8. FIELD CENTER HEMATOLOGY SERVICES

8.1 Clinical Significance

Quantitation of the formed elements of the blood (erythrocytes -RBCs, leukocytes - WBCs, and platelets) is important in the ARIC study primarily so that the associations of the formed elements with atherosclerosis and its clinical manifestations can be studied. The association of elevated WBC count with the risk of cardiovascular disease requires confirmation. These determinations are also of value in recognizing asymptomatic disorders (e.g., anemia, leucocytosis, and thrombocytopenia) which may require the ARIC participant's referral to his usual source of care for further medical evaluation.

8.2 Principles of Quantitative Hematologic Determinations (1)

Procedure for counting circulating blood cells, whether manual or automated, all involve a sequence of (1) diluting the blood specimen, (2) aliquotting the diluted specimen into a measured volume, and (3) counting the cells in that volume.

All hospital based and independent laboratories now use automated instruments to count blood cells. These instruments work on either of two basic principles. In the first type of instrument employing electronic particle counting (e.g., the Coulter counter, Coulter Diagnostics, Hialeah, FL), blood cells pass through an aperture through which an electrical current is passed. The change in electrical resistance caused by the cell's passage is counted as a voltage pulse. Combinations of aperture size and threshold/window discrimination of voltage pulse height allow distinctions between erythrocytes, leukocytes, and platelets. The second type of instrument (e.g., Hemalog H-6000, Technicon Corporation, Tarrytown, NY) uses light-scattering from cells flowing through a counting chamber. Scattered light is detected by a photomultiplier tube, and cell number and size are evaluated as voltage pulses.

Automated hematology analyzers directly measure the cell counts for total RBCs, WBCs, and platelets. Total hemoglobin (Hb) is measured by the formation of hemoglobincyanide (HICN). The hematocrit (Hct) is calculated from the measurement of RBCs and either the calculated erythrocyte mean cell volume (Coulter Counter) or pattern of light-scattering (Hemalog H-6000). The hematocrit, as calculated by these automated analyzers, may differ from the hematocrit as determined directly by centrifugation ("Packed cell volume"). These differences are usually not significant in normal subjects with correct handling of samples.

Three red cell indices are calculated by automated hematology analyzers: (1) the mean cell volume (MCV), (2) the mean cell hemoglobin (MCH), and (3) the mean cell hemoglobin concentration (MCHC). The following formulas are used in these derivations:

$$(1) \quad \text{MCV (ul or fl)} = \frac{\text{Hct} \times 1000}{\text{RBCs (106/ul)}}$$

$$(2)\quad \text{MCH (pg)} = \frac{\text{Hb (g/L)}}{\text{RBC (106/ul)}}$$

$$(3)\quad \text{MCHC (g/dL)} = \frac{\text{Hb (g/Dl)}}{\text{Hct}}$$

fl = femtoliter
pg = picograms
g/Dl = grams per deciliter
ul = microliter
g/L = grams per liter

These indices are clinically useful in recognizing and classifying various types of anemias. If the primary erythrocytic measurements (Hct, RBC count and Hb) are normal, these indices will also be essentially normal.

8.3 General Operation of Field Center Hematology Studies

In contrast to the other types of laboratory determinations in the ARIC study which are performed at a central laboratory (e.g., coagulation, lipids), hematology procedures use specimens collected in EDTA which cannot be shipped to distant sites without jeopardizing sample stability and reducing reliability.

Each ARIC Field Center uses a local reference laboratory to perform the routine hematology procedures specified by the ARIC protocol. These laboratories are responsible for prompt specimen pickup, analysis, and result reporting. Although whole blood specimens collected in EDTA are stable for up to 24 hours at 4°C, it is desirable that ARIC specimens collected in the morning at the Field Center be analyzed that day by the reference laboratory. Specimens collected by the Field Centers in the afternoon are analyzed promptly after storage at 4°C. (EDTA is the only acceptable anticoagulant for samples to be analyzed for cell counts. [Heparin produces variable artifacts of cell size.] The professional staff at each Field Center periodically review the performance of the laboratory performing ARIC hematology studies, particularly in terms of the laboratory's quality control program for automated hematology.

8.4 Calibration and Interlaboratory Standardization

Each field center utilizes the services of one or more local hematology laboratories. Jackson uses the hematology laboratory at the University of Mississippi Medical Center seven days a week. The Jackson laboratory runs all ARIC hematology specimens on two instruments, the Coulter S + IV and the Technicon H-6000. Washington County uses the services of one hematology laboratory, the Hagerstown Medical Laboratory, to process samples during the week. The Minneapolis field center sends blood samples to one hematology laboratory which uses a Coulter S + IV. Forsyth County uses one hematology laboratory, Roche-Biomedical Laboratories, Inc., which uses a Technicon H-6000 for processing ARIC samples. A technical summary of type of instruments, calibration and quality control is provided in Table 1. Three of the four laboratories use the same type of hematology analyzer with similar calibration procedures (as shown in Table 1), thus reducing problems with interlaboratory standardization. Standardization of the processing of hematology specimens, however, remains problematic as illustrated (Savage; RA, 1985).

No stable reference materials are available for standardizing cell counts. The International Committee for Standardization in Hematology and the College of American Pathologists have both recognized that an automated hematology calibrator material that possesses the physical and chemical characteristics of fresh whole blood and is of sufficient stability to be analyzed by reference methods and distributed to hospital and outpatient laboratories of recalibration purposes is not now available nor is likely to appear because of the technical inadequacies of surrogates for white blood cells and platelets and to the compromises in matrix composition that are necessary in rendering the product stable for long-term analysis (Savage, RA, 1985).

Table 1. ARIC Field Center Hematology: Technical Summary

Field Center	Instrument	Calibration	Quality Control
Jackson, MS	Coulter S + IV	S-Cal	CAP Survey[1] Patient Samples
Washington County, MD	Coulter S + IV	S-Cal	CAP Survey Patient Samples
Minneapolis, MN	Coulter S + IV	S-Cal	CAP Survey
Forsyth County, NC	Technicon H-6000	Fisher Computrol	CAP Survey Medicare Survey Staff Samples

[1] CAP Survey - College of American Pathologists Survey

Laboratories currently use stabilized materials prepared by the manufacturers of automated hematology analyzers to calibrate their instruments. Thus, interlaboratory standardization in this area depends upon the widespread use of the calibrator.

8.5 Precision

Precision of cell counts within the laboratory relies upon (1) replicate determinations performed on the same specimen over a 24-hour period, (2) use for stabilized cell suspensions, (3) calculation of a "moving average" of all patient results, or (4) some combination of two or more of the preceding quality control methods (3). The precision (expressed as coefficient of variation (CV) of routine automated hematology assays and the minimum bias which can be detected by either replicate assays of fresh blood or serial assays of stabilized blood are summarized in Table 2 (modified from Bull, BS, 1982). Precision is better (and thus minimum detectable bias is less) for erythrocyte than for leucocyte or platelet counts.

Table 2. Precision of Routine Automated Hematology Assays

Routine Assay (CV)	Type of Whole Blood Control	Minimum Detectable Bias % Day	Week	Month
1. Cyanmethemoglobin (1%)	Fresh	2		
	Stabilized	2	2	2
2. RBC, by automated (<1%) counter	Fresh	2		
	Stabilized	2	4	7
3. Hct or MCV by automated (<1%) counter	Fresh	2		
	Stabilized	2	4	7
4. WBC by automated (2%) counter	Fresh	6		
	Stabilized	6	8	8
5. Platelet count by (4%) automated counter	Fresh	12		
	Stabilized	12	15	15

Source: modification from Bull BS

Markedly abnormal hematology results are to be telephoned back to the Field Center, according to the criteria specified in ARIC Manual 2.

8.6 Accuracy of Automated Hematology Procedures

Interlaboratory comparability of these data can also be evaluated by mailed proficiency-testing samples in programs operated by external organizations. All the laboratories selected by the Field Centers participate in the hematology proficiency survey of the College of American Pathologists (CAP). Table 1 also shows the other quality control procedures used by each laboratory.

The major interlaboratory variable in automated hematology which these external proficiency programs have identified is that of the material used for calibration. As indicated previously, three of the four laboratories participating in the ARIC Field Center hematology studies use the same material.

8.7 Reporting of Results

The laboratories performing automated hematology for the ARIC Field Centers have the responsibility for reporting results formatted (either manually or electronically) for incorporation into the ARIC data base. Five data elements are included as hematology results in this data base:

1) Total hemoglobin (Hb)
2) Leucocyte (WBC) count
3) Platelet count
4) Hematocrit
5) Differential

8.8 References

1. Henry JB, ed. Clinical Diagnosis and Management by Laboratory Methods. Philadelphia: W.B. Saunders, 1985, pp. 586-601.

2. Savage RA. Calibration bias and imprecision for automated hematology analyzers. An evaluation of short-term bias resulting from calibration of an analyzer with S-Cal. AM J Clin Pathol 84:186-190, 1985.

3. Lappin TRJ, Farrington CL, Nelson MG, Merrett JD. Intralaboratory quality control of hematology. Comparison of two system. Am J Clin Pathol 72:426-431, 1979.

4. Bull BS. The use of patient values, calibrator and control materials in the routine laboratory. In Advances in Hematological Methods: The Blood Count. Van Assenfeldt OW, England JM, eds. Boca Raton: CRC Press, 1982, pp. 217-227.