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

07.28.2015

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.

For this sample checklist, the following are requirements taken from the Hematology Checklist to illustrate the scope covered under the discipline of Hematology.

SPECIMEN COLLECTION AND HANDLING - HEMATOLOGY

Inspector Instructions:

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

HEM.22000 Collection in Anticoagulant **Phase II**

All blood specimens collected in anticoagulant for hematology testing are mixed thoroughly immediately before analysis.

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

Evidence of Compliance:

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

REFERENCES
1) CLSI. *Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition*. CLSI

Document H4-A6 (ISBN 1-56238-677-8). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008
2) Clinical and Laboratory Standards Institute (CLSI). *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard - Sixth Edition*. CLSI Document H3-A6 (ISBN 1-56238-650-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2007.

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 (CLSI). *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard - Sixth Edition*. CLSI Document H3-A6 (ISBN 1-56238-650-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2007.
5) Clinical and Laboratory Standards Institute (CLSI). *Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition*. CLSI Document H4-A6 (ISBN 1-56238-677-8). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
6) Clinical and Laboratory Standards Institute. *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard.* 6th ed. CLSI Document GP41-A6. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.
7) Broden PN. Anticoagulant and tube effect on selected blood cell parameters using Sysmex NE-series instruments. *Sysmex J Intl.* 1992;2:112-119
8) Brunson D, *et al*. Comparing hematology anticoagulants: K2EDTA vs K3EDTA. *Lab Hematol.* 1995;1:112-119
9) Boos MS, *et al*. Temperature- and storage-dependent changes in hematologic variable and peripheral blood morphology. *Am J Clin Pathol.* 1998;110:537
10) 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

*****REVISED**** ***07/28/2015***

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.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.

SPECIMEN COLLECTION AND HANDLING - COAGULATION

Inspector Instructions:

READ	• Sampling of coagulation specimen collection and handling policies and procedures • Sampling of specimen rejection records/log
OBSERVE	• Sampling of patient coagulation specimens (anticoagulant, labeling)
ASK	• 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 (CLSI). *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard - Sixth Edition*. CLSI Document H3-A6 (ISBN 1-56238-650-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2007.

HEM.22748 Anticoagulant - Coagulation — Phase I

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

NOTE: Sodium citrate is effective as an anticoagulant due to its mild calcium-chelating properties. Of the 2 commercially available forms of citrate, 3.2% buffered sodium citrate (105-109 mmol/L of the dihydrate form of trisodium citrate $Na_3C_6H_5O_7 \cdot 2H_2O$) 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. Coagulation testing cannot be performed in samples collected in EDTA due to the more potent calcium chelation. Heparinized tubes are not appropriate due to the inhibitory effect of heparin on multiple coagulation proteins. Testing for platelet function 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 **OR** procedure with an alternative anticoagulant defined with records of validation data

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.

HEM.22789 Specimen Rejection Criteria - Coagulation — Phase I

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

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

Evidence of Compliance:

✓ Records of rejected specimens

REFERENCES

1) Peterson P, Gottfried EL. The effects of inaccurate blood sample volume on prothrombin time (PT) and activated partial thromboplastin time. *Thromb Haemost.* 1982;47:101-103
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.

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

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

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

REFERENCES
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

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

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.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 a reference 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

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.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.