampler, or manual inversions) for reproducibility of results, as applicable

REFERENCES
1) Clinical and Laboratory Standards Institute. *Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard;* 6th ed. CLSI document GP42-A6. CLSI, Wayne, PA, 2008.
2) Clinical and Laboratory Standards Institute. *Collection of Diagnostic Venous Blood Specimens;* 7th ed. CLSI standard GP41-ED7. Clinical and Laboratory Standards Institute, Wayne, PA, 2017.

HEM.22050 CBC Anticoagulant — Phase II

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

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

REFERENCES
1) Cohle SD, *et al*. Effects of storage of blood on stability of hematologic parameters. *Am J Clin Pathol.* 1981;76:67-79
2) Savage RA. Pseudoleukocytosis due to EDTA-induced platelet clumping. *Am J Clin Pathol.* 1984;82:132-133
3) Rabinovitch A. Anticoagulants, platelets and instrument problems. *Am J Clin Pathol.* 1984;82:132
4) Clinical and Laboratory Standards Institute. *Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard;* 6th ed. CLSI document GP42-A6. CLSI, Wayne, PA, 2008.
5) Clinical and Laboratory Standards Institute. *Collection of Diagnostic Venous Blood Specimens;* 7th ed. CLSI standard GP41-ED7. Clinical and Laboratory Standards Institute, Wayne, PA, 2017.
6) Broden PN. Anticoagulant and tube effect on selected blood cell parameters using Sysmex NE-series instruments. *Sysmex J Intl.* 1992;2:112-119
7) Brunson D, *et al*. Comparing hematology anticoagulants: K2EDTA vs K3EDTA. *Lab Hematol.* 1995;1:112-119
8) Boos MS, *et al*. Temperature- and storage-dependent changes in hematologic variable and peripheral blood morphology. *Am J Clin Pathol.* 1998;110:537
9) Wood BL, *et al*. Refrigerated storage improves the stability of the complete blood cell count and automated differential. *Am J Clin Pathol.* 1999;112:687-695

HEM.22100 Capillary Tube Collection Criteria — Phase II

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 capillary blood CBC parameter determinations need not be collected in duplicate. Because of the risk of injury, glass capillary tubes are not used, or are used with measures to reduce risk or injury.

Evidence of Compliance:

✓ Written procedure for collection in capillary tubes

REFERENCES
1) Clinical and Laboratory Standards Institute. *Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard.* 6th ed. CLSI Document GP42-A6. Clinical and Laboratory Standards Institute, Wayne, PA; 2008.
2) Occupational Safety and Health Administration. Toxic and hazardous substances. Bloodborne pathogens. Washington, DC: US Government Printing Office, 1999(Jul 1): [29CFR1910.1030].

HEM.22150 Specimen Quality Assessment - CBC — Phase II

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

NOTE: This may be done visually or with applicator sticks before testing. Additionally, microclots will often present themselves histographically on automated and semi-automated particle counters or by flagging, and the 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
1) Clinical and Laboratory Standards Institute. *Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard.* 2nd ed. CLSI Document H26-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.

HEM.22200 Hemolyzed or Lipemic Specimens - CBC — Phase II

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

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

Evidence of Compliance:

✓ Written procedure defining method for checking specimens for in vitro hemolysis and lipemia

REFERENCES
1) Cantero M, *et al*. Interference from lipemia in cell count by hematology. *Clin Chem.* 1996;42:987-988
2) Clinical and Laboratory Standards Institute. *Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard.* 2nd ed. CLSI Document H26-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.

HEM.22625 Storage and Stability - Hematology — Phase I

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

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

Evidence of Compliance:

✓ Written policy defining specimen stability and storage requirements

REFERENCES
1) Boos MS, *et al*. Temperature- and storage-dependent changes in hematologic variable and peripheral blood morphology. *Am J Clin Pathol.* 1998;110:537
2) Gulati GL, *et al.* Changes in automated complete blood cell count and differential leukocyte count results induced by storage of blood at room temperature. *Arch Pathol Lab Med.* 2002;126:336-342
3) Clinical and Laboratory Standards Institute. *Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard.* 2nd ed. CLSI Document H26-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.

SPECIMEN COLLECTION AND HANDLING - COAGULATION

Inspector Instructions:

	• Sampling of coagulation specimen collection and handling policies and procedures • Sampling of specimen rejection records/log
	• Sampling of patient coagulation specimens (anticoagulant, labeling)
	• How do you know if the specimen is clotted? • What further actions are necessary if the specimen has a hematocrit of 60%? • What is your course of action when you receive unacceptable coagulation specimens?

HEM.22707 Specimen Collection - Intravenous Lines **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 discarded before the coagulation tube is filled. For those samples collected from a normal saline lock (capped off venous port) twice the dead space volume of the catheter and extension set should be discarded.

REFERENCES
1) Lew JKL, *et al*. Intra-arterial blood sampling for clotting studies. Effects of heparin contamination. *Anesthesia.* 1991;46:719-721
2) Konopad E, *et al*. Comparison of PT and aPTT values drawn by venipuncture and arterial line using three discard volumes. *Am J Crit Care.* 1992;3:94-101
3) Laxson CJ, Titler MG. Drawing coagulation studies from arterial lines; an integrative literature review. *Am J Critical Care*. 1994; 1:16-24
4) Adcock DM, *et al.* Are discard tubes necessary in coagulation studies? *Lab Med.* 1997;28:530-533
5) Brigden ML, *et al.* Prothrombin time determination. The lack of need for a discard tube and 24-hour stability. *Lab Med*. 1997;108:422-426
6) Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition*. CLSI Document H21-A5 (ISBN 1-56238-657-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
7) Clinical and Laboratory Standards Institute. *Collection of Diagnostic Venous Blood Specimens;* 7th ed. CLSI standard GP41-ED7. Clinical and Laboratory Standards Institute, Wayne, PA, 2017.

*****REVISED** 08/21/2017***

HEM.22748 Anticoagulant - Coagulation **Phase I**

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 $Na_3C_6H_5O_7 \cdot 2H_2O$) is the recommended anticoagulant for coagulation testing. Reference intervals for clot-based assays should be determined using the same concentration of sodium citrate that the laboratory uses for patient testing. The higher citrate concentration in 3.8% sodium citrate, may result in falsely lengthened clotting times (more so than 3.2% sodium citrate) for calcium-dependent coagulation tests (i.e. PT and aPTT) performed on slightly underfilled samples and samples with high hematocrits. 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 nm/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

1) Adcock DM, *et al*. Effect of 3.2% *vs* 3.8% sodium citrate concentration on routine coagulation testing. *Am J Clin Pathol.* 1997;107:105-110
2) Reneke, J *et al.* Prolonged prothrombin time and activated partial thromboplastin time due to underfilled specimen tubes with 109 mmol/L (3.2%) citrate anticoagulant. *Am J Clin Pathol.* 1998;109:754-757
3) Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition*. CLSI Document H21-A5 (ISBN 1-56238-657-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.

*****REVISED** 08/21/2017***

HEM.22789 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
2) Adcock DM, Kressin D, Mariar PA. Minimum specimen volume requirements for routine coagulation testing. Dependence on citrate concentration. *Am J Clin Pathol.* 1998;109:595-599
3) Reneke J, *et al*. Prolonged prothrombin time and activated partial thromboplastin time due to underfilled specimen tubes with 109 mmol/L (3.2%) citrate anticoagulant. *Am J Clin Pathol.* 1998;109:754-757
4) Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition*. CLSI Document H21-A5 (ISBN 1-56238-657-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.

*****REVISED** 08/21/2017***

HEM.22830 Elevated Hematocrits - Coagulation **Phase I**

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 polycythemic 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

1) Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition*. CLSI Document H21-A5 (ISBN 1-56238-657-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
2) Siegel JE, *et al*. Effect (or lack of it) of severe anemia on PT and APTT results. *Am J Clin Pathol.* 1998; 110:106-110
3) Siegel JE, *et al*. Monitoring heparin therapy. APTT results from partial- *vs* full-draw tubes. *Am J Clin Pathol.* 1998;110:184-187
4) Mariar RA, *et al*. Effect on routine and special coagulation testing values of citrate anticoagulant adjustment in patients with high hematocrit values. *Am J Clin Pathol.* 2006: 126:400-405
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.

HEM.22871 Specimen Quality Assessment - Coagulation **Phase II**

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

Evidence of Compliance:

- ✓ Written policy to assess quality of coagulation specimens **AND**
- ✓ Records of rejection for clotted specimens

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition*. CLSI Document H21-A5 (ISBN 1-56238-657-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
2) Arkin CF. Collection, handling, storage of coagulation specimens. Advance/Lab. 2002;11(1);33-38

HEM.22912 Specimen Handling - Coagulation — Phase II

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

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

1. *PT specimens, uncentrifuged or centrifuged with plasma remaining in the capped tube above the packed cells, 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 (e.g. thrombin time, protein C, factor V, factor VIII) have variable stability and should be kept in the same manner as aPTT samples*

If 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

1) Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition*. CLSI Document H21-A5 (ISBN 1-56238-657-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
2) Adcock DM, *et al*. The effect of time and temperature variables on routine coagulation tests. *Blood Coag Fibrinolysis.* 1998;9:463-470
3) Neofotistos D, *et al*. Stability of plasma for add-on PT and aPTT tests. *Am J Clin Pathol.* 1998;109:758-763
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

HEM.22953 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 approximately 3-4 hours. The laboratory must ensure that platelet aggregation studies are completed within 3-4 hours from the time of phlebotomy, or erroneous results could be obtained.

Evidence of Compliance:

- ✓ Written policy defining specimen stability for platelet function studies **AND**
- ✓ Records reflecting completion of testing within defined time period

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Platelet Function Testing by Aggregometry; Approved Guideline*. CLSI document H58-A. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.

RESULTS REPORTING - GENERAL

Inspector Instructions:

	• Sampling of reporting policies and procedures • Sampling of patient reports (reference intervals)
	• How have you established or verified reference intervals?

***REVISED** 08/21/2017*

HEM.23050 Reference Intervals **Phase II**

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

NOTE: The results of commercial quality control plasmas that may be used in coagulation assays are internal data for quality assurance purposes, and must NOT be externally reported; if reported with patient results, they may be confused as normal values.

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 should 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.

REFERENCES
1) Brigden ML, Johnston M. A survey of aPTT reporting in Canadian medical laboratories. The need for increased standardization. *Am J Clin Pathol.* 2000;114:276-282
2) Trost DC, *et al*. Probability-based construction of reference ranges for ratios of log-Gaussian analytes: an example from automated leukocyte counts. *Am J Clin Pathol*. 2002;117:851-856
3) Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory - Approved Guideline- Third Edition - CLSI Document EP28-A3C. (ISBN 1-56238-682-4) Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, PA, 19087-1898, USA, 2010.
4) Etzell, JE. For WBC differentials reporting absolute numbers. *CAP Today.* 2010; 3:12
5) Richardson-Jones A, Twedt D, Hellman R. Absolute versus proportional differential leukocyte counts. *Clin Lab. Haem.* 1995:17(2), 115-123

RESULTS REPORTING - COAGULATION

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

Inspector Instructions:

	• Sampling of reporting policies and procedures • Sampling of patient PT/aPTT reports
	• How have you established or validated your PT and aPTT reference 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?
	• Examine the current PT reagent lot package insert for the ISI value, lot number and instrument model, and verify that the reagent lot number and ISI value is programmed for the correct instrument model • Review the data used to establish the geometric mean, and compare to that entered into the instrument • Verify calculations for the current reagent lot in use and examine the lot number validation records for the correct INR calculations • Check patient reports to ensure INR and reference interval correlate with the data obtained in the lot number conversion

HEM.23220 ISI **Phase II**

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

1) Clinical and Laboratory Standards Institute (CLSI). *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (aPTT) Test; Approved Guideline-Second Edition*. CLSI Document H47-A2. (ISBN 1-56238-672-7). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2008.
2) Fairweather RB, *et al*. College of American Pathologists Conference XXXI on laboratory monitoring of oral anticoagulant therapy. *Arch Pathol Lab Med.* 1998;122:768-781
3) Clinical and Laboratory Standards Institute (CLSI). *Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline*. CLSI document H54-A. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2005.
4) 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): [42CFR493.1252(a)]

HEM.23290 INR Calculation Adjustment for ISI **Phase II**

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

1) Clinical and Laboratory Standards Institute (CLSI). *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (aPTT) Test; Approved Guideline-Second Edition*. CLSI Document H47-A2. (ISBN 1-56238-672-7). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2008.
2) Fairweather RB, *et al*. College of American Pathologists Conference XXXI on laboratory monitoring of oral anticoagulant therapy. *Arch Pathol Lab Med.* 1998;122:768-781
3) Clinical and Laboratory Standards Institute (CLSI). *Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline*. CLSI document H54-A. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2005.

HEM.23360 INR Geometric Mean — Phase II

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

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

$$INR=(PT\ of\ patient\ /\ PT\ of\ geometric\ mean\ normal\ population)^{ISI}$$

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.

$$GM = antilog\ [(log(X1) + log(X2) + log(X3) + \ldots log(Xn))/n].$$

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

1) Clinical and Laboratory Standards Institute (CLSI). *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (aPTT) Test; Approved Guideline-Second Edition*. CLSI Document H47-A2. (ISBN 1-56238-672-7). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2008.
2) Fairweather RB, *et al*. College of American Pathologists Conference XXXI on laboratory monitoring of oral anticoagulant therapy. *Arch Pathol Lab Med.* 1998;122:768-781
3) Ansell J, *et al*. Managing oral anticoagulant therapy. *Chest* 2001;119:22s-38s
4) Critchfield GC, Bennett ST. The influence of the reference mean prothrombin time on the international normalized ratio. *Am J Clin Pathol*. 1994 Dec;102(6):806-11

5) Clinical and Laboratory Standards Institute (CLSI). *Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline*. CLSI document H54-A. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2005.

HEM.23430 Report Verification Criteria — Phase II

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

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

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

Evidence of Compliance:

✓ Records of patient report checks at defined frequency

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (aPTT) Test; Approved Guideline-Second Edition*. CLSI Document H47-A2. (ISBN 1-56238-672-7). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2008.
2) Fairweather RB, *et al*. College of American Pathologists Conference XXXI on laboratory monitoring of oral anticoagulant therapy. *Arch Pathol Lab Med.* 1998;122:768-781
3) Ansell J, *et al*. Managing oral anticoagulant therapy. *Chest* 2001;119:22s-38s
4) Clinical and Laboratory Standards Institute (CLSI). *Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline*. CLSI document H54-A. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2005.

HEM.23453 Heparin Therapeutic Range — Phase I

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.

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

1) Rosborough TK. Comparison of anti-factor Xa heparin activity and activated partial thromboplastin time in 2,773 plasma samples from unfractionated heparin-treated patients. *Am J Clin Pathol.* 1997;108:662-668

2) Olson JD, *et al*. College of American Pathologists conference XXXI on laboratory monitoring of anticoagulant therapy. Laboratory monitoring of unfractionated heparin therapy. *Arch Pathol Lab Med.* 1998;122:782-798
3) Smythe MA, *et al*. Use of the activated partial thromboplastin time for heparin monitoring. *Am J Clin Pathol.* 2001;115:148-155
4) Smythe MA, *et al*. Different heparin lots. Does it matter? *Arch Pathol Lab Med.* 2001;125:1458-1462
5) Hirsh J, *et al*. Guide to anticoagulant therapy. Heparin: a statement for healthcare officials from the American Heart Association. *Circulation.* 2001 19:2994-3018
6) Hirsh J, Raschke R. Heparin and low-molecular-weight heparin: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest.* 2004 Sep;126(3 Suppl):188S-203S
7) Clinical and Laboratory Standards Institute (CLSI). *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (aPTT) Test; Approved Guideline-Second Edition*. CLSI Document H47-A2. (ISBN 1-56238-672-7). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2008.

HEM.23500 Reference Intervals — Phase II

Reference intervals for PT and aPTT are current for the reagent or lot number, and are appropriately determined.

NOTE: Because of the variability between different types of PT and aPTT reagents, and even different lots of PT and aPTT reagents, there may be significant changes in the reference interval after a change of the type or lot of reagent. For this reason, the laboratory should establish 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

1) Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory - Approved Guideline- Third Edition - CLSI Document EP28-A3C. (ISBN 1-56238-682-4) Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, PA, 19087-1898, USA, 2010.

HEM.23575 Coagulation Test Recommendations — Phase I

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

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

Evidence of Compliance:

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

REFERENCES

1) Rosborough TK. Comparison of anti-factor Xa heparin activity and activated partial thromboplastin time in 2,773 plasma samples from unfractionated heparin-treated patients. *Am J Clin Pathol.* 1997;108:662-668
2) Haraldsson HM, *et al.* Performance of prothrombin-proconvertin time as a monitoring test of oral anticoagulation therapy. *Am J Clin Pathol.* 1997;108:662-668

3) Baker BE, *et al*. Inability of the activated partial thromboplastin time to predict heparin levels: time to reassess guidelines for heparin assays. *Arch Intern Med.* 1997;157:2475-2479
4) Brigden, ML *et al*. INR reporting in Canadian medical laboratories. An update. *Am J Clin Pathol.* 1998;109:589-594
5) Leech BF, Carter CJ. Falsely elevated INR results due to the sensitivity of a thromboplastin reagent to heparin. *Am J Clin Pathol.* 1998;109:764-768
6) Fairweather RB, *et al*. College of American Pathologists conference XXXI on laboratory monitoring of oral anticoagulant therapy. *Arch Pathol Lab Med.* 1998;122:768-781
7) Olson JD, *et al*. College of American Pathologists conference XXXI on laboratory monitoring of oral anticoagulant therapy. Laboratory monitoring of unfractionated heparin therapy. *Arch Pathol Lab Med.* 1998;122:782-798
8) Lousberg TR, *et al*. Evaluation of excessive anticoagulation in a group model health maintenance organization. *Arch Intern Med.* 1998;158:528-534
9) Davis KD, *et al*. Use of different thromboplastin reagents causes greater variability in international normalized ratio results than prolonged room temperature storage of specimens. *Arch Pathol Lab Med.* 1998;122:972-977
10) Koerner SD, Fuller RE. Comparison of a portable capillary whole blood coagulation monitor and standard laboratory methods for determining international normalized ratio. *Mil Med.* 1998;163:820-825
11) Laposata M, *et al*. College of American Pathologists conference XXXI on laboratory monitoring of low-molecular-weight heparin, danaparoid, hirudin and related compounds, and argatroban. *Arch Pathol Lab Med.* 1998;122:799-807
12) Smythe MA, *et al*. Use of the activated partial thromboplastin time for heparin monitoring. *Am J Clin Pathol.* 2001;115:148-155
13) Smythe MA, *et al*. Different heparin lots. Does it matter? *Arch Pathol Lab Med.* 2001;125:1458-1462
14) Ansell J, *et al*. Managing oral anticoagulant therapy. *Chest*. 2001;119:22s-38s
15) Hirsh J, *et al*. Heparin and low-molecular-weight heparin: Mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy and safety. *Chest.* 2001; 119:64s-94s
16) Hirsh J, *et al*. Guide to anticoagulant therapy. Heparin: a statement for healthcare officials from the American Heart Association. *Circulation.* 2001 19:2994-3018

INSTRUMENTS AND EQUIPMENT

The checklist requirements in this section should be used in conjunction with the requirements in the All Common Checklist relating to instruments and equipment.

Inspector Instructions:

READ	• Sampling of pipette/dilutor checks
ASK	• How are you assured your automatic pipetting systems exhibit no carryover effects?

HEM.25150 Pipettors and Dilutors **Phase II**

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

NOTE: Pipette checks must be performed following manufacturer's instructions, at minimum, and as defined in laboratory procedure.

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

Evidence of Compliance:

✓ Written procedure for checking the accuracy and reproducibility of pipettes

REFERENCES

1) Curtis RH. Performance verification of manual action pipets. Part I. *Am Clin Lab. 1994*;12(7):8-9
2) Curtis RH. Performance verification of manual action pipets. Part II. *Am Clin Lab. 1994*;12(9):16-17

3) Perrier S, *et al*. Micro-pipette calibration using a ratiometric photometer-reagent system as compared to the gravimetric method. *Clin Chem.* 1995;41:S183
4) Johnson B. Calibration to dye for: Artel's new pipette calibration system. *Scientist.* 1999;13(12):14
5) Connors M, Curtis R. Pipetting error: a real problem with a simple solution. Parts I and II. *Am Lab News.* 1999;31(13):20-22
6) Skeen GA, Ashwood ER. Using spectrophotometry to evaluate volumetric devices. *Lab Med.* 2000;31:478-479

HEM.25175 Pipette Carryover **Phase II**

The laboratory has evaluated its automatic pipetting systems for carryover.

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

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

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

This requirement is not applicable to coagulation.

Evidence of Compliance:

✓ Records of reassessment of samples with potential carryover

REFERENCES

1) Clinical and Laboratory Standards Institute. *Laboratory Instrument Implementation, Verification, and Maintenance; Approved Guideline.* CLSI Document GP31-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2009.

HEM.25250 Glassware Accuracy **Phase II**

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

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

Evidence of Compliance:

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

REFERENCES

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

COMPLETE BLOOD COUNT (CBC) INSTRUMENTS

CALIBRATION

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

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

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

Inspector Instructions:

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

HEM.25400 Precalibrated Instrument Verification **Phase II**

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

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

Evidence of Compliance:

✓ Records of calibration verification following manufacturer's instructions

REFERENCES
1) van Assendelft OW, Buursma A. Reference method for the measurement of hemoglobin. *Lab Hematol.* 1995;1:154-155
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1255]

FRESH WHOLE BLOOD

HEM.25500 Calibration - Whole Blood **Phase II**

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

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

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition. CLSI document H26-A2 (ISBN 1-56238-728-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2010.

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

***REVISED** 08/21/2017*

HEM.25570 Calibration Verification Criteria **Phase II**

Criteria are established for calibration verification.

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

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

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

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7165 [42CFR493.1255]

HEM.25600 Recalibration **Phase II**

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

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

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

Evidence of Compliance:

- ✓ Written procedure defining the criteria for recalibration

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition. CLSI document H26-A2 (ISBN 1-56238-728-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2010.
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1255]

HEM.25625 Verification Following Whole Blood Calibration **Phase I**

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

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

Evidence of Compliance:

✓ Records of acceptable calibration verification

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition. CLSI document H26-A2 (ISBN 1-56238-728-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2010.

COMMERCIAL CALIBRATORS

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

HEM.25700 Calibration - Stabilized Materials **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

1) Gilmer PR, Williams LJ. The status of methods of calibration in hematology. *Am J Clin Pathol.* 1980;74:600-605
2) Lewis SM, *et al*. Current concepts in haematology 3: blood count calibration. *J Clin Pathol.* 1991;144:881-884
3) Clinical and Laboratory Standards Institute (CLSI). Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition. CLSI document H26-A2 (ISBN 1-56238-728-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2010.
4) 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): [42CFR493.1255]

*****REVISED** 08/21/2017***

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

Criteria are established for calibration verification.

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

1. *At complete changes of reagents (i.e. change in type of reagent from same vendor, 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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7165 [42CFR493.1255]

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

HEM.25785 Verification Following Commercial Calibrator Calibration **Phase I**

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

Evidence of Compliance:
✓ Records of acceptable calibration verification

CBC INSTRUMENT QUALITY CONTROL

Longitudinal process quality control (QC) procedures for individual instruments 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
	• How do you determine when QC is unacceptable and when corrective actions are needed? • How does your laboratory establish or verify acceptable QC ranges?

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 (i.e., "normal" and "high"). Three levels of control is a conceptual carryover from clinical chemistry, and does not apply to hematology particle counting. Dilute, "low-level" (e.g. leukopenic and thrombocytopenic) "oncology" controls are less informative indicators of calibration status, and are neither required nor recommended. For example, a 10% calibration bias will be numerically most apparent in a high-level control, less apparent in a normal-level control, and perhaps inapparent in a low-level control; it would be quite extraordinary for a low-level control to indicate a calibration problem that is not revealed by the other controls. There should be some relationship between the frequency of control runs and the numbers of patient specimens processed. If the frequency of commercial control use is less than 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
1) Lott JA, *et al*. Synthetic materials for platelet quality control. *Am J Med Technol.* 1983;49:43-48
2) Yacko M, *et al*. Multiple methods for platelet enumeration. Observation of a newly introduced bias. *Am J Clin Pathol.* 1987;87:109112
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)]
4) Dotson MA. Methods to monitor and control systematic error. In: clinical hematology: principles, procedures, correlations, 2nd edition. Stiene-Martin EA, *et al*, eds. Philadelphia, PA: Lippincott, 1998:579-590
5) Fink NE, *et al*. Evaluation and additional recommendations for preparing a whole blood control material. *Rev Saude Publica.* 1998;32:107-111
6) Springer W, *et al*. Evaluation of a new reagent for preserving fresh blood samples and its potential usefulness for internal quality control of multichannel hematology analyzers. *Am J Clin Pathol.* 1999;111:387-396

HEM.25870 Commercially Assayed Controls — Phase II

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

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

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

HEM.25880 Unassayed Controls — Phase II

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

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

REFERENCES
1) Fink NE, *et al*. Evaluation and additional recommendations for preparing a whole blood control material. *Rev Saude Publica.* 1998;32:107-111
2) Springer W, *et al*. Evaluation of a new reagent for preserving fresh blood samples and its potential usefulness for internal quality control of multichannel hematology analyzers. *Am J Clin Pathol.* 1999;111:387-396

MOVING AVERAGES

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

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

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

HEM.25920 QC - Moving Averages **Phase II**

Control limits for moving averages are appropriately sensitive.

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

Evidence of Compliance:

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

REFERENCES
1) Bull BS, *et al*. A study of various estimators for the derivation of quality control procedures from patient erythrocyte indices. *Am J Clin Pathol.* 1974;61:473-481
2) Talamo TS, *et al*. Microcomputer assisted hematology quality control using a modified average of normals program. *Am J Clin Pathol.* 1981; 76:707-712
3) Bull BS, Korpman RA. Autocalibration of hematology analyzers. *J Clin Lab Automation.* 1983;3:111-116
4) Cembrowski GS, Westgard JO. Quality control of multichannel hematology analyzers: evaluation of Bull's algorithm. *Am J Clin Pathol.* 1985;83:337-345
5) Bull BS, Hay KL. Are red blood cells indexes international? *Arch Pathol Lab Med.* 1985;109:604-606
6) Levy WC, *et al*. Preserved blood versus patient data for quality control - Bull's algorithm revisited. *Am J Clin Pathol.* 1986;85:719-721
7) Levy WC, *et al*. The incorporation of red blood cell index mean data into quality control programs. *Am J Clin Pathol.* 1986;86:193-199
8) Lunetzky ES, Cembrowski GS. Performance characteristics of Bull's multirule algorithm for the quality control of multichannel hematology analyzers. *Am J Clin Pathol.* 1987;88:634-638
9) Clinical and Laboratory Standards Institute (CLSI). Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition. CLSI document H26-A2 (ISBN 1-56238-728-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2010.

HEM.25990 QC Procedure **Phase II**

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

Evidence of Compliance:

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

REFERENCES
1) Bull BS, *et al*. A study of various estimators for the derivation of quality control procedures from patient erythrocyte indices. *Am J Clin Pathol.* 1974;61:473-481
2) Talamo TS, *et al*. Microcomputer assisted hematology quality control using a modified average of normals program. *Am J Clin Pathol.* 1981; 76:707-712
3) Bull BS, Korpman RA. Autocalibration of hematology analyzers. *J Clin Lab Automation.* 1983;3:111-116
4) Cembrowski GS, Westgard JO. Quality control of multichannel hematology analyzers: evaluation of Bull's algorithm. *Am J Clin Pathol.* 1985;83:337-345
5) Bull BS, Hay KL. Are red blood cells indexes international? *Arch Pathol Lab Med.* 1985;109:604-606
6) Levy WC, *et al*. Preserved blood versus patient data for quality control - Bull's algorithm revisited. *Am J Clin Pathol.* 1986;85:719-721
7) Levy WC, *et al*. The incorporation of red blood cell index mean data into quality control programs. *Am J Clin Pathol.* 1986;86:193-199
8) Lunetzky ES, Cembrowski GS. Performance characteristics of Bull's multirule algorithm for the quality control of multichannel hematology analyzers. *Am J Clin Pathol.* 1987;88:634-638
9) Hackney JR, Cembrowski GS. Evaluation of intralaboratory quality control schemes, In Cavil I. Quality Control. Edinburgh, UK: Churchill Livingstone, 1990
10) Lewis SM, *et al*. Current concepts in haematology 3: blood count calibration. *J Clin Pathol.* 1991;144:881-884

RETAINED PATIENT SPECIMENS

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

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

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

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

Evidence of Compliance:
- ✓ Written QC procedure defining the control limits for repeat analysis of retained patient specimens **AND**
- ✓ QC records showing the use of the defined control limits

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

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

Inspector Instructions:

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

HEM.30070 Sampling Mode Comparison **Phase I**

There are records that at least annually compare all results obtained for patient specimens analyzed in the multiple sampling modes of the CBC analyzer (*e.g.* "open" and "closed" modes) to ensure that they are in agreement.

NOTE: Different modes may involve a different sample path before analysis. When samples are analyzed in more than one mode, it is important to ensure that all modes function properly. Re-analysis of a previously analyzed sample should be performed in the alternate mode(s), and results should agree with the initial mode within the tolerance limits established for agreement by the hematology laboratory's quality control program, and any recommendations by the instrument manufacturer. Mode-to-mode correlation is not necessary for those analyzers which use the same pathway for all modes.

Evidence of Compliance:

✓ Written procedure for sampling mode comparison with defined criteria for agreement **AND**
✓ Records of sampling mode comparison studies

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

There is a written procedure available and in use for detecting and correcting automated WBC counts for the presence of nucleated red cells or megakaryocytes.

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

Evidence of Compliance:

✓ Records showing actions taken to verify CBC concentration prior to reporting

REFERENCES

1) Culp NB, Wallace J. Correcting the NE-series WBC count for nucleated RBCs. *Sysmex J Intl.* 1991;1:62-65
2) Bridgen ML, Dalal BI. Cell counter-related abnormalities. *Lab Med.* 1999;30:325-334

HEM.30150 Spurious CBC Results — Phase II

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

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

Evidence of Compliance:

✓ Written procedure to detect spurious CBC results **AND**

✓ Record of action taken when spurious CBC instrument results are detected

REFERENCES

1) Gagne CC, *et al*. Effect of hyperchylomicronemia on the measurement of hemoglobin. *Am J Clin Pathol.* 1977;68:584-586
2) Cornbleet J. Spurious results from automated hematology cell counters. *Lab Med.* 1983;14:509-514
3) Gloster ES, *et al*. Spurious platelet counts associated with bacteremia. *Am J Hematol.* 1985;18:329-332
4) Savage RA. Analytic inaccuracy resulting from hematology specimen characteristics. Three cases of clinically misleading artifacts affecting white blood cell and platelet counts. *Am J Clin Pathol.* 1989;92:295-299
5) Rohr LR, Rivers FM. Spurious automated leukopenia due to in vitro granulocyte aggregation. *Am J Clin Pathol.* 1990;93:572-574
6) Robbins SH, *et al*. Cold-induced granulocyte aggregation. A cause of pseudoleukopenia. *Arch Pathol Lab Med.* 1991;115:155-157
7) Gulati GG, *et al*. Interference by cryoproteins in the blood with automated CBCs. *Lab Med.* 1995;26:138-142
8) Cantero M, *et al*. Interference from lipemia in cell count by hematology. *Clin Chem.* 1996;42:987-988
9) Bowen KL. Clinical pathology rounds. Erroneous leukocyte counts and cold agglutinins. *Lab Med.* 1997;28:247-250
10) Bridgen ML, Dalal BI. Cell counter-related abnormalities. *Lab Med.* 1999;30:325-334

HEM.30200 Red Cell Indices — Phase I

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

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

Evidence of Compliance:

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

REFERENCES
1) Cornbleet J. How laboratories use RBC indices. *Arch Intern Med.* 1983;143:1490
2) Houwen B. Random errors in haematology tests: a process control approach. *Clin Lab Haemat.* 1990;12:157-168
3) Gulati GG, *et al*. Interference by cryoproteins in the blood with automated CBCs. *Lab Med.* 1995;26:138-142
4) Cantero M, *et al*. Interference from lipemia in cell count by hematology. *Clin Chem.* 1996;42:987-988
5) Bridgen ML, Dalal BI. Cell counter-related abnormalities. *Lab Med.* 1999;30:325-334

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
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7164 [42CFR493.1253]
2) Hanseler E, *et al*. Estimation of the lower limits of manual and automated platelet counting. *Am J Clin Pathol.* 1996;105:782-787
3) Ault KA. Implementation of the immunological platelet count on a hematology analyzer - the Abbott Cell-Dyn 4000. *Lab Hematol.* 1997;3:125-128

HEM.30300 Platelet Abnormalities — Phase II

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

NOTE: When 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 should be quantified from blood collected directly into a counting diluent, or by use of a different anticoagulant (e.g. liquid sodium citrate with subsequent adjustment for dilution) or by estimation from a non-anticoagulated blood film.

Evidence of Compliance:

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

REFERENCES
1) Hyun BH, *et al*. Platelet satellitosis. Chicago, IL: American Society of Clinical Pathology Check Sample H-78, 1976
2) Veenhoven WA, *et al*. Pseudothrombocytopenia due to agglutinins. *Am J Clin Pathol.* 1979;72:1005-1008

3) Gloster ES, *et al*. Spurious platelet counts associated with bacteremia. *Am J Hematol.* 1985;18:329-332
4) Cunningham VL, Brandt JT. Spurious thrombocytopenia due to EDTA-independent cold-reactive agglutinins. *Am J Clin Pathol.* 1992;97:359-362
5) Hanseler E, *et al*. Estimation of the lower limits of manual and automated platelet counting. *Am J Clin Pathol.* 1996;105:782-787
6) Bridgen ML, Dalal BU. Cell counter-related abnormalities. *Lab Med.* 1999;30:325-334
7) Kunicka JE, *et al.* Improved platelet counting using two-dimensional laser light scatter. *Am J Clin Pathol.* 2000;114;114:283-289

HEM.30350 Spuriously High WBC Concentration — Phase II

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

NOTE: When unlysed RBC, PLT satellitosis, significant numbers of giant PLT and/or PLT clumps are suspected/detected by histographic abnormalities or instrument rejection of 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 of falsely elevated WBC counts and to correct results **AND**
- ✓ Record showing actions taken to verify WBC concentration prior to reporting

REFERENCES

1) Savage RA. Pseudoleukocytosis due to EDTA-induced platelet clumping. *Am J Clin Pathol.* 1984;81:317-322
2) Rabinovitch A. Anticoagulants, platelets, and instrument problems. *Am J Clin Pathol.* 1984;82:132-133
3) Cunningham VL, Brandt JT. Spurious thrombocytopenia due to EDTA-independent cold-reactive agglutinins. *Am J Clin Pathol.* 1992;97:359-362
4) Dorner K, *et al*. Improved automated leukocyte counting and differential in newborns achieved by the haematology analyser Cell-Dyn 3500. *Clin Lab Haem.* 1995;17:23-30
5) Bridgen ML, Dalal BU. Cell counter-related abnormalities. *Lab Med.* 1999;30:325-334
6) Kunicka JE, *et al.* Improved platelet counting using two-dimensional laser light scatter. *Am J Clin Pathol.* 2000;114;114:283-289

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

1) Morton BD, *et al*. Pappenheimer bodies: an additional cause for a spurious platelet count. *Am J Clin Pathol.* 1980;74:310-311
2) Akware AM, *et al*. Spuriously elevated platelet counts due to microspherocytosis. *Am J Clin Pathol.* 1982;77:220-221
3) Gloster ES, *et al*. Spurious elevated platelet counts associated with bacteremia. *Am J Hematol.* 1985;18:329-332
4) Bridgen ML, Dalal BI. Cell counter-related abnormalities. *Lab Med.* 1999;30:325-334
5) Li S, Salhany KE. Spurious elevation of automated platelet counts in secondary acute monocytic leukemia associated with tumor lysis syndrome. *Arch Pathol Lab Med.* 1999;123:1111-1114
6) Kunicka JE, *et al.* Improved platelet counting using two-dimensional laser light scatter. *Am J Clin Pathol.* 2000;114;114:283-289

MANUAL HEMATOCRIT (MICROHEMATOCRIT, PACKED CELL VOLUME)

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 a relative centrifugal field (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

1) Clinical and Laboratory Standards Institute. *Procedure for Determining Packed Cell Volume by the Microhematocrit Method; Approved Standard;* 3rd ed. CLSI document H07-A3. CLSI, Wayne, PA, 2000.

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:

- ✓ Mechanical timer accuracy 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

1) Clinical and Laboratory Standards Institute. *Procedure for Determining Packed Cell Volume by the Microhematocrit Method; Approved Standard;* 3rd ed. CLSI document H07-A3. CLSI, Wayne, PA, 2000.

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

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

Inspector Instructions:

	• 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

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1269(a)(2)]

HEM.33250 Manual Counts - PLT/WBC **Phase I**

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 (e.g. increased number of hemocytometer squares enumerated or a lesser specimen dilution) when there is leukopenia or thrombocytopenia, in order to avoid increasing the imprecision of particle counting, which is governed by binomial distributions and Poisson statistics.

Evidence of Compliance:

✓ Records or worksheets for manual counts on leukopenic or thrombocytopenic specimens

REFERENCES

1) Barnett RN. Clinical laboratory statistics, 2nd ed. Boston, MA: Little, Brown, 1979:30-33, 101-103
2) Miale JB. Laboratory medicine hematology, 6th ed. St Louis, MO: CV Mosby, 1982:373-374

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

HEM.33300 Contamination Checks **Phase II**

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

*****REVISED** 08/17/2016***

HEM.33330 Cell Count Controls **Phase II**

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

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24): [42CFR493.1269(a)(1)]

HEM.33350 PLT Estimate **Phase I**

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

Evidence of Compliance:
- ✓ Records of slide review/correlation

REFERENCES

1) Abbey AP, Belliveau RR. Enumeration of platelets. *Am J Clin Pathol.* 1978;69:55-56
2) Nosanchuk JS, *et al*. The analytic basis for the use of platelet estimates from peripheral blood smears. Laboratory and clinical applications. *Am J Clin Pathol.* 1978;69:383-387
3) Mogadam L. Application of the Miller's disc for the estimation and quality control of the platelet count. *Lab Med.* 1980;11:131-132

AUTOMATED DIFFERENTIAL COUNTERS

Inspector Instructions:

- Automated differential procedure
- Sampling of QC records

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

HEM.34100 Limits 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 (e.g. "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 (e.g. nucleated RBC, blasts), then the QC material must contain surrogate particles to evaluate accuracy.

Evidence of Compliance:

✓ Written procedure defining quality control requirements for automated WBC differentials

REFERENCES

1) Rümke CL. The statistically expected variability in differential leukocyte counts. In: Differential leukocyte counting, CAP conference/ Aspen. Northfield, IL: CAP, 1977:39-45
2) Kalish RJ, Becker K. Evaluation of the Coulter S-Plus V three-part differential in a community hospital, including criteria for its use. *Am J Clin Pathol.* 1986;86:751-755
3) Etzell, JE. For WBC differentials reporting absolute numbers. *CAP Today*, March 2010
4) Richardson-Jones A, Twedt D, Hellman R. Absolute versus proportional differential leukocyte counts. *Clin Lab. Haem.* 1995:17, 115-123
5) Ross DW, Bentley SA. Evaluation of an automated hematology system (Technicon H1). *Arch Pathol Lab Med.* 1986;110:803-808
6) Clinical and Laboratory Standards Institute (CLSI). *Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition*. CLSI document H20-A2 (ISBN 1-56238-628-X). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007
7) Miers MK, *et al*. White blood cell differentials as performed by the Technicon H-1; evaluation and implementation in a tertiary care hospital. *Lab Med.* 1991;22:99-106
8) Hallawell R, *et al*. An evaluation of the Sysmex NE8000 hematology analyzer. *Am J Clin Pathol.* 1991;96:594-601
9) Cornbleet PJ, *et al*. Evaluation of the CellDyn 3000 differential. *Am J Clin Pathol.* 1992;98:603-614
10) Clinical and Laboratory Standards Institute. *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline.* 3rd ed. CLSI Document EP09-A3. Clinical and Laboratory Standards Institute, Wayne, PA; 2013.
11) Krause JR. The automated white blood cell differential. A current perspective. *Hematol Oncol Clin North Am.* 1994;8:605-16
12) Goyzueta FG, *et al*. Automated differential white blood cell counts in the young pediatric population. *Lab Med.* 1996;27:48-52
13) Gulati GL, *et al*. Suspect flags and regional flags on the Coulter-STKS. An assessment. *Lab Med.* 1999;30:675-680
14) Grimaldi E, Scopacasa F. Evaluation of the Abbott CELL-DYN 4000 hematology analyzer. *Am J Clin Pathol.* 2000;113:497-505

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

1) Rümke CL. The statistically expected variability in differential leukocyte counts. In: Differential leukocyte counting, CAP conference/ Aspen. Northfield, IL: CAP, 1977:39-45
2) Payne BA, Pierre RV. Using the three-part differential: part II. Implementation of the system. *Lab Med.* 1986;17:517-522
3) Kalish RJ, Becker K. Evaluation of the Coulter S-Plus V three-part differential in a community hospital, including criteria for its use. *Am J Clin Pathol.* 1986;86:751-755
4) Ross DW, Bentley SA. Evaluation of an automated hematology system (Technicon H-1). *Arch Pathol Lab Med.* 1986;110:803-808
5) Clinical and Laboratory Standards Institute (CLSI). *Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition*. CLSI document H20-A2 (ISBN 1-56238-628-X). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007
6) Miers MK, *et al*. White blood cell differentials as performed by the Technicon H-1; evaluation and implementation in a tertiary care hospital. *Lab Med.* 1991;22:99-106
7) Hallawell R, *et al*. An evaluation of the Sysmex NE-8000 hematology analyzer. *Am J Clin Pathol.* 1991;96:594-601
8) Cornbleet PJ, *et al*. Evaluation of the Cell-Dyn 3000 differential. *Am J Clin Pathol.* 1992;98:603-614
9) Clinical and Laboratory Standards Institute. *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline*. 3rd ed. CLSI Document EP09-A3. Clinical and Laboratory Standards Institute, Wayne, PA; 2013.
10) Krause JR. The automated white blood cell differential. A current perspective. *Hematol Oncol Clin North Am.* 1994;8:605-16
11) Goyzueta FG, *et al*. Automated differential white blood cell counts in the young pediatric population. *Lab Med.* 1996;27:48-52
12) Gulati GL, *et al*. Suspect flags and regional flags on the Coulter-STKS. An assessment. *Lab Med.* 1999;30:675-680

MANUAL BLOOD FILM EXAMINATION (DIFFERENTIAL COUNT)

Inspector Instructions:

READ	• Manual differential policies and procedures
OBSERVE	• Sampling of patient peripheral blood smears (uniquely identified, properly stained, free of precipitate, good cell distribution) • File of unusual slides
ASK	• How do you ensure consistency among personnel performing blood cell microscopy?

HEM.34300 Blood Film Quality **Phase I**

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

REFERENCES

1) Wenk RE. Comparison of five methods for preparing blood smears. *Am J Med Technol.* 1976;42:71-78
2) College of American Pathologists. Differential leukocyte counting. CAP conference/aspen. Northfield, IL: CAP, 1977
3) Stiene-Martihn EA. Causes for poor leukocyte distribution in manual spreaderslide blood films. *Am J Med Technol.* 1980;46:624-632
4) Lewis SM. Blood film evaluations as a quality control activity. *Clin Lab Haematol.* 1990;12:119-127
5) Turgeon ML. Clinical hematology, theories and procedures, 2nd ed. Boston, MA: Little, Brown, 1993;16-25
6) Dacie JV, Lewis SM. Practical hematology, 8th ed. New York, NY: Churchill Livingstone, 1995;83-89

***NEW** 08/21/2017*

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

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

HEM.34400 Morphologic Observation Assessment - CBC **Phase II**

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

NOTE: Suggested methods to accomplish this include:

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

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

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

Evidence of Compliance:

- ✓ Written procedure defining the method and criteria used for evaluation of consistency **AND**
- ✓ Employee records of morphology assessment

REFERENCES

1) Rümke CL. The statistically expected variability in differential leukocyte counts, In: Differential leukocyte counting, CAP conference/ Aspen. Northfield, IL: CAP, 1977:39-45
2) Wood B, *et al*. Teaching the clinical interpretation of peripheral blood smears to second-year medical school class using the peripheral blood-tutor computer program. *Am J Clin Pathol.* 1998;109:514-520
3) College of American Pathologists. Surveys hematology glossary. Northfield, IL: CAP, 1999:1-26
4) Brigden ML, Dalal BI. Morphologic abnormalities, pseudosyndromes, and spurious test results. *Lab Med.* 1999;30:397-405
5) Haun DE, *et al*. A better way to assess WBC differential counting skills. *Lab Med.* 2000;31:329-333

HEM.34450 Slide Retention **Phase I**

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

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

HEM.34500 Morphology Assessment - PLT/RBC **Phase II**

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

NOTE: The laboratory must have a system to ensure that technical personnel have fully assessed all morphologic findings in each patient film. Each laboratory director should, in consultation with the medical staff, determine which morphologic findings are reportable. For example, minor degrees of anisocytosis and poikilocytosis without specific types of RBC

abnormalities may be considered within the normal spectrum and not reportable to the chart. For RBC abnormalities that are reported, the laboratory must define a qualitative or semiquantitative grading system. When defined abnormalities (e.g. spherocytes, target cells, fragments, etc.) are present, non-specific listings of "anisocytosis" and/or "poikilocytosis" may not provide additional clinically useful information.

Evidence of Compliance:

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

REFERENCES
1) Napoli V, *et al*. A semiquantitative estimate method for reporting abnormal RBC morphology. *Lab Med.* 1980;11:111-116
2) Krause JR. Redcell abnormalities in the blood smear: disease correlations. *Lab Mgmt.* 1985;23(10):29-35
3) Bell A, Lofsness KG. A photo essay on red cell morphology. *J Med Tech.* 1986;3:85-93
4) Lewis SM. Blood film evaluations as a quality control activity. *Clin Lab Haematol.* 1990;12:119-127

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
1) Peterson P, *et al*. Physician review of the peripheral blood smear: when and why. An opinion. *Lab Hematol.* 2001;7:175-179
2) Gulati GL, *et al.* Criteria for blood smear review. *Lab Med.* 2002;33:374-377

BLOOD FILMS FOR MALARIA AND OTHER MICROORGANISMS

Inspector Instructions:

READ 	• Sampling of blood parasite and microorganism policies and procedures • Sampling of patient reports • Buffer pH records
OBSERVE 	• Ocular micrometer

*****REVISED** 08/21/2017***

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 (e.g. 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

1) Clinical and Laboratory Standards Institute. *Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline.* CLSI document M15-A. CLSI, Wayne, PA, 2000.
2) Pritt BS. Parasitology Benchtop Reference Guide: An Illustrated Guide for Commonly Encountered Parasites. Northfield, IL; College of American Pathologists, 2014.

HEM.34660 Ocular Micrometer — Phase I

An ocular micrometer is available for determining the size of bloodborne parasites.

HEM.34665 Calibration/Recalibration - Ocular Micrometer — Phase I

The ocular micrometer is calibrated for the microscope(s) in which it is used and it is recalibrated each time the eyepieces or objectives are changed.

NOTE: Calibrations can be checked against a micrometer or other objects of known dimensions. If there are no changes to a particular microscope's optical components, there is no need to recheck calibration.

Evidence of Compliance:

- ✓ Records of initial calibration and recalibration, if applicable

HEM.34687 Percentage Parasitemia Reporting — Phase I

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

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

Evidence of Compliance:

- ✓ Written procedure for performing and reporting parasitemia percentage with identification **AND**
- ✓ Patient reports

REFERENCES

1) Clinical and Laboratory Standards Institute. *Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline.* CLSI document M15-A. CLSI, Wayne, PA, 2000.
2) Garcia LS, *Diagnostic Medical Parasitology*. Washington, DC, ASM Press, 2001
3) http://www.dpd.cdc.gov/DPDx/HTML/DiagnosticProcedures.htm
4) Pritt BS. Parasitology Benchtop Reference Guide: An Illustrated Guide for Commonly Encountered Parasites. Northfield, IL; College of American Pathologists, 2014.

HEM.34724 Thick and Thin Films — Phase II

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

Evidence of Compliance:

- ✓ Written procedure with instructions for preparing thin and thick films

REFERENCES

1) Clinical and Laboratory Standards Institute. *Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline.* CLSI document M15-A. CLSI, Wayne, PA, 2000.
2) Thomson S, *et al.* External quality assessment in the examination of blood films for malHelvetica parasites within Ontario, Canada. *Arch Pathol Lab Med.* 2000;124:57-60
3) Pritt BS. Parasitology Benchtop Reference Guide: An Illustrated Guide for Commonly Encountered Parasites. Northfield, IL; College of American Pathologists, 2014.

HEM.34798 Malaria Stain Procedure **Phase I**

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

REFERENCES

1) Clinical and Laboratory Standards Institute. *Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline.* CLSI document M15-A. CLSI, Wayne, PA, 2000.
2) Garcia LS, Bruckner DA. Diagnostic medical parasitology. Washington, DC: American Society for Microbiology, 1997:702-703
3) Pritt BS. Parasitology Benchtop Reference Guide: An Illustrated Guide for Commonly Encountered Parasites. Northfield, IL; College of American Pathologists, 2014.

HEM.34872 Slide Review Procedure **Phase I**

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

Evidence of Compliance:

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

REFERENCES

1) Clinical and Laboratory Standards Institute. *Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline.* CLSI document M15-A. CLSI, Wayne, PA, 2000.
2) Pritt BS. Parasitology Benchtop Reference Guide: An Illustrated Guide for Commonly Encountered Parasites. Northfield, IL; College of American Pathologists, 2014.

AUTOMATED RETICULOCYTES

Inspector Instructions:

READ

- Automated reticulocyte procedure

***REVISED** 08/21/2017*

HEM.35150 Spurious Reticulocyte Results **Phase I**

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

1) Jacobberger HW, *et al*. Flow cytometric analysis of blood cells stained with the cyanine dye Dioc1[3]: reticulocyte quantification. *Cytometry.* 1984;5:589-600
2) Davis BH, *et al*. Utility of flow cytometric reticulocyte quantification as a predictor of engraftment in autologous bone marrow transplantation. *Am J Hematol.* 1989;32:81-87
3) Davis BH, Bigelow NC. Flow cytometric quantification using thiazole orange provides clinically useful reticulocyte maturity index. *Arch Pathol Lab Med.* 1989;113:684-689
4) Hackney JR, *et al*. Automated reticulocyte counting by image analysis and flow cytometry. *Lab Med.* 1989;20:551-555
5) Coulet M, Bezou MJ. Utilization of the automated reticulocyte counter Sysmex R-1000. *Sysmex J.* 1990;13:393-406
6) Wells DA, *et al*. Effect of iron status on reticulocyte mean channel fluorescence. *Am J Clin Pathol.* 1992;97:130-134
7) Riley RS, Ross W. Reticulocyte enumeration, In: Riley RS, Makin EJ, Ross W, eds. Clinical applications of flow cytometry. New York, NY: Igaku-shoin, 1993:582-611
8) Batjer JD, *et al*. Predicting bone marrow transplant engraftment by automated flow cytometric reticulocyte analysis. *Lab Med.* 1994;25:22-26
9) Lofsness KG, *et al*. Evaluation of automated reticulocyte counts and their reliability in the presence of Howell-Jolly bodies. *Am J Clin Pathol.* 1994;101:85-90

MANUAL RETICULOCYTES

Inspector Instructions:

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

HEM.35250 Blood Film Examination **Phase I**

Examine a blood film stained for reticulocytes. It is satisfactory.

REFERENCES

1) Fannon M, *et al*. Effect of staining and storage times on reticulocyte counts. *Lab Med.* 1982;13:431-433

HEM.35300 Reporting - Retics **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

1) Greenberg ER, Beck R. The effects of sample size on reticulocyte counting and stool examination. The binomial and Poisson distributions in laboratory medicine. *Arch Pathol Lab Med.* 1984;108:396-398
2) Savage RA, *et al*. Analytic inaccuracy and imprecision in reticulocyte counting: a preliminary report from the College of American Pathologists reticulocyte project. *Blood Cells.* 1985;11:97-112
3) Koepke JA. Update on reticulocyte counting. *Lab Med.* 1999;30:339-343

BODY FLUIDS

Inspector Instructions:

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

BODY FLUID CELL COUNTING - MANUAL

HEM.35319 Diluting Equipment **Phase II**

Certified pipettes or commercial dilution systems are used when diluting body fluid samples.

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

*****REVISED** 08/21/2017***

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

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2004(Oct 1):1041 [42CFR493.1269(a)

***REVISED** 08/21/2017*

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.

***REVISED** 08/21/2017*

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

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1269(a)(2)]

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

BODY FLUID CELL COUNTING - INSTRUMENTAL

HEM.35414 Background Checks - Automated Counts **Phase II**

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

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

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

HEM.35452 Acceptable Limits **Phase II**

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

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

Evidence of Compliance:
- ✓ Written 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

1) International Committee for Standardization in Haematology (ICSH). Protocol for evaluation of automated blood cell counters. *Clin Lab Haemat*. 1984;6:69-84
2) Subira D, *et al*. Flow cytometric analysis of cerebrospinal fluid samples and its usefulness in routine clinical practice. *Am J Clin Pathol.* 2002;117:952-958

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.

BODY FLUID NUCLEATED CELL DIFFERENTIALS

HEM.35528 Quantitative Differentials — Phase I

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

NOTE: The laboratory should use stained cytocentrifuge preparations to facilitate quantitative differentials and complete classification of nucleated cell types in body fluids, as opposed to performing differentials of unstained hemocytometer preparations. Differentials based on 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 cytospin slides show excellent morphologic correlation with blood and bone marrow smears. If the laboratory uses an alternate slide preparation method or stain for sub-classification of body fluid mononuclear cells and/or detection of malignant cells, it must demonstrate from literature or in-house studies that this technique is equivalent in cell yield/recovery and morphologic detail to Romanowsky-stained cytocentrifuge preparations.

Evidence of Compliance:

- ✓ Written procedure defining method for performing cell differentiation on body fluid **AND**
- ✓ Records showing in-house or literature validation of techniques other than Romanowsky-stained cytocentrifuge preparations

REFERENCES

1) Mengel M. The use of the cytocentrifuge in the diagnosis of meningitis. *Am J Clin Pathol.* 1985;84:212-216
2) Ricevuti G, *et al.* Meningeal leukemia diagnosed by cytocentrifuge study of cerebrospinal fluid. *Arch Neurol.* 1986;43:466-470
3) Davey DD, *et al.* Millipore filter *vs* cytocentrifuge for detection of childhood central nervous system leukemia. *Arch Pathol Lab Med.* 1986;110:705-708
4) Clare N, Rone R. Detection of malignancy in body fluids. *Lab Med.* 1986;17:147-150
5) Odom LF, *et al.* Significance of blasts in low-cell cerebrospin*al spe*cimens from children with acute lymphoblastic leukemia. *Cancer.* 1990;66:1748-1754
6) Craver RD, Carson TH. Hematopoietic elements in cerebrospinal fluid in children. *Am J Clin Pathol.* 1991;95:532-535
7) Rippin KP, *et al.* Clinical evaluation of the slide centrifuge (cytospin) gram's stained smear for the detection of bacteriuria and comparison with the Filtracheck-UTI and UTIscreen. *Am J Clin Pathol.* 1995;103:316-319
8) Jones CD, Cornbleet PJ. Wright-Giemsa cytology of body fluids. Techniques for optimal cytocentrifuge slide preparation. *Lab Med.* 1997;28:713-716
9) Kleine TO, Lehmitz R. Evaluation of cytodiagnosis of cerebrospinal fluid (CSF) cells. *Clin Chem*. 2000;46:A137

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

1) Jones CD, Cornbleet PJ. Wright-Giemsa cytology of body fluids. Techniques for optimal cytocentrifuge slide preparation. *Lab Med.* 1997;28:713-716

HEM.35566 Morphologic Observation Assessment - Body Fluid — Phase II

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

NOTE: Suggested methods to accomplish this include:

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

Evidence of Compliance:

- ✓ Written procedure defining the method and criteria used for evaluation of consistency **AND**
- ✓ Employee records of morphology assessment

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). Body Fluid Analysis for Cellular Composition; Approved Guideline. CLSI document H56-A (ISBN 1-56238-614-X). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006.

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
2) Walts AE, Strigle S. Toward optimal use of the cytology laboratory: quality improvement and cerebrospinal fluid specimens. *Diagn Cytopathol.* 1995;13:357-361
3) Clinical and Laboratory Standards Institute (CLSI). *Body Fluid Analysis for Cellular Composition; Approved Guideline*. CLSI document H56-A (ISBN 1-56238-614-X). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006.

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

1) Kolmer HW. Atlas of cerebrospinal fluid cells, 2nd ed. New York, NY: Springer-Verlag, 1977
2) Dieppe PA, *et al.* Synovial fluid crystals. *Quart J Med.* 1979;192:533-553
3) Glasser L. Body fluids II. Reading the signs in synovia. *Diag Med.* 1980;3(6):35-50
4) Glasser L. Body fluids III. Tapping the wealth of information in CSF. *Diag Med.* 1981;4:23-33
5) Greening SE, *et al.* Differential diagnosis in effusion cytology. *J Med Tech.* 1984;1:885-895
6) Strasinger SK. Urinalysis and body fluids. A self instructional text. Philadelphia: FA Davis, 1985:134-186
7) Hyun BH, Salazar GH. Cerebrospinal fluid cells in leukemias, lymphomas, and myeloma. *Lab Med.* 1985;16: 667-670
8) Kjeldsberg CR, Knight JA. Body fluids, 3rd ed. Chicago, IL: American Society of Clinical Pathology, 1993
9) Galagan KA, Blomberg D, Cornbleet PJ, Glassy EF, Color Atlas of Body Fluids, CAP, 2006.

HEM.35642 Slide Retention **Phase I**

Slides are retained for future reference.

NOTE: All body fluid smears must be retained for at least one week for possible review/ reference. The laboratory may choose to retain significantly abnormal smears (e.g. 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.

SEMEN ANALYSIS

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

Inspector Instructions:

	• Sampling of manual and automated semen analysis policies and procedures • Sampling of 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 QC/calibration records
	• Stained smear (properly stained, free of precipitate, uniform cell distribution, recognition of reportable cell types) • File of unusual slides
	• What do you do if there is difficulty distinguishing leukocytes from other round cells when performing sperm counts using bright field microscopy? • How is the sperm motility method in use verified? • How do you ensure that morphologic observations are consistent among all personnel who report sperm differential results?
	• Follow a semen analysis from requisition, collection information, testing, reporting and recording of results. Determine if practice follows laboratory procedure.

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)
2) Diagnostic Evaluation of the Infertile Male: A Committee Opinion. *Fertil Steril.* 2012; 98:294-301
3) American Urological Association (AUA) Guideline. American Urological Association Education and Research, Inc. 2012; amended 2015. https://www.auanet.org/guidelines/vasectomy-(2012-amended-2015)
4) Vasectomy Update 2010. *Can Urol Assoc J*. 2010 October; 4(5):306-309

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

HEM.35680 Specimen Collection/Handling **Phase I**

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

1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press

HEM.35699 Specimen Collection/Handling **Phase I**

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

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

REFERENCES

1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press

HEM.35718 Liquefaction **Phase I**

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

Evidence of Compliance:
- ✓ Written policy defining criteria for liquefaction

REFERENCES
1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press

HEM.35737 Specimen Handling - Pre-analytic **Phase I**

Semen specimens are mixed thoroughly before testing.

REFERENCES
1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press

HEM.35756 Specimen Characteristics - Analytic **Phase I**

All characteristics of the semen specimens are noted and reported (*e.g.* 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 (i.e. fourth or fifth edition).

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

REFERENCES
1) Haugen TB, Grotmol T. pH of human semen. *Int J. Androl.* 1998;21:105-108
2) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press

SPERM MOTILITY

HEM.35762 Motility Method Verification **Phase I**

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

Evidence of Compliance:
- ✓ Records of method verification

REFERENCES
1) Mortimer D. Practical laboratory andrology. New York, NY: Oxford University Press, 1994
2) Yeung CH, *et al*. A technique for standardization and quality control of subjective sperm motility assessments in semen analysis. *Fertil Steril.* 1997;67:1156-1158

HEM.35765 Motility Quantification **Phase II**

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 (i.e. fourth or fifth edition).

REFERENCES

1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press
2) Yeung CH, *et al*. A technique for standardization and quality control of subjective sperm motility assessments in semen analysis. *Fertil Steril.* 1997;67:1156-1158

HEM.35768 Forward Progression **Phase II**

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
1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press
2) Vulcano GJ, *et al*. A lineal equation for the classification of progressive and hyperactive spermatozoa. *Math Biosci*. 1998;149:77-93

HEM.35775 Motility/Progression Evaluation **Phase II**

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

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

REFERENCES
1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press

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
1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press

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
1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press

HEM.35822 Viability Testing Criteria **Phase I**

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

NOTE: Non-motile sperm may represent forms that were originally non-viable in the ejaculate, or previously motile forms that have subsequently lost motility. Thus, viability assessment is

useful in making the distinction, and is commonly performed with a dye-exclusion method such as eosin-nigrosin.

Evidence of Compliance:
- ✓ Written procedure for viability testing **AND**
- ✓ Patient records or worksheet with results of viability testing **OR** patient report with cautionary verbiage

REFERENCES
1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press
2) Gunalp S, *et al.* A study of semen parameters with emphasis on sperm morphology in a fertile population: an attempt to develop clinical thresholds. *Hum Repro.* 2001;16:110-114

STAINED SMEAR - SPERM DIFFERENTIAL

HEM.35832 Morphology Classification **Phase I**

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 (i.e. fourth or fifth edition), and the Kruger classification system, and discontinuing the use of older classification systems.

REFERENCES
1) Kruger, T.F., *et al*. Sperm morphology features as a prognostic factor in vitro fertilization. *Fertility and Sterility* 46:1118-1123, 1986
2) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press
3) Gunalp S, *et al.* A study of semen parameters with emphasis on sperm morphology in a fertile population: an attempt to develop clinical thresholds. *Hum Repro.* 2001;16:110-114

HEM.35851 Morphologic Observation Assessment - Sperm **Phase II**

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

NOTE: Suggested methods to accomplish this include:

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

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

REFERENCES
1) Souter VL, *et al.* Laboratory techniques for semen analysis; a Scottish survey. *Health Bull (Edinb).* 1997;55:140-149
2) Baker DJ, Witmyer J. Semen analysis training tool. Chicago, IL: American Society of Clinical Pathology, 1998
3) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press
4) Kruger T, Frenken D. Atlas of Human Sperm Morphology Evaluation; Taylor & Frances, 2004
5) Glassy E. CAP Color Atlas of Hematology, 1998

HEM.35870 Consultation **Phase II**

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

REFERENCES
1) Revised guidelines for human embryology and andrology laboratories. *Fertil Steril.* 2008;90 (suppl 3):S45-S59

HEM.35889 Sperm Morphology Reference — Phase I

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

REFERENCES
1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press
2) Kruger T, Frenken, D. Atlas of Human Sperm Morphology Evaluation, Taylor & Frances, 2004

HEM.35892 Stain Usage — Phase I

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
1) Coetzee K, *et al.* Predictive value of normal sperm morphology: a structured literature review. *Hum Reprod Update*. 1998;4:73-82

HEM.35893 White Cell Confirmation Techniques — Phase I

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

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
1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press
2) Fishel TJ, *et al*. Increased polymorphonuclear granulocytes in seminal plasma in relation to sperm morphology. *Hum Reprod.* 1997;12:2418-2421
3) Zimmermann BS, *et al*. Relationship of bacteriological characteristics to semen indices and its influence on fertilization and pregnancy rates after IVF. *Acta Obstet Gynecol Scand*. 1997;76:964-968
4) Trum JW, *et al.* Value of detecting leukocytospermia in the diagnosis of genital tract infection in subfertile men. *Fertil Steril.* 1998;70:315-319

*****REVISED** 08/21/2017***

HEM.35895 Stain QC — Phase II

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

HEM.35902 Stain Quality — Phase II

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

*****NEW** 08/21/2017***

HEM.35905 Calibration/Recalibration - Ocular Micrometer **Phase II**

The ocular micrometer (when required) has been calibrated for the microscope(s) in which it is used, and it is recalibrated each time the eyepieces or objectives are changed.

NOTE: An ocular micrometer is required for certain sperm morphology classification methods, including Kruger Strict and World Health Organization (WHO) sperm morphology methods, as referenced in the 3rd, 4th, and 5th editions of the WHO laboratory manual for the examination and processing of human semen. Calibrations can be checked against a micrometer or other objects of known dimensions. If there are no changes to a particular microscope's optical components, there is no need to recheck calibration.

Evidence of Compliance:
- ✓ Written policy for ocular micrometer calibration and recalibration **AND**
- ✓ Records of initial calibration and recalibration, if 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 **AND**
- ✓ Records of QC results

REFERENCES
1) Mortimer D. Practical laboratory andrology. New York, NY: Oxford University Press, 1994

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, 19RLM.185

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
1) Mortimer D. Practical laboratory andrology. New York, NY: Oxford University Press, 1994

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 **AND**
✓ 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
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7166 [42CFR493. 1256(d)(iii)]
3) Evans ML, *et al.* A convenient mixed immunobeads screen for antisperm antibodies during routine semen analysis. *Fertil Steril.* 1998;70:344-349

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 (e.g. fixed or preserved)*
3. *Sperm surrogates (e.g. latex particles)*
4. *Digital images/videotaped sperm specimens*

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

HEM.35914 Calibration Materials **Phase II**

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
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1255]
2) Revised guidelines for human embryology and andrology laboratories. *Fertil Steril.* 2008;90 (suppl 3):S45-S59

HEM.35915 Daily QC **Phase II**

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
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1256(d)]
2) Revised guidelines for human embryology and andrology laboratories. *Fertil Steril.* 2008;90 (suppl 3):S45-S59

*****REVISED** 08/17/2016***
HEM.35916 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
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1255(a)(3)]
2) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press

HEM.35918 Calibration Material Validation **Phase II**

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

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

REFERENCES
1) WHO laboratory manual for the examination and processing of human semen, most recent editions (i.e. fourth edition 1999 and fifth edition 2010). New York, NY: Cambridge University Press
2) Krause W. [Value of computer-assisted sperm analysis (CASA). reproducibility--online documentation--prognostic value]. [Article in German]. *Fortschr Med.* 1996;114:470-473
3) Tsuji T, *et al.* Automated sperm concentration analysis with a new flow cytometry-based device, S_FCM. *Am J Clin Pathol.* 2002;117:401-408

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
1) Mortimer D. Practical laboratory andrology. New York, NY: Oxford University Press. 1994
2) Lenzi A. Computer-aided semen analysis (CASA) 10 years later: a test-bed for the European scientific andrological community. *Int J Androl.* 1997;20:1-2
3) Mahmoud AM, *et al*. Performance of the sperm quality analyser in predicting the outcome of assisted reproduction. *Int J Androl*. 1998;21:41-46

4) Tsuji T, *et al.* Automated sperm concentration analysis with a new flow cytometry-based device, S_FCM. *Am J Clin Pathol.* 2002;117:401-408

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
1) Vantman DD, *et al*. Computer assisted semen analysis: evaluation of method and assessment of the influence of sperm concentration on linear velocity determination. *Fertil Steril.* 1988;49:510-515
2) Yeung CH, *et al.* A technique for standardization and quality control of subjective sperm motility assessments in semen analysis. *Fertil Steril*. 1997;67:1156-1158
3) Sidhu RS, *et al.* accuracy of computer-assisted semen analysis in prefreeze and post-thaw specimens with high and low sperm counts and motility. *Urology*. 1998;51:306-312
4) Tsuji T, *et al.* Automated sperm concentration analysis with a new flow cytometry-based device, S_FCM. *Am J Clin Pathol.* 2002;117:401-408

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
1) Mortimer D. Practical laboratory andrology. New York, NY: Oxford University Press. 1994

*****REVISED** 08/21/2017***
HEM.35924 Calibration Verification Criteria **Phase II**

There are written criteria for method calibration verification.

NOTE: Criteria for determining the need for calibration verification typically include:

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

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

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7165 [42 CFR 493.1255]

ABNORMAL HEMOGLOBIN DETECTION

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

Inspector Instructions:

	• Sampling of abnormal hemoglobin policies 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 (*e.g.* A, F, and S) are performed with the patient specimen(s) and separations are satisfactory.

Evidence of Compliance:

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

REFERENCES

1) Fairbanks VF. Hemoglobinopathies and thalassemias. Laboratory methods and case studies. New York, NY: BC Decker, 1980
2) Beuzard Y, *et al*. Isoelectric focusing of human hemoglobins, In Hanash, Brewer, eds. Advances in hemoglobin analysis. New York, NY: Alan R. Liss, 1981:177-195
3) Cossu G, *et al*. Neonatal screening of beta-thalassemias by thin layer isoelectric focusing. *Am J Hematol*. 1982;13:149
4) Bunn HF, Forget BG. Hemoglobin: molecular, genetic and clinical aspects. Philadelphia, PA: WB Saunders, 1986
5) Honig GR, Adams JG III. Human hemoglobin genetics. Vienna, Austria: Springer-Verlag, 1986
6) Jacobs S, *et al*. Newborn screening for hemoglobin abnormalities. A comparison of methods. *Am J Clin Pathol*. 1986;85:713-715
7) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part I. The introduction and thalassemia syndromes. *Lab Med*. 1987;18:368-372
8) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part II. The sickle cell disorders. *Lab Med*. 1987;18:441-443
9) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part III. Nonsickling disorders and cord blood screening. *Lab Med*. 1987;18:513-518
10) Armbruster DA. Neonatal hemoglobinopathy screening. Lab Med. 1990;21:815-822
11) Adams JG III, Steinberg MH. Analysis of hemoglobins, In Hoffman R, *et al*, eds. Hematology: basic principles and practice. New York, NY: ChurchillLivingstone, 1991:1815-1827
12) Mallory PA, *et al*. Comparison of isoelectric focusing and cellulose acetate electrophoresis for hemoglobin separation. *Clin Lab Sci*. 1994;7:348-352
13) Awalt E, *et al*. Tandem mass spectrometry (MS) – A screening tool for hemoglobinopathies. *Clin Chem*. 2001;47(suppl):A165
14) Bradley CA, Kelly A. Comparison of high performance liquid chromatography with electrophoresis for measurement of hemoglobins A, A2, S, F, and C. *Clin Chem*. 2001;47(suppl):A172
15) Bradley CA, Kelly A. Calibration verification of hemoglobins A, A2, S, and F with an automated chromatography system. *Clin Chem*. 2001;47(suppl):A17315)
16) Hoyer JD, *et al*. Flow cytometric measurement of hemoglobin F in RBCs: diagnostic usefulness in the distinction of hereditary persistence of fetal hemoglobin (HPFH) and hemoglobin S-HPFH from other conditions with elevated levels of hemoglobin F. *Am J Clin Pathol*. 2002;117:857-863

HEM.35946 Hemoglobin Variants — Phase II

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

NOTE: If all clinically significant variants are not clearly separated by the primary method, additional testing is performed to further characterize these hemoglobin variants. This may include, but is not limited to: performance by a complementary, separate methodology or increasing the duration of the assay (for HPLC) where the hemoglobins migrate/elute at different configurations. Further workup of such variants, including referral to 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

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

13) Bradley CA, Kelly A. Calibration verification of hemoglobins A, A2, S, and F with an automated chromatography system. *Clin Chem*. 2001;47(suppl):A173
14) Hoyer JD, *et al*. Flow cytometric measurement of hemoglobin F in RBCs: diagnostic usefulness in the distinction of hereditary persistence of fetal hemoglobin (HPFH) and hemoglobin S-HPFH from other conditions with elevated levels of hemoglobin F. *Am J Clin Pathol*. 2002;117:857-863

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
2) Bunn HF, Forget BG. Hemoglobin: molecular, genetic and clinical aspects. Philadelphia, PA: WB Saunders, 1986
3) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part I. The introduction and thalassemia syndromes. *Lab Med*. 1987;18:368-372
4) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part II. The sickle cell disorders. *Lab Med*. 1987;18:441-443
5) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part III. Nonsickling disorders and cord blood screening. *Lab Med*. 1987;18:513-518
6) Adams JG III, Steinberg MH. Analysis of hemoglobins, In Hoffman R, et al, eds. Hematology: basic principles and practice. New York, NY: Churchill-Livingstone, 1991:1815-1827
7) Mallory PA, *et al*. Comparison of isoelectric focusing and cellulose acetate electrophoresis for hemoglobin separation. *Clin Lab Sci*. 1994;7:348-352

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HEM.35986 Calibration and Calibration Verification — Phase II

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 **Phase II**

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

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 slide (uniquely identified, satisfactory staining and cell distribution)
	• How do you reconcile clinically significant discrepancies between the bone marrow morphologic diagnosis and the results of ancillary studies?

HEM.36100 Slide Review **Phase I**

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

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

1) Krause JR, ed. Bone marrow biopsy. New York, NY: Churchill Livingstone, 1981:1-9
2) Bartl R, *et al*. Bone marrow biopsies revisited. Basel, Switzerland: Karger, 1982
3) Brunning RD. Bone marrow, In Rosai J, ed. Ackerman's surgical pathology. St Louis, MO: CV Mosby, 1989:1379-1454
4) Brunning RD. Bone marrow specimen processing, In Knowles DM, ed. Neoplastic hematopathology. Baltimore, MD: Williams & Wilkins, 1992:1081-1095
5) Dacie JV, Lewis SM. Practical hematology, 8th ed. New York, NY: Churchill Livingstone, 1995:178-184
6) Foucar K. Bone marrow pathology. Chicago, IL: American Society of Clinical Pathology, 1995

HEM.36200 Fixed Tissue Quality **Phase II**

The quality of fixed tissue sections of bone marrow is conducive to a reliable diagnosis.

HEM.36250 Fixed Tissue Correlation **Phase 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

HEM.36270 Record Retention **Phase II**

Bone marrow reports and smears are retained for 10 years.

HEM.36300 Bone Marrow Evaluation **Phase II**

Bone marrow specimens are evaluated by a pathologist or qualified hematologist and formal reports prepared.

REFERENCES

1) Peterson LC, *et al*. Protocol for the examination of specimens from patients with hematopoietic neoplasms of the bone marrow: a basis for checklists. *Arch Pathol Lab Med.* 2002;126:1050-1056

HEM.36325 Correlation of Results **Phase I**

There is a mechanism to correlate the results of ancillary studies (immunohistochemistry, cytogenetics, flow cytometry, *etc.*) with the morphologic diagnosis.

NOTE: The pathologist or qualified hematologist should correlate all of the special studies, reconcile conflicting data, and render a final interpretation of all correlated studies where appropriate. A mechanism should exist in the laboratory that 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

1) Peterson LC, *et al*. Protocol for the examination of specimens from patients with hematopoietic neoplasms of the bone marrow: a basis for checklists. *Arch Pathol Lab Med.* 2002;126:1050-1056

*****REVISED** 08/21/2017***

HEM.36350 Iron Stain **Phase I**

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

HEM.36800 Stain Reactivity **Phase II**

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

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

Evidence of Compliance:

✓ Written procedures for stain QC **AND**

✓ Records of stain QC at defined frequency

REFERENCES

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

BLOOD COAGULATION STUDIES

Inspector Instructions:

READ	• Sampling of coagulation/D-dimer/platelet function policies and procedures • Sampling of QC records • Sampling of patient reports • Sampling of calibration/calibration verification/recalibration records
ASK	• How do you know the PPP is really platelet-poor? • How do you know if an increased aPTT is not due to heparin? • What is your course of action if results fall outside the AMR?
DISCOVER	• Track a PT and aPTT specimen from testing in the laboratory to results reporting. Assess the following: proper ID on tube, proper anticoagulant, acceptable QC for that run, if critical result is called, reference intervals on patient report, recommendation

for laboratory tests to monitor coumadin and heparin, INR calculation and all associated parameters are correct.
- When was the last time you performed a calibration procedure for directly measured coagulation analytes? How did you verify the calibration?

HEM.37150 DIC **Phase II**

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

REFERENCES
1) Bovill EG. Laboratory diagnosis of disseminated intravascular coagulation. Sem Hematol. 1994;31(2;suppl)35-29
2) Clinical and Laboratory Standards Institute. *Procedure for Determining Packed Cell Volume by the Microhematocrit Method; Approved Standard;* 3rd ed. CLSI document H07-A3. CLSI, Wayne, PA, 2000.
3) Clinical and Laboratory Standards Institute. *Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline;* 2nd ed. CLSI document H30-A2. CLSI, Wayne, PA, 2001.
4) Yu M, *et al*. Screening tests of disseminated intravascular coagulation: guidelines for rapid and specific laboratory diagnosis. *Crit Care Med.* 2000;28:1777-1780
5) Kotke-Marchant K (ed). An Algorithmic Approach to Hemostasis Testing. CAP Press: 2008

HEM.37175 Platelet-poor Plasma **Phase I**

At least annually, 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 X 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, XII, and vWF; thus filtration of plasma to achieve a platelet-poor specimen is discouraged. aPTT, prothrombin time/international normalized ratio (PT/NR) and thrombin clotting time (TT) performed on fresh plasma samples are not affected by platelet counts of at least up to 200 x 10^9/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
1) Lupus Anticoagulant Working Party. Guidelines on testing for the lupus anticoagulant. *J Clin Pathol.* 1991;44:885-889
2) Middleton AL, Oakley E. Activated partial thromboplastin time (aPTT): Review of Methods. Chicago, IL: American Society of Clinical Pathology Check Sample PTS 91-8, 1991
3) Brien W, *et al.* Lupus anticoagulant testing: effect of the platelet count on the activated partial thromboplastin time. *Brit J Biomed Sci* 1993;50:114-116
4) Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition*. CLSI Document H21-A5 (ISBN 1-56238-657-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
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
6) Favaloro EJ, Lippi B, Adcock DM. Preanalytical and postanalytical variables: The leading causes of diagnostic error in hemostasis? *Sem in Thromb Haem*, 2008; 34:612-634

7) van den Besselaar AMHP, *et al.* Monitoring heparin therapy by the activated partial thromboplastin time--the effect of pre-analytical conditions. *Thromb Haemost*, 1990; 57(2):226-231

HEM.37200 Coagulation Tests - 37°C — Phase II

Coagulation tests (*e.g.* PT, aPTT, fibrinogen, and factor assays) are performed at 37°C.

Evidence of Compliance:

✓ Records of temperature checks or automated internal instrument temperature monitoring

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (aPTT) Test; Approved Guideline-Second Edition*. CLSI Document H47-A2. (ISBN 1-56238-672-7). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2008.
2) Clinical and Laboratory Standards Institute. *Determination of Coagulant Factor Activities Using the One-State Clotting Assay; Approved Guideline.* 2nd ed. CLSI document H48-ED2. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.

COAGULATION TEST SYSTEMS

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 (i.e. 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 (e.g. 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 to address the use of the alternative control system. Please refer to the Individualized Quality Control Plan 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)].
2) Steindel SJ, Tetrault G. Quality control practices for calcium, cholesterol, digoxin, and hemoglobin. A College of American Pathologists Q-Probes study in 505 hospital laboratories. *Arch Pathol Lab Med* 1998;122:401-408
3) Voss EM, *et al*. Determining acceptability of blood glucose meters. Statistical methods of determining error. *Lab Med.* 1996;27:601-606
4) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline.* 4th ed. CLSI document C24-ED4. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.
5) Ye JJ, *et al.* Performance evaluation and planning for patient/client-based quality control procedures. *Am J Clin Pathol.*2000;113:240-248
6) LaBeau KM, *et al.* Quality control of test systems waived by the clinical laboratory improvement amendments of 1988. Perceptions and practices. *Arch Pathol Lab Med.* 2000;124:1122-1127
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) & 42CFR.493.1269(c)(2)]

HEM.37350 QC Acceptable Limits — Phase II

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

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

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Protocol for the Evaluation, Validation, and Implementation of Coagulometers; Approved Guideline.* CLSI document H57-A (ISBN 1-56238-656-5). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2008.
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1256(d)]

HEM.37400 Alternative Method Criteria **Phase I**

For photo-optical coagulation systems, guidelines are established for determining when alternative procedures are performed (*e.g.* lipemia, hyperbilirubinemia, turbidity, *etc.*).

NOTE: Very long clotting times may not be reproducible on an automated coagulation instrument. Criteria should be established by each laboratory for performance of the PT or aPTT by an alternate technique (e.g. manual method) when the readable range of the instrument is exceeded. In addition, criteria should be provided for performance of alternate procedures in the presence of significant hyperbilirubinemia or lipemia, paradoxically short aPTTs and non-duplicating 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
1) Favaloro EJ, Lippi B, Adcock DM. Preanalytical and postanalytical variables: The leading causes of diagnostic error in hemostasis? *Sem in Thromb Haem*, 2008; 34:612-634

HEM.37600 Clot Detection **Phase II**

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 **Phase II**

For manual coagulation testing (e.g. 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
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7168 [42CFR493.1269(c)(2)]
2) Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition*. CLSI Document H21-A5 (ISBN 1-56238-657-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.

COAGULATION STUDIES BY ELECTROPHORESIS

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

HEM.37905 Daily QC - Electrophoresis **Phase 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.37910 Electrophoretic Separation **Phase II**

Electrophoretic separations are satisfactory.

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

D-DIMER STUDIES

***REVISED** 08/17/2016*

HEM.37924 D-dimer Unit Results **Phase II**

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 maintained.

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:

Manufacturer Units	Final Units	Correct Conversion Factor	Equivalency Equation
FEU ng/mL	D-DU ng/mL	0.5	1 FEU ng/mL = 0.5 D-DU ng/mL
FEU ng/mL	D-DU µg/mL	0.0005	1 FEU ng/mL = 0.0005 D-DU µg/mL
FEU µg/mL	FEU ng/mL	1000	1 FEU µg/mL = 1000 FEU ng/mL
D-DU ng/mL	FEU ng/mL	2	1 D-DU ng/mL = 2 FEU ng/mL
D-DU µg/mL	FEU ng/mL	2000	1 D-DU µg/mL = 2000 FEU ng/mL
D-DU µg/mL	D-DU ng/mL	1000	1 D-DU µg/mL = 1000 D-DU ng/mL

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

1) Clinical and Laboratory Standards Institute (CLSI). *Quantitative D-dimer for the Exclusion of Venous Thromboembolic Disease; Approved Guideline.* CLSI document H59-A (ISBN 1-56238-747-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA, 2011.

2) Olson JD, Cunninghan 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 (i.e. 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

1) Olson J, Cunningham M, Brandt J, *et al*. Use of the D-Dimer for Exclusion of VTE: Difficulties Uncovered through the Proficiency Testing Program of the College of American Pathologists (CAP). *J Thromb Hemostasis*, Abstract, August 2005
2) Spannagl M, Haverkate F, Reinauer H, Meijer P. The performance of quantitative D-dimer assays in laboratory routine. *Blood Coagul Fibrinolysis*. 2005 Sep;16(6):439-43
3) Goodacre S, Sampson FC, Sutton AJ, *et al*. Variation in the diagnostic performance of D-dimer for suspected deep vein thrombosis. QJM. 2005 Jul;98(7):513-27. Epub 2005 Jun 13
4) Gardiner C, Pennaneac'h C, Walford C, *et al*. An evaluation of rapid D-dimer assays for the exclusion of deep vein thrombosis. *Br J Haematol*. 2005 Mar;128(6):842-8
5) Diamond S, Goldweber R, Katz S. Use of D-dimer to aid in excluding deep venous thrombosis in ambulatory patients. *Am J Surg*. 2005 Jan;189(1):23-6
6) Wolf SJ, McCubbin TR, Feldhaus KM, *et al*. Prospective validation of Wells Criteria in the evaluation of patients with suspected pulmonary embolism. *Ann Emerg Med*. 2004 Nov;44(5):503-10
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
8) Stein PD, Hull RD, Patel KC, *et al*. D-dimer for the exclusion of acute venous thrombosis and pulmonary embolism: a systematic review. *Ann Intern Med*. 2004 Apr 20;140(8):589-602
9) Clinical and Laboratory Standards Institute (CLSI). *Quantitative D-dimer for the Exclusion of Venous Thromboembolic Disease; Approved Guideline.* CLSI document H59-A (ISBN 1-56238-747-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA, 2011.

HEM.37930 D-dimer Reporting **Phase II**

If a D-dimer test is used for evaluation of venous thromboembolism (VTE), the laboratory reports both the cut-off value and reference interval.

NOTE: This requirement only applies to quantitative D-dimer tests used for evaluation of VTE.

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. Both the cut-off value (evaluation of VTE) and the reference interval (e.g. DIC evaluation) must be reported. The cut-off value, reference interval, and patient results must be reported in identical units, including both unit type (FEU or D-DU) and unit of magnitude (e.g. ng/mL).

Evidence of Compliance:

✓ Patient reports including both the reference interval and the cut-off value for VTE evaluation

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Quantitative D-dimer for the Exclusion of Venous Thromboembolic Disease; Approved Guideline.* CLSI document H59-A (ISBN 1-56238-747-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA, 2011.

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.

COAGULATION FACTOR ASSAYS (INCLUDING FIBRINOGEN)

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

HEM.37940 Standard Curve **Phase II**

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

NOTE: Plotting less than 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

1) Arkin CF, *et al*. Factors affecting the performance of Factor VIII coagulant activity assays. Results of proficiency surveys of the College of American Pathologists. *Arch Pathol Lab Med.* 1992;116:908-915

2) Clinical and Laboratory Standards Institute. *Determination of Coagulant Factor Activities Using the One-State Clotting Assay; Approved Guideline.* 2nd ed. CLSI document H48-ED2. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.

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 (e.g. two or more points with assayed reference plasma) each time ensures accuracy of the result.

Evidence of Compliance:

✓ Written procedure describing the verification of standard curves with two reference points **AND**

✓ Records of QC at defined frequency

REFERENCES

1) Clinical and Laboratory Standards Institute. *Determination of Coagulant Factor Activities Using the One-State Clotting Assay; Approved Guideline.* 2nd ed. CLSI document H48-ED2. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.

HEM.37980 Factor Assay Criteria **Phase II**

Three or more dilutions are plotted for each factor assay.

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

This requirement does not apply to fibrinogen assays.

Evidence of Compliance:

✓ Records or worksheets showing patient data analyzed at three or more dilutions

REFERENCES

1) Clinical and Laboratory Standards Institute. *Determination of Coagulant Factor Activities Using the One-State Clotting Assay; Approved Guideline.* 2nd ed. CLSI document H48-ED2. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.
2) Clinical and Laboratory Standards Institute. *Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline;* 2nd ed. CLSI document H30-A2. CLSI, Wayne, PA, 2001.
3) Acquired Hemophilia. Kessler C, Garvey MB, Green D, Kasper C, Luster J (editors), Excerpta Medica, Inc. Princeton, NJ, 1995. Chapter 2, Laboratory Diagnosis of FVIIII Inhibitors. C.K. Kaspar

HEM.37982 Inhibitor Effect **Phase II**

When factor assays are performed, the laboratory reports apparent inhibitor effects.

NOTE: The goal is to provide clinically useful data when a non-specific inhibitor activity is detected, e.g. 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. Inhibitor effect is not applicable to fibrinogen assays.

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: Inhibitor interference is not applicable to fibrinogen assays.

REFERENCES

1) Acquired Hemophilia. Kessler C, Garvey MB, Green D, Kasper C, Luster J (editors), Excerpta Medica, Inc. Princeton, NJ, 1995. Chapter 2, Laboratory Diagnosis of FVIIII Inhibitors. C.K. Kaspar

MIXING STUDIES

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

HEM.37991 Mixing Studies Procedure **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

1) Kaczor DA, *et al.* Evaluation of different mixing study reagents and dilution effect in lupus anticoagulant testing. *Am J Clin Pathol.* 1991;95:408-411
2) Clinical and Laboratory Standards Institute (CLSI). *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (aPTT) Test; Approved Guideline-Second Edition*. CLSI Document H47-A2. (ISBN 1-56238-672-7). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2008.

HEM.38002 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 (e.g. heparin, factor Xa inhibitors or direct thrombin inhibitors) may give positive results in mixing study assays. Laboratories must have procedures established to screen mixing study samples with elevated PT and/or aPTT results for these anticoagulant drugs. For heparin, performing a thrombin time assay, heparin Xa inhibition assay, repeating the aPTT with polybrene, or treating the sample with heparinase may be acceptable. For direct thrombin inhibitors, performing a thrombin time should detect the presence of the inhibitor. A thrombin time should be greatly prolonged (or even give a "clot undetected" result) in the presence of a direct thrombin inhibitor. A thrombin time has the advantage of detecting not only heparin, but also the presence of direct thrombin inhibitors such as lepirudin, bivalirudin and argatroban. Alternately, the test result from a positive mixing study 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
2) Harsfalvi J, *et al*. The use of polybrene for heparin neutralization in protein C activity assay. *Blood Coag Fibrinolysis*. 1990;1:357-361
3) Haynes SR, *et al*. Accuracy of coagulation studies performed on blood samples obtained from arterial cannulae. *Brit J Anaesth*. 1992;69:599-601
4) Carlsson SC, Mattsson C, Eriksson UG, *et al*. A review of the effects of the oral direct thrombin inhibitor ximelagatran on coagulation assays. *Thromb Res*. 2005;115(1-2):9-18
5) Chang SH, Tillema V, Scherr D.A "percent correction" formula for evaluation of mixing studies. *Am J Clin Pathol*. 2002 Jan;117(1):62-73
6) Kaczor DA, Bickford NN, Triplett DA. Evaluation of different mixing study reagents and dilution effect in lupus anticoagulant testing. *Am J Clin Pathol*. 1991 Mar;95(3):408-11

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 verification (AMR) 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 chromagenic assays for antithrombin activity, protein C activity, and heparin or low molecular weight heparin. This list is not exhaustive, and laboratory directors should review their laboratory's test menu to identify additional tests which 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 AND CALIBRATION VERIFICATION

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

CALIBRATION VERIFICATION denotes the process of confirming that the current calibration settings for each analyte remain valid for a test system. If calibration verification confirms that the current calibration settings for each analyte are valid, it is not necessary to perform a complete calibration or recalibration of the test system. Each laboratory must define limits for accepting or rejecting tests of calibration verification. 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 method calibration materials as unknown specimens, and determination that the correct target values are recovered, and (2) assay of matrix-appropriate materials with target values that are specific for the test system.

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

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

4. *When recommended by the manufacturer*

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

1. *Calibrators used to calibrate the analytical system*
2. *Materials provided by the analytical measurement system vendor for the purpose of calibration verification*
3. *Previously tested unaltered patient/client specimens*
4. *Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method,*
5. *Third party general purpose reference materials that are suitable for verification of calibration following reagent lot changes if the material is listed in the package insert or claimed by the method manufacturer to be commutable with patient specimens for the method. A commutable reference material is one that gives the same numeric result as would a patient specimen containing the same quantity of analyte in the analytic method under discussion; i.e. matrix effects are absent. Commutability between a reference material and patient specimens can be demonstrated using the protocol in CLSI EP14-A3,*
6. *Proficiency testing material or proficiency testing validated material with matrix characteristics and target values appropriate for the method*

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

ANALYTICAL MEASUREMENT RANGE

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

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

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

AMR VERIFICATION

Minimum requirements for AMR verification can be met by using matrix appropriate materials, which include the low, mid and high concentration or activity range of the AMR and recovering appropriate target values, within defined acceptance criteria. Records of the AMR verification process must be available.

The best practice for AMR verification is to demonstrate a linear relationship, within defined acceptance criteria, between measured concentrations of analytes and expected values for a set of four or more matrix-appropriate samples that cover the AMR.

AMR verification may be accomplished through the calibration process under certain circumstances. It is not necessary to perform a separate AMR verification if the process of calibration of an assay includes calibrators that span the full range of the AMR, with low, midpoint and high values (i.e. three points) included. A one-point or two-point calibration does not include all of the necessary points to validate the AMR.

REQUIRED FREQUENCY OF AMR VERIFICATION
When initially introducing a new method, it is necessary to verify the AMR independently from the calibration process. In this situation, suitable materials for the AMR verification include those listed below (see OTHER MATERIALS SUITABLE FOR AMR VERIFICATION). Additionally, when multipoint calibration that spans the AMR is utilized, a set of calibrators from a different lot number than that used to calibrate the system may be suitable for independent AMR verification.

The AMR must be verified at least every six months after a method is initially placed in service and following the criteria defined in the checklist. If multipoint calibrators that span the AMR are used for calibration/calibration verification, it is not necessary to independently verify the AMR, as long as the system is calibrated at least every six months.

OTHER MATERIALS SUITABLE FOR AMR VERIFICATION
The materials used for AMR verification must be known to have matrix characteristics appropriate for the method. The matrix of the sample (i.e. the environment in which the sample is suspended or dissolved) may influence the measurement of the analyte. In many cases, the method manufacturer will recommend suitable materials. The verification must include specimens, which at a minimum, are near the low, midpoint, and high values of the AMR. Suitable materials for AMR verification include the following:

1. *Linearity material of appropriate matrix, e.g. CAP CVL Survey-based or other suitable linearity verification material*
2. *Previously tested patient/client specimens, that may be altered by admixture with other specimens, dilution, spiking in known amounts of an analyte, or other technique*
3. *Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method*
4. *Patient samples that have reference method assigned target values*
5. *Control materials, if they adequately span the AMR and have method specific target values*

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 method manufacturer's instructions for verifying the AMR should be followed, when available. Specimen target values can be established by comparison with peer group values for reference materials, by assignment of reference or comparison method values, and by dilution ratios of one or more specimens with known values. The Laboratory Director must define limits for accepting or rejecting tests of the AMR. One option is to establish imprecision and percent recovery limits for an assay and then determine at what point at the top and bottom of the AMR these limits fail.

PATIENT SAMPLES WITH UNUSUALLY HIGH CONCENTRATIONS OF ANALYTE
In the case of samples with very high concentrations or activities of an analyte, very large dilutions may be required to bring the concentration or activity into the AMR. Making large dilutions of patient samples can

introduce error, and the Laboratory Director should establish appropriate volumes of sample and diluent to be used to minimize dilution errors. For example, pipetting 1 µL of a sample is difficult to do accurately and larger sample and diluent volumes should be specified. Note that for some analytes, an acceptable dilution protocol may not exist because dilution would alter the analyte or the matrix causing erroneous results, e.g. free drugs or free hormones. Also note that for some analytes, there may be no clinical relevance to reporting a numeric result greater than a stated value. If it is not possible to achieve a measured value that is within the AMR by using allowable dilutions, or there is no clinical value to reporting a higher value, then the result may be reported as "greater than" the value of the highest allowable dilution.

HEM.38003 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 and criteria for acceptable performance.

REFERENCES

1) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 1992(Feb 28):7165 [42CFR493.1217]
2) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register*. 2003(Jan 24):3707 [42CFR493.1255]
3) Kroll MH, Emancipator K. A theoretical evaluation of linearity. *Clin Chem.* 1993;39:405-413
4) Clinical and Laboratory Standards Institute. *Evaluation of Matrix Effects; Approved Guideline.* 3rd ed. CLSI Document EP14-A3. Clinical and Laboratory Standards Institute, Wayne, PA; 2014
5) Miller WG. "Quality control." Professional Practice in Clinical Chemistry: A Companion Text, ed DR Dufour. Washington, DC: AACC Press, 1999:12-1 to 12-22
6) Kroll MH, *et al*. Evaluation of the extent of nonlinearity in reportable range studies. *Arch Pathol Lab Med.* 2000;124:1331-1338

HEM.38006 Recalibration/Calibration Verification Criteria **Phase II**

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

NOTE: Criteria typically include:

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

Materials that may be used for calibration verification include, but are not limited to:

1. *Calibrators used to calibrate the analytical measurement system*
2. *Materials provided by the analytical measurement system vendor for the purpose of calibration verification*
3. *Previously tested unaltered patient/client specimens*
4. *Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method*
5. *Third party general purpose reference materials that are suitable for verification of calibration following reagent lot changes if the material is listed in the package insert*

or known by the method manufacturer to be commutable with patient specimens for the method. A commutable reference material is one that gives the same numeric result as a patient specimen containing the same quantity of analyte in the analytic method under discussion; i.e. matrix effects are absent. Commutability between a reference material and patient specimens can be demonstrated using the protocol in CLSI EP14-A3.

6. *Proficiency testing material or proficiency testing validated material with matrix characteristics and target values appropriate for the method.*

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

Evidence of Compliance:

- ✓ Written procedure defining the method, frequency and limits of acceptability of calibration verification **AND**
- ✓ Records of calibration verification and/or recalibration at defined frequency

REFERENCES

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

HEM.38007 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

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1255(a)(3)]

HEM.38009 AMR Verification — Phase II

Verification of the analytical measurement range (AMR) is performed with matrix-appropriate materials, which, at a minimum, include the low, mid and high range of the AMR, appropriate acceptance criteria are defined, and the process is recorded and reviewed.

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

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

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

1. *A change of reagent lots for chemically or physically active or critical components, unless the laboratory can demonstrate that the use of different lots does not affect*

the accuracy of patient/client test results, and the range used to report patient/client test data.

2. *If QC 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*
3. *After major preventive maintenance or change of a critical instrument component*
4. *When recommended by the manufacturer*

Evidence of Compliance:

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

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):3707 [42CFR493.1255]
2) Anne Ford. As coag tests evolve, so do checklist requirements. Northfield, IL; College of American Pathologists. *CAP Today* November 2012

HEM.38010 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

HEM.38011 Maximum Dilution — Phase II

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

NOTE:

1. *For each analyte, the laboratory protocol should define the maximum dilution that falls within the AMR and that can be subsequently corrected by the dilution factor*

to obtain a reportable numeric result. Note that for some analytes, an acceptable dilution protocol may not exist because dilution would alter the analyte or the matrix causing erroneous results. Also note that, for some analytes, there may be no clinical relevance to reporting a numeric result greater than a stated value.

2. *Analytes for which a dilution protocol is unable to bring the activity or concentration into the AMR should be reported as "greater than" the highest estimated values.*
3. *Establishment of allowable dilutions is performed when a method is first placed into service and is reviewed biennially thereafter as part of the procedure manual review by the Laboratory Director or designee. The laboratory director is responsible for establishing the maximum allowable dilution of samples that will yield a credible laboratory result for clinical use.*

Evidence of Compliance:

✓ Patient reports or worksheets

PLATELET FUNCTION STUDIES

HEM.38013 Specimen Handling - Platelets **Phase II**

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

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

Evidence of Compliance:

✓ Written policy defining the specimen handling requirements prior to analysis

REFERENCES

1) Winokur R, Hartwig JH. Mechanism of shape change in chilled human platelets. *Blood.* 1995; 85:1796-1804
2) Clinical and Laboratory Standards Institute (CLSI). *Platelet Function Testing by Aggregometry; Approved Guideline*. CLSI document H58-A. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
3) Mani H, Kitchmayr K, Klaffling C, et al. Influence of blood collection techniques on platelet function. *Platelets.* 2004;15(5):315-318
4) Kattlove HE, Alexander B. The effect of cold on platelets. I. Cold-induced platelet aggregation. *Blood.* 1971;38(1):39-48
5) Kattlove HE, Alexander B, White F. The effect of cold on Platelets. II. Platelet function after short-term storage at cold temperatures. *Blood*. 1972;40(5):688-695

HEM.38024 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.38028 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_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
1) Clinical and Laboratory Standards Institute (CLSI). *Platelet Function Testing by Aggregometry; Approved Guideline*. CLSI document H58-A. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
2) Silver WP, Keller MP, Teel R, Silver D. Effects of donor characteristics and platelet in vitro time and temperature on platelet aggregometry, *J Vasc Surg.* 1993;17(4):7826-733
3) Zucker MB. Platelet aggregation measured by the photometric method. *Methods Enzymol.* 1989;169:117-133
4) Elijah W. Muriithi, *et al*. Heparin-induced platelet dysfunction and cardiopulmonary bypass. *Am J Clin Path* 1989; 91:155-164

HEM.38035 Optical Aggregation Method — Phase I

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

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

Evidence of Compliance:

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

REFERENCES
1) Cattaneo M, *et al*. Platelet aggregation studies: autologous platelet-poor plasma inhibits aggregation when added to platelet-rich plasma to normalize platelet count. *Haematologica.* 2007;92:694-7
2) Linnemann B, *et al*. Standardization of light transmittance aggregometry for monitoring antiplatelet therapy: and adjustment for platelet count is not necessary. *J Thromb Haemost.* 2008;6:677-83
3) Favaloro EF, *et al*. Platelet function testing: auditing local practice and broader implications. *Clinical Laboratory Science.* 2010;23:21-31
4) Hayward CPM, *et al*. Development of North American Consensus Guidelines for medical laboratories that perform and interpret platelet function testing using light transmission aggregometry. *Am J Clin Pathol*. 2010;134:955-63
5) Castiloux JF, *et al*. A prospective cohort study of light transmission platelet aggregometry for bleeding disorders: Is testing native platelet-rich plasma non-inferior to testing platelet count adjusted samples? *Thromb Haemost*. 2011;106:675-82