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:

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

HEM.22000  Collection in Anticoagulant

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
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HEM.22050  CBC Anticoagulant

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
3) Rabinovitch A. Anticoagulants, platelets and instrument problems. Am J Clin Pathol. 1984;82:133

HEM.22100  Capillary Tube Collection Criteria

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

NOTE: Microspecimen containers such as those used for 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

HEM.22200  Hemolyzed or Lipemic Specimens - CBC

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

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

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

REFERENCES

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
HEM.22789 Specimen Rejection Criteria - Coagulation  

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
HEM.22871 Specimen Quality Assessment - Coagulation Phase II

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

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

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

AUTOMATED DIFFERENTIAL COUNTERS

Inspector Instructions:

- Automated differential procedure
- Sampling of GC 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
3) Etzell JE. For WBC differentials reporting absolute numbers. CAP Today, March 2001
5) Ross DW, Bentley SA. Evaluation of an automated hematology system (Technicon H1). Arch Pathol Lab Med. 1986;110:803-808

HEM.34200 WBC Differential Verification Phase II
The laboratory establishes criteria for checking and reviewing leukocyte differential counter data, histograms, and/or blood films for clinically important results flagged by the automated differential counter.

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

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

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

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

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

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

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

HEM.35984 Hb S Predominant Band Phase II

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

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

Evidence of Compliance:
✓ Written 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
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
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**

**Bone marrow reports and smears are retained for 10 years.**