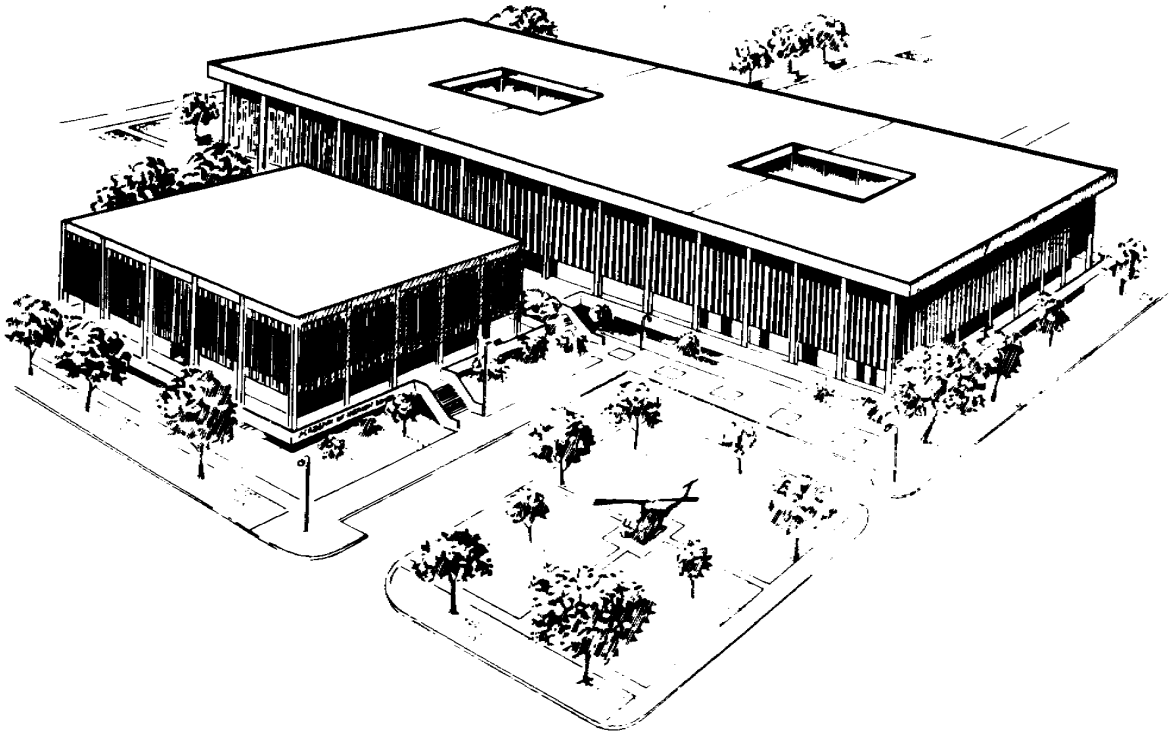
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
FORT SAM HOUSTON, TEXAS 78234-6100



HEMATOLOGY II

SUBCOURSE MD0857 EDITION 100

DEVELOPMENT

This subcourse is approved for resident and correspondence course instruction. It reflects the current thought of the Academy of Health Sciences and conforms to printed Department of the Army doctrine as closely as currently possible. Development and progress render such doctrine continuously subject to change.

ADMINISTRATION

Students who desire credit hours for this correspondence subcourse must enroll in the subcourse. Application for enrollment should be made at the Internet website: http://www.atrrs.army.mil. You can access the course catalog in the upper right corner. Enter School Code 555 for medical correspondence courses. Copy down the course number and title. To apply for enrollment, return to the main ATRRS screen and scroll down the right side for ATRRS Channels. Click on SELF DEVELOPMENT to open the application; then follow the on-screen instructions.

For comments or questions regarding enrollment, student records, or examination shipments, contact the Nonresident Instruction Branch at DSN 471-5877, commercial (210) 221-5877, toll-free 1-800-344-2380; fax: 210-221-4012 or DSN 471-4012, e-mail accp@amedd.army.mil, or write to:

NONRESIDENT INSTRUCTION BRANCH
AMEDDC&S
ATTN: MCCS-HSN
2105 11TH STREET SUITE 4191
FORT SAM HOUSTON TX 78234-5064

Be sure your social security number is on all correspondence sent to the Academy of Health Sciences.

CLARIFICATION OF TERMINOLOGY

When used in this publication, words such as "he," "him," "his," and "men" 'are intended to include both the masculine and feminine genders, unless specifically stated otherwise or when obvious in context.

USE OF PROPRIETARY NAMES

The initial letters of the names of some products may be capitalized in this subcourse. Such names are proprietary names, that is, brand names or trademarks. Proprietary names have been used in this subcourse only to make it a more effective learning aid. The use of any name, proprietary or otherwise, should not be interpreted as endorsement, deprecation, or criticism of a product; nor should such use be considered to interpret the validity of proprietary rights in a name, whether it is registered or not.

TABLE OF CONTENTS

CORRESPONDENCE COURSE OF
THE U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL

SUBCOURSE MD0857

HEMATOLOGY II

INTRODUCTION

This subcourse is concerned with the blood tests performed in the hematology section of the laboratory. The purpose of these tests is to aid the physician in diagnosis. Thus, these tests are important and often essential to the health and life of the patient. Thorough study of this subcourse should enable you to better fulfill your role in health care.

ACKNOWLEDGEMENT

Portions of this subcourse are extracted from TM 8-227-4, Clinical Laboratory Procedure--Hematology, dated 5 December 1973; from Brown, Barbara, Hematology Principles and Procedures, 4th ed., Lea and Febiger; and from Operator's Manual for QBC II Centrifugal Hematology System, Clay Division of Becton Dickinson Company. Written consent of the copyright owner has been obtained. Under no circumstances will this material be sold, commercially used, or copied.

Subcourse Components:

The subcourse instructional material consists of seven lessons as follows:

Lesson 1, Differential Leukocytes Count and Other Procedures
Lesson 2, Blood Coagulation.
Lesson 3, Automation.
Glossary of terms

Here are some suggestions that may be helpful to you in completing this subcourse:

--Read and study each lesson carefully.

--Complete the subcourse lesson by lesson. After completing each lesson, work the exercises at the end of the lesson, marking your answers in this booklet.

--After completing each set of lesson exercises, compare your answers with those on the solution sheet that follows the exercises. If you have answered an exercise incorrectly, check the reference cited after the answer on the solution sheet to determine why your response was not the correct one.

Credit Awarded:

Upon successful completion of the examination for this subcourse, you will be awarded 8 credit hours.

To receive credit hours, you must be officially enrolled and complete an examination furnished by the Nonresident Instruction Branch at Fort Sam Houston, Texas.

You can enroll by going to the web site http://atrrs.army.mil and enrolling under "Self Development" (School Code 555).

A listing of correspondence courses and subcourses available through the Nonresident Instruction Section is found in Chapter 4 of DA Pamphlet 350-59, Army Correspondence Course Program Catalog. The DA PAM is available at the following website: http://www.usapa.army.mil/pdffiles/p350-59.pdf.

LESSON ASSIGNMENT

LESSON 1	Differential Leukocyte Count and Other Procedures.
TEXT ASSIGNMENT	Paragraphs 1-1 through 1-12.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

1-1. Select the statement that best describes the appropriate materials and procedures used to specially stain and examine blood smears.

1-2. Select the correct principles and steps used in the calculation of erythrocyte indices and in performing the various erythrocyte fragility tests.

1-3. Select the statement that best describes the principles used in the demonstration of LE cells.

1-4. Select the materials and procedures used in performing a screening test for G-6-PD deficiency.

SUGGESTION After completing the assignment, complete the exercises of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 1

DIFFERENTIAL LEUKOCYTE COUNT AND OTHER PROCEDURES

Section I. DIFFERENTIAL LEUKOCYTE COUNT

1-1. INTRODUCTION

a. The critical examination of a blood smear includes the following: quantitative and qualitative study of platelets, differential count quantitating the three types of leukocytes (granulocytes, lymphocytes, monocytes), and morphological characteristics of erythrocytes and leukocytes. Staining the blood smears is a critical part of the examination. The procedure for staining is described in Lesson 3 of Subcourse MD0853. To accurately perform the differential count it is necessary for a technician to recognize all the characteristics of normal blood cells. This includes normal biological variation. For instance, not every lymphocyte is exactly the same size, nor do all lymphocytes have exactly the same number of azurophilic granules.

b. Certain morphological and histochemical characteristics are utilized to differentiate blood cells. A review of the significant features promotes a better understanding of blood differentials. Cellular characteristics such as relative size, shape, cytoplasmic granulation, nuclear- cytoplasmic ratio, nuclear configuration, chromatin or nucleoli are very important. These features are discussed in Subcourse MD0853, lesson 4.

c. Experience is the foremost teacher in hematology. It is readily acquired in a busy hematology section where the opportunity for differential analysis occurs frequently. Experience can be diversified and interesting if proficiency slides and material from cases of confirmed diagnoses are maintained as study sets. This study material should be available to all technicians in the laboratory.

d. All routine blood smears should be kept until the physicians have reviewed the differential reports. A 1-week period is usually adequate. Occasionally, a review of a specific problem slide results in findings that were not originally apparent and reinforces confidence in the laboratory by the medical staff. This practice also adds to the experience and proficiency of the technician.

1-2. EXAMINATION OF PERIPHERAL BLOOD SMEARS

a. **Principle.** The stained blood smear permits the study of the appearance and the identification of the different kinds of leukocytes, and the appearance of erythrocytes and thrombocytes (blood platelets).

b. **Differential Leukocyte Count.**

(1) Inspect the smear under low power magnification. Locate the thin end of the smear where there is no overlapping of erythrocytes.

(2) Switch to oil immersion. Identify and count 100 consecutive leukocytes and record each cell type separately on the differential counter. Begin at the thin end of the smear and count the white cells observed as the slide is moved in a vertical direction. When near the edges of the smear, move the slide horizontally for a distance of about two fields, then proceed vertically back across the smear. See figure 1-1. Continue this "snake-like" movement until 100 leukocytes have been counted and classified.

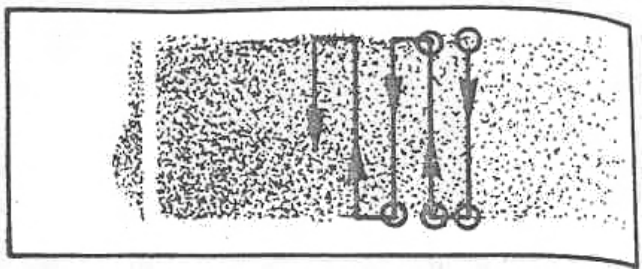


Figure 1-1. Examination of and peripheral blood smears.

(3) If the WBC count is between 20,000 and 50,000 per cu mm of blood, count and classify 300 leukocytes. When the count is greater than 50,000 per cu mm of blood, count and classify 500 leukocytes.

(4) The number of each type of leukocyte is expressed as a percent of the total number of white cells counted. Absolute values may be calculated by multiplying the percent value by the total leukocytic count.

c. **Erythrocyte Morphology.**

(1) Study the erythrocytes and report any evidence of rouleaux formation or signs of immaturity.

(2) Report the erythrocyte morphology with reference to size, shape, staining characteristics, and inclusions. Report the degree of the specific morphological characteristic (for example, moderate hypochromia).

(3) If nucleated erythrocytes are found (usually these are metarubricytes), report the number per 100 leukocytes counted.

d. **Qualitative Platelet Evaluation.**

(1) Observe the thrombocytes in several oil immersion fields to obtain a rough estimation of their numbers (normal, increased, or decreased). Normal is an average of 8-10 per oil immersion field.

(2) Note any abnormality in morphology (giant platelets, etc.) If the thrombocytes appear to be significantly decreased, a thrombocyte count and/or a clot retraction test may be indicated.

e. **Discussion.**

(1) All abnormal white cells (for example, immature, hypersegmented, toxic, atypical lymphocytes, etc.) should be classified or described and reported in percent, separately. Cells that are ruptured, fragmented, or degenerated are not included in the differential count, but should be noted separately and reported as the number seen per 100 leukocytes.

(2) In view of the gradual transition from the metamyelocyte to the band neutrophil and then to the segmented neutrophil, exact classification is sometimes difficult. In such cases, classify the cell according to the more mature form.

(3) Size considerations in differentiating blood cells require a defined linear standard. The micron (.001 mm) is usually used in reference to microscopic dimensions. Ocular micrometers are available through Federal medical supply channels and are easily calibrated, using a hemacytometer that has standardized dimensions. In routine screening of blood smears, an experienced technician relates the size of a normocytic erythrocyte (seven to eight microns) to the size of the white cell to be differentiated, since erythrocytes are usually present throughout the microscopic field. Finally, it should be understood that personal visual discrimination is an inaccurate gauge of linear measure. Some reference measure should be employed.

(4) The shape of blood cells often depends upon the smear and staining technique. Variations that have no clinical significance occur from physical and chemical distortion that result from technical error. These variations are avoided with careful technique. Each routine smear should be scanned initially to evaluate the smear and stain quality before differential analysis.

(5) Cytoplasmic granulation--neutrophilic, basophilic, or eosinophilic--is an important morphological observation. Differences in granule color in Wright-stained preparations are caused by the variable dye affinity of specific granules. The intensity of colors and the relative blueness or redness of the erythrocytes is used to evaluate the quality of the stain. The familiar basophilic (blue), eosinophilic (red), and neutrophilic (pink) granules are quite obvious in routine blood smears. The presence, absence, type, and quantity of granules are characteristic attributes used to differentiate leukocytes.

(6) The size ratio of nucleus to cytoplasm (N:C) is a differentiating characteristic. For instance, a cell with a nuclear mass equal to the cytoplasmic mass would have an N:C ratio of 1:1. The total cell mass is usually greater in the more immature cells and decreases as the cell matures. The nuclear mass usually decreases also as the cell matures. Of course, lymphocytes are the exception to this generality.

(7) The nuclear configurations of leukocytes help distinguish these cells. Round, oval indented, band, or segmented are terms used to describe variations in shape. These normal configurations can be distorted by physical and chemical factors. Some of the leukocytes are so fragile that in thick blood smears their normal configuration may be distorted by the pressure of erythrocytes forced against them. These artifacts should be recognized as such in an intelligent evaluation of blood differentials.

(8) In addition to nuclear shape and size, the internal nuclear morphology shows differential inclusions. The chromatin appears finely reticulated in some cells, or as a coarse network, or even clumped, in others. The parachromatin, a lighter staining material beside the chromatin, is scant or abundant. The appearance of the chromatin and the quality of parachromatin are utilized to differentiate blood cells. The presence, absence, and number of nucleoli in the nucleus are the most distinctive characteristics of immature nuclei in blood cells.

(9) All abnormal blood smears should be examined by another trained person for confirmation of the results.

f. **Normal Differential Values.**

(1) Banded neutrophil: 0 to 1 percent

(2) Segmented neutrophil: 50 to 65 percent

(3) Eosinophils: 1 to 3 percent

(4) Basophils: 0 to 1 percent

(5) Lymphocytes: 25 to 40 percent

(6) Monocytes: 4 to 10 percent

1-3. EXAMINATION OF BLOOD MARROW SMEARS

a. **Principle.** Nucleated blood cells are counted and classified from a bone marrow smear stained with Modified Wright's stain.

b. **Procedure.**

(1) Using oil immersion magnification, count and classify 300-500 nucleated cells.

(2) Classify all blood cells according to cell type and various stages of maturation.

(3) Calculate myeloid-erythroid ratio by dividing the number of nucleated erythrocytes into the number of granulocytic (myeloid) cells.

(4) A peripheral blood evaluation usually accompanies the bone marrow reports. This evaluation usually includes an erythrocyte count, leukocyte count, differential count, hemoglobin, hematocrit, and a reticulocyte count.

c. **Discussion.**

(1) The differential cell count on a bone marrow smear is carried out by a hematologist, pathologist, or trained technician.

(2) Since interpretation of findings in bone marrow examinations is very difficult, it is of utmost importance that the smears and stains are carefully prepared using scrupulously clean equipment.

(3) The laboratory technician is usually responsible for preparing bone marrow smears, staining the smears, checking the quality of the stained smear, and coverslipping the slides.

d. **Normal Values.**

(1) Leukocytes.

(a) Myeloblast: 0-5 percent

(b) Promyelocytes: 2-8 percent

(c) Neutrophilic myelocytes: 4-16 percent

(d) Neutrophilic metamyelocytes: 5-20 percent

(e) Neutrophilic bands: 10-35 percent

(f) Neutrophilic segmented cells: 7-30 percent

(g) Eosinophilic cells: 1-4 percent

(h) Basophilic cells: 0-1 percent

(i) Lymphocytes: 5-15 percent

(j) Monocytes: 0-5 percent

(k) Plasmocytes: 0-1 percent

(2) Erythrocytes.

(a) Rubriblasts: 0-1 percent

(b) Prorubricytes: 1-4 percent

(c) Rubricytes: 3-10 percent

(d) Metarubricytes: 5-25 percent

(3) Megakaryocytes. 0-3 percent

(4) Myeloid-Erythroid Ratio (M:E). 3-4:1.

Section II. ERYTHROCYTE INDICES AND FRAGILITY TESTS

1-4. ERYTHROCYTE INDICES

a. **Principle.** By using accurately determined red blood cell counts, hematocrits, and hemoglobin values, the size and hemoglobin content of the average red cell in a given blood sample is calculated. The values obtained are the erythrocyte indices which aid in the classification and study of anemias. They consist of the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

b. **Calculation of Erythrocyte Indices.**

(1) Mean corpuscular volume (MCV)--The average volume of the individual red blood cell. Femtoliter (fl) or 10^{-15} liter = 1 fl.

$$\frac{\text{Hematocrit (percent)} \times 10}{\text{Red cell count (in millions)}} = \text{fl}$$

Example: Hematocrit 45 percent
Red count5,000,000 per cu mm

45 X 10
---------- = 90 fl
5.0

(2) Mean corpuscular hemoglobin (MCH)--The average weight of hemoglobin of the individual red cell.

Hemoglobin (gm per dl) x 10
---------- = micromicrograms
Red cell count (in millions)

Example: Hemoglobin 15 gm per dl
Red count5,000,000 per cu mm

15 x 10
---------- = 30 micromicrograms (normal)
5.0

(3) Mean corpuscular hemoglobin concentration (MCHC)--The percent of hemoglobin in the average red cell.

Hemoglobin (gm per dl) x 100
---------- = percent
Hematocrit (percent)

Example: Hemoglobin 15 g per dl
Hematocrit 45 percent

15 x 100
---------- = 33 percent (normal)
45

c. **Discussion.**

(1) Accurate individual determinations of hemoglobin, hematocrit, and erythrocyte count ensure reliable indices. The following procedures are recommended:

(a) Erythrocyte count--two separate pipets and 2-4 counting chambers or electronic cell counting.

(b) Hemoglobin--precise reagent standards and accurate instrument calibration.

(c) Hematocrit--Wintrobe, centrifuge at 3,000 rpm for 55 minutes.

(2) It is useful to compare the calculated indices with a stained peripheral blood smear.

(3) Wintrobe classified anemias into the following groups on the basis of the indices.

Anemia	MCV	MCH	MCHC
Macrocytic	95-160	32-50	32-36
Microcytic	72-79	21-24	24-36
Microcytic hypochromic	50-79	19-29	24-30
Normocytic	80-94	26-32	32-36

(4) The MCHC cannot exceed the normal value since the erythrocyte cannot be supersaturated with hemoglobin. The MCHC is the most valid of the indices since it does not require the erythrocyte count in its deviation. It is a good index of iron deficiency.

(5) The MCV and MCH are increased at birth and fall to low values during childhood. The MCHC is fairly constant for all ages.

d. **Normal Values.**

(1) Mean corpuscular volume: 80-97 fl.

(2) Mean corpuscular hemoglobin: 27-31 micromicrograms.

(3) Mean corpuscular hemoglobin concentration: 32-36 percent

1-5. ERYTHROCYTE OSMOTIC FRAGILITY TEST (DACIE)

a. **Principle.** A specific amount of blood is introduced into a series of tubes containing different concentrations of buffered salt solutions. The ability of the erythrocytes to resist hemolysis in such solutions is determined spectrophotometrically on the basis of free hemoglobin present.

b. **Sources of Error.**

(1) The concentration of the NaCl in the solutions is critical. The salt must be chemically pure and dried before weighing. It is advisable to dry the salt in a 100ºC oven and store it in a desiccator. Store the NaCl solutions in a glass-stoppered, tightly sealed bottle.

(2) Inaccurate preparation of the dilutions causes inaccurate results.

(3) Maintain the pH of the solution at an interval of 7.35-7.50. A different pH range causes invalid results.

(4) Rough handling of the blood specimen causes hemolys is which leads to invalid results.

c. **Discussion.**

(1) In hypotonic salt solutions, erythrocytes take up water, swell to a spheroid shape and burst. In congenital spherocytic anemia the red cells with defective structure more rapidly rupture at salt concentrations closer to isotonicity (0.85 percent). These cells thus show an increased osmotic fragility. In contrast, the flat or thin but otherwise normal red cells of hypochromic anemia show a decreased osmotic fragility and do not hemolyze until lower salt concentrations are reached.

(2) When hemolysis begins beyond the range of the prepared solutions or when intermediate dilutions are desired, the additional dilutions are readily prepared using the 1 percent sodium chloride stock solution.

(3) In cases where the results of the fragility test are borderline, the following procedure is recommended to enhance any latent abnormality in fragility. Incubate samples of defibrinated blood (control and patient's) at 37ºC for 24 hours under sterile conditions and controlled pH (7.35-7.50). The test is then performed as described above.

(4) Decreases in pH increase osmotic fragility. The reagents are buffered to maintain a constant pH of 7.35-7.50.

(5) This test may also be run visually, with some sacrifice of accuracy, by allowing the blood-saline dilutions to stand at 20ºC for 45 minutes. The tubes are then lightly centrifuged (1,000 rpm for 3 minutes) and observed for signs of initial and complete hemolysis. A slight pink coloration of the supernatant fluid indicates initial hemolysis and a clear red solution, free of sediment, indicates complete hemolysis. The salt concentrations in these two tubes are noted and recorded. The control should always be reported along with results of patient's tubes.

d. **Normal Values.**

(1) 0.30 percent saline: 97-100 percent hemolysis.

(2) 0.35 percent saline: 90-99 percent hemolysis.

(3) 0.40 percent saline: 50-95 percent hemolysis.

(4) 0.45 percent Saline: 4-45 percent hemolysis.

(5) 0.50% Saline: 0-6% hemolysis.

(6) 0.55% Saline: 0% hemolysis.

1-6. HAM TEST FOR ERYTHOCYTE FRAGILITY

a. **Principle.** This test is positive in paroxysmal nocturnal hemoglobinuria (PNH). Erythrocytes in this form of anemia lyse easily in slight variations in the pH (acid). In this test, the erythrocytes are subjected to pH values ranging from 6.5 to 7.0 at 37ºC.

b. **Discussion.**

(1) With a positive test the tubes containing acidified sera and patient's cells should show considerable hemolysis.

(2) Normally no tubes should show hemolysis.

(3) Occasionally, tubes with unacidified sera and patient's cells may show moderate hemolysis.

(4) A false positive test is sometimes seen in congenital spherocytic anemia.

(5) If congenital spherocytic anemia is suspected, the test should be repeated, using acidified serum previously inactivated at 56ºC for 30 minutes.

(6) Since erythrocytes of PNH require complement for hemolysis, the modified test (item 5 above) will be negative in PNH and will remain positive in spherocytosis.

c. **Interpretation**. Hemolysis in the acidified tube is indicative of paroxysmal nocturnal hemoglobinuria.

Section III. DEMONSTRATION OF L.E. CELLS

1-7. GENERAL INFORMATION

a. Persons having lupus erythematosus, one of the "collagen" diseases, have an abnormal plasma protein that causes swelling and breakdown of certain blood cell nuclei in vitro. This degenerated nuclear material attracts phagocytic cells, particularly segmented neutrophils, which engulf this nuclear mass. The resulting phagocyte and inclusion material is termed an "L.E." cell.

b. Two methods of demonstrating the L.E. cell and antinuclear antibodies are the rotary bead method and fluorescent antibody method. The rotary bead method is positive in 75-80 erythematosus. The fluorescent antibody method is positive in 95-100 patients with lupus erythematosus. The rotary bead method is presented in the next paragraph. The fluorescent antibody method requires equipment that limits its use to larger laboratories.

1-8. ROTARY BEAD METHOD

a**. Principle.** Leukocytes are broken down in vitro allowing the abnormal plasma protein to react on the altered nuclear material. Incubation enhances the nuclear deterioration and phagocytosis. Slides are prepared and examined for the peculiar "L.E." cell.

b. **Discussion.**

(1) Lupus erythematosus is a chronic, sometimes fatal, disease of unknown etiology. The peculiar skin eruption across the nose and cheeks (butterfly rash) and arthritis can be accompanied by various visceral manifestations. Often the rash is not present, and diagnosis depends on demonstration of the L.E. cell. Frequently the earliest symptoms appear after intense exposure to sunlight. Leukopenia, thrombocytopenia, and an elevated sedimentation rate are some of the clinical signs of the disease.

(2) Free masses of lysed nuclear material, with or without polymorphonuclear leukocytes clustered about them (rosette formation), are suggestive of the L.E. phenomenon. Observing "rosettes" should encourage the technician to repeat examinations and further search for the true "L.E." cells. A positive report should not be made without the identification of this cell. The inclusion body with the leukocyte is homogeneous and has no chromatin pattern. This feature distinguishes the true "L.E." cell from the "tart" cell (nucleophagocytosis). This latter cell contains an engulfed, damaged nucleus, usually that of a lymphocyte, which still contains a recognizable chromatin pattern and a distinct nuclear membrane.

c. **Interpretation.**

(1) These cells are seen as large polymorphonuclear (segmented) leukocytes which contain large ingested nuclear fragments in their cytoplasm.

(2) The inclusion body is a purplish-staining, smoky, homogeneous mass of material that is so large that it usually pushes the nucleus to one side of the cell.

Section IV. SPECIAL STAINS

1-9. PEROXIDASE STAIN (KAPLOW)

a. **Principle.** The members of the granulocytic series contain an enzyme, peroxidase, which liberates the oxygen from hydrogen peroxide. This enzyme is more prominent in mature forms. A benzidine derivative is used as an indicator of peroxidase activity. The indicator is oxidized and precipitates in the form of brown to blue granules. This stain is used to help differentiate leukemias.

NOTE: Follow manufacture's instructions for all special stains.

b. **Interpretation.** Peroxidase positive cells are identified by yellow-green to blue and brown-green granules. Cells of the granulocyte series from the promyelocyte through the segmented neutrophil are peroxidase positive. The degree of peroxidase activity increases as the granulocytes mature. Monocytes may show a weak reaction. All other cells are negative.

c. **Discussion.**

(1) The oxidizing enzyme in the granules of the granulocytic leukocytes deteriorates rapidly in vitro. It is, therefore, necessary to use fresh blood in making the preparation.

(2) Smears should be prepared within 1 hour of obtaining the specimen and stained within 3 hours after they are prepared.

(3) The monocyte is thought to be slightly peroxidase positive through the phagocytization of peroxidase positive granules of ruptured cells.

(4) Myeloblasts can show weak peroxidase activity using this method.

(5) Addition of 4.9 mg of sodium cyanide to the stain inhibits peroxidase activity in all granulocytes except eosinophils.

(6) If greater nuclear detail is required, counter stain with 1aqueous cresyl violet acetate for 1 minute or in freshly prepared Giemsa stain for 10 minutes.

(7) Giemsa stain is prepared as follows: Mix 3.8 g Giemsa stain powder and 200 ml glycerin. Incubate at 60ºC for two hours. Add 312 ml absolute methanol; dilute the staining solution 1:10 with 5 sodium carbonate before use.

1-10. LEUKOCYTE ALKALINE PHOSPHATASE

a. **Principle.** Blood smears are fixed and stained for alkaline phosphatase activity.

b. **Scoring.**

(1) Count two slides (100 cells per slide) on each patient, rating the segmented neutrophils according to how much black staining of the granules is observed. If no staining is noted, the rating is 0; if slight black staining is noted, the rating is 1+, if a medium amount of black staining is noted, the rating is 2+, if a heavy amount of dark black staining is observed, the rating is 3+, and if there is heavy black staining covering all the cytoplasm, the rating is 4+.

(2) After 100 cells per slide are rated, figure the score-giving cells counted as 0--no score; cells rated as 1+ get a score of 1 each; cells rated as 2+ get a score of 2 each, etc.

(3) Report the total number of cells, giving their ratings and score. Report the total score for each individual slide. Average the two total scores and report the average. Also, always report the normal score range.

c. **Discussion.**

(1) Patients with infections, polycythemia, and myeloproliferative disorders demonstrate increased alkaline phosphatase activity.

(2) In patients with acute or chronic granulocytic leukemia, alkaline phosphatase activity is decreased.

d. **Normal Values.** Scores of 13-130 have been obtained in healthy adults. However, the attending physician should interpret whether values are normal or abnormal.

1-11. HEINZ-BODY STAIN

a. **Principle**. Blood is mixed with methyl violet solution and a smear is prepared. Heinz-bodies stained with methyl violet are purple, round or oval granules, one-two microns in diameter within the erythrocytes.

b. **Interpretation**. Iron granules present in erythrocytes stain blue.

c. **Discussion**.

(1) Heinz-bodies are invisible in Wright-stained preparation.

(2) They can be observed in reticulocyte preparations and by the use of phase microscopy.

(3) Heinz-bodies are thought to be denatured hemoglobin. They are usually demonstrated in hemolytic anemias caused by toxic agents, including vegetable and animal poisons.

1-12. SIDEROCYTE STAIN

a. **Principle.** Siderocytes are erythrocytes containing iron granules. The granules are blue when stained with Prussian blue.

b. **Interpretation.** Iron granules present in erythrocytes stain blue.

c. **Discussion.**

(1) Siderocytes occur in several anemias, lead poisoning, and after splenectomy.

(2) On Wright-stained preparation, the granules are bluish-purple and are called Pappenheimer bodies.

Continue with Exercises

EXERCISES, LESSON 1

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. A critical examination of a stained blood smear includes the differential count that quantitates the three types of:

   a. Thrombocytes.

   b. Granulocytes.

   c. Lympocytes.

   d. Leukocytes.

2. Which area of the blood smear is used for the differential leukocyte count?

   a. Thin end.

   b. Thick end.

   c. Inner portion.

   d. Peripheral area.

3. The _____ objective lens is used to perform the differential leukocyte count.

   a. 10X (low power).

   b. 43X (high power).

   c. 97X (oil immersion).

4. When nucleated erythrocytes are located on a blood smear, they are reported by the _____ counted.

   a. Number per 100 leukocytes.

   b. Number per 100 erythrocytes.

   c. Percentage of all leukocytes.

   d. Percentage of all erythrocytes.

5. What is the normal average number of thrombocytes counted per oil immersion field when performing a qualitative platelet evaluation of a blood smear?

   a. 0-2.

   b. 4-6.

   c. 6-8

   d. 8-10.

6. Which test is indicated when the amount of thrombocytes appear to be decreasing significantly on an oil immersion field blood smear?

   a. Rosettes.

   b. Alkali denaturation.

   c. Clot retraction.

   d. pH acid.

7. Which white blood cells are counted as part of the 100 in a differential leukocyte count and reported in a separate category in percent?

   a. Immature leukocyte.

   b. Ruptured leukocyte.

   c. Fragmented leukocyte.

   d. Degenerated leukocyte.

8. A cell with a nuclear mass twice as great as the cytoplasmic mass would have an N:C ratio of:

    a. 1:2.

    b. 2:3.

    c. 1:1.

    d. 2:1.

9. What has the second highest value in the normal differential count?

    a. Monocytes.

    b. Eosinophils.

    c. Lymphocytes.

    d. Segmented neutrophils.

    e. Neutrophilic band cells.

10. The myeloid-erythroid (M:E) ratio of the bone marrow is the ratio of the granulocytic white blood cells to the:

    a. Red blood cells.

    b. Mature red blood cells.

    c. Nucleated red blood cells.

    d. Bone marrow cells other than granulocytes.

11. The bone marrow study should be accompanied by a:

    a. Hematocrit.

    b. Red blood cell count.

    c. Peripheral blood evaluation.

    d. Total white blood cell count.

12. What is the normal M:E (myeloid-erythroid) ratio of the bone marrow?

    a. 1:1.

    b. 2:1 to 3:1.

    c. 3:1 to 4:1.

    d. 4:1 to 7:1.

13. The hematocrit and the RBC count are needed to calculate the:

    a. MCV.

    b. MCH.

    c. MCHC.

14. When calculating the MCV in femtoliters, what is the divisor after multiplying the hemotocrit and 10?

    a. RBC count (millions).

    b. Hematocrit (percent).

    c. WBC count (thousands).

    d. Hemoglobin concentration (g/dl).

15. What is the MCV if the hematocrit is 44%, the RBC count is 5.2 million per cu mm, and the hemoglobin concentration is 14 g/dl?

    a. 1.2 fl.

    b. 8.5 fl.

    c. 12 fl.

    d. 85 fl.

16. What is the MCV if the hematocrit is 36%. the RBC is 4.6 million per cu mm, and the hemoglobin concentration is ll g/dl?

    a. 1.2 fl.

    b. 78 fl.

    c. 118 fl.

    d. 783 fl.

17. The RBC count and the hemoglobin concentration are needed to calculate the:

    a. MCV.

    b. MCH.

    c. MCHC.

18. To calculate the MCH in micromicrograms, _____ is multiplied by to 10.

    a. RBC count (millions).

    b. WBC count (thousands).

    c. Hematocrit (percent).

    d. Hemoglobin concentration (g/dl).

19. If the hematocrit is 44%, the RBC is 5.2 million per cu mm, and the hemoglobin concentration is 14 g/dl, what is the MCH?

    a. 12 micromicrograms.

    b. 27 micromicrograms.

    c. 37 micromicrograms.

    d. 85 micromicrograms.

20. If the hematocrit is 36%, the RBC is 4.6 million per cu mm, and the hemoglobin concentration is 11 g/dl, what is the MCH?

    a. 24 micromicrograms.

    b. 31 micromicrograms.

    c. 33 micromicrograms.

    d. 1/2 micromicrogram.

21. To calculate the MCHC, _____ is multiplied by 100, then divided by the Hemocrit. The result equals the percent of hemoglobin in the average RBC.

    a. RBC count (millions).

    b. WBC count (thousands).

    c. Hematocrit (percent).

    d. Hemoglobin concentration (g/dl).

22. If the hematocrit is 44%, the RBC is 5.2 million per cu mm, and the hemoglobin concentration is 14 g/dl, what is the MCHC?

    a. 12%.

    b. 27%.

    c. 32%.

    d. 37%.

23. If the hematocrit is 36%, the RBC is 4.6 million per cu mm, and the hemoglobin concentration is 11 g/dl, what is the MCHC?

    a. 24%.

    b. 31%.

    c. 33%.

    d. 42%.

24. A mean corpuscular volume below 80 fl indicates that the erythrocytes are:

    a. Macrocytic.

    b. Normocytic.

    c. Microcytic.

    d. Megaloblastic.

25. The maximum value for the _____ is included in its normal range.

    a. MCV.

    b. MCH.

    c. MCHC.

26. The normal range for the mean corpuscular volume of an erythrocyte is approximately:

    a. 62-82 fl.

    b. 70-80 fl.

    c. 80-97 fl.

    d. 90-100 fl.

27. The osmotic fragility of erythrocytes is increased in:

    a. Thalassemia major.

    b. Sickle cell anemia.

    c. Iron deficiency (hypochromic) anemia.

    d. Congenital spherocytic (hemolytic) anemia.

28. When the osmotic fragility test is performed visually, the salt concentrations are recorded for the two tubes that show:

    a. 0% and 50% hemolysis.

    b. 0% and 100% hemolysis.

    c. Least and greatest hemolysis.

    d. Initial hemolysis and first complete hemolysis.

29. What is the normal percentage of hemolysis in 0.55% saline?

    a. 0%.

    b. 40%.

    c. 65%.

    d. 100%.

30. The Ham test is positive in:

    a. Polycythemia.

    b. Paroxysmal nocturnal hemoglobinuria.

    c. Chronic lymphocytic leukemia.

    d. All hemoglobinopathies.

31. Erythrocytes in paroxysmal nocturnal hemoglobinuria lyse easily in serum which is slightly:

    a. Basic.

    b. Acidic.

    c. Hypotonic.

    d. Hypertonic.

32. A false-positive Ham test may occur in:

    a. Sickle cell anemia.

    b. Congenital spherocytic anemia.

    c. Severe iron deficiency anemia.

    d. Paroxysmal nocturnal hemoglobinuria.

33. When demonstrating "L.D." cells, which of the following has degenerative nuclear material that attracts phagocytic cells, particular segmented neutrophis?

    a. Jaundice.

    b. Leukemia.

    c. Lupus erythematossus.

    d. Pernicious amenia.

34. Which method is used to determine L.E. cell and antinuclear antibodies with a 75-80% accuracy rate?

    a. Rotary bead.

    b. Fluorescent antibody.

    c. a and b.

    d. None of the above.

35. Lupus erythematosus is:

    a. A chronic, sometimes fatal, disease of unknown etiology.

    b. A regular skin eruption across the nose and mouth (butterfly rash), with arthritis that can be accompanied by various visceral manifestations.

    c. A rash, which is sometimes not present. Diagnosis depends on demonstration of the L.E. cell.

    d. Sometimes not diagnosed early because the early symptoms do not appear after intense exposure to sunlight.

36. Which statement is correct for the erythrocyte osmotic fragility test?

    a. In hypertonic salt solutions, erythrocytes take up water, swell to a spheroid shape and burst.

    b. In congenital spherocytic anemia, the WBCs with defective structure, will more rapidly rupture at salt concentrations closer to isotonicity (0.85 percent).

    c. The RBCs cells thus show an increased osmotic fragility. In contrast, the flat or thin but otherwise normal red cells of hypochromic anemia show a decreased osmotic fragility and do not hemolyze until lower salt concentrations are reached.

    d. When hemolysis begins within the normal range of the prepared solutions or when intermediate dilutions are desired, the additional dilutions are readily prepared using the 1 percent sodium chloride stock solution.

37. Which statement is correct for the erythrocyte osmotic fragility test?

    a. When the results of the fragility test are normal, one procedure is immediately followed to enhance any latent abnormality in fragility.

    b. Incubate samples of defibrinated blood (control and patient's) at 37ºC for 24 hours under sterile conditions and controlled pH (7.35-7.50).

    c. The test does not need to be performed.

    d. Increases in pH decrease osmotic fragility. The reagents are buffered to maintain a constant pH of 7.35-7.50.

38. Which statement is correct for the erythrocyte osmotic fragility test?

    a. This test may also be run visually, with some sacrifice of accuracy, by allowing the blood-saline dilutions to stand at 20ºC for 45 minutes.

    b. The tubes are then vigorously centrifuged (1,000 rpm for 3 minutes) and observed for signs of initial and complete hemolysis.

    c. A rich dark pink coloration of the supernatant fluid indicates initial hemolysis and a cloudy red solution indicates complete hemolysis.

    d. Salt free concentrations in these two tubes are noted and recorded.

    e. The control does not have to be reported along with results of patient's tubes.

39. When there is no "butterfly rash," diagnosis of lupus erythematosus often depends upon demonstration of:

    a. Collagen.

    b. L.E. cells.

    c. Leukocytosis.

    d. Polycythemia.

40. In addition to L.E. cells, two characteristic phenomena in lupus erythematosus are:

    a. Free nuclear masses and rosettes.

    b. Rouleaux and Cabot rings.

    c. Basket cells and toxic granulation.

    d. Distorted lymphocytes and smudge cells.

41. Which of the following is easily mistaken for an L.E. cell?

    a. Tart cell.

    b. Monocyte.

    c. Plasmocyte.

    d. Segmented neutrophil.

42. Which of the following is peroxidase negative?

    a. Lymphocytes.

    b. Promyelocytes.

    c. Neutrophilic myelocytes.

    d. Neutrophilic metamyelocytes.

    e. Neutrophilic band cells.

    f. Neutrophilic segmented cells.

43. Which of the following is peroxidase positive?

    a. Lymphocytes.

    b. Plasmocytes.

    c. Segmented neutrophils.

44. With the alkaline phosphatase stain, a segmented neutrophil exhibiting no darkly stained granules is rated:

    a. 0.

    b. 1+.

    c. 2+.

    d. 3+.

    e. 4+.

45. Generally speaking, when leukocyte alkaline phosphatase is used, a patient with what score is considered to be a healthy adult?

    a. 10-50.

    b. 10-80.

    c. 13-100.

    d. 13-130.

46. Heinz bodies are often present in the erythrocytes of hemolytic anemia caused by:

    a. Toxic agents.

    b. Spherocytosis.

    c. Thalassemia major.

    d. Paroxysmal nocturnal hemoglobinuria.

47. What color are Heinz-bodies when stained with methyl violet?

    a. Colorless.

    b. Blue-green.

    c. Purple.

    d. Black.

48. Heienz-bodies prepared with Wright-stain are what color?

    a. Green.

    b. Purple.

    c. Colorless.

    d. Orange.

49. Siderocytes are:

    a. Iron granules.

    b. Denatured hemoglobin.

    c. Nucleated erythrocytes.

    d. Erythrocytes containing iron granules.

50. Siderocytes may be observed:

    a. All of the below.

    b. In several anemias.

    c. After splenectomy.

    d. In lead poisoning.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 1

1. d (para 1-1a)
2. a (para 1-2b(1), (2))
3. d (para 1-2b(2))
4. a (para 1-2c(3))
5. d (para 1-2d(1))
6. c (para 1-2d(2))
7. a (para 1-2e(1)
8. d (para 1-2e(6))
9. c (para 1-2f)
10. c (para 1-3b(3))
11. c (para 1-3b(4))
12. c (para 1-3d(4))
13. a (para 1-4b(1))
14. a (para 1-4b(1))
15. d (para 1-4b(2))
16. b (para 1-4b(2))
17. b (para 1-4b(2))
18. d (para 1-4b(2))
19. b (para 1-4b(2))
20. a (para 1-4b(2))

21. d (para 1-4b(3))

22. c (para 1-4b(3))

23. b (para 1-4b(3))

24. c (para 1-4c(3))

25. c (para 1-4c(4))

26. c (para 1-4d(1))

27. d (para 1-5c(1)

28. d (para 1-5c(5))

29. a (para 1-5d(6))

30. b (para 1-6a)

31. b (para 1-6a)

32. b (para 1-6b(4))

33. c (para 1-7a)

34. a (para 1-7b)

35. a (para 1-8b(1))

36. c (para 1-5c(1))

37. b (para 1-5c(3))

38. a (para 1-5c(5))

39. b (para 1-8b(1))

40. a (para 1-8b(2))

41. a (para 1-8b(2))

42. a (para 1-9b)

43. c (para 1-9b)

44. a (para 1-10b(1))

45. d (para 1-10d)

46. a (para 1-11c(3))

47. c (para 1-11a)

48. c (para 1-11c(3))

49. d (para 1-12a)

50. a (para 1-12c(1))

End of Lesson 1

LESSON ASSIGNMENT

LESSON 2 Blood Coagulation.

TEXT ASSIGNMENT Paragraphs 2-1 through 2-30.

LESSON OBJECTIVES After completing this lesson, you should be able to:

2-1. Select the statement that best describes the process of hemostasis and its relationship to the vascular system.

2-2. Select the statement that correctly describes the process and system of coagulation.

2-3. Select the statement that best describes the four components or stages of blood clotting.

2-4. Select the correct materials and procedures used in the different techniques and methods of blood collection for coagulation studies.

2-5. Select the statement that correctly describes the procedures, tests, and events during the stages of coagulation.

2-6. Select the statement that accurately describes normal value standards to follow and sources of errors that could occur during the coagulation process.

2-7. Select the statement that determines the adequacy of the thromboplastic complex.

2-8. Select the appropriate materials and procedures used to test for other coagulation deficiencies.

SUGGESTION After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 2

BLOOD COAGULATION

Section I. INTRODUCTION

2-1. HEMOSTASIS

a. Coagulation of the blood is only one of the components in the larger function of stopping blood flow known as hemostasis. Hemostasis is a complex process in which several factors work together or in sequence to stop the flow of blood from an injured blood vessel. It may be viewed as the combination of clotting and lysing mechanisms that maintain the integrity of the vascular system.

b. Platelets play a major role in the hemostatic process. Within 1 to 2 seconds after injury to a blood vessel, platelets come in contact with and adhere to the injured tissues (platelet adhesiveness). As a result, the platelets become swollen and extend pseudopodia. Serotonin (5-hydroxytryptamine), ADP, catecholamines, and platelet factor 4 (a glycoprotein with antiheparin activity) are released by the platelets. The ADP released by the platelets and also by the injured tissues causes the platelets to stick to one another (known as platelet aggregation; when platelets attach to non-platelet surfaces, this is called platelet adhesion). Platelets continue to aggregate until the site of injury is healed.

c. The vascular system also affects the hemostatic process through the function of vasoconstriction. The vascular mechanism involves the veins, arteries, and capillaries themselves. Their effectiveness depends on thickness of the vessel wall and its structure, contractibility, and retractibility. Bleeding into the tissues surrounding a wound increases perivascular pressure about small vessels, causing collapse and reduction of blood flow in larger vessels. Following the formation of a clot, clot retraction begins due to the action of actomyosin (thrombosthenin, the platelet contractile protein), which represents 15-20% of platelet protein.

2-2. COAGULATION SYSTEM

a. Blood coagulation is the formation of a clot from liquid blood. It is a complex subject and many aspects of nomenclature, physiology, and interpretation of tests remain controversial. When bleeding occurs, clotting is initiated by aggregation of platelets (see figure 2-1). The platelets congeal to plug the site of the injury. The congealing (viscous metamorphosis) process is stimulated by contact with collagen (the supporting tissue surrounding blood vessels) or by the formation of thrombin. Hemostasis is not achieved without the simultaneous formation of fibrin. Platelet and plasma factors are activated, and by a complex process, a fibrin clot is formed. The arrest of bleeding is attained when a firm fibrin network seals the blood vessel wound with enough strength to withstand the impact of intravascular pressure.

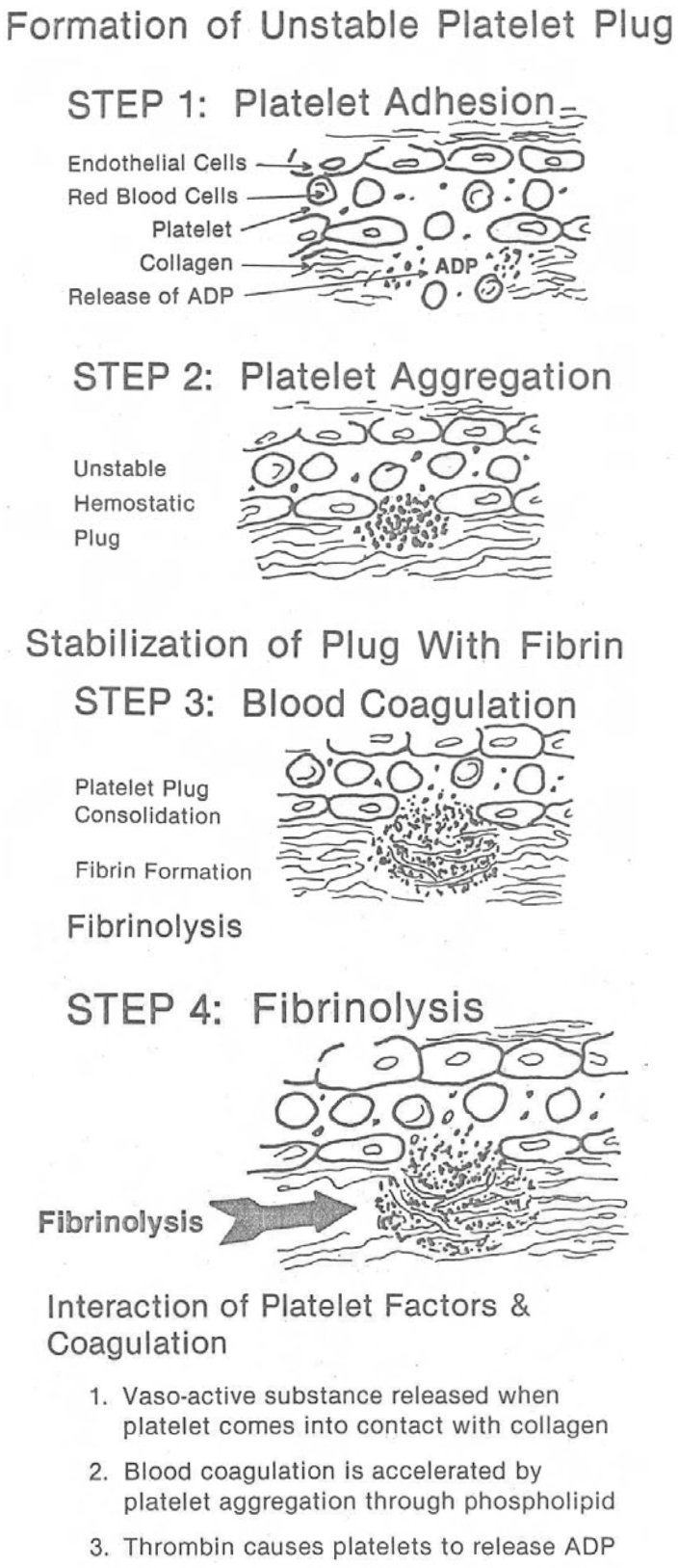


Figure 2-1. Formation of unstable platelet plug.

b. Bleeding disorders occur in the following instances: injury to the vascular system, inadequate number of platelets and/or dysfunctional platelets, inadequate fibrin clotting mechanisms, and inadequate fibroblastic repair. The laboratory performs a variety of tests that assist the physician in his investigation of blood coagulation. Several of these tests measure the overall coagulation process. The bleeding disorders are due to one or several of the many factors involved in this process. In most instances, prolonged bleeding is due to a deficiency of one factor or another. However, in some instances it is due to therapeutic anticoagulants that are intentionally injected to interfere with the coagulation mechanism. In a few rare instances, prolonged bleeding is due to a natural or antigenically stimulated increase in circulating anticoagulants produced in the body.

c. The coagulation process is divided into two systems: the intrinsic system and the extrinsic system. All factors required for the intrinsic system are contained within the blood. The extrinsic system relies on thromboplastin (factor III), which is released from the damaged cells and tissues (see figure 2-2). The distinction between the intrinsic and extrinsic pathways becomes blurred upon deeper analysis. As more and more information is gathered, it shows how each interacts with the other and feedback mechanisms work in combination. Examples of such is how activated factor XII, will trigger factor VII to its active form. Additional crossovers show with the activation of factor XI by activated factor VII.

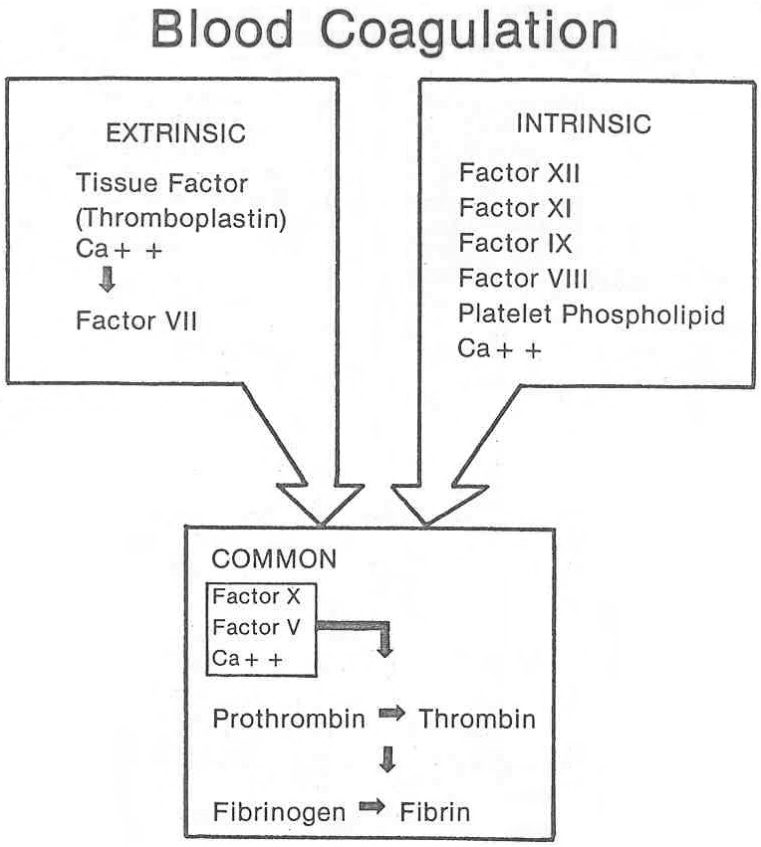


Figure 2-2. Coagulation systems.

Section II. COAGULATION SYSTEM

2-3. COAGULATION FACTORS

Table 2-1 contains a complete list of coagulation factors and their synonyms.

Factor Numerical	Description Name
I	Fibrinogen
II	Prothrombin
III	Tissue thromboplastin
IV	Calcium
V	Labile factor, proaccelerin, AC-globulin
VI	No factor is designated by this numeral
VII	Proconvertin, stable factor
VIII	Antihemophilic factor (AHF) Antihemophilic globulin (AHG)
IX	Plasma thromboplastin component (PTC), Christmas factor
X	Stuart-prower factor, autoprothrombin III
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor, glass factor, contact factor
XIII	Fibrin stabilizing factor (FSF), fibrinase
Prekallikrein	Fletcher factor
High molecular weight kininogen	Fitzgerald factor, Williams factor, Flau Jeac factor, Contact activation cofactor
(Designation) a	A factor that has been activated and is now functional

Table 2-1. Nomenclature of coagulation factors.

a. **Factor I (Fibrinogen).** Fibrinogen, a plasma glycoprotein, is converted into fibrin in the presence of thrombin. The major source of fibrinogen is the liver. Bleeding due to a fibrinogen deficiency does not usually become manifest until the plasma concentration is below 75 mg per dl. Decreased levels of fibrinogen can be caused by several reasons -- decreased liver production is due to acute hepatitis or cirrosis; fibrinolysins, which attack both fibrin and fibrinogen molecules; and massive production of fibrin, as seen in disseminated intravascular coagulation (DIC). Replenishment can be achieved by administration of fresh frozen plasma or cryoprecipitates.

b. **Factor II (Prothrombin).** This substance is a stable glycoprotein, synthesized in the liver if an adequate amount of vitamin K is available. Prothrombin (a proenzyme) is the inactive precursor of thrombin. When Vitamin K is absent, various types of prothrombin molecules still form, but the site on the molecule that would react in the coagulation scheme is left inactive and nonfunctional. Factors II, VII, IX, and X are part of the prothrombin complex; all are vitamin K dependent, meaning that these factors cannot be produced without the presence of vitamin K in the liver in adequate amounts. These factors are absorbed by both $BaSO_4$ (barium sulfate) and $Al(OH)_3$ (aluminum hydroxide). With the exception of Factor II, all are found in serum.

c. **Factor III (Tissue Thromboplastin).** Tissue thromboplastin is a high-molecular-weight lipoprotein found in most of the body tissues with increased concentrations in the lungs and brain. It is probably the phospholipid content of tissue thromboplastin that makes platelets unnecessary in stage I of the coagulation process. Tissue thromboplastin requires calcium and factors V, VII, and X to convert prothrombin to thrombin.

d. **Factor IV (Calcium).** Calcium is an inorganic ion that is necessary for clotting to occur. The exact mechanism by which calcium acts in the coagulation process is not known. The fact that it is essential for coagulation makes possible the use of anticoagulants, which merely bind up the calcium and, therefore, completely inhibit coagulation. It is unlikely, however, that a bleeding tendency is ever caused by a deficiency of calcium, since clinical tetany occurs with higher levels of calcium than are necessary for coagulation.

e. **Factor V (Labile Factor Proaccelerin, Accelerator Globulin).** Factor V is derived from plasma globulin, and it acts as an accelerator in the conversion of prothrombin to thrombin in the presence of tissue thromboplastin. Factor V is not present in serum because it is consumed during the clotting of blood. In addition, it rapidly is inactivated during storage. It is not absorbed by $BaSO_4$ (barium sulfate).

f. **Factor VI (Accelerin).** Factor VI has been eliminated as an entity by the International Committee on Blood Clotting Factors.

g. **Factor VII (Stable Factor, Proconvertin).** Factor VII is stable to both heat and storage. It is thought to act as an accelerator in the conversion of prothrombin to thrombin. Factor VII is not consumed in the clotting process; therefore, it has a high concentration in serum and plasma. Factor VII activity may actually increase the coagulation process. A vitamin K dependent factor is manufactured in the liver.

h. **Factor VIII (Antihemophilic Factor, Antihemophilic Globulin).** Factor VIII is essential to the formation of intrinsic blood thromboplastin in the first stage of clotting. Deficiency of factor VIII results in the reduction of thromboplastin as well as decreased conversion of prothrombin. Factor VIII deficiency is a hereditary sex-linked disorder, which is transmitted by females and manifested almost exclusively in males (hemophilia A).

i. **Factor IX (Plasma Thromboplastin Component, Christmas Factor)**. Factor IX influences the amount of thromboplastin formed. This factor is not consumed in the clotting process; therefore, it is present in serum. Deficiency of factor IX is either hereditary or acquired and is known as hemophilia B or Christmas disease.

j. **Factor X (Stuart Prower Factor).** Factor X aids in the prompt conversion of prothrombin to thrombin. Deficiency of factor X is either acquired or hereditary.

k. **Factor XI (Plasma Thromboplastin Antecedent).** Factor XI aids in the formation of plasma thromboplastin. This factor is stable and is found in plasma or serum. Deficiency of factor XI is probably hereditary and results in a mild hemophilia.

l. **Factor XII (Hageman Factor, Glass Factor).** This factor is not required for normal hemostasis, but it is important in the various in vitro assays of the clotting mechanisms. It is a plasma contact factor with glass and is absorbed onto glass. Factor XII is related to factor XI in the activation of thromboplastin, and behaves like an enzyme for which one substrate is factor XI.

m. **Factor XIII (Fibrin Stabilizing Factor, Fibrinase).** Factor XIII converts a loosely linked, fibrin clot (in the presence of the calcium ions) into a tough gel. Its activity is greatly reduced in serum (as compared with plasma) because of its strong adsorption of fibrin.

2-4. PLATELET FACTORS

Platelets are active in blood coagulation. They perform the following functions: aid in vasoconstriction and the formation of a hemostatic plug, thromboplastic activity, and clot retraction. When platelets contact a wettable surface, at first they adhere to one another and then rupture, releasing chemical factors.

a. **Platelet Factor 1.** This factor accelerates prothrombin conversion and is actually blood factor V adsorbed on platelets. (No longer used conventionally.)

b. **Platelet Factor 2.** Factor 2 accelerates fibrinogen clotting of thrombin. (No longer used conventionally.)

c. **Platelet Factor 3.** A phospholipid substance, found in the platelet membrane, is involved in prothrombin activation. This is the most important factor and probably is an actual intrinsic component of platelets.

d. **Platelet Factor 4.** This factor reacts to neutralize heparin.

e. **Platelet Factor 5.** This factor is an adsorbed intrinsic fibrinogen. (No longer used conventionally.)

f. **Platelet Factor 6.** This factor reduces fibrinolytic activity. (No longer used conventionally.)

g. **Platelet Factor 7.** This factor is adsorbed blood factor VII. (No longer used conventionally.)

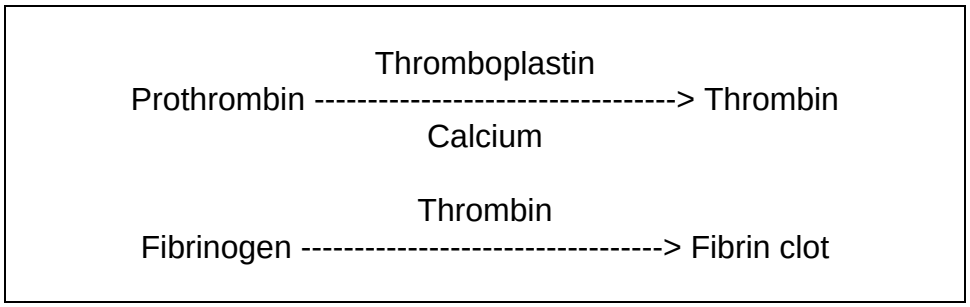
2-5. FIBRINOLYTIC FACTORS

Fibrinolysis is the dissolution of a fibrin clot. The process is a necessary activity following clot formation. The mechanism of clot dissolution is complex and involves a variety of factors. In active circulating plasma profibrinolysin (plasminogen) is converted to its active form, fibrinolysin (plasmin), by tissue activators, streptokinase, urokinase, and other unknown activators. Fibrinolysin acts locally to dissolve the clot.

Section III. THE COAGULATION MECHANISM

2-6. INTRODUCTION

a. The classical theory of Morowitz proposed that four components interact to form a clot as follows:



b. From this concept, the modern theory was devised. The modern theory is based on four stages: (I) the formation of thromboplastin, (II) the conversion of prothrombin to thrombin, (III) formation of an insoluble fibrin clot through the interaction of fibrinogen and thrombin, and (IV) the lysis of the fibrin clot by fibrinolysin. These stages are illustrated in figure 2-3.

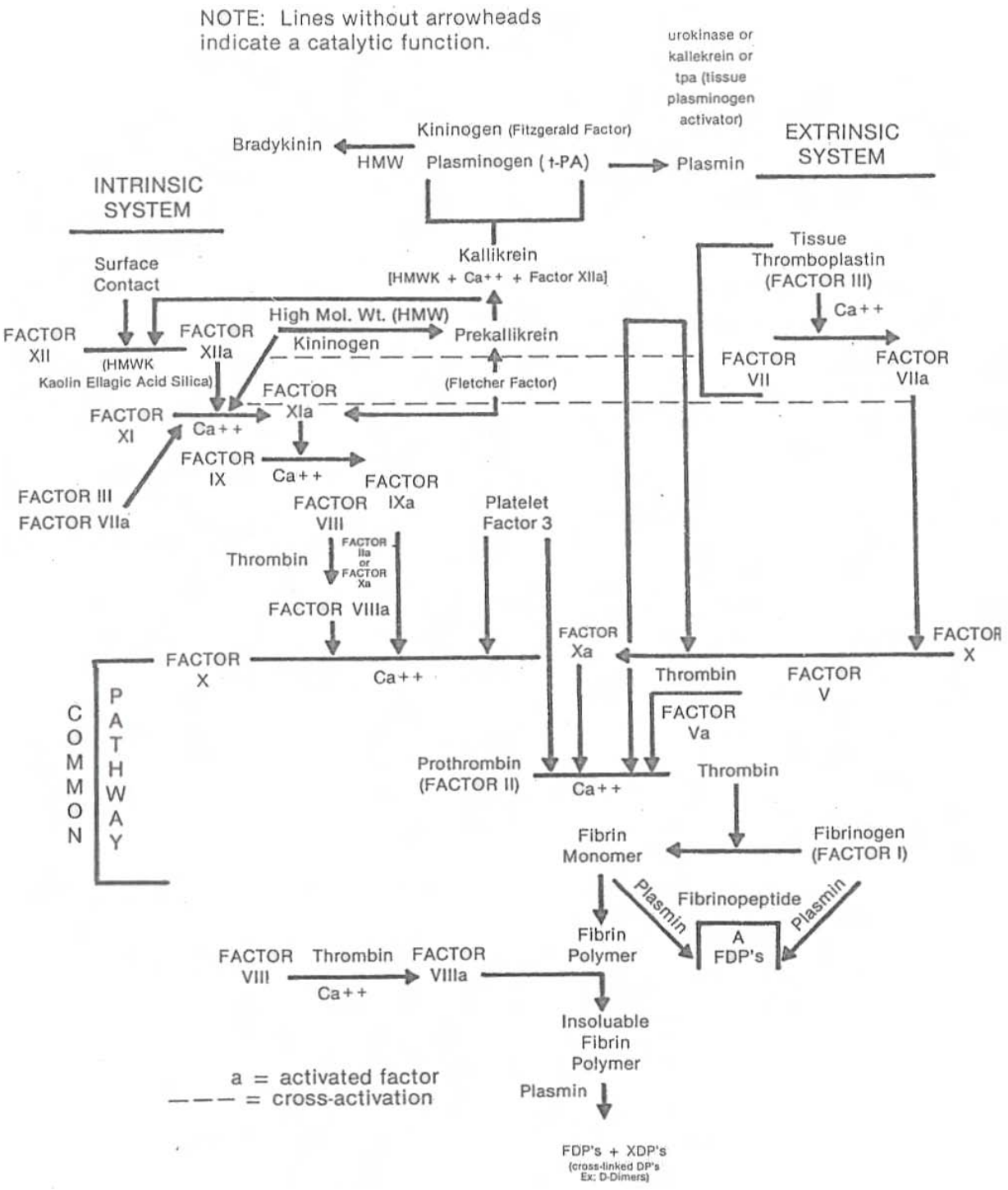


Figure 2-3. Stages of coagulation.

2-7. STAGES OF COAGULATION

a. **Stage I--The Generation of Plasma Thromboplastin.** Stage I involves the information of intrinsic (plasma) thromboplastin. This stage is initiated by the platelets adhering and rupturing, releasing platelet factor 3. Platelet factor 3 reacts with factor XII, along with prekallikrein and high molecular weight kininogen (HMWK), PTA (factor XI), PTC (factor IX), AHF (factor VIII) and in the presence of calcium (factor IV), factor X, and factor V to form intrinsic thromboplastin. Tissue thromboplastin or extrinsic thromboplastin is released by the affected tissues.

b. **Stage II--The Formation of Thrombin from Prothrombin.** In the intrinsic system, prothrombin is converted to thrombin in the presence of plasma thromboplastin, calcium, and factors V and X, and platelets. The extrinsic system requires the presence of an additional factor, factor VII, for the conversion of prothrombin to thrombin.

c. **Stage III--The Formation of Fibrin from Fibrinogen.** After the thrombin is generated, it quickly reacts with fibrinogen to form a fine fibrin fiber. The fibrin fibers polymerize (form a mesh with other fibers with disulphide bonds) in conjunction with calcium ions and factor XIII, the fibrin-stabilizing factor, to form a stabilized clot. The stabilized clot is characterized by its insolubility in 5 M urea.

d. **Stage IV--Clot Lysis.** This stage involves the fibrinolytic dissolution of the clot. Circulating plasminogen can be converted to its active form plasmin, by both intrinsic and extrinsic factors. The intrinsic factors, which are present in circulation plasma include: factor XII, prekallikrein, HMWK, and pro-urokinase. The extrinsic factors, which are present in body tissues are: tissue plasminogen activator (t-PA), urokinase (UK), and streptokinase (SK). Plasmin, which is a serine protease, hydrolyzes arginine and lysine bonds that are present in fibrin, fibrinogen, and factors V and VIII. Thus, the clot in the presence of plasmin is dissolved, forming a series of fragments called fibrin or fibrinogen degredation products (FDP) or fibrin or fibrinogen split products (FSP). These fragments FDP-FSP will inhibit platelet aggregation and conversion of fibrinogen by thrombin.

2-8. COAGULATION INHIBITORS

In addition to the factors necessary for clot formation, inhibitors are present which control but do not prevent coagulation. Natural inhibitors have been described for virtually every clotting factor. Clotting activity is also inhibited by the administration of anticoagulants such as heparin and coumarin derivatives.

Section IV. COAGULATION STUDIES

2-9. INTRODUCTION

a. The activated partial thromboplastin time, prothrombin time, thrombin time (or quantitative fibrinogen), bleeding time, platelet count, and clot retraction constitute a satisfactory coagulation screen to perform on the bleeding patient or on one who is suspected of having a bleeding disorder. The activated partial thromboplastin test is the most useful single laboratory test available. This procedure detects deficiencies present in the intrinsic coagulation system, except for platelets and factor XIII. The prothrombin time is the method of choice for detecting disorders in the extrinsic system. The thrombin time is useful as a test for functional fibrinogen and to test the conversion of fibrinogen to fibrin. It is also sensitive to the presence of thrombin-inhibitors such as heparin. The most widely used screening tests for platelets are the platelet count, the bleeding time, and clot retraction.

b. Once it has been established through screening procedures that a coagulation disorder exists, a systematic approach should be followed in identifying the exact deficiency or disorder. Specific tests to measure the various coagulation functions are listed in table 2-2.

Coagulation Function	Test
Vascular function	Bleeding time, tourniquet test
Platelet function	Bleeding time, clot retraction, tourniquet test, platelet count, platelet aggregation
Clotting ability	Activated partial thromboplastin time, thrombin time, prothrombin time
Fibrinolytic function	Clot retraction, thrombin time, fibrinolysin assay

Table 2-2. Diagnostic coagulation tests.

2-10. BLOOD COLLECTION

a. **Principle.** There are general rules for using a "Vacutainer-type" blood collection system. Tests requiring sterility are always drawn first (blood culture). Next, obtain those tubes for specimens which do not require anticoagulants. This is followed by those specimens which do require anticoagulants. Coagulation specimens come first.

b. **Two-Tube Technique.** To avoid contamination of blood by tissue juices, the two-tube (Vacutainer-type) venipuncture is usually employed for specialized coagulation tests using venous blood. The technique is as follows:

(1) Place a tourniquet on the arm.

(2) Observe the area for the most accessible vein.

(3) Cleanse the site with 70 percent isopropyl alcohol and allow to dry.

(4) Insert the needle and remove the tourniquet. (Quick removal of the tourniquet prevents stasis.)

(5) Obtain specimens for all tests that require no anticoagulants first. If only coagulation work is specified, use a "red" top tube to withdraw about 2 ml of blood, and dispose of the "red" top tube.

(6) Draw off the required specimen using a "blue" top coagulation tube. The ratio of blood to enclosed anticoagulant is 9:1. Allow the tube to fill to its capacity, do not remove until filling stops completely.

(7) Blood samples needing anticoagulation should be mixed immediately following withdrawal from the needle.

c. **Anticoagulants.** Anticoagulants for coagulation studies can be obtained in commercially-prepared vacuum tubes or prepared in the laboratory. The preparation and use of anticoagulants is as follows:

(1) Sodium citrate, 3.8 percent. Dissolve 3.8 g of sodium citrate in 100 ml distilled water. Refrigerate. As an anticoagulant, combine one part 3.8 percent sodium citrate with 9 parts blood.

(2) Sodium oxalate, 0.1 M. Dissolve 1.34 g sodium oxalate in 100 ml distilled water. Refrigerate. As an anticoagulant, combine one part 0.1 m. sodium oxalate with 9 parts blood.

(3) EDTA. Dissolve 10 g of EDTA salt in 100 ml distilled water. Pipet 1 ml of this solution into a suitable test tube. Allow to dry in the oven at low temperature. For use as an anticoagulant, add 5 ml of venous blood and mix well.

d. **Glassware.** All glassware for coagulation studies must be scrupulously clean. Used glassware should be free of chemicals and any traces of human blood components. All glassware should be cleaned in detergents free of organic solvents and rinsed several times with distilled water. The use of disposable syringes and needles eliminates the need for siliconizing glassware.

CAUTION: Never use test tubes or pipets that are damaged.

2-11. IVY BLEEDING TIME

a. **Principle.**

(1) The bleeding time is an in vivo measurement of platelet participation in small vessel hemostasis. The introduction of the template bleeding time technique has improved the reproducibility and sensitivity of the test by controlling the length and depth of the incision. Control of the depth is important so that only the smaller vessels (capillaries) are incised.

(2) The platelets initially adhere to the cut surface of the vessel wall; aggregation takes place. The platelets interdigitate and release the contents of their granules. This is followed by the formation of fibrin that stabilizes the hemostatic plug.

(3) The purpose of the bleeding time test is to provide a measure of such platelet functions in small vessel hemostasis. A prolonged bleeding time does not in itself diagnose underlying platelet disorders, either qualitatively or quantitatively. It indicates the need for more quantifiable testing.

b. **Reagents and Materials.**

(1) 1 surgicutt.

(2) 1 70 percent alcohol pad.

(3) Sphygmomanometer.

(4) Stopwatch.

(5) Filter paper (2 - 3 Sheets).

(6) Band-Aid ® or other bandage.

c. **Procedure (Ivy Method)**.

(1) Position the patient's arm with the volar surface exposed.

(2) Select a site avoiding surface veins, bruises, and edematous areas.

(3) Place the sphygmomanometer on the upper arm.

(4) Cleanse the test area with the 70 percent alcohol pad and let air-dry.

(5) Inflate the cuff to 40 mm Hg., and hold at this exact pressure for the duration of the test.

NOTE: The time between inflation and incision should be 30 to 60 seconds.

(6) Open the sterile package and gently rest the Surgicutt surface on the patient's forearm.

(7) Apply minimal pressure so that both ends of the instrument lightly touch the skin.

(8) Gently push the trigger, starting the stopwatch simultaneously.

(9) Remove the Surgicutt immediately after triggering.

(10) After 30 seconds, wipe the flow of blood with the filter paper. (Bring the paper close to the incision, but do not touch the paper directly to the incision, so as not to disturb the formation of the platelet plug.)

(11) Wipe the blood every 30 seconds thereafter, until no blood stains the paper.

(12) Stop the timer when only clear fluid is absorbed onto the filter paper. The bleeding time is determined to the nearest 30 seconds.

(13) Release the pressure of the sphygmomanometer.

(14) Record the bleeding time.

d. **Sources of Error.**

(1) A horizontal incision, approximately 5 cm below the antecubital crease, gives the best reproducibility.

(2) If body hair will interfere, lightly shave the area.

(3) Patient should be advised of a potential to produce a scar. This can usually be avoided by the use of a butterfly bandage applied for 24 hours.

(4) Aspirin and aspirin-containing products may cause a prolonged bleeding time for up to two (2) weeks.

(5) A standardized cut is necessary for valid results. Too little pressure on the device and the wound will be shallow or nonexistent. Too much pressure and the wound will be too deep. This is the one area where standardization has not been completely controlled.

(6) Low skin temperature produces a constriction of the capillary vessels, resulting in decreased blood flow.

e. **Discussion.**

(1) The Ivy Bleeding Time is the method of choice because the blood pressure on the vessels is constant, the incision is uniform, and the arm offers an area for multiple determinations.

(2) The bleeding time depends primarily on extravascular and vascular factors and, to a lesser degree, on the factors of coagulation. The chief factor controlling bleeding from a small cut is the constriction of the minute vessels following injury. Accuracy in this test is enhanced by blotting the drops of blood at shorter intervals of time as the drops of blood become progressively smaller.

(3) Thrombocytes play an important part in the formation of the hemostatic plug that seals off a wound. In thrombocytopenic purpura there is a decrease in platelets resulting in a prolonged bleeding time due to a defective platelet plug. An additional factor prolonging the bleeding time in this condition is a defect in capillary contraction.

(4) In hemophilia, the bleeding time is normal. This is explained by the fact that there are no vascular or extravascular abnormalities. However, the test should not be performed on a known hemophiliac, for delayed oozing of blood is a real hazard.

f. **Range of Values.**

(1) Normal values: 2 - 8 minutes.

(2) Critical values:

Less Than	Greater Than
Not applicable	15 minutes

2-12. WHOLE BLOOD CLOTTING TIME (LEE-WHITE)

a. **Principle.** The whole blood clotting time is a rough measure of all intrinsic clotting factors in the absence of tissue factors. Variations are wide and the test sensitivity is limited. Whole blood, when removed from the vascular system and exposed to a foreign surface, will form a solid clot. Within limits, the time required for the formation of the solid clot is a measure of the coagulation system.

b. **Reagents and Materials.**

(1) Stop watch equipment for collection of blood.

(2) 2 plastic syringes.

(3) 3 clean, dry glass test tubes (10 x 75 mm).

(4) Water/dry bath (at 37^{o}C).

c. **Procedure.**

(1) Label glass tube numbers one, two, and three.

(2) Collect at least 1--2 ml of blood in a plastic syringe. Discard this blood. (This prevents tissue thromboplastin from entering the blood sample.) Change syringes.

(3) Collect at least 5 ml of blood in the second plastic syringe.

(4) Approximately 1 ml of blood is placed in each of the three glass test tubes. (number three first, then number two, then number one)

(5) The stopwatch is started as soon as the blood enters the first tube number three.

(6) All tubes are placed into the 37ºC water bath.

(7) Gently tilt tube number three(45 angle) every 30 seconds, until the blood in it clots.

(8) Thirty seconds after tube number three clots, proceed with tube number two, tilting eve seconds, until a clot is formed.

(9) Thirty seconds after tube number two is clotted, tube number one is tilted until no flow blood is observed on tilting.

(10) Record the time. The coagulation time is the time required for the blood to clot in the last tube. (Tube number one)

NOTE: This range should be between 5 to 10 minutes.

d. **Range of Values**.

(1) Normal values: 5--5 minutes.

(2) Critical value:

Less Than	Greater Than
Not applicable	15 minutes

NOTES: 1. The following variables tend to decrease the clotting time: rough handling of the blood specimen, presence of tissue fluids (traumatic venipuncture), frequent tilting of the tube, and unclean tubes.

2. The following variables tend to increase the clotting time: extreme increases in temperature, variation in pH, and performance of the test at room temperature.

3. This test is of value primarily as it was used to follow heparin therapy. Its use as a screening procedure is limited due to its poor sensitivity.

4. The whole blood clotting time is affected mainly by defects in the intrinsic pathway factors and by defects in fibrin and fibrinogen. It is not sensitive to platelet abnormalities.

5. A prolonged clotting time immediately indicates impaired coagulation, but a normal clotting time does not exclude many serious clotting defects.

6. The disadvantages of the whole blood clotting time is its relative lack of reproducibility.

7. This procedure has been replaced in most laboratories with the APTT, which is more reproducible and easily controlled.

8. The coagulation time is normal in thrombocytopenic purpura. This is explained by the fact that only a small number of thrombocytes need be present for normal coagulation to take place.

2-13. CLOT RETRACTION TEST

a. **Principle.** When blood coagulation is complete, the clot retracts and expresses serum as the clot becomes denser. Thrombosthenin, released by the platelets, is responsible for clot retraction. The number of platelets present also affects the clot retraction time.

b. **Reagents and Equipment.**

(1) Venous specimen, approximately three ml, using the two-tube technique.

(2) 12 x 75 mm glass test tubes, three.

(3) 37ºC waterbath.

(4) Timer.

(5) One ml Pipettor or volumetric pipet.

c. **Procedure.**

(1) Withdraw three ml of venous blood using the two-tube technique.

(2) Place one ml of blood into each of three glass test tubes and immediately incubate in a 37ºC water bath.

(3) Set a timer for 1 hour.

(4) At 1 hour, observe the clot and record results.

(5) Inspect the tubes at 2, 4, and 24 hours, observe and record results.

(6) Examine the tubes for retraction after incubation. Separation of the clot from the test tube is complete retraction (+4).

d. **Calculations.** Approximate amount of shrinkage of clot. Either in percentage or using (+1 to +4) grading.

e. **Sources of Error.**

(1) Shaking or jarring of the tube of blood should be avoided. This may lead to a shortened clot retraction time.

(2) Certain anemic patients with a low hematocrit value show increased clot retraction due to the formation of a small clot. Polycythemia vera may also affects results.

f. **Discussion.**

(1) Poor clot retraction occurs in thrombocytopenia, qualitative platelet deficiency, and in cases of increased red cell mass.

(2) The clot retraction is normal in hemophilia since there are a normal number of platelets. However, the onset of contraction is often delayed in blood samples from hemophiliac patients.

(3) Clot retraction varies inversely with the plasma fibrinogen concentration. That is, if the plasma fibrinogen level is elevated, clot retraction may be poor.

(4) Generally, there is a small amount of what is termed red cell fallout during clot retraction. This is seen as a few red cells at the bottom of the tube that have fallen from the clot. The significance of an increased amount of red cell fallout is not known. Whenever red cell fallout is increased, a notation on the patient's report should be made.

(5) Tubes from a completed Lee-White clotting time can be used to perform this test.

g. **Normal Values**. Results are reported as the length of time it took for the clotted blood to retract. A normal clot retracts from the sides and bottom of the test tube within 1 to 2 hours.

2-14. TOURNIQUET TEST

a. **Principle.** The fragility of capillaries is determined under increased pressure due to a sphygmomanometer. The pressure partially obstructs the venous return from the arm and increases intracapillary pressure. The number of petechial hemorrhages reflects the degree of capillary fragility.

b. **Equipment**. Sphygmomanometer.

c. **Procedure.**

(1) Place a blood pressure cuff on the patient's arm.

(2) Inflate it to a point midway between the systolic and diastolic pressure.

(3) Maintain this pressure for 5 minutes.

(4) Remove the cuff and wait 2 minutes.

(5) Examine a representative area (a circle about 2.5 centimeters in diameter) on the hand or arm for the presence of petechiae.

d. **Calculations.** Grade the number of petechiae as follows:

0 -10	=	1+
10 -20	=	2+
20 -50	=	3+
over 50	=	4+

e. **Sources of Error.**

(1) Mistaking skin blemishes for petechia increases the number.

NOTE: Check for skin blemishes before the test.

(2) Capillary fragility varies at different sites.

(3) Maintaining the pressure too long causes false positives.

f. **Normal Values.** 0-10 petechiae per 2.5 cm area.

g. **Discussion.**

(1) Increased petechiae are observed with vascular purpura.

(2) Platelet disorders also cause increased petechial formation.

(3) The tourniquet test is a crude test to determine the ability of blood vessels to withstand trauma and should not be used as a screening test for surgery.

(4) Increased vascular fragility is sometimes found in qualitative and quantitative platelet defects, vitamin C deficiency, dietary ascorbic acid deficiency, and in the various purpuras. The term "purpura" is not specific but applies to a number of affections characterized by bleeding into tissue.

(5) The tourniquet test is most often performed by the physician.

2-15. PARTIAL THROMBOPLASTIN TIME (ACTIVATED)

a. **Principle.** Normal citrated plasma contains all clotting factors except calcium ions and platelets. Calcium ions and a partial thromboplastin (platelet-like substance, a phospholipid) are added to the plasma and the clotting time recorded. An activator (such as ellagic acid, celite, or kaolin) is added to make activation of the plasma independent of the surface contact of the tube. As a result of optimal activation of the contact factors, the activated partial thromboplastin time is shorter and less variable than the partial thromboplastin time. The time required for the plasma to clot is the activated partial thromboplastin time.

NOTE: The tube must have the exact required blood volume. A "short" draw will affect results. Ratio of blood to anticoagulant must be 9:1. Most tubes draw 4.5 ml of whole blood to be mixed with 0.5 ml anticoagulant, usually citrate. Tube should be placed on ice as soon as possible, and preferably centrifuged in a refrigerated centrifuge.

b. **Reagents and Equipment.**

(1) Blue top, sodium citrated Vacutainer tube.

(2) Kontact APTT (Curtin Matheson Supply (CMS)).

(3) Liquid reagent calcium chloride, CMS.

(4) Thromboscreen controls.

(5) Levels I & II MLA 800 cuvettes 0.1 ml pipettor.

(6) Pipettor tips.

(7) MLA 800 automatic coagulation timer.

c. **Procedure.**

(1) Obtain the coagulation specimen by the two-tube method.

(2) Centrifuge the specimen, at 2500 RPM, for 5 minutes, to obtain a platelet poor plasma (PPP) specimen.

(3) Remove the specimen and place in an ice bath or cold block.

(4) Make sure that the correct reagents on set up on the instrument, since it performs both PT and APTT testing, using the same pumps.

(5) Pump #2 should contain the activated partial thromboplastin reagent.

(6) Pump #1 should contain the calcium chloride reagent.

(7) Place a cuvette in the first well.

(8) Place pipette into wells A and B, 0.1 ml of the same patient's PPP specimen.

(9) Place the instrument in the APPT mode.

(10) Press run on the instrument's touch panel.

(11) Print out the results on both the screen and hard copy from wells A and B, in addition to the average, following the incubation period that the instrument is set up for, either 3 or 5 minutes.

d. **Calculations.** All done by the instrument.

e. **Sources of Error.**

(1) Plasma must be prepared with care without disturbing the buffy coat.

(2) If plasma is left at room temperature, the test must be performed within 45 minutes. Otherwise plasma can be stored at 4ºC for 3 hours.

(3) Accurate pipetting is essential for valid results.

(4) Do not use sodium oxalate as an anticoagulant. Sodium citrate is a better preservative and activates plasma faster.

(5) Paired APTT values must be within + or - 10 percent of each other in the normal range. If not, instrument will beep and flag the results.

f. **Discussion**.

(1) This test is an overall screening test for all three stages of coagulation with the exception of factor VII, calcium, and platelet factor 3.

(2) The partial thromboplastin time is prolonged in all deficiencies of prothrombin and factor V, as well as deficiencies of all the plasma factors in the intrinsic system. To exclude an abnormality in the extrinsic system, a prothrombin time test should be performed on all abnormal plasma.

(3) In conjunction with the prothrombin time the following deficiencies can be determined:

PTT	PT	Deficiency
Abnormal	Normal	Stage I: factors VIII, IX, XI, XII
Abnormal	Abnormal	Fibrinogen, prothrombin, factor V, and X
Normal	Abnormal	Factor VII

(4) A differential partial thromboplastin time is performed to detect the specific deficiency.

(5) The partial thromboplastin test is much more sensitive to coagulation factor deficiencies than is the whole blood clotting time.

g. **Range of Values.** Both Normal and Abnormal controls should be run on a daily or shift basis. Controls should come in within 2 S.D.'s of the established mean.

(1) Normal range: 23 to 30 seconds.

(2) Critical Values:

Less Than	Greater Than
APTT 20 secs.	100 secs.

2-16. ONE-STAGE PROTHROMBIN TIME

a. **Principle**. When optimal amounts of thromboplastin, calcium, and citrated plasma are mixed under carefully controlled conditions, fibrin strands will normally form within a matter of seconds. The interval during which timed reaction occurs is the prothrombin time. This test measures the overall prothrombin activity of plasma. The prothrombin time measures the integrity of the extrinsic system. It is the most widely used measurement for monitoring patients on coumarin (oral anticoagulant) therapy.

b. **Reagents and Equipment**.

(1) Blue top, sodium citrated Vacutainer tube thromboscreen.

(2) Thromboplastin (CMS),liquid thromboscreen controls, levels I & II MLA 800 cuvettes, and 0.1 ml pipettor pipettor tips.

(3) MLA 800 automatic coagulation timer.

(4) One tube of citrated blood (blue top tube), drawn by the two-tube method.

NOTE: The tube must have the exact required blood volume. A "short" draw will affect results. Ratio of blood to anticoagulant must be 9:1. Most tubes draw 4.5 ml of whole blood to be mixed with 0.5 ml anticoagulant, usually citrate. Tube should be placed on ice as soon as possible, and preferably centrifuged in a refrigerated centrifuge.

c. **Procedure.**

(1) Obtain the coagulation specimen by the two-tube method.

(2) Centrifuge the specimen, at 2500 RPM, for 5 minutes, to obtain a platelet poor plasma (PPP) specimen.

(3) Remove the specimen and place in an ice bath or cold block.

(4) Make sure that the correct reagents on set up on the instrument, since it performs both PT and APTT testing, using the same pumps.

(5) Pump number one should contain the thromboplastin reagent.

(6) Pump number two may contain the activated partial thromboplastin reagent, it does not matter. It will not be used during this procedure.

(7) Place a cuvette in the first well.

(8) Pipette into wells A and B, 0.1 ml of the same patient's PPP specimen.

(9) Place the instrument in the PT mode.

(10) Press run on the instrument's touch panel.

(11) Following the incubation period that the instrument is set up for, 2 minutes, the results will be printed out on both the screen and on a hard copy.

(12) The instrument will print out both results, from wells A and B. In addition, it will print out the average plus a ratio from a stored normal range value.

d. **Calculations.** All done by the instrument.

e. **Source of Error.**

(1) Avoid traumatic venipuncture.

(2) Tourniquet application must not be prolonged.

(3) The test must be accomplished within 3 hours after collection of the blood specimen. If the test is not run immediately, refrigerate the plasma and thromboplastin. Refrigerated plasma is stable for a maximum of 3 hours.

(4) The blood must be added to the anticoagulant immediately to avoid clot formation.

(5) The use of sodium oxalate as anticoagulant decreases the activity of factor V. Use sodium citrate as stated in the procedure.

(6) Hemolyzed plasma must not be used.

f. **Discussion.**

(1) Paired PT values must be within + or - 0.5 secs of each other in the normal range, or the instrument will beep and flag the results.

(2) The prothrombin activity may also be reported as a ratio as well as in seconds by applying the patient's prothrombin time (in seconds) to a stored normal range prothrombin value.

(3) The prothrombin activity of a patient's plasma has important diagnostic and prognostic significance in diseases of the liver, in vitamin K deficiency, specific coagulation deficiencies, and in the use of coumarin drugs as therapeutic anticoagulants.

(4) Patients receiving coumarin drugs for thromboembolic disorders generally have prothrombin times of 20 to 30 seconds or 1.5 to 2.5 times their normal prothrombin time.

(5) Prolongation of the one-stage prothrombin time does not measure prothrombin deficiency alone but rather indicates some failure of conversion of prothrombin to thrombin. Specifically, the test detects deficiencies of factors I, II, V, VII, or X. Varying reductions in any one, or combination, of these factors prolong the one-stage prothrombin time.

(6) This test is well adapted for fibrometer use also.

Prothrombin time	Variation
12-20 seconds	0.5 second
20-30 seconds	1.0 second
Over 30 seconds	2.0 second

g. **Range of Values.** Both normal and abnormal controls should be run on a daily or shift basis. Controls should come in within 2 S.D.'s of the established mean.

(1) Normal value: 12 to 14 seconds.

(2) Critical value:

	Less Than	Greater Than
PT	Not applicable	40 secs.

2-17. PROTHROMBIN CONSUMPTION TIME

a. **Principle.** Prothrombin is partially used up in the normal clotting process. The amount of prothrombin remaining in serum indicates the adequacy of the thromboplastin complex. The time required for a clot to form when optimal amounts of thromboplastin, fibrinogen, factor V, calcium, and serum are mixed is the prothrombin consumption time. The test is less sensitive than either TGT or the APTT. The test is not sensitive enough to exclude minor defects in the intrinsic pathway as for mildly deficient patients. Thrombocytopenia and certain other platelet defects will cause the PCT to be abnormal.

b. **Reagents and Specimen Required.**

(1) Thromboplastin, liquid thromboscreen controls, levels I & II MLA 800 cuvettes, and 0.1 ml pipettor pipettor tips.

(2) MLA 800 automatic coagulation timer.

(3) One tube of citrated blood (blue top tube), drawn by the two-tube method.

(4) Normal adsorbed plasma reagent.

(a) Mix 9 parts of freshly collected, normal blood with 1 part 3.8 percent sodium citrate.

(b) Centrifuge at 2,000 rpm for 5 minutes and remove supernatant plasma.

(c) For each ml of plasma, add 100 mg of chemically pure barium sulfate (see Federal Supply Catalog).

(d) Mix well for 3 minutes and allow to stand at room temperature for an additional 2 minutes to complete adsorption.

(e) Centrifuge the material and the upper three-quarters of the plasma.

(f) Perform a prothrombin time on the adsorbed plasma. The prothrombin time should be greater than 60 seconds; if not, repeat the absorption.

NOTE: Barium sulfate-adsorbed plasma is available commercially. Also available is a reagent containing fibrinogen, factor V, thromboplastin, and calcium.

c. **Procedure.**

(1) Draw 5 ml of blood by a nontraumatic venipuncture.

(2) Place the blood in a test tube and allow it to clot at room temperature.

(3) Place the clotted blood in a 37ºC water bath for 1 hour.

(4) Centrifuge the specimen at 3,000 rpm for 3 minutes and transfer the serum to another test tube.

(5) Place the serum, thromboplastin reagent, and barium sulfate- adsorbed plasma in a 37ºC water bath for 5 minutes to equilibrate to that temperature.

(6) Pipet 0.1 ml of serum, 0.1 ml of barium sulfate-adsorbed plasma, and 0.2 ml of thromboplastin reagent into a Kahn tube in that order, Once items are added, start a stopwatch.

(7) Determine the time for the clot to form as in the one-stage prothrombin time.

(8) Repeat the procedure, commencing with step 6, for two other determinations.

(9) The three determinations should agree as follows:

Time	Variation
Over 30 seconds	2.0 seconds
20-30 seconds	1.0 second
12-20 seconds	0.5 second

d. **Sources of Error.** See paragraph 2-16d.

e. **Disscussion.**

(1) Patients having decreases or defects in the thromboplastin complex (thrombocytopenia, hemophilia, deficiency in plasma thromboplastin antecedent or component) will not use up normal amounts of prothrombin in the clotting process. The excess of residual prothrombin in the serum will result in a serum prothrombin time of less than 20 seconds. If the content of any of the factors in the first phase is low, the rate will be decreased and, therefore, the prothrombin will not be consumed as rapidly as normal.

(2) Administration of heparin will serve to shorten the prothrombin consumption time as well as prolong the "Lee-White" clotting time. Coumarin, on the other hand, will have no effect upon the serum prothrombin time, even while prolonging the plasma prothrombin time. This indicates that coumarin has no influence upon the thromboplastin complex.

(3) Normal results are considered valid only if the one-stage prothrombin time is normal.

(4) The test is abnormal in platelet deficiencies and deficiencies of factors VIII, IX, X, XI, and XII.

(5) It is important that the barium sulfate-adsorbed plasma be deprothrombinated sufficiently to give a prothrombin time of over 1 minute. This reagent is a source of fibrinogen and factor V since they are used up in the formation of a clot.

f. **Range of Values.**

(1) Normal value: Longer than 30 seconds.

(2) Doubtful: 20-30 seconds.

(3) Abnormal: Less than 20 seconds.

2-18. THROMBOPLASTIN GENERATION TIME

a. **Principle**. A potent thromboplastin is generated when platelets, prothrombin-free plasma, serum, and calcium are mixed. After generation of the thromboplastin, all factors necessary to produce a clot are present except for factor I and factor II. When these factors are added to a normal thromboplastin generation mixture, a clot is detected within 7-16 seconds. With an abnormal time, deficient factors are identified by substituting adsorbed patient plasma or aged patient serum. This procedure, which is quite complicated, tests the ability of the patient to generate adequate prothrombin activation through the intrinsic system pathway.

b. **Reagents and Specimen Required.**

(1) Normal plasma substrate. Available from the Federal Supply Catalog. This control is used as the source of factors I, II, V, VII, and X.

(2) Partial thromboplastin (platelet-like substance). Available from commercial sources.

(3) Normal adsorbed plasma reagent. See paragraph 6-17b(2). The adsorbed plasma is the source of factors V, VIII, XI, and XII. Dilute normal adsorbed plasma 1:5 in saline (0.85 percent NaCl) and allow to stand for 1 hour at 4ºC or place in an ice bath before use.

(4) Normal aged serum. Add 2 ml of freshly-drawn blood to a clean test tube and allow to clot at 37ºC for 4 hours. After the incubation, centrifuge and remove the clot. Dilute the serum 1:10 with 0.85 percent NaCl (saline) and allow to stand for 1 hour before using.

(5) Calcium chloride, 0.025 M. Add 0.277 g anhydrous calcium chloride to a 100 ml volumetric flask. Dilute to the mark with distilled water.

(6) Patient's adsorbed plasma reagent. Prepare in the same manner as normal adsorbed plasma, substituting patient's plasma for normal plasma.

(7) Patient's aged serum reagent. Prepare in the same manner as normal aged serum substituting patient's serum for normal serum.

c. **Procedure for Standardization of Control Reagents.**

(1) Pipet 0.1 ml of 0.025 M calcium chloride into three 12 x 75 mm test tubes.

(2) Place test tubes of the following reagents in a 37ºC water bath:

(a) Partial thromboplastin reagent.

(b) Normal plasma substrate.

(c) Normal adsorbed plasma.

(d) Normal aged serum.

(e) 2.0 ml of 0.025 M calcium chloride.

(f) Four tubes containing 0.1 ml of 0.025 M calcium chloride.

(3) Prepare a generation mixture by adding the following reagents to a clear 12 x 75 mm test tube:

(a) 0.2 ml partial thromboplastin reagent.

(b) 0.2 ml normal adsorbed plasma reagent.

(c) 0.2 ml normal aged serum reagent.

(d) 0.2 ml of 0.025 M calcium chloride, simultaneously starting a stopwatch or automatic timer.

(4) At 2 minutes, pipet 0.1 ml of the generation mixture into the first tube containing 0.1 ml of 0.025 M calcium chloride. Immediately add 0.1 ml of normal plasma substrate to the tube, simultaneously starting a stopwatch.

(5) Check for the first formation of a clot using the tilt-tube method. (If available, substitute a Fibrometer for detection of clot formation.)

(6) Repeat steps 4-5 at 4, 6, and 8 minutes.

(7) The normal range for the control reagents is 7-16 seconds for clot formation. This range is obtained within the 2 to 8 minute generation time.

d. **Procedure for Test for Patient Deficiencies.**

(1) Repeat the procedure for standardization of control reagents, substituting patient's aged serum for normal aged serum and patient's adsorbed plasma for normal adsorbed plasma.

(2) If there is a marked difference between the normal generation time and the patient's generation time, repeat the test, substituting normal aged serum and normal adsorbed plasma, one at a time, for the patient's reagent in the generation mixture.

e. **Interpretation.** See chart below.

Adsorbed Plasma	Aged Serum	PT	PTT	Deficient Factor
Abnormal	Normal	Abnormal	Abnormal	V
Normal	Normal	Abnormal	Normal	VII
Abnormal	Normal	Normal	Abnormal	VIII
Normal	Abnormal	Normal	Abnormal	IX
Normal	Abnormal	Abnormal	Abnormal	X
Abnormal	Abnormal	Normal	Abnormal	XI
Abnormal	Abnormal	Normal	Abnormal	XII
Abnormal	Abnormal	Abnormal	Abnormal	Inhibitor

f. **Sources of Error.**

(1) All reagents must be freshly prepared each day.

(2) Tubes of plasma substrate left at room temperature are unreliable.

(3) All U.S.P. barium sulfate preparations are not standard in reaction. Factor IX at times may be incompletely removed.

(4) Antithromboplastin activity in blood prolongs TGT, and accounts for some double deficiencies.

(5) This is not a routine laboratory procedure; considerable technologist skill is required.

(6) A clot can form when the generation mixture is prepared. This is removed with a wooden applicator stick.

(7) Other sources of error can be found in paragraphs 2-15d and 2-16d.

g. **Discussion.**

(1) A platelet deficiency can be detected by the TGT. This is done by preparing platelet-rich plasma and substituting it for the partial thromboplastin reagent. An abnormal generation time is noted in a patient with platelet deficiency.

(2) The TGT reveals abnormalities essential to the development of thromboplastin activity in the intrinsic blood system.

(3) The TGT should be correlated with the PT and PTT to determine the specific abnormality.

(4) Adsorbed plasma contains factors V, VIII, XI, and XII. If the TGT is prolonged when patient's adsorbed plasma is substituted for normal adsorbed plasma, a deficiency in one or more of these factors is indicated.

(5) Aged serum contains factors IX, X, XI, and XII. If the TGT is prolonged when patient's aged serum is substituted for normal aged serum, a deficiency in one or more of these factors is indicated.

(6) Factors XI and XII are present in adsorbed plasma and aged serum. A deficiency in either of these factors prolongs the TGT. Determination of the specific factor deficiency is done by the physician on the basis of clinical grounds.

h. **Normal Values.** Clot formation time of 7-16 seconds within 8 minutes generation time.

2-19. THROMBIN TIME

a. **Principle.** A known amount of thrombin is added to plasma and the time required for clot formation is recorded. Results are affected by abnormal fibrinogen levels, heparin, and antithrombins. This is useful in the diagnosis of DIC.

b. **Reagents.**

(1) Thrombin, 5000 units per ml. Available from the Federal Supply Catalog.

(2) Stock Thrombin Solution, 500 Units per ml. Reconstitute the thrombin with 10 ml of saline (0.85 percent NaCl).

(3) Working thrombin solution, 10 Units per ml. Dilute the stock solution 1:50 with saline (0.85 percent NaCl).

(4) Control plasma. Available from the Federal Supply Catalog.

c. **Procedure.**

(1) Obtain 4.5 ml of venous blood and add to 0.5 ml sodium citrate. Mix well.

(2) Centrifuge and separate the plasma.

(3) Incubate patient plasma, control plasma, and thrombin solution in 37ºC water bath for 3 minutes.

(4) Pipet 0.1 ml of control plasma and 0.1 ml of thrombin solution into a clean test-tube, simultaneously starting a stopwatch. Observe for clot formation.

(5) Repeat step (4) for the patient's plasma.

d. **Sources of Error**. See paragraph 6-16d.

e. **Discussion.**

(1) The thrombin time is prolonged in fibrinogen levels below 100 mg per dl, presence of fibrinolysins, and presence of circulating anticoagulants.

(2) Low concentrations of heparin in the patient's plasma may not be observed by this test. To detect small amounts of heparin, thrombin is diluted, and normal patient's plasma thrombin times are determined. A prolongation of the patient's thrombin time over that of the normal at some dilution indicates an antithrombic substance.

(3) To perform the antithrombin test, dilute the working thrombin 1:2, 1:4, 1:8, 1:16, and 1:32 with saline. Then follow the procedure for the thrombin time test using the dilutions.

f. **Normal Values.** 11-15 seconds.

2-20. FIBRINOGEN ASSAY (SEMIQUANTITATIVE)

a. **Principle.** Fibrinogen, a plasma globulin formed in the liver, is salted out by ammonium sulfate and measured with a spectrophotometer.

b. **Reagents.**

(1) Parfentjev reagent. Add 133.33 g ammonium sulfate, 10.0 g NaCl, and 0.025 g merthiolate to a 1-liter volumetric flask. Dilute to the mark with distilled water.

(2) Saline (0.85 percent NaCl). Add 8.5 g sodium chloride to a 1-liter volumetric flask. Dilute to the mark with distilled water.

(3) Fibrinogen, U.S.P. Available from the Federal Supply Catalog.

(4) Fibrinogen stock standard, 500 mg per dl. Add 500 mg of fibrinogen to a 100-ml volumetric flask. Dilute to the mark with saline.

c. **Calibration Curve.**

(1) Prepare the following dilutions of the stock standard:

Fibrogen Stock Standard	Saline	Concentration
10.0 ml	0	500 mg per dl
7.5 ml	2.5 ml	350 mg per dl
5.0 ml	5.0 ml	250 mg per dl
2.5 ml	7.5 ml	125 mg per dl

(2) Set up the following cuvettes for each standard:

Blank Cuvet	Test Cuvette
0.5 ml standard	0.5 ml standard
4.5 ml saline	4.5 Parfentjev reagent

(3) Three minutes after addition of the Parfentjev reagent, shake the cuvettes vigorously, and read the absorbances of the test cuvettes at 510 nm with the blank set at zero absorbance.

(4) Plot absorbance versus concentration on linear graph paper.

d. **Procedure.**

(1) Draw 4.5 ml of fresh venous blood and add to a test tube containing 0.5 ml of 3.8 percent sodium citrate.

(2) Centrifuge and separate the plasma.

(3) Set up the following cuvets for each unknown plasma:

Blank	Unknown
0.5 ml plasma	0.5 ml plasma
4.5 saline	4.5 Parfentjev reagent

(4) Three minutes after addition of the Parfentjev reagent, shake the cuvets vigorously, and read the absorbances of the test cuvets at 510 nm with the blank set at zero absorbance.

(5) Obtain the fibrinogen concentration from the calibration curve.

e. **Sources of Error.**

(1) The Parfentjev reagent deteriorates after two weeks of storage. Prepare fresh every 2 weeks.

(2) The fibrinogen has a potency for 60 months. Do not use outdated fibrinogen to prepare standards.

(3) The procedure is limited as it is dependent on time, temperature, anticoagulant, and concentration of other proteins.

f. **Discussion.**

(1) Although the procedure is limited, a rapid determination can be obtained when adequate fibrinogen levels are present.

(2) Screening procedures, in kit form, are available commercially.

(3) Fibrinogen is essential to clot formation in stage 3 of the clotting mechanism. Bleeding is encountered when the plasma fibrinogen level falls below 75 mg per dl.

(4) A colorimetric fibrinogen procedure is outlined in AFM 160-49.

g. **Normal Values.** 200-400 mg per dl.

2-21. PLASMA RECALCIFICATION TIME

a. **Principle.** Calcium chloride is added to plasma and the clotting time recorded.

b. **Reagents.**

(1) Calcium chloride, 0.025 M, information is at paragraph 2-18b(5).

(2) Sodium citrate, 3.8 percent, is available from the Federal Supply Catalog.

(3) Sodium chloride, 0.85 percent (w/v).

c. **Procedure.**

(1) Draw 4.5 ml of fresh venous blood and add to 0.5 ml of 3.8 percent sodium citrate.

(2) Centrifuge at 2,500 rpm for 20 minutes and separate the plasma.

(3) Incubate, at 37ºC for 2 to 3 minutes prior to each test, each of the following, in separate test tubes:

(a) Patient's platelet-poor plasma.

(b) Normal platelet-poor control plasma.

(c) Calcium chloride, 0.025 M.

(d) Sodium chloride, 0.85 percent.

(4) Into a 13 x 100 mm test tube, in the 37ºC water bath, pipet 0.1 ml 0.85 percent sodium chloride and 0.1 ml of patient's plasma. Mix.

(5) Blow in 0.1 ml 0.025 M calcium chloride and simultaneously start a stopwatch.

(6) Allow the tube to remain in the 37ºC water bath for 90 seconds, tilting the tube gently every 30 seconds.

(7) After 90 seconds, remove the tube from the water bath and gently tilt. Stop the watch as soon as a clot forms, and record the results.

d. **Sources of Error.**

(1) Platelet-poor plasma must be used in the test. Platelet-rich plasma shortens the recalcification time.

(2) The test is dependent on the platelet count, concentration of plasma clotting factors, time of storage in glass, and the presence of circulating anticoagulants.

(3) See paragraph 2-15d for other sources of error.

e. **Discussion.**

(1) This test is the basis for other coagulation procedures.

(2) A decrease in any of the clotting factors present in the intrinsic system will cause a prolonged clotting time.

f. **Normal Values.** 90 to 250 seconds.

2-22. DETECTION OF A CIRCULATING ANTICOAGULANT

a. **Principle.** An abnormal recalcification time is not corrected by the addition of normal plasma if a circulating anticoagulant is present.

b. **Reagents.** See paragraph 2-21b.

c. **Procedure.**

(1) Obtain citrated plasma from a normal donor and from the patient.

(2) Set up the following mixture in 12 x 75 mm test tubes:

Tube No.	Patient's Plasma	Normal Plasma	% Patient's Plasma
1	0.20 ml	0.00	100%
2	0.15 ml	0.05 ml	75%
3	0.10 ml	0.10 ml	50%
4	0.05 ml	0.15 ml	25%
5	0.02 ml	0.18 ml	10%
6	0.00	0.20 ml	Control

(3) Add 0.2 ml of 0.025 M. $CaCl_2$ to each tube and determine the plasma recalcification time (see para 2-21) on each tube.

d. **Interpretation.**

(1) The abnormal recalcification time is corrected by normal plasma if no circulating anticoagulant is present.

(2) A circulating anticoagulant present in patient's plasma prolongs the recalcification time of normal plasma.

2-23. DETECTION OF FACTOR XIII DEFICIENCY

a. **Principle.** Factor XIII converts a loosely-linked, fibrin clot (in the presence of calcium ions) into a tough gel. The well-formed clot is insoluble in 5 M urea. In the absence of factor XIII, the clot lyses within 3 hours.

b. **Reagents.**

(1) Sodium citrate, 3.8 percent. Available from the Federal Supply Catalog.

(2) Calcium chloride, 0.05 M. Add 0.555 g anhydrous calcium chloride to a 100 ml volumetric flask. Dilute to the mark with distilled water.

(3) Urea solution, 5 M. Add 30 g of urea to a 100-ml volumetric flask. Dilute to the mark with distilled water.

c. **Procedure.**

(1) Draw 4.5 ml of fresh venous blood and add to 0.5 ml of 3.8 percent sodium citrate.

(2) Centrifuge at 2,500 rpm for 5 minutes and separate plasma.

(3) Pipet 0.5 ml of patient's plasma into each of two test tubes. Repeat, pipeting 0.5 ml of normal control plasma into each of two additional tubes.

(4) Add 0.5 ml of 0.05 M calcium chloride to the four tubes.

(5) Incubate the resulting fibrin clots at 37ºC for 30 minutes.

(6) Loosen the clots from the sides of the test tubes by gently tapping the sides of the tube.

(7) Transfer one of the patient's clots and one of the normal control clots to respective tubes containing 5 ml of 5 M urea. Transfer both the remaining patient clot and the normal control clot to a third tube containing 5 ml of 5 M urea.

(8) Allow the mixtures to stand at room temperature.

(9) Check the clots at the end of 1 hour, 2 hours, 3 hours, and 24 hours, and note if the clots have dissolved.

d. **Interpretation**. A clot that lyses within 3 hours is deficient in factor XIII.

2-24. PLATELET COUNT (REES-ECKER)

a. **Principle.** A sample of blood is diluted in an isotonic anticoagulant. The anticoagulant employed contains a stain and serves the dual purpose of being both a fixing diluent and a staining medium. After mixing, the specimen is introduced into the counting chamber and the number of thrombocytes in a known volume is counted.

b. **Reagent.** Rees-Ecker diluting fluid. Add 3.8 g sodium citrate, 0.2 ml 40Dilute to the mark with distilled water. Filter the solution just prior to use. Keep in a glass-stoppered bottle in the refrigerator. Prepare the solution frequently since in old solutions the formaldehyde oxidizes to formic acid, hemolyzing the red cells.

c. **Procedure.**

(1) Fill a red cell diluting pipet with freshly-filtered Rees-Ecker solution and expel it, leaving a film of the solution inside the pipet.

(2) Draw blood to the 0.5 mark in the red cell pipet.

(3) Wipe the excess blood from the outside of the pipet and dilute to the 101 mark with Rees-Ecker diluting fluid.

(4) Shake the pipet for several minutes.

(5) Prepare a moist chamber by placing a moist piece of filter paper in the top of a Petri dish. Paper should be moist enough to adhere to the Petri dish.

(6) Discharge a few drops from the pipet. Then charge both chambers of a hemacytometer.

(7) Allow the thrombocytes to settle for 15 minutes. To prevent drying, place the moist chamber over the hemacytometer.

(8) Under high-power magnification, count all of the thrombocytes seen in the center 1-sq-mm area of each of the two charged chambers of the hemacytometer. With proper light adjustment and continual fine focusing, the thrombocytes are seen as blue, highly-refractile bodies, which may be round, oval, or comma-shaped. They vary in size from one to five microns.

d. **Calculations.** The volume in both chambers is 0.2 cu mm (area x depth = 2 sq mm x 0.1 mm = 0.2 cu mm). The formula for calculation of indirect platelet count is as follows.

$$\frac{\text{Total platelets counted} \times \text{dilution}}{\text{volume}} = \text{platelets per cu mm}$$

or

$$\frac{\text{Total platelets counted} \times 200}{0.2} = \text{platelets per cu mm}$$

e. **Sources of Error.**

(1) Platelet agglutinates invalidate the count. This is prevented by using scrupulously clean glassware. Therefore, the following precautions are recommended:

(a) Boil counting chambers and coverslips for 2 hours in distilled water to which a small amount of sodium bicarbonate has been added.

(b) Pipets must be very carefully cleaned.

(c) The diluting fluid must be frequently filtered or centrifuged and stored at 2^{o} to 4^{o}C.

(2) Other sources of error as listed under red and white blood cell counts also apply, including pipetting errors. These procedures should be consulted.

(3) More accuracy may be obtained through use of the phase contrast microscope.

f. **Discussion.**

(1) No one method for the enumeration of thrombocytes is satisfactory in every respect. In experienced hands the direct procedure is more accurate than the indirect method, and venous blood samples are more representative and preferable to those obtained by capillary puncture. Direct procedures, however, require greater skill and experience for proper performance and interpretation. The error in a single count has been estimated to be about 10 percent and this margin of error can be reduced by multiple counts.

(2) A major disadvantage of the direct thrombocyte count is that the platelets must be counted under high-power rather than oil-immersion magnification as is used in the indirect method. Because of the extremely small size of blood platelets, it is a common error to confuse them with yeasts, debris, and precipitated stain.

(3) Due to the inherent errors in this procedure, it is recommended that a thrombocyte count be performed on a normal person as a control.

g. **Normal Values.** 150,000-350,000 platelets per cu mm.

NOTE: This range is generally acceptable, but it must be realized that there is a great variance in normal values which differ with each technique, laboratory, and technician.

2-25. PLATELET COUNT (PHASE MICROSCOPY)

a. **Principle.** Blood is diluted with ammonium oxalate, and the diluted specimen is introduced into a counting chamber. The platelets appear round or oval, pink, purple, or even black under a phase condenser.

b. **Reagent.** Ammonium oxalate, 1%. Add 1g of ammonium oxalate to a 100-ml volumetric flask. Dilute to the mark with distilled water. Store in the refrigerator and filter before use.

c. **Procedure.**

(1) Draw 5 ml of venous blood and immediately place in a test tube containing EDTA. (If capillary blood is used, immediately fill a red blood cell diluting pipet to the 1 mark.)

(2) Fill a red blood cell diluting pipet to the 1 mark and then draw ammonium oxalate to the 101 mark.

(3) Shake the pipet 10 to 15 minutes.

(4) Discard from 1/3 to 1/2 the volume from an RBC pipet, then charge a special flat bottom-phase counting chamber. Place the counting chamber in a Petri dish containing moist gauze and let stand 15 to 20 minutes to ensure complete settling of platelets.

(5) Count the platelets in all 25 squares of the area normally used for the red cell count. Multiply the results by 1,000.

(6) Both sides of each chamber are filled with the same sample; the count from either side should not deviate more than 10% from the other count. If a greater than 10% deviation occurs, repeat the count using a fresh dilution and a second chamber.

(7) Report the average of the two sides counted.

d. **Sources of Error.**

(1) The diluting fluid must be fresh and free of bacterial contamination.

(2) Platelet clumping occurs if there is delay in adding blood to the anticoagulant or if the mixing is inadequate. Platelet clumps cause invalid results.

(3) Occasionally extraneous material is mistaken for platelets.

(4) Glassware that is not scrupulously clean causes platelets to attach to the debris on the glassware.

(5) See paragraph 2-24e for other sources of error.

e. **Discussion.**

(1) Platelets sometimes show dendritic processes. Structures such as dirt, crystals, and WBCs are refractile. Platelets are not. The white cells are normally lysed; however, with patients that demonstrate excessive high white counts some cells will be observed in the platelet counting prep.

(2) Ammonium oxalate ensures clearing of the background by hemolysis.

(3) This method is more accurate than the Rees-Ecker. The main drawback to this method is that special equipment is required.

(4) See paragraph 2-24f for further discussion.

f. **Normal Values.** 150,000 to 350,000 per cu mm.

2-26. MACROGLOBULIN DISTILLED WATER SCREENING TEST

a. **Principle.** Macroglobulins are precipitated when brought into contact with distilled water.

b. **Procedure.**

(1) Collect 5 ml of blood from the patient by venipuncture.

(2) Set the blood aside for 1 hour to clot, and then separate the serum by centrifuging at 2,000 rpm for 5 minutes.

NOTE: Oxalated, citrated, or heparinized plasma is also satisfactory for the performance of this test.

(3) Place 5 ml of distilled water in a Wassermann tube.

(4) With a 1 ml pipet, draw up 0.5 ml of patient's serum or plasma. Holding the pipet tip close to the meniscus of the water, add 0.2 ml of the patient's serum or plasma to the distilled water.

(5) Interpret results.

c. **Interpretation.**

(1) Observe the behavior of the drop of serum or plasma sinking in the water and the modifications of color of the solution.

(2) If macroglobins are present:

(a) The drop will sink leaving a smoky trace.

(b) The entire distilled water medium will promptly assume a whitish color, due to the condensation of transparent, slimy masses forming a precipitate which quickly sinks to the bottom.

(3) The separated precipitate will dissolve well when transferred to a test tube containing normal saline.

2-27. CRYOGLOBULIN SCREENING TEST

a. **Principle.** Cryoglobulins in serum or plasma are precipitated at 4ºC and dissolved by temperature elevation to 37^{o}C.

b. **Procedure.**

(1) Collect 10 ml of blood by venipuncture and place a 5 ml portion in a tube containing anticoagulant (citrate, oxalate, or heparin) and place the other 5 ml portion into a clean, sterile test tube.

(2) Incubate both tubes immediately for 1 hour at 37^{o}C.

(3) One hour later, separate the supernatant plasma from the tube containing the anticoagulant and the serum from the clot in the untreated tube.

(4) Transfer aliquots of 1 ml of serum into 2 clean sterile Wassermann tubes.

(5) Transfer aliquots of 1 ml of plasma into 2 clean sterile Wassermann tubes.

(6) Incubate 1 serum specimen and 1 plasma specimen at 4ºC (refrigerator temperature) for 4 hours.

(7) Incubate 1 serum specimen and 1 plasma specimen at 37ºC for 4 hours.

(8) Read results.

c. **Interpretation.**

(1) The test is positive if the serum or plasma incubated at 37ºC shows no changes and the serum or plasma incubated at 4ºC shows the following changes:

(a) The serum or plasma appear clotted in toto.

(b) The plasma or serum appear divided into two layers; the upper one containing normal plasma or serum, faintly stained by bilirubin; the lower one represented by the cryoglobulin, whitish in color, which has precipitated to the bottom of the test tube.

(2) The test is confirmed if the serum or plasma incubated at 4ºC is brought back to a temperature of 37ºC and its appearance returns to normal.

2-28. FIBRIN/FIBRINOGEN DEGREDATION (SPLIT) PRODUCTS

a. **Principle.** In the normal production of clot formation, fibrinogen is acted upon by thrombin, breaking off small molecules from the fibrinogen molecule. These small molecules are fibrinopeptides D and E (fibrinogen split products). Once these molecules are broken off, the main molecule that remains is the fibrin monomer (A & B). It is this monomer that polymerizes to form the fibrin threads of a clot. In addition to the split products from the action of thrombin on fibrinogen, additional split products are produced by the action of fibrinolysin on fibrinogen and plasma in both fibrinogen and fibrin clots. When large amounts of FSP's are produced or are present, prior to adsorption antifibrinogen antiserum with an affected individual's serum, they may interfere with polymerization of fibrin monomers, preventing clot formation (DIC).

b. **Specimen and Reagents.**

(1) Venous blood in a special blue top vacutainer-type tube does not contain the anticoagulant found in blue top tubes, but a clot activator, specific for FDP assay. The tube contains thrombin to promote clotting, and soybean trypsin inhibitor to prevent in vitro fibrinolysis. This is sufficient to collect 2 ml whole blood or urine.

(2) Use the FDP Assay Kit from Wellcome Reagents Div., Burroughs Wellcome Co., Research Triangle Pk, NC 27709

c. **Procedure.**

(1) Prepare dilutions of serum samples. Take 2- 12 x 75 mm test tubes and label them #1 and #2.

(2) Mark 2 of the rings on the glass slide #1 and #2.

(3) Using the graduated dropper provided with the bottle of buffer solution in the kit, place 0.75 ml of glycine buffer into each test tube.

(4) Using 1 of the plastic droppers (provided with the kit), with the teat provided (blue), aspirate clear serum.

(5) Place 5 drops of serum into test tube #1 and 1 drop of serum into test tube #2.

(6) Mix the contents of each test tube:

Dilution #1 = 1:5.
#2 = 1:20.

(7) Rinse the plastic dropper with glycine buffer.

(8) Transfer 1 drop from test tube #2 to position #2 on the slide.

(9) Now take 1 drop from test tube #1 and place it in position #1 on the slide.

NOTE: Since you're going from a lesser to greater concentration, you must pipette in this order.

(10) Mix the latex suspension by shaking the container vigorously for a few minutes.

(11) Add 1 drop of latex suspension to each position on the slide.

(12) Stir the serum/latex mixture with a wooded applicator, spreading the mixture to fill the entire circle.

(13) Rock the slide gently back and forth for a maximum of 2 minutes, while observing for agglutination. Agglutination means that there is at least 2 µg/ml present.

d. **Calculations.**

(1) If agglutination is present, there is at least 2 µg/ml present in that position.

(2) If agglutination occurs in #1, concentration is greater than 10 ug/ml. If agglutination occurs in #2, concentration is greater than 40 µg/ml.

NOTE: Additional serial dilutions may be made and tested accordingly.

		10 µg/ml	40 µg/ml
Dilutions:	1:2	20	80
	1:4	40	160

e. **Controls.** Controls should be run with each batch of tests. Positive control should contain 5 to 10 µg/ml FDP. Negative control should contain less than 2 µg/ml of FDP.

f. **Range of Values.** Normal values are below 10 µg/ml. In cases of DIC, levels of FDP exceed 10 µg/ml, and in severe cases can exceed 40 µg/ml.

2-29. FACTOR V ASSAY (SIMILAR FOR FACTORS VII & X)

a. **Principle.** The patient's plasma is mixed with a commercially known deficient plasma. It is then tested, by doing a Prothrombin Time test on the mixture, and compared on a logarithmic chart. Factors V, VII, and X are synthesized in the liver and are decreased in severe liver disease.

b. **Material and Equipment Required.**

(1) Citrated plasma from a blue top, vacutainer-type tube.

(2) Commercially prepared factor (specific) deficient plasma and Log-Log chart paper.

(3) Any device for performing prothrombin time testing.

c. **Procedure.**

(1) Prepare the graph from normal citrated plasma (see chart below).

Control for making graph aliquots of normal citrated plasma.

100% -- Undiluted (100% = 0.2 ml plasma).

50% -- Diluted with imidazole buffered saline (50% = 0.2 ml plasma + 0.2 ml IBS).

25% -- Diluted with imidazole buffered saline (25% = 0.1 ml plasma + 0.3 ml IBS).

10% -- Diluted with imidazole buffered saline (10% = 0.1 ml plasma + 0.9 ml IBS).

(2) Set up a series of five 10 x 75 mm test tubes.

a) 1st tube -- 0.01 ml undiluted plasma.

(b) 2nd tube -- 0.01 ml 50% dilution.

(c) 3rd tube -- 0.01 ml 25% dilution.

(d) 4th tube -- 0.01 ml 10% dilution.

(e) 5th tube -- no normal plasma (optional).

(3) To the first 4 tubes, add 0.09 ml factor V (or VII or X) deficient plasma, and mix.

(4) Proceed with performing a regular prothrombin time analysis.

(5) The above mixtures are in a ratio of 1:9, where 1 is normal and 9 is deficient.

(6) Record the results on a double-logarithmic (Log-Log) scale.

(7) For each patient to be analyzed, use 100% and a 50% dilution.

(8) Read the results off the graph.

d. **Controls.** Any normal coagulation controls will do. Any abnormal coagulation controls will do.

e. **Normal Values.** Normal patients should show values from 80 to 120 of the normal control.

NOTES: 1. Refrigeration preserves factor V, but after 24 hours, even at $4^{o}C$, factor V is greatly reduced.

2. Factor VII is a vitamin K dependent factor.

2-30. FACTOR VIII ASSAY (SIMILAR FOR FACTORS IX, XI, & XII)

a. **Principle.** The patient's plasma is mixed with a commercially known deficient plasma. It is then tested, by doing a activated partial thromboplastin test on the mixture, and compared on a logarithmic chart. Factors VIII, IX, XI, and XII are analyzed using this procedure.

b. **Materials, Equipment, and Specimen Required**.

(1) Citrated plasma from a blue top, vacutainer-type tube.

(2) Commercially prepared factor (specific) deficient plasma and Log-Log chart paper.

(3) Any device for performing activated partial thromboplastin time testing.

c. **Procedure.**

(1) Prepare the graph from normal citrated plasma (see chart below).

Control for making graph aliquots of normal citrated plasma.

100% --	Undiluted (100% = 0.2 ml plasma).
50% --	Undiluted with imidazole buffered saline (50% = 0.2 ml plasma + 0.2 ml IBS).
25% --	Undiluted with imidazole buffered saline (25% = 0.1 ml plasma + 0.3 ml IBS).
10% --	Undiluted with imidazole buffered saline (10% = 0.1 ml plasma + 0.9 ml IBS).

(2) Set up a series of five, 10 x 75 mm test tubes.

(a) 1st tube -- 0.01 ml undiluted plasma.

(b) 2nd tube -- 0.01 ml 50% dilution.

(c) 3rd tube -- 0.01 ml 25% dilution.

(d) 4th tube -- 0.01 ml 10% dilution.

(e) 5th tube -- No normal plasma (optional).

(3) To the first 4 tubes, add 0.09 ml factor VIII (or IX, XI, or XII) and deficient plasma. Mix.

(4) Proceed with performing a regular activated partial thromboplastin time analysis.

(5) The above mixtures are in a ratio of 1:9, where 1 is normal and 9 is deficient.

(6) Record the results on a double-logarithmic (Log-Log) scale.

(7) For each patient to be analyzed, use 100% and a 50% dilution.

(8) Read the results off the graph.

d. **Control.** Any normal coagulation controls will do. Any abnormal coagulation controls will do.

e. **Normal Values.** Normal patients should show values from 80 to 120% of the normal control.

Continue with Exercises

EXERCISES, LESSON 2

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Hemostasis:

   a. Plays a small part in stopping the flow of blood.

   b. Plays a large part in stopping the flow of blood.

   c. Is a vascular system.

   d. Represents 15-20% of platelet protein.

2. Coagulation is one component in:

   a. Fibrinolysis.

   b. Hemostasis.

   c. Disruption of the intrinsic system.

   d. Vascular dilatation.

3. What major role does blood platelets perform?

   a. In the hemostatic process, blood platelets swell, congeal, and adhere to blood vessel wall to plug the site of the injury.

   b. They contract.

   c. They stimulate collagen.

   d. Platelets extend pseudopodia.

4. Which occurs last in the clotting process?

    a. Formation of thrombin.

    b. Formation of thromboplastin.

    c. Aggregation of platelets.

    d. Formation of fibrin network.

5. Coagulation factors I through IV are, respectively:

    a. Calcium, fibrinogen, prothrombin, and Christmas.

    b. Fibrinogen, prothrombin, thromboplastin, and calcium.

    c. Prothrombin, calcium, fibrinogen, and thromboplastin.

    d. Thromboplastin, prothrombin, calcium, and fibrinogen.

6. Which is the Stuart Prower Factor?

    a. Factor V.

    b. Factor VII.

    c. Factor X.

    d. Factor XIII.

7. Thromboplastin eliminates the need for what item in stage I?

    a. Globulin.

    b. The accelerator, proconvertin.

    c. High concentration of serum and plasma.

    d. Platelets.

8. What are the two systems that make up the coagulation process?

    a. Vascular and hemolysis.

    b. Extrinsic and intravascular.

    c. Intravascular and vascular.

    d. Intrinsic and extrinsic.

9. Coagulation factor XIII produces a tough gel through:

    a. Increasing the serum content.

    b. Stabilizing serum.

    c. Converting a loosely linked fibrin clot with the help of calcium ions.

    d. Decreasing the conversion of prothrombin with the help of calcium ions.

10. The synthesis of prothrombin takes place in the liver and requires the presence of:

    a. Folic acid.

    b. Vitamin A.

    c. Vitamin B12.

    d. Vitamin K.

11. Prothrombin is the precursor of:

    a. Fibrin.

    b. Fibrinogen.

    c. Thrombin.

    d. Thromboplastin.

12. A _____ deficiency is unlikely to interfere with coagulation because clinical tetany would intervene.

    a. Calcium.

    b. Fibrinogen.

    c. Prothrombin.

    d. Factor VIII.

13. Which factor is thought to be an accelerator in the conversion of prothrombin to thrombin?

    a. Factor VII.

    b. Factor VIII.

    c. Factor IX.

    d. Factor XII.

14. Which is consumed during the clotting process and is therefore not present in serum?

    a. Factor V.

    b. Factor VII.

    c. Factor IX.

    d. Factor XI.

15. Which platelet factor activates prothrombin?

    a. 2.

    b. 3.

    c. 4.

    d. 7.

16. Hemophilias A and B are hereditary deficiencies of which coagulation factors, respectively?

    a. VII and IX.

    b. VIII and IX.

    c. XI and VIII.

    d. XI and IX.

17. Platelet factor 3 is found in the platelet _____ and is a _____ component of platelets.

    a. Membrane; extrinsic.

    b. Plasma; extrinsic.

    c. Membrane; intrinsic.

    d. Plasma; intrinsic.

18. A fibrin clot is ultimately formed by the interaction of fibrinogen and:

    a. Calcium.

    b. Thrombin.

    c. Thromboplastin.

    d. Platelets.

19. The formation of thrombin from prothrombin occurs in what stage?

    a. Stage I.

    b. Stage II.

    c. Stage III.

    d. Stage IV.

20. During the clot lysis stage, what happens to the blood clot when plasmin interacts with it?

    a. Enlarges.

    b. Shrivels up like an old red blood cell.

    c. Enlongates like an ameoba.

    d. Dissolves into fragments.

21. One anticoagulant that inhibits the clotting activity of blood is:

    a. Coumarin derivitives.

    b. Fibrin.

    c. Throboplastin.

    d. Proconvertin.

22. In the Ivy method for determining the bleeding time, the blood pressure cuff is inflated to:

    a. 20 mm Hg.

    b. 30 mm Hg.

    c. 40 mm Hg.

    d. 50 mm Hg.

23. The normal bleeding time with the Ivy method is:

    a. Up to 8 minutes.

    b. 3-10 minutes.

    c. 10-15 minutes.

    d. 15-20 minutes.

24. What should be the temperature of the water bath for the whole blood clotting time? (NOTE: The whole blood clotting time discussed in paragraph 2-12 of the text is also referred to as the coagulation time or clotting time.)

    a. 10^{o}C.

    b. 20^{o}C.

    c. 37^{o}C.

    d. 56^{o}C.

25. All of the variables listed below decrease the clotting time of whole blood except:

    a. Excessive agitation of the blood specimen.

    b. Air bubbles in the blood.

    c. Tissue fluid in the blood.

    d. Performing test at room temperature.

26. Using the Lee-White method for whole blood clotting, what should be done to tube number one after tube number two's blood has clotted for 30 seconds?

    a. Place it straight up for no blood to flow.

    b. Tilt it until no flow blood is observed.

    c. Tilt it upside down for blood to flow.

    d. Gently tilt it upward.

27. The clot retraction is normal in hemophilia because of:

    a. A reduced number of platelets.

    b. A normal number of platelets.

    c. Increased prothrombin activity.

    d. The presence of calcium.

28. To measure clot retraction, we can use tubes previously used to determine:

    a. Coagulation time.

    b. Prothrombin time.

    c. Prothrombin consumption time.

    d. Thromboplastin generation time.

29. The tourniquet test is positive in:

    a. Purpuras.

    b. Hemophilia.

    c. Polycythemia.

    d. Agranulocytosis.

30. The partial thromboplastin time cannot be used to reveal a deficiency of:

    a. Factors V or X.

    b. Factors XI or XII.

    c. Prothrombin or fibrinogen.

    d. Factor VII or platelet factor 3.

31. Which of the following values for the activated partial thromboplastin time is normal?

    a. 30 seconds.

    b. 50 seconds.

    c. 1 minute, 10 seconds.

    d. 1 minute, 30 seconds.

32. The anticoagulant used in preparing the patient's plasma for the one-stage prothrombin time is:

    a. 0.2 ml of EDTA.

    b. 0.5 ml of 3.8 percent sodium citrate.

    c. 0.5 ml of 1.34 percent sodium citrate.

    d. 0.1 ml of 5 percent potassium oxalate.

33. The prothrombin time is sensitive to a deficiency of factor:

    a. VII.

    b. VIII.

    c. IX.

    d. XI.

    e. XII.

34. A prothrombin consumption time of less than 20 seconds indicates a deficiency in:

    a. Fibrinogen.

    b. Prothrombin.

    c. Factor V, VII, or X.

    d. Platelet and factors VIII, IX, XI, or XII.

35. What is the most important difference between the procedures for the one-stage prothrombin time and the prothrombin consumption time?

    a. Sensitivity.

    b. Sources of error.

    c. Blood fraction tested.

36. Deficiencies of which two factors cannot be distinguished from each other by using the thromboplastin generation time, the prothrombin time, and the partial thromboplastin time?

    a. V and VII.

    b. VIII and IX.

    c. X and XI.

    d. XI and XII.

37. Which of the following is **NOT** used to determine the thrombin time?

    a. Patient's plasma.

    b. Thrombin solution.

    c. Thromboplastin reagent.

    d. A 37ºC water bath.

38. The thrombin time is sensitive to a deficiency of:

    a. Factor V.

    b. Factor VII.

    c. Fibrinogen.

    d. Prothrombin.

39. Which of the following plasma concentrations of fibrinogen falls in the normal range?

    a. 50 mg/dl.

    b. 100 mg/dl.

    c. 300 mg/dl.

    d. 500 mg/dl.

40. The plasma recalcification time is used in which of the following?

    a. Coagulation time.

    b. Thrombin time.

    c. Detection of factor XIII deficiency.

    d. Detection of a circulating anticoagulant.

41. If a clot lyses within 3 hours after being placed in 5M urea, which factor is deficient?

    a. Fibrin.

    b. Platelets.

    c. Factor VIII.

    d. Factor XIII.

42. In the Rees-Ecker platelet count, what is the platelet count if a total of 100 platelets are counted in the two center 1-sq mm areas?

    a. 50,000 per cu mm.

    b. 100,000 per cu mm.

    c. 150,000 per cu mm.

    d. 200,000 per cu mm.

43. During the microscopy phase of the platelet count, what is the appearance of the platelets in the counting chamber after the blood has been diluted with ammonium oxalate?

    a. Round or oval.

    b. Pink.

    c. Purple.

    d. Or even black under a phase condenser.

    e. All of the above.

    f. None of the above.

44. Once the diluted blood has been left to stand for 15 to 20 minutes, what method is used to count the platelets and what are the results multiplied by?

    a. Count the platelets in all 25 squares of the area normally used for the RBCs and then multiply those results by 1,000.

    b. Count the platelets in 6 squares of the area normally used for the WBCs and then multiply those results by 1,000.

    c. Count the platelets and then multiply those results by 2,000.

    d. Count the platelets in all 40 squares of the area normally used for the RBCs and then multiply those results by 500.

45. What other items could be used to precipitate macroglobulins besides using distilled water?

    a. Oxalated or heparinized plasma or calcium dioxide.

    b. Oxalated, citrated, or heparinized plasma.

    c. Cresyl blue, citrated, or heparinized plasma.

    d. Oxalated, citrated, or heparinized plasma or formaldehyde.

46. When interpretating the results of the macroglobulin distilled water screening test, what do you observe?

    a. The behavior of the drop of serum or plasma sinking in the water and the modifications of color of the solution.

    b. The behavior of the drop of serum or plasma remaining stationary in the water and the modifications of color of the solution.

    c. The behavior of the drop of serum or plasma rising in the water and the modifications of color of the solution.

    d. The behavior of the drop of serum or plasma sinking in the water and the retention of the color of the solution.

47. What confirms that serum or plasma tests positive when incubated at 4ºC during the cryoglobulin screening test?

    a. If the serum or plasma turns a bright green color upon returning to a temperature of 37^{o}C, then the test is confirmed.

    b. The test is confirmed if the serum or plasma is brought back to a temperature of 37ºC and its appearance returns to normal.

    c. If the serum or plasma turns a dull yellow color upon returning to a temperature of 37^{o}C, then the test is confirmed.

    d. The test is confirmed if the serum or plasma is brought back to a temperature of 44ºC and its appearance returns to normal.

48. What is a range of values for the fibrin/frininogen degredation products?

    a. Normal is above 10 ug/ml.

    b. DIC levels of FDP are below 8 ug/ml.

    c. Severe cases exceed 40 ug/ml.

    d. Normal levels are at 10ug/ml.

49. In performing a factor V assay, what percentage of dilution should the second tube be with 0.01 ml of imidazole buffered saline?

    a. 100.

    b. 60.

    c. 50.

    d. 25.

50. Which factors are analyzed using the factor VIII assay procedure?

    a. Factors II and III.

    b. Factors V, VI, and VII.

    c. Factors I and IX.

    d. Factors VIII, IX, XI, and XII.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 2

1. b (para 2-1a)
2. b (para 2-2a)
3. a (para 2-1(b))
4. d (para 2-2a)
5. b (paras 2-3a-d, table 2-1)
6. c (para 2-3j)
7. d (para 2-3c)
8. d (para 2-2c)
9. c (para 2-3m)
10. d (para 2-3b)
11. c (para 2-3b)
12. a (para 2-3d)
13. a (para 2-3g)
14. a (para 2-3e)
15. b (para 2-4c)
16. b (paras 2-3h, i)
17. c (para 2-4c)
18. b (para 2-7c, figure 2-1)
19. b (para 2-7b)
20. d (para 2-7d)
21. a (para 2-8)

22. c (para 2-11c(5))

23. a (para 2-11f)

24. c (para 2-12b)

25. d (para 2-12d NOTES (1), (2))

26. b (para 2-12c(9))

27. b (para 2-13f(2))

28. c (para 2-13(a))

29. a (para 2-14g(4))

30. c (para 2-15f)

31. a (para 2-15g(1))

32. b (para 2-16b)

33. a (para 2-16f(5))

34. d (paras 2-17e(1), (4))

35. c (paras 2-16f(5), 2-17)

36. d (paras 2-18e, g(6))

37. c (paras 2-19b, c)

38. c (para 2-19e(1))

39. c (para 2-20g)

40. d (paras 2-22a, c(3))

41. d (para 2-23d)

42. b (para 2-24d)

43. e (para 2-25a)

44. a (para 2-25c)

45. b (para 2-26b(1) NOTE)

46. a (para 2-26c(1))

47. b (para 2-27c(2))

48. c (para 2-28f)

49. c (para 2-29c(2)(b))

50. d (para 2-30)

End of Lesson 2

LESSON ASSIGNMENT

LESSON 3	Automation.
TEXT ASSIGNMENT	Paragraphs 3-1 through 3-40.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

3-1. Identify the important characteristics and functions of automatic diluters.

3-2. Identify the important characteristics and functions of slide stainers.

3-3. Identify the important characteristics and functions of the Coulter Model FN Counter.

3-4. Identify the important characteristics and functions of a fibrometer.

3-5. Identify the important characteristics and functions of the QBC II System.

3-6. Identify the important characteristics and functions of the various types of cell counters.

SUGGESTION After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 3

AUTOMATION

Section I. SAMPLE PROCESSING

3-1. INTRODUCTION

In recent years, instrumentation has been developed to automate many hematological analyses. The use of automated equipment in the laboratory has increased the number of analyses performed by decreasing the man-hours spent on the analyses. These instruments perform various functions such as: dilutions, staining, cell counts, hematocrit, hemoglobin, coagulation studies, and indices. The type of instrument used by a particular laboratory depends on the size of the daily workload, the complexity of the method used, and the technical ability of the technician performing the analysis. If the number of hematology requests received is 30-40 a day, it would be advantageous to have automatic diluters, pipetters, and electronic cell counters for blood cell counts and hemoglobin determinations. When the number of routine requests approaches 100-150 per day, an automatic hematology system should be considered.

3-2. DILUTERS

Automatic dilutors assist in the processing of large numbers of specimens by reducing technician time per test. Repetitive dilutions can be accomplished rapidly with instruments similar to the one illustrated in figure 3-1. The concept of an automatic dilutor involves the sampling from a larger volume and the dilution of the sample. The automatic dilutor is essentially two syringes that can be operated in a systematic manner. One syringe measures and draws the sample; the other syringe measures the diluent. Both syringes can be set to draw a metered amount repeatedly within an allowable range of error. A daily calibration check of this instrument is recommended to ensure accuracy.

3-3. SLIDE STAINER

The automatic slide stainer is a coordinated three-phase operation that fixes, stains, buffers, rinses, and dries differential slides at a rate of approximately one per minute. Stain packs are available with this instrument, but satisfactory Wright stain and buffer can be prepared in the laboratory. The slide stainer is illustrated in figure 3-2.

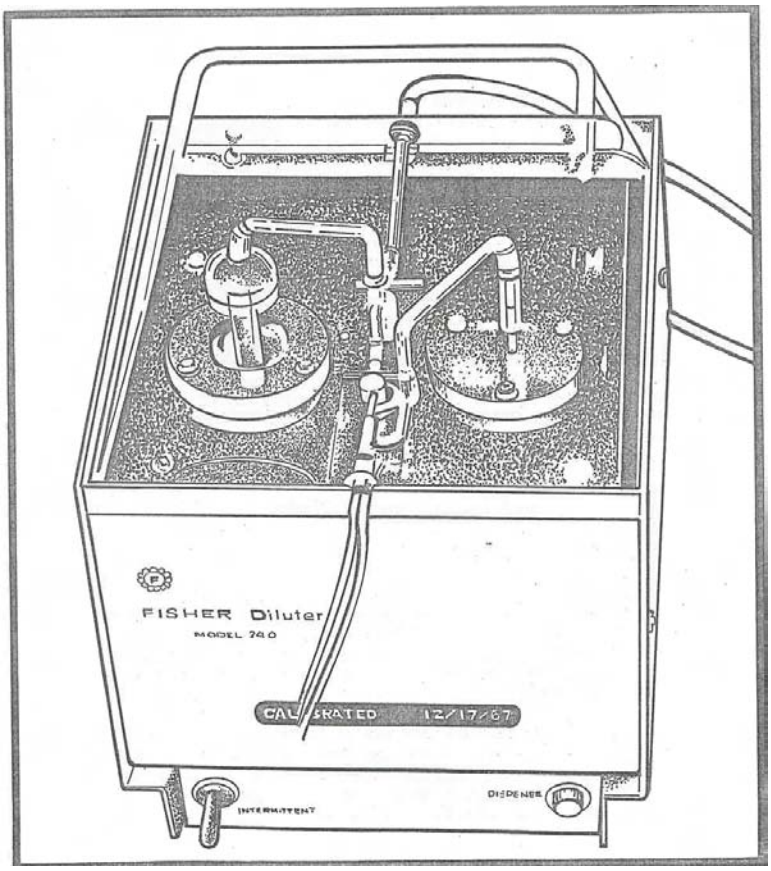


Figure 3-1. Automatic diluter.

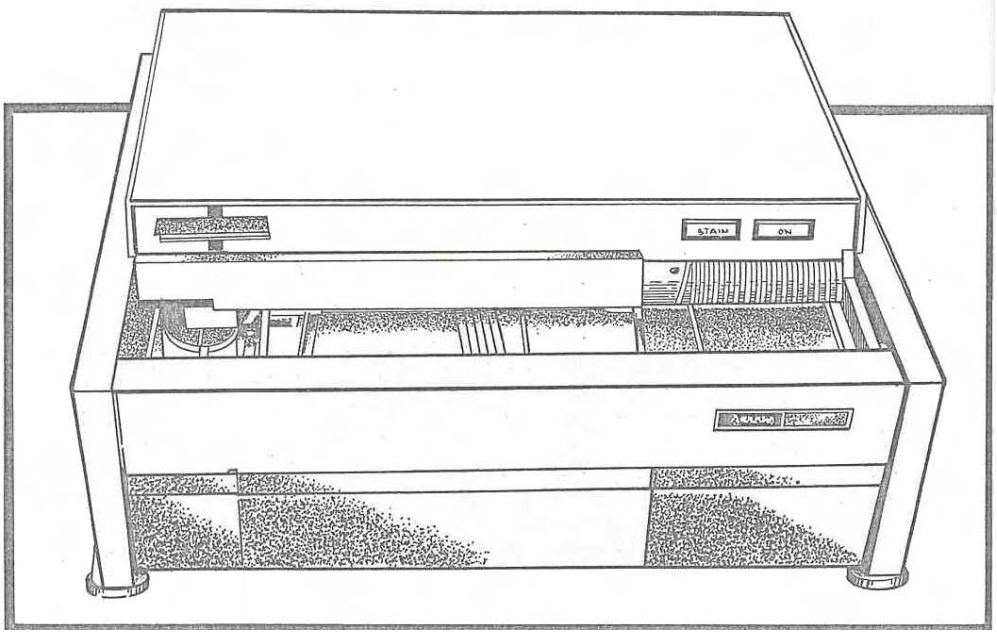


Figure 3-2. Automatic slide stainer.

Section II. SAMPLE TESTING

3-4. INTRODUCTION

a. Automatic cell counters have become a part of the hematology section. The accuracy and rapidity of their operation has been, and will continue to be, a boon to the patient, the physician, and the technician. In order for you to use the machines effectively, you must become familiar with their systems and operations.

b. Electronic cell counters can easily handle more than 800 individual counts per day, which is a decided advantage over the 30 to 50 counts that are routinely done manually. These machines actually count an average of 30,000 to 50,000 cells per specimen, which is equivalent of up to 100 chamber counts per specimen. An additional advantage is the uniformity in the amount of specimen counted by the automatic counter; it is always the same. The large number of cells counted plus the uniformity of samples tested reduces error to only one-tenth of the error encountered in the visual methods; electronic cell counts allow an accuracy and reproducibility factor of about 2 percent. The reason that one individual can handle so many counts utilizing the electronic cell counter greatly reduces the manpower needed to operate a hematology section, so that technicians are freed for other duties.

3-5. COULTER MODEL FN COUNTER

a. The Coulter Counter (see figure 3-3) operates on the principle of electrical resistance. Since blood cells are poor electrical conductors, they act as resistors to current flow. As more cells pass into the electrical field they offer correspondingly more resistance. The change in the current flow caused by the change in resistance is sensed and counted by a digital counting apparatus.

b. A suspension of blood cells in an electrolyte (that is, saline) is drawn through an aperture with electrodes on each side to form an electrical circuit. As the blood cells pass through the aperture, the mass of the cell changes the resistance between the electrodes. The change of resistance alters the current flow and causes electronic pulses as a result of the variation in the field. The changes are amplified, inspected, and counted electronically. The end product is a number which represents the number of blood cells and their volume in the sample being counted. By arranging the pulses, the sampling volume, and the sampling time, the counter reports directly the number of cells per cubic millimeter. A schematic of the operation of the Coulter Cell Counter is illustrated in figure 3-4. When the valve is opened, the mercury falls, thus creating a vacuum in the sampler. This change in pressure causes cells in the sample to be sucked through the aperture and into the sampler. The removal of cells from the sample causes a change in electrical conductivity between the two electrodes. This change is amplified and displayed on the scope. It is further amplified and registered on the digital counter. The higher the count, the greater the probability that more than one cell will enter the aperture at one time (coincidence passage). For this reason, WBCs over 10,000 and all RBCs are corrected for coincidence passage.

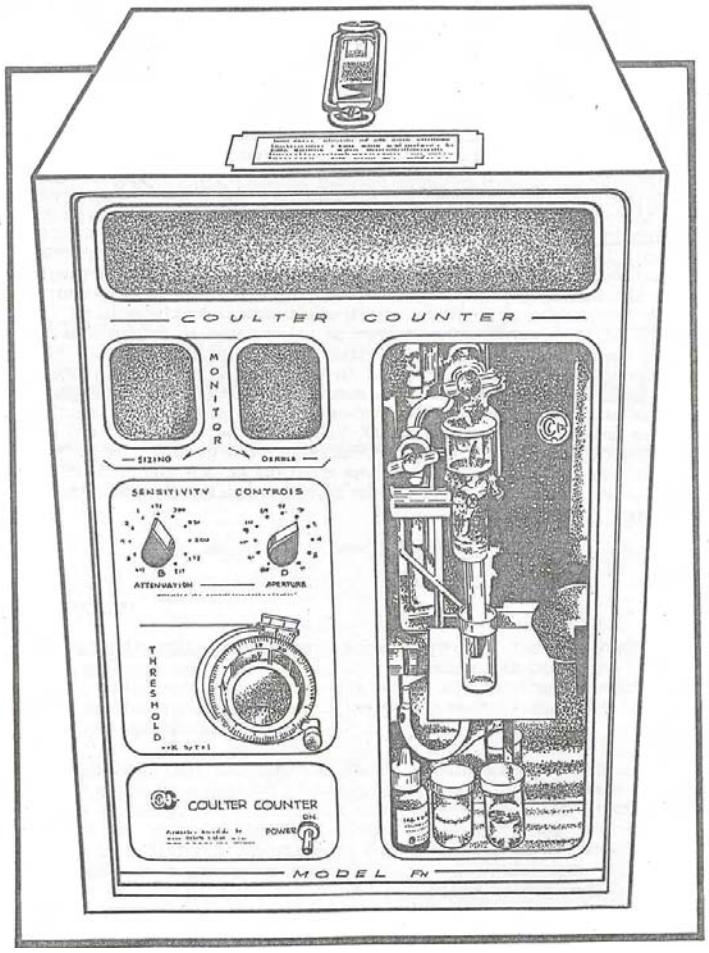


Figure 3-3. Coulter Model FN Counter.

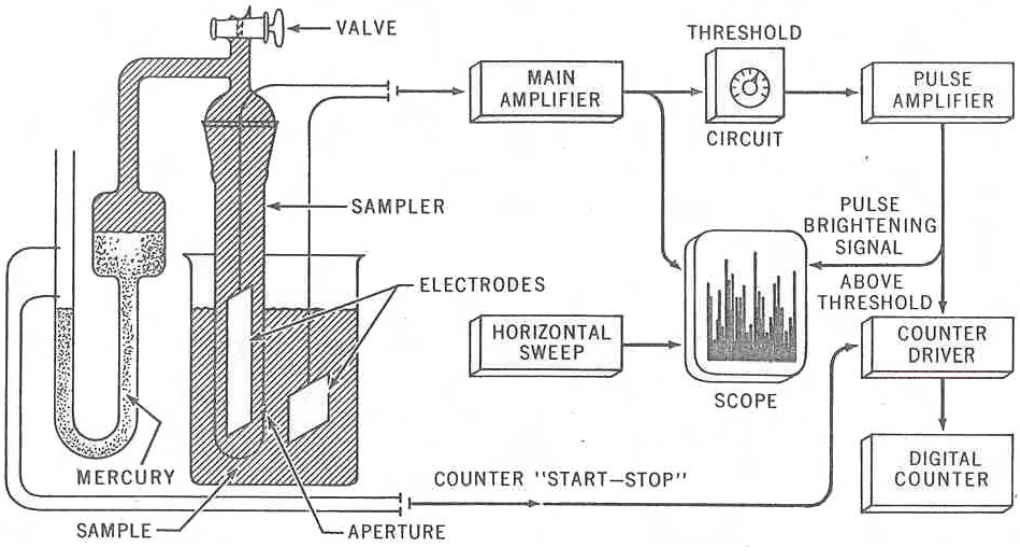


Figure 3-4. Schematic diagram of the Coulter Counter.

c. Adequate maintenance of the Coulter Counter will reduce incidence of instrument failure. The following should be done on a daily basis:

(1) Observe the mercury travel time in the manometer. If the mercury column does not move, moves erratically, or flows quickly into the aperture tube, the manometer needs cleaning.

(2) Record a background count with diluent and diluent plus saponin at the WBC threshold setting.

(3) Record the control suspension counted at the RBC and WBC setting.

(4) Flush orifice with dilute sodium hypochlorite (bleach). Flush the system thoroughly with distilled water and then saline.

d. In addition to the daily maintenance, the following maintenance should be performed once a week to reduce the incidence of instrument failure:

(1) Oil the vacuum pump.

(2) Clean the orifice; be very careful with the orifice insert.

(3) Check threshold zero.

e. Check the threshold plateau every month.

f. Once every 6 months perform the following maintenance:

(1) Change the latex tubing.

(2) Clean the manometer.

(3) Check the calibration factor for the instrument.

g. Problems of electronic counters.

(1) Dilution inaccuracies often result in erroneous results.

(2) False impulses resulting from electronic problems such as interference from other machines can cause erroneous results.

(3) Contaminating particles in apparently clear diluent are another source of impulse. Background counts must be made daily on all diluents.

(4) White blood cell counts are not accurate over 10,000 per cu mm and must be diluted with saline.

(5) High platelet counts or nucleated erythrocytes can cause erroneously elevated white blood cell counts. A correction must be made if these cells are observed on the blood film.

(6) Standardization is required with hemacytometer results.

(7) Bubbles must be avoided since they will be counted as cells.

(8) The orifice of the counter can become plugged. All equipment used with the counter should be cleaned.

(9) The equipment has a high initial cost; meticulous care is required for the proper maintenance of the counter.

3-6. HEMATOLOGICAL SYSTEMS

Complete hematological systems have been developed that perform fifteen hematology parameters. These systems include: red blood cell count, white blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, mean platelet volume, and a five-part differential, reporting neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The hematological instruments flag high and low values, and along with PC computer analysis, user-defined flags can be set with probable disease states. Some of these instruments provide automatic sampling of the specimen and patient sample identification using bar code readers. In addition to digital printouts of parameter values, RBC and platelet histograms, WBC scattergrams are provided on top of the line models.

Section III. COULTER COUNTER MODEL LINE

3-7. COULTER MODEL S COUNTER

a. The Coulter Model S Counter (see figure 3-5) operates on the same principle of differences in electrical conductivity as the Coulter Models F and FN. There are, however, certain significant improvements inherent in the Model S. These include the running of samples in triplicate by the instrument. If data from one aperture disagrees, it is "voted out" and data from the remaining two are averaged. If all three disagree, no result is reported and the instrument requests a new sample to repeat the test. The Coulter Model S runs the WBC count and the RBC count simultaneously eliminating the need for switching back and forth, making dilutions of samples, etc., thereby saving even more time for the technician. In addition, the Coulter Model S also performs a

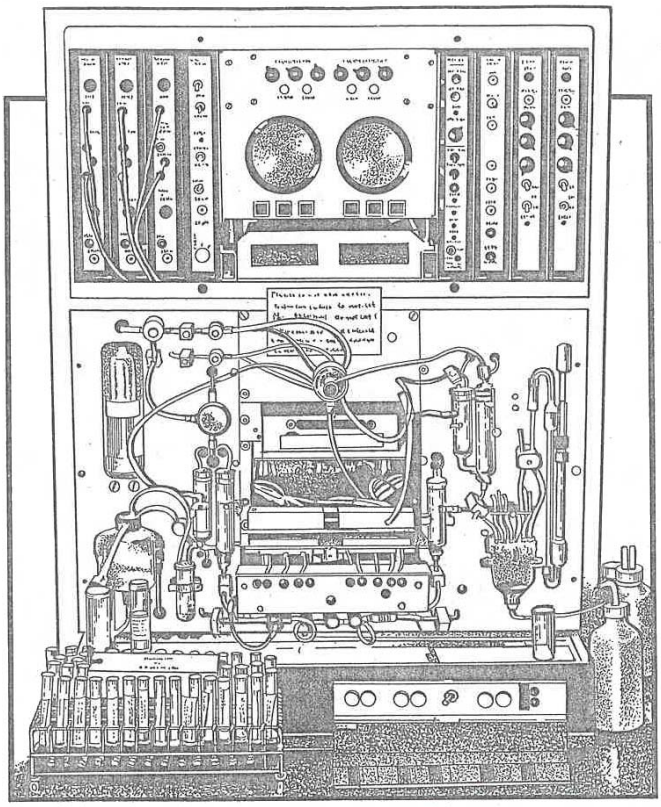


Figure 3-5. Coulter Model S Counter.

hemoglobin determination by the Drabkins method, calculates the hematocrit and indices, and prints these results on the request slip or report card. The Coulter Model S also has the ability to be linked to a computer network so that the results may be sent to the patient's ward, central records area, or any other desired locations as the tests are completed. The Coulter Model S may also be attached to an automatic differential device, the results being correlated with the CBC indices. The Coulter Model S accepts microcapillary samples for complete CBC workups as well as unopettes. This greatly lessens the amount of sample necessary and enables testing for pediatric patients and for other related procedures.

b. The Coulter Model S counter requires one full milliliter of undiluted blood for a normal sample. Maintenance on the Coulter Model S is at times very intricate. The manufacturer of the machine rigidly adheres to recommended cleaning reagents, that only commercial reagents from its stock be used with this instrument, and that a maintenance contract be purchased from the parent company. There are now additional suppliers of both reagents and equipment repair so one sole supplier is not the norm.

c. The Coulter Model S utilizes automatic dilution analysis to perform seven hematology parameters. The RBC and WBC counts are performed in triplicate. If there is any disagreement due to debris or other error, the technician will be alerted by an alarm system. The patient sample is introduced to the sample aspirator manually and the patient identification is submitted to an automatic printer. The instrument is activated, aspirates a sample, performs the necessary dilutions, and the results are printed automatically on the patient's identification card. The Coulter Model S analyzes and records the data quickly and accurately.

3-8. COULTER MODEL S PLUS COUNTER

In 1978, with the introduction of the Coulter Model S Plus (see figure 3-6), the main improvement over the Model S was the additional capability of performing platelet counts, providing mean platelet volumes, and plotted histograms. Asthetically, the instrument is now fully enclosed. The electronics section is shielded against electromagnetic interference, by a metal cover panel. The sampling area is encased behind a safety plate glass door. The only items remaining outside, are the two sampling tips, and a diluent dispenser. One tip (on the left side of the door), is for whole blood sampling, which still requires a full 1 ml of blood. The other sample tip is for a prediluted specimen, and is located on the right side of the door. For the prediluted specimen, only 44.7 µl of whole blood is needed. It is then diluted into 10 ml of the saline diluent. The diluent dispenser is located at the top center of the door. Once the white diluent dispense button is pressed, 10 ml of the saline diluent is discharged. Routine recalibration is recommended on a quarterly basis. This procedure is quite intricate, and takes approximately two hours total time.

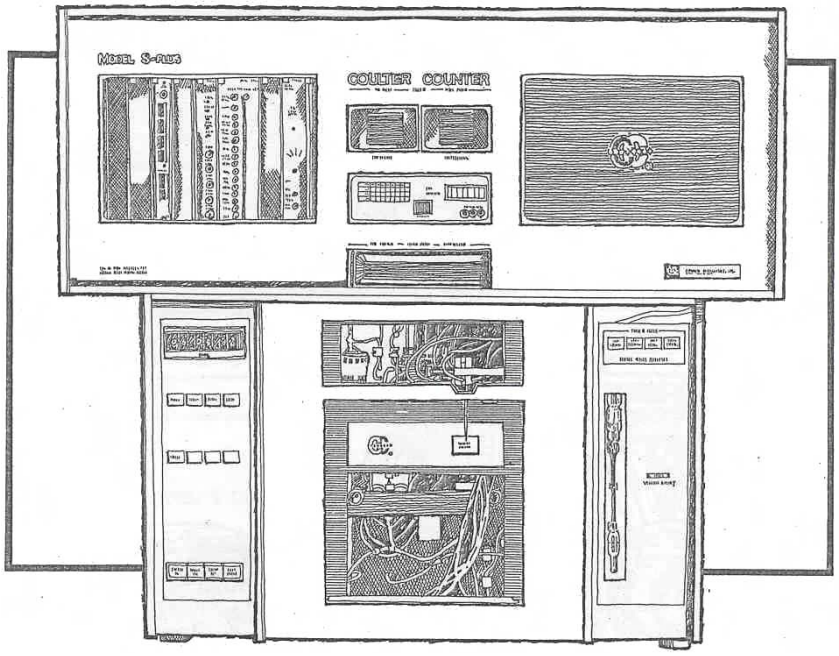


Figure 3-6. Coulter Model S Plus.

3-9. COULTER MODEL S PLUS II COUNTER

In 1980, Coulter once again improved upon their instrument. The instrument now was capable of providing the total lymphocyte count and a percentage evaluation, bringing the total number of parameters provided to 12. In addition, Coulter introduced the Data Terminal. On this piece of hardware, the CRT would display all results, operator alerts, and histograms. With improvements in the computer section, routine recalibration is no longer necessary, the exception is when a major component is replaced.

3-10. COULTER MODEL S PLUS III COUNTER

The Coulter Model S Plus III, which came out around 1982, added two more white cell parameters to its differential count, provided with the optional Data Terminal. These values were printed out in both total number and percentage. The differential now consisted of lymphocytes, granulocytes, and mononuclear cells. The instrument now has but one aspirator tip with a major improvement in sample volume size. Sample size was now dropped down to 0.1 ml. This had an advantageous side effect, that reagent consumption was also reduced. Automatic sample handling could be obtained by using the optional Coulter Automatic Sample Handler (CASH) system (see figure 3-7). This peripheral device would pierce the specimen cap and aspirate the sample. This device has two 32-specimen trays, which would rotate to mix the samples. It included separate areas for stat specimens and control vials. Analysis rates were also increased to 135 samples per hour. With a Data Terminal (see figure 3-8), control results could be displayed in either tabular form or by means of Levey-Jennings graphs.

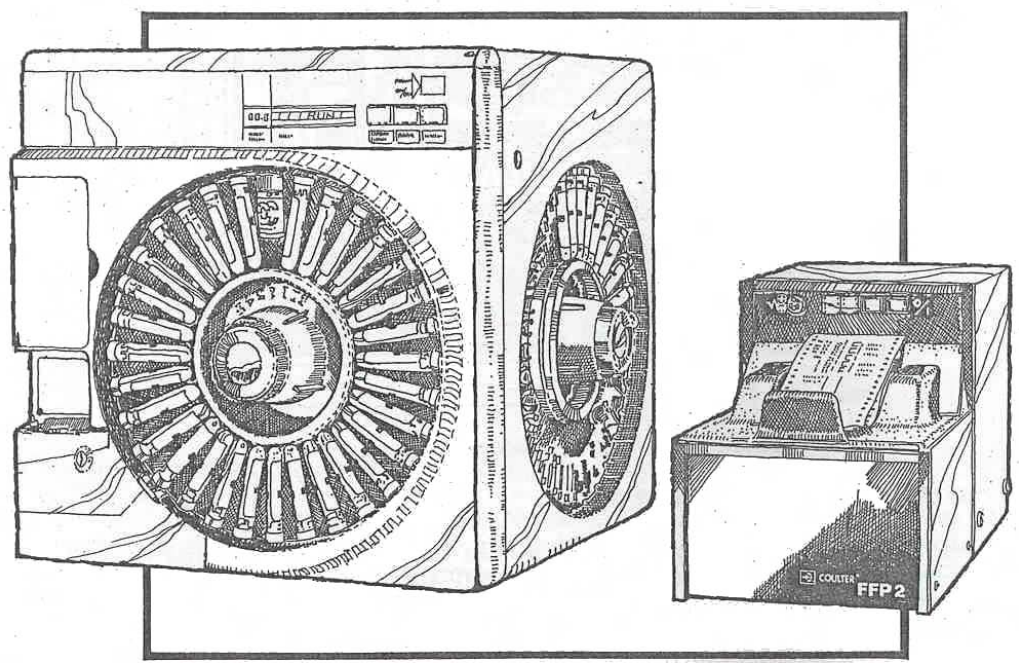


Figure 3-7. CASH system.

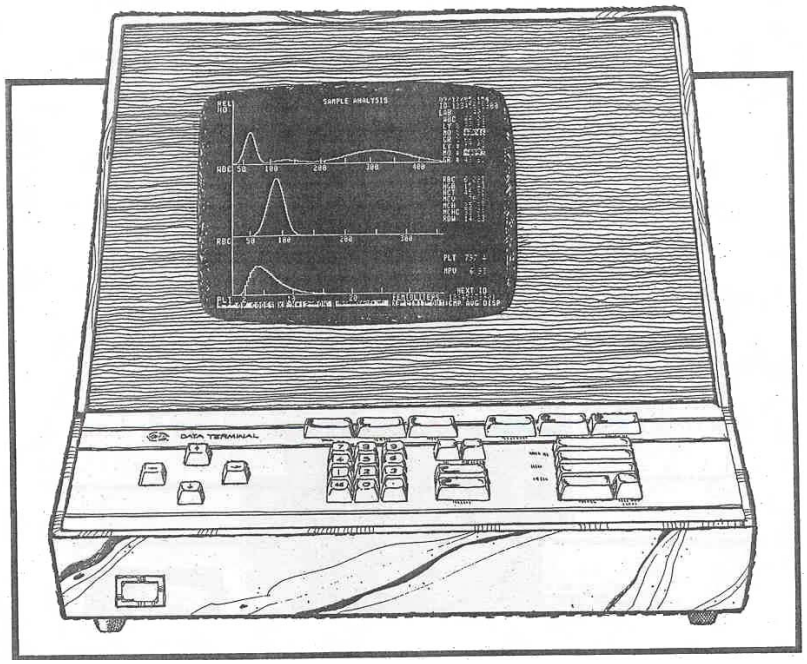


Figure 3-8. Coulter Data Terminal.

3-11. COULTER MODEL S PLUS IV COUNTER

The Coulter Model S Plus IV had only minor changes from the Coulter Model S Plus III. Optional accessories included the Bar Code Reader which would match results with request forms, and an Auto-Reporter printer which could match results to the correct bar coded slip. Throughput increase was only up to 138 samples per hour. Auto sampling was not a feature of this model, so that it is considered only a semiautomated instrument. Differentials were performed by a process called volumetric cytochemistry. This method was based on a cytochemical reaction that altered white cell volumes in predictable ways. The resulting volume-distribution histogram was then analyzed by the computer's program to determine numerical differential results and the detection of abnormalities. Data storage was increased by 2000 profile records, which could be further increased by its capability to control two data storage packages at once.

3-12. COULTER MODEL S PLUS V COUNTER

The Coulter Model S Plus V has many of the same features of the other members of the S Plus family (see figure 3-9). The S Plus V operates mainly with individual samples so that it is a semiautomated device, not a fully automated instrument. The main sample aspirator collects 125 µl samples from an automatic cap piercer, which eliminates the hazard of opening and closing collection tubes. The instrument has a secondary sample aspirator tip which requires only 100 µl of whole blood. As an option, the instrument can come with a bar-code reader to match up results.

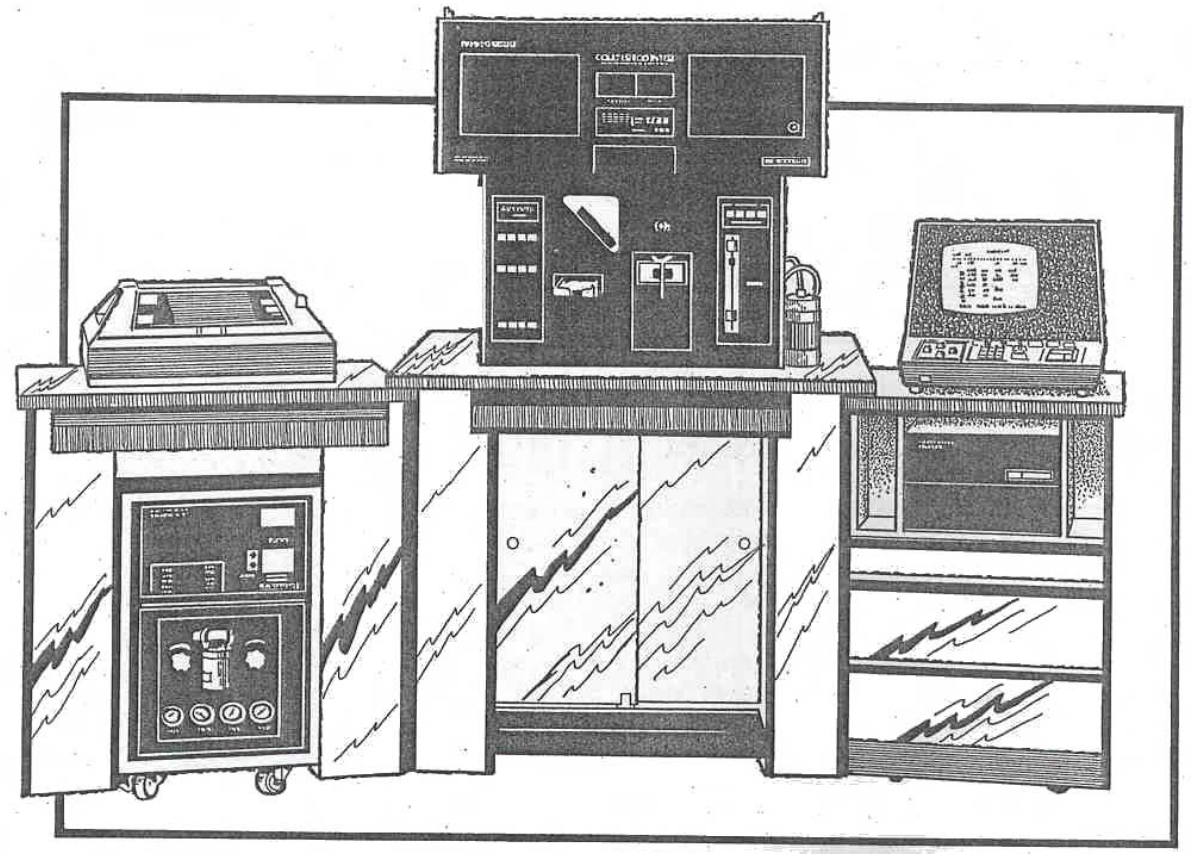


Figure 3-9. Coulter Model S Plus V Counter.

3-13. COULTER MODEL S PLUS VI COUNTER

a. This Model S Plus VI provides the user with many valuable options of which auto-sampling from sealed collection tubes making the instrument totally automated is the most important (see figure 3-10). Ten parameter profiles are provided: these include WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, Plt, and MPV. With the addition of the optional printer/plotter, a full 16-parameter profile is available. This includes volume referenced histograms for WBC, RBC, and Plt's, plus Levey-Jennings graphs.

b. Models S Plus IV and S Plus V can be upgraded to the S Plus VI with the addition of the auto-sampler. With the use of the Data Terminal option, the instrument is capable of auto-calibrating, using the S-Cal Calibrators. This feature saves the technologist several hours of work, making calibration as easy as running normal patient specimens. Another feature is its ability to handle statistical analyses at the touch of a button. As soon as the statistics are processed, automatic sampling resumes. In its primary mode, sampling size is 750 µl. Secondary mode or manual analysis requires only 125 µl.

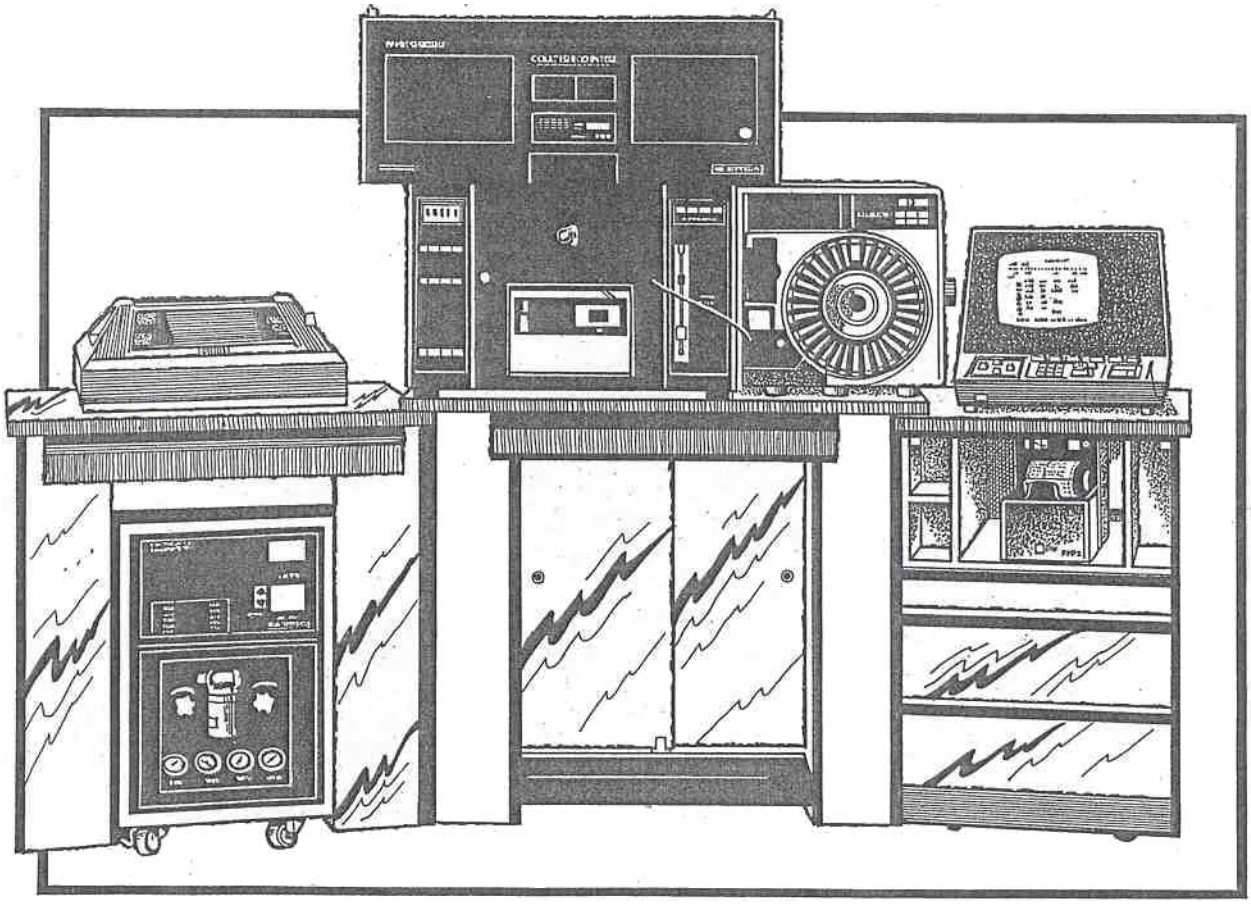


Figure 3-10. Coulter Model S Plus VI Counter.

3-14. COULTER MODEL CBC5 COUNTER

This instrument was designed for small labs doing only a few tests per day. It is a compact unit that provides the most frequently asked for parameters such as: WBC, RBC, Hgb, Hct, and MCV. It comes with premeasured reagents in color coded vials. Samples are collected in exact-fill pipets. A "How-to" audio-tutorial comes with the instrument.

3-15. COULTER MODEL M430 COUNTER

The Coulter Model M430 Counter is a compact, semi-automated system for small labs doing 30 to 50 samples per day. This system provides 7 parameters: WBC, RBC, Hgb, Hct, MCV, MCH, and MCHC. Some of the features provided include aperture viewing, audible counts, automatic coincidence correction, low reagent indicators, electronic aperture cleaning, and operator alerts. Each sample takes only 50 seconds to analyze and print results.

3-16. COULTER* JR MODEL

For the larger laboratory, the Model Coulter* JR is capable of running 80 samples per hour providing for the following parameters: WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, MPV, Plt and a complete WBC differential (see figure 3-11). Additionally, the instrument is capable of interpreting suspected abnormal differential results. The Data Terminal (an option) provides the following: data storage, management, quality control, XB analysis, maintenance program, and limits flagging. A bar code reader and printer are other options. Sample size is only 100 µl of whole blood.

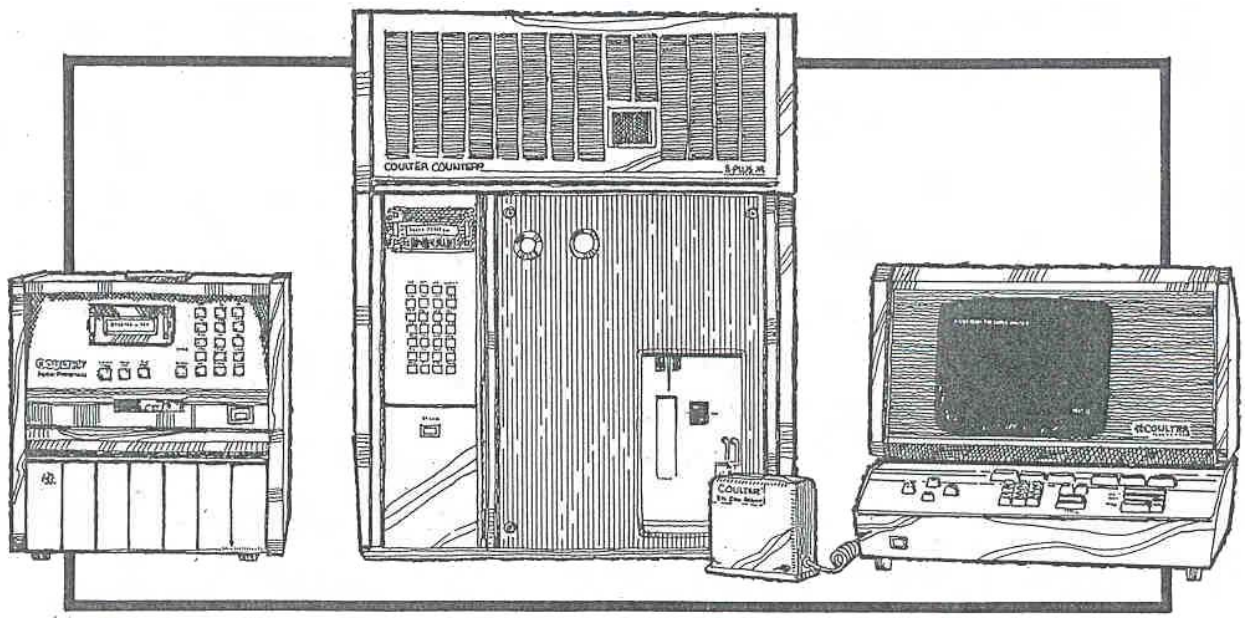


Figure 3-11. Coulter* Jr.

3-17. COULTER* JS MODEL

This model is a totally automated, walkaway system (see figure 3-12). It provides 16 parameter hematology profiles. Included in every profile are: red and white blood cell and platelet counts, red cell indices, and the complete Coulter Histogram Differential with interpretive report (see figure 3-13). Some of the features are: closed-tube auto-sampling at 80 samples per hour, automatic differentials, detection, and flagging of significant abnormalities with less than 1 of the instrument can store 270 patient results or 37 composite histograms and patient results. Optional software can increase this storage to 3800 profiles or 550 complete reports with histograms. The quality control package provides multiple file storage, automatic generation of Levey-Jennings graphs, and continuous X_B analysis.

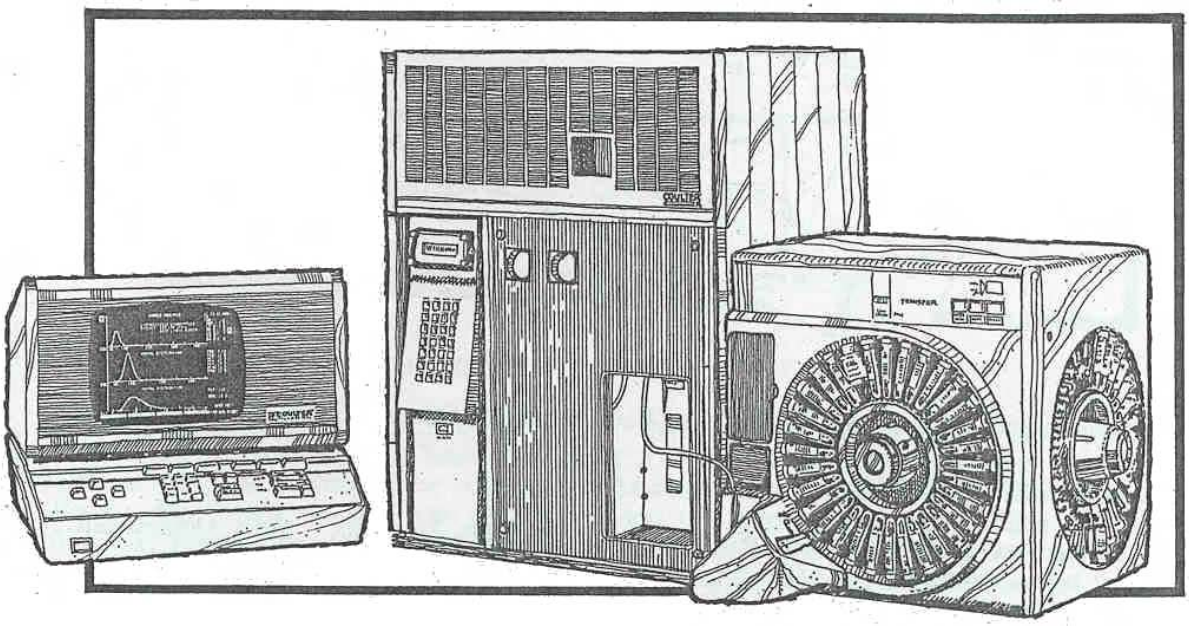


Figure 3-12. Coulter* Js.

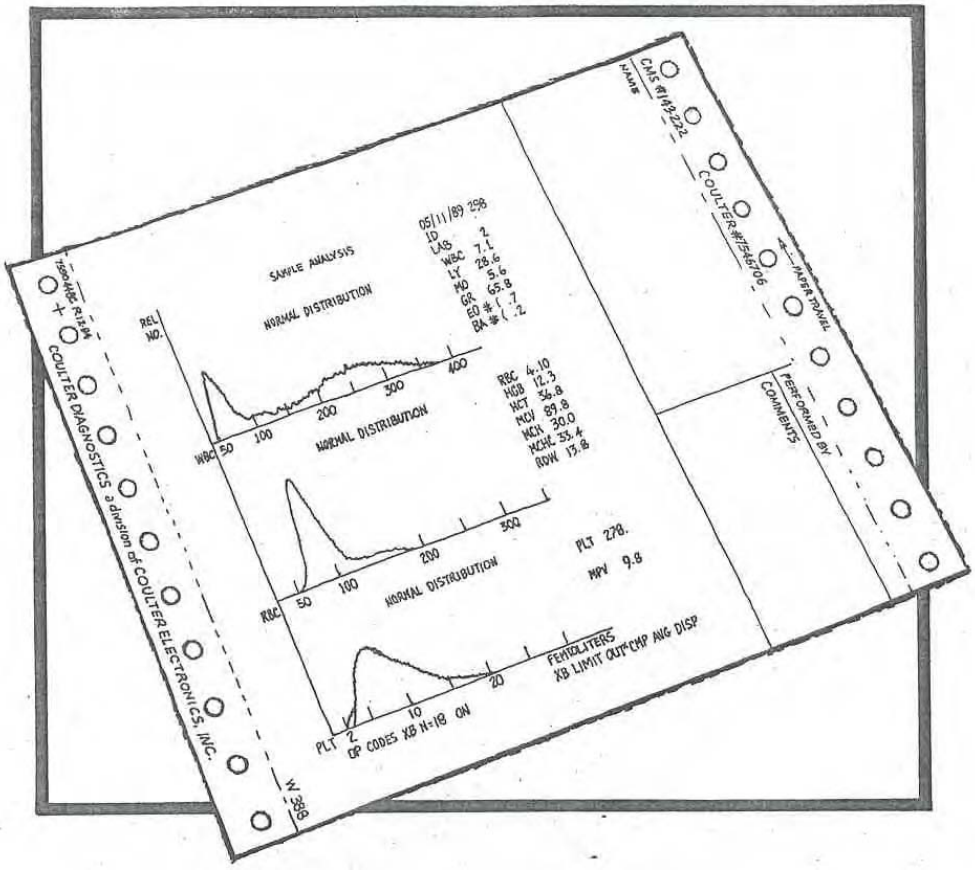


Figure 3-13. Coulter Histogram Differential.

3-18. COULTER* STKR MODEL

This was Coulter's top of the line from 1986 to 1989. This instrument featured high throughput (138 samples per hour, in 12 portable cassettes holding 12 samples apiece), totally automated closed-vial sampling, with auto-mixing, and auto-feed (see figure 3-14). A 16 part hematologic report is rendered with the Coulter Histogram and Interpretive Report. Bar code identification of patient specimens provided work list generation and positive sample identification. Reports include red cell morphology and suspected presence of eosinophilia and basophilia. Automatic delta checks are performed on all patients. Interfacing to a laboratory computer is available. Autoprocessing can be interrupted for statistics at any time. The Coulter Histogram Differential (CHD) is capable of indicating abnormalities such as: eosinophilia, basophilia, monocytosis and blastocytosis, NRBC's, atypical lymphocytes, increased bands, and other abnormal cells.

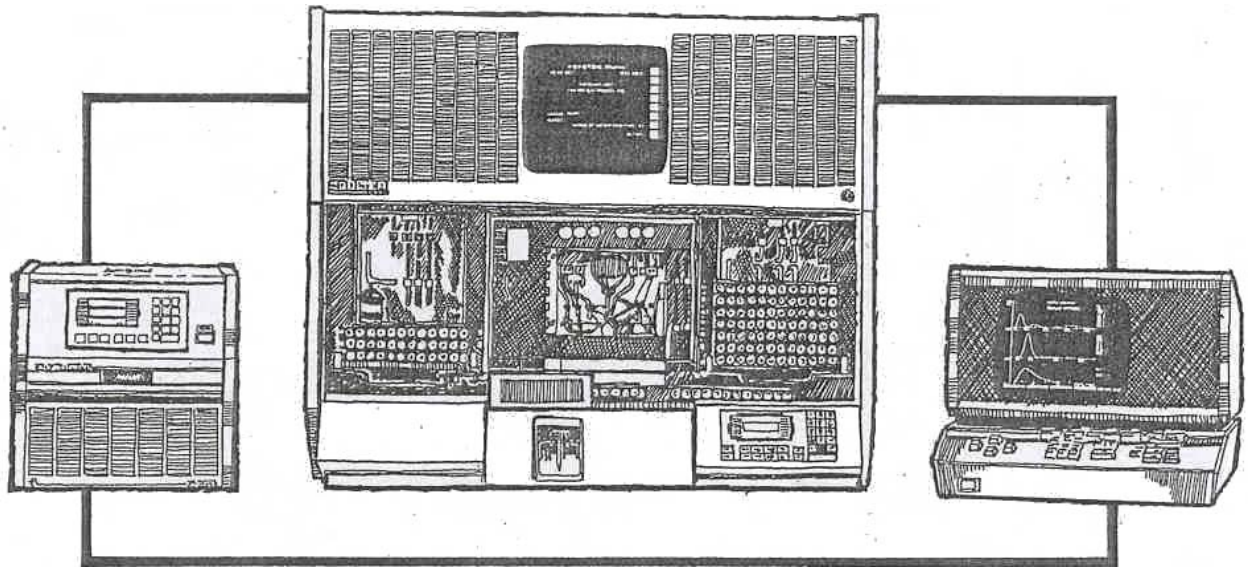


Figure 3-14. Coulter STKR.

Section IV. LATEST GENERATION COULTER COUNTERS

3-19. COULTER T-SERIES COUNTERS

This next series consisted of three instruments, the Models T540, T660, and T890. These instruments were designed for the small laboratory or private physicians' office. The major difference between the models was in throughput per hour. The basic model, the T540 provided the capability of running 40 samples per hour, providing 7 parameters: WBC, RBC, Hgb, Hct, Plt and total lymphocyte count with percentage. The model T660, provided 60 samples to be run per hour and added MCV to its capability. The model T890 was able to run 90 samples per hour and provided MCH and MCHC in addition. System operation was simplified by using automatic whole blood microsample aspiration, with push button start-up and shutdown. The self-cleaning blood sampling valve required no routine maintenance. Precision and accuracy were improved with automated backwash, rinsing, and electronic aperture cleaning. All reagents came in a simplified system in one container, called a UNI-T-PAK (see figure 3-15). This provided simple, clean, nonhazardous disposal with reagent and specimen waste automatically stored in the UNI-T-PAK.

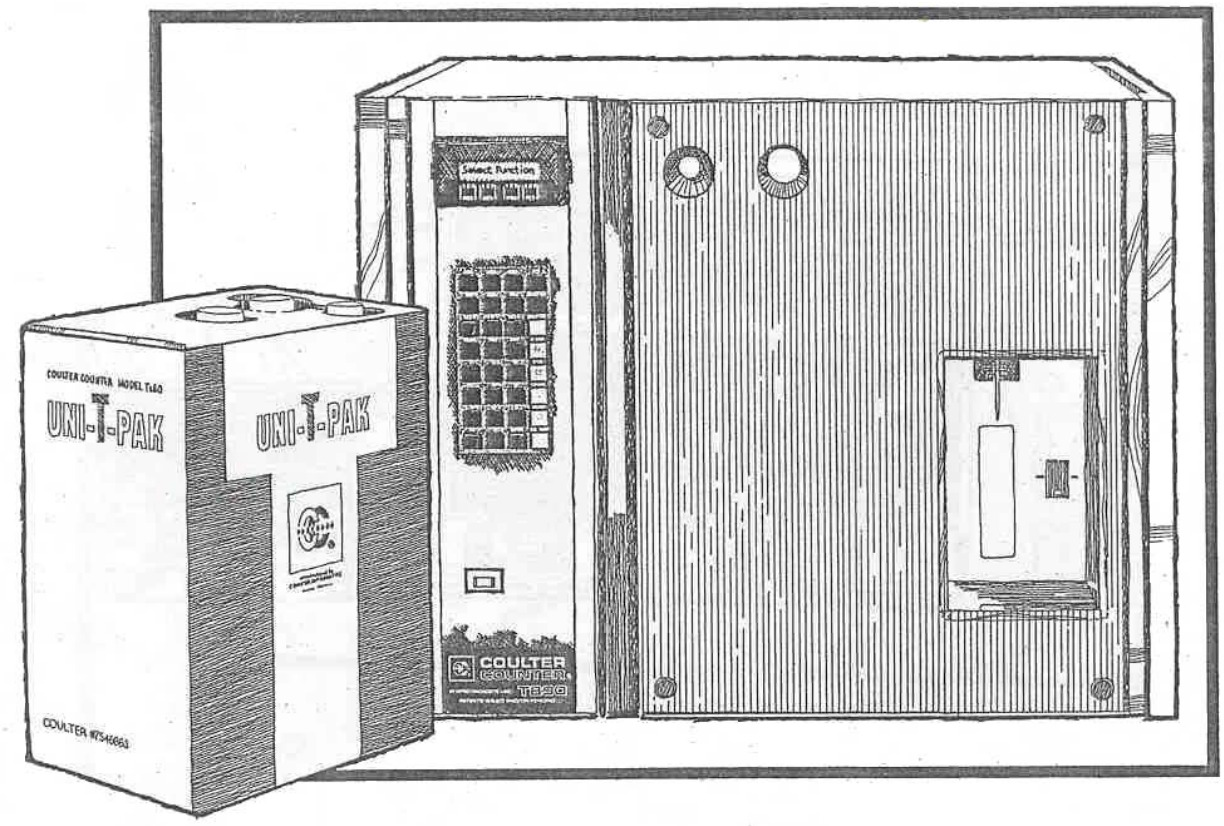


Figure 3-15. Coulter T-Series and UNI-T-PAK Counters.

3-20. COULTER JT-SERIES

The Coulter JT-Series or group was developed to handle moderate volume facilities, with throughput of 50 samples per hour for the JT and JT2, and 60 samples for the JT3. Each includes the data terminal which gives the instrument the capability of performing differentials by the CHD method. Sample volumes are small at 110 µl in the manual mode and 270 µl for automatic modes on the JT2 and JT3. The JT2 and JT3 also provide for single closed-vial sampling. The JT runs on manual sampling. Patient data storage is up to 3900 sample results or up to 540 with histograms. Bar-code readers are standard on the JT2 and JT3 but is optional on the JT. Pushbutton control of all diluter functions, including start-up and shutdown, are features of all JT-Series (see figure 3-16).

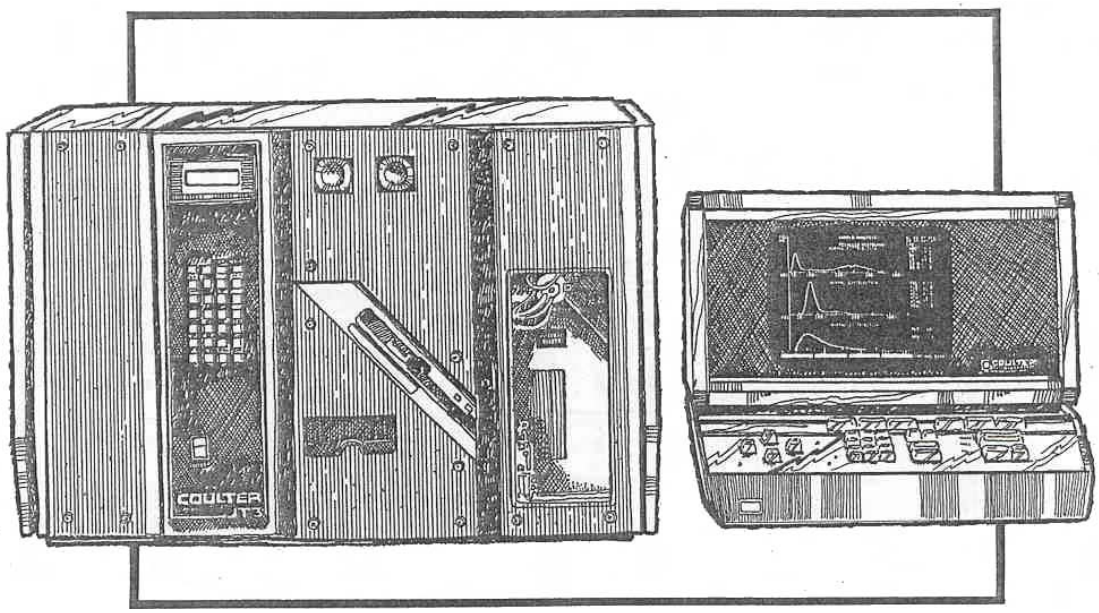


Figure 3-16. Coulter JT-Series.

3-21. COULTER MAXM AND MAXM AL (AUTO-LOADER)

a. These moderate volume throughput instruments (75 samples per hour) work on a new Coulter WBC differential methodology, called VCS. This represents cell volume, conductivity, and laser light scatter. To determine the white cell differential, the instrument examines more than 8,000 white cells in their native form. All measurements are taken from a single flow cell in which the cell is subjected to 3 simultaneous independent assessments (see figure 3-17 for MAXM). In total, over 16 million data points are plotted for analysis on each sample. The results of these readings are plotted onto a scatterplot diagram or scattergram. This diagram has designated fields that represent different cell types (see figures 3-18 and 3-19 for printouts and scattergram). The instrument itself provides 22 printed parameters.

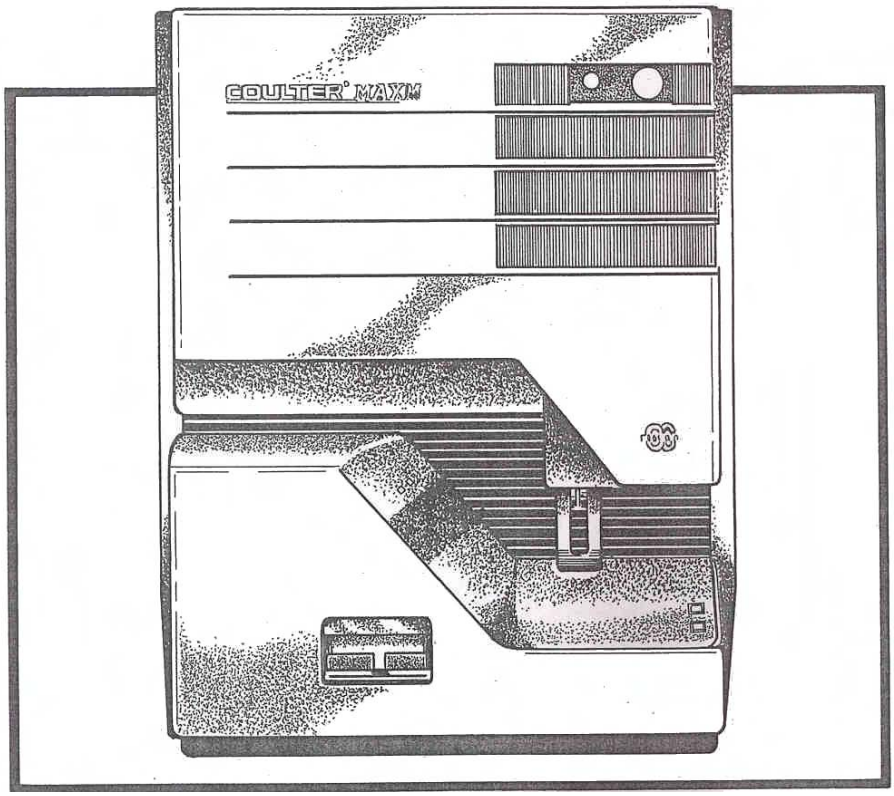


Figure 3-17. Coulter MAXM.

Sample volume requirements are small, 185 µl for the primary autosampling mode, 125 µl for the secondary manual aspirating mode, and 50 µl, for the prediluted mode. An optional auto-loader is available which provides walkaway automation. This sampler handles up to 25 specimens in five cassettes of 5 apiece. The samples are rocked and moved to the sampling mechanism automatically. Closed-vial samples are pierced and bar coded labels are read simultaneously for positive sample ID.

b. Some of the other convenience features are a self-cleaning blood sampling valve, and automatic maintenance of the flow cell eliminate routine system bleaching. Coulter Clenz keeps the fluidics and optics fully cleaned at all times. The cap-piercing needle, which obtains the sample, never needs maintenance. Bar codes are read by laser. Calibration is totally automatic. The instrument also wipes the probes automatically for secondary, manual microsamples, and predilutes. Easy operation is assisted by on-line help instructions. Single button start-up initiates a full system check with self-diagnostics.

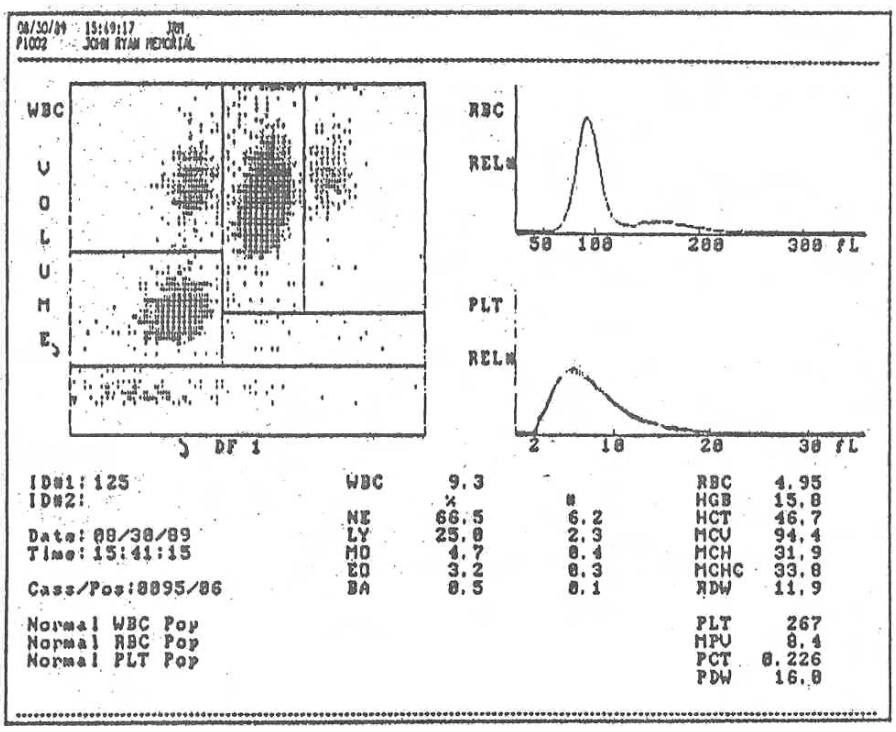
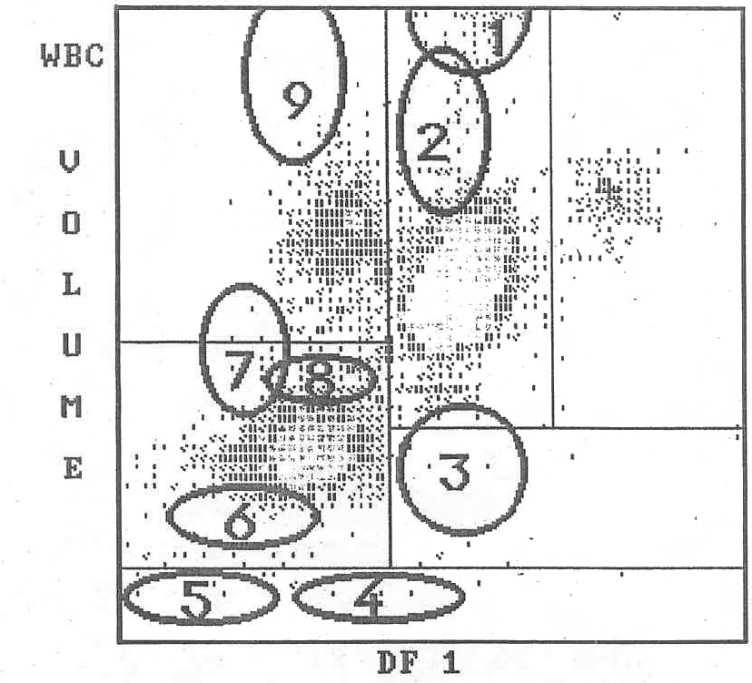


Figure 3-18. Coulter STKS and MAXM printouts.



1. Suspect Blasts
2. Suspect Immature Granulocytes
3. Aged and Damaged Neutrophils
4. Giant Platelets
5. Nucleated Red Blood Cells
6. Variant Lymphocytes
7. Suspect Blasts
8. Variant Lymphocytes
9. Suspect Blasts

Figure 3-19. Scattergram enlargement.

3-22. COULTER STKS

a. Model STKS is Coulter's top of the line in high volume, auto-sampling, differential analyzing instruments (see figure 3-20). The methodology of doing the differentials on this instrument is called VCS Technology by Coulter Corporation. This process entails three simultaneous measurements of leukocytes properties using impedance (Coulter principle) and Conductivity and Laser Light Scatter principles with the Coulter Hematology Cell Classification Report.

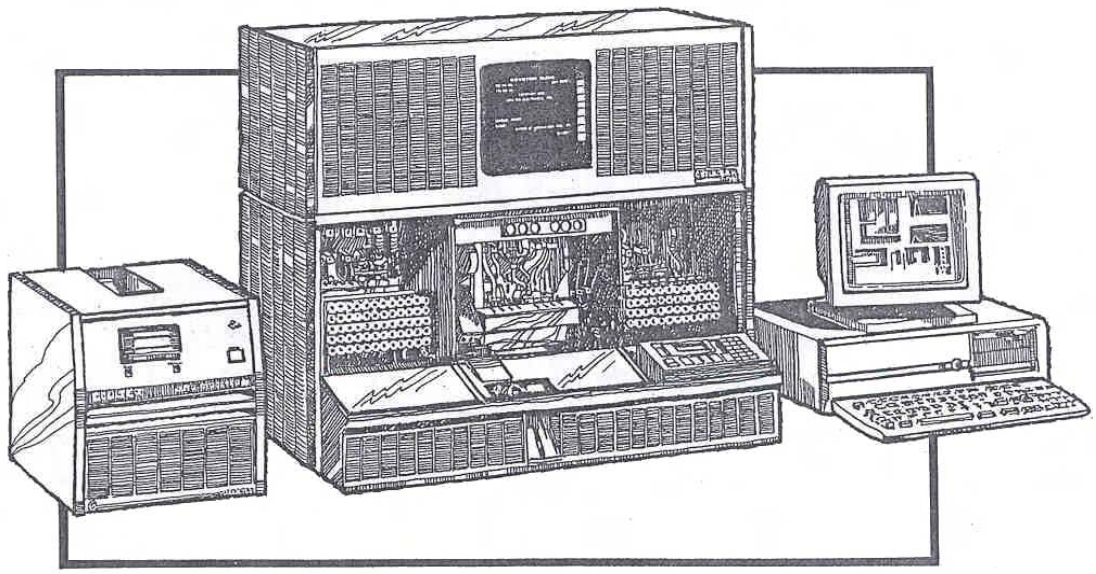


Figure 3-20. Coulter STKS.

b. The printout is the same as that of the MAXM. Specimens with bar coded labels are placed into cassettes in groups of 12 with 144 specimens maximum at a time. Each cassette is also bar coded. As the cassette is moved automatically toward the cap-piercing needle for sample aspiration, the cassette is rocked, to thoroughly mix the samples. Dual sample sensors, one before and one after the blood sampling valve, ensure aspiration integrity. The sampling robotics are the same as that of the STKR. As with the MAXM, the STKS studies over 8,000 white cells for each differential. The STKS has 2-sets of 3 viewing apertures, one set for WBC's and one set for RBC and PLT counts. Due to Coulter developments, the instrument has no routine maintenance requirements. With the optional printer, the Auto-Reporter 3, normal and abnormal reports can be separated automatically, allowing for retrieval of abnormals for later follow-up. The Data Management system consists of a PC computer with a graphics printer. This allows the continuous monitoring of the instrument and the ability to offer help windows and menu screens for easy operation and training. The CRT screen provides a clear, high-resolution view of the VCS differential and histograms. Sample throughput is at 109 samples per hour, which includes differentials. Only 5 reagents are required to run the STKS. The instrument is also capable of quantifying red cell morphology (see figure 3-21 chart for available classifications).

Coulter Classification Chart

Suspect and Definitive flag information is operator selectable.

	WBCs	RBCs	Platelets
Instrument-Defined Suspect Classifications: (Lab Report Only)	Immature Grans/Bands Variant Lymphs Blasts Review Slide	Nucleated RBCs Dimorphic RBC Population Micro RBCs/RBC Fragments RBC Agglutination	Platelet Clumps Giant Platelets
User-Defined Abnormalities: Definitive Flags (Lab Report Only)	Leukopenia Leukocytosis Neutropenia Neutrophilia Lymphopenia Lymphocytosis Monocytosis Eosinophilia Basophilia	Anisocytosis (Quantitative +, ++, +++) Microcytosis (Quantitative +, ++, +++) Macrocytosis (Quantitative +, ++, +++) Hypochromia (Quantitative +, ++, +++) Poikilocytosis (Quantitative +, ++, +++) Anemia Erythrocytosis Pancytopenia	Thrombocytopenia Thrombocytosis Large Platelets Small Platelets
User-Defined High and Low Action Limits (Chartable Report Only)	All WBC Parameters	All RBC Parameters	All Platelet Parameters

Figure 3-21. Coulter classification chart.

3-23. SYSMEX CC-130 HEMATOLOGY ANALYZER

This analyzer is a small, compact, desktop, semiautomated instrument that provides for WBC, RBC, and Hgb analysis. Sample size is only 20 µl of whole blood using the Model AD-241 Automatic Dilutor. A built in microprocessor monitors the instruments operation, alerting the operator to functional irregularities. Hct measurements are corrected for temperature. Approximately 3 samples can be run each minute (see figure 3-22).

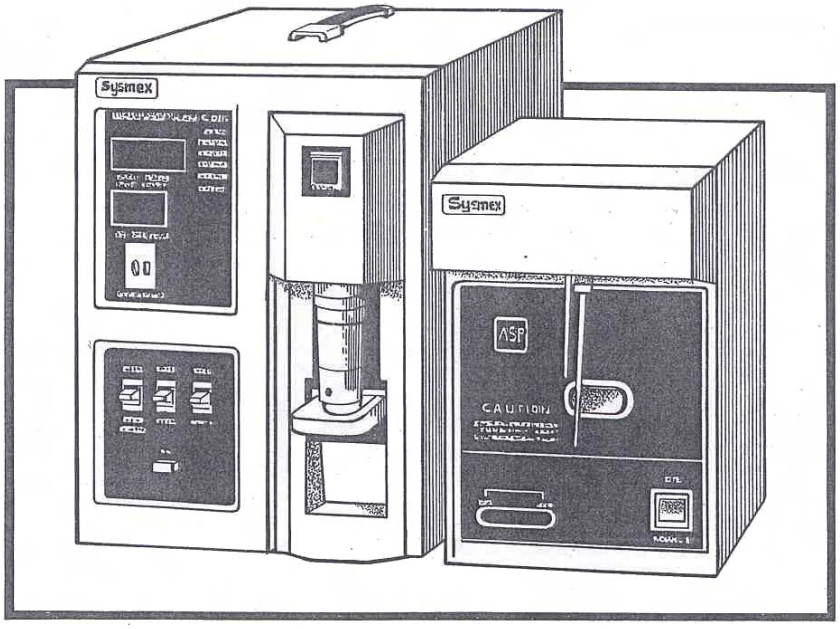


Figure 3-22. Sysmex Model CC-130.

3-24. SYSMEX MODEL CC-150

A rapid, semiautomated analyzer which can test for 5 parameters: WBC, RBC, Hgb, Hct, and MCV (see figure 3-23). WBC and Hgb are analyzed together, while RBC's are handled separately. Samples are run on 20 µl of whole blood. The AD-241 Automatic Diluter comes with the instrument.

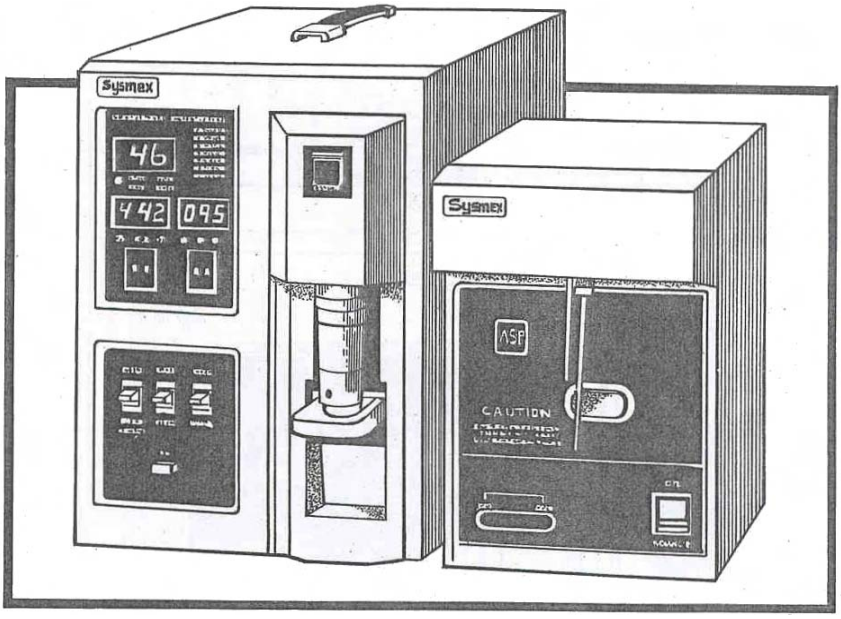


Figure 3-23. Sysmex Model CC-150.

3-25. SYSMEX CC-170 HEMATOLOGY ANALYZER

This tabletop system from Sysmex will simultaneously measure 7 parameters. The instrument offers a dual measuring system for the simultaneous measurement of WBC and RBC within 20 seconds of count activation. Its built in microprocessor can store data on up to 100 samples, which includes date sample ID number and analysis values. The instrument includes a built in quality control (QC) and operational monitoring program. The instrument includes an automatic dilutor, and can come with an optional card printer (see figure 3-24).

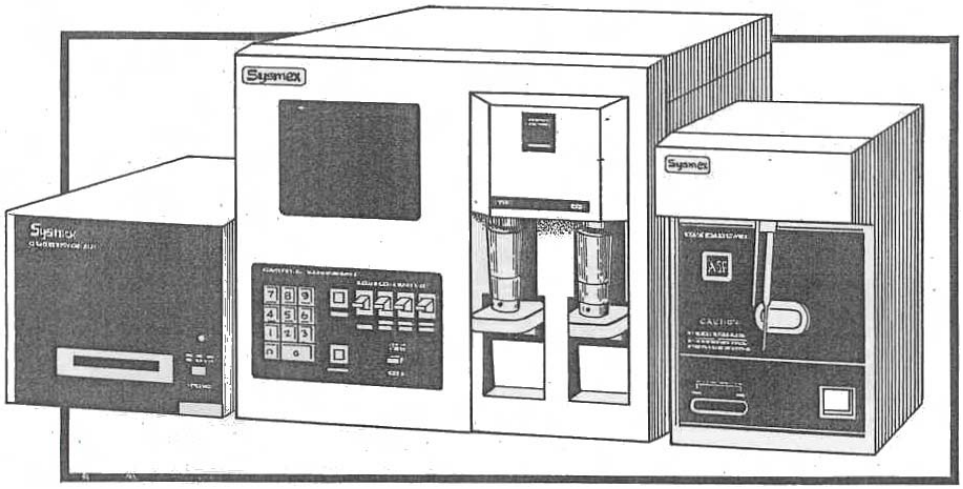


Figure 3-24. Sysmex Model CC-170.

3-26. SYSMEX MODEL CC-180

This semiautomated instrument has dual apertures and can run prediluted samples at a rate of 120 per hour. The instrument reports out with 8 parameters: WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, and PLT. Prediluted samples can be run at 120 per hour. Abnormal results are detected and automatic recounts are made for values that are outside of established ranges. Data from the last 10 samples may be displayed on the CRT screen simultaneously. The microprocessor in the instrument monitors instrument performance, self-diagnostic checks, analyzes irregularity of performance, dilute temperature checks, diluent temperature, and aperture clogging (see figure 3-25). Additional features include auto calibration and storage of up to 100 samples.

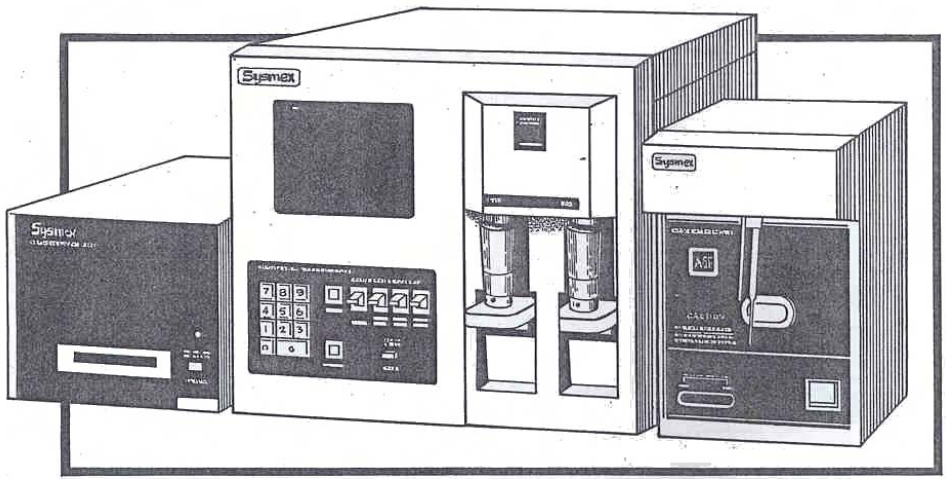


Figure 3-25. Sysmex Model CC-180.

3-27. SYSMEX MODEL CC-780 HEMATOLOGY ANALYZER

This is a fully automated hematology analyzer, providing 8 separate parameters. Sample size is 0.5 ml for whole blood and 40 µl of prediluted blood. Complete processing of a sample takes only 45 seconds and will run approximately 80 samples per hour. A built in roll printer or an optional card printer provides results. Flow system rinses the entire hydraulic system between samples (see figure 3-26).

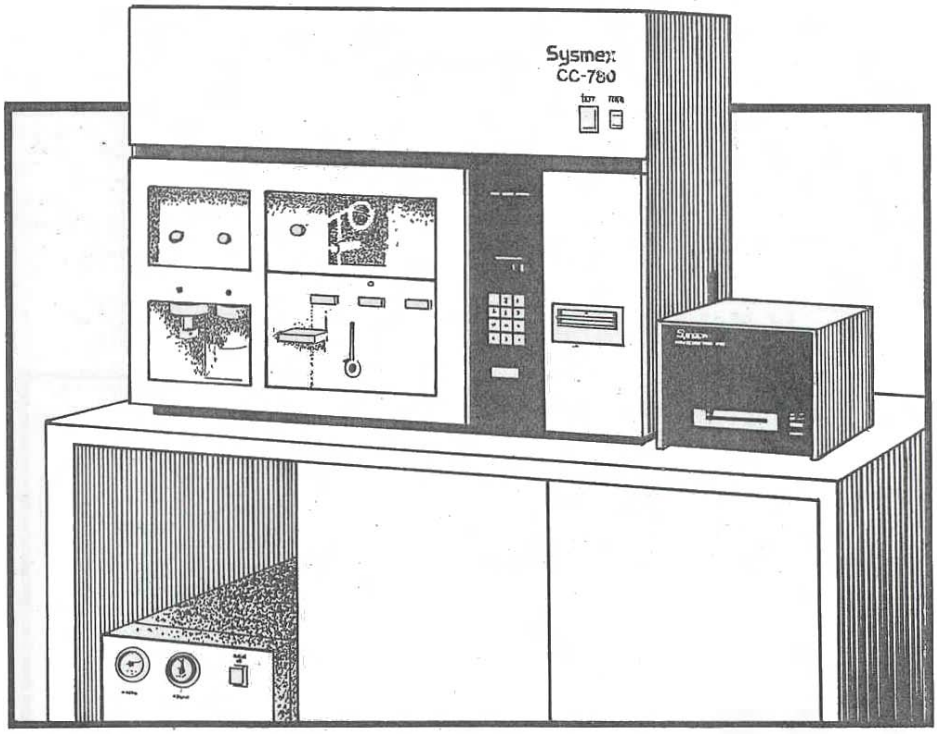


Figure 3-26. Sysmex Model CC-780.

3-28. SYSMEX MODEL CC-800 HEMATOLOGY ANALYZER

This fully automated analyzer can run up to 100 samples unattended at a rate of 80 samples per hour (see figure 3-27). Results are stored in memory for future reference. The autosampler turntable automatically mixes, aspirates, dilutes, and analyzes sequentially, all unattended. Three modes are available: autosampling, whole blood, and individual, which requires 0.5 ml of whole blood and prediluted, which only requires 0.04 ml of whole blood. An auto-rinse cycle prevents sample carryover. Extensive QC programs monitor instrument performance and accuracy. Quality control includes Levey-Jennings charts which can be displayed on the CRT. Results can be printed out on either optional printers, the Z-fold continuous feed, or the card printer.

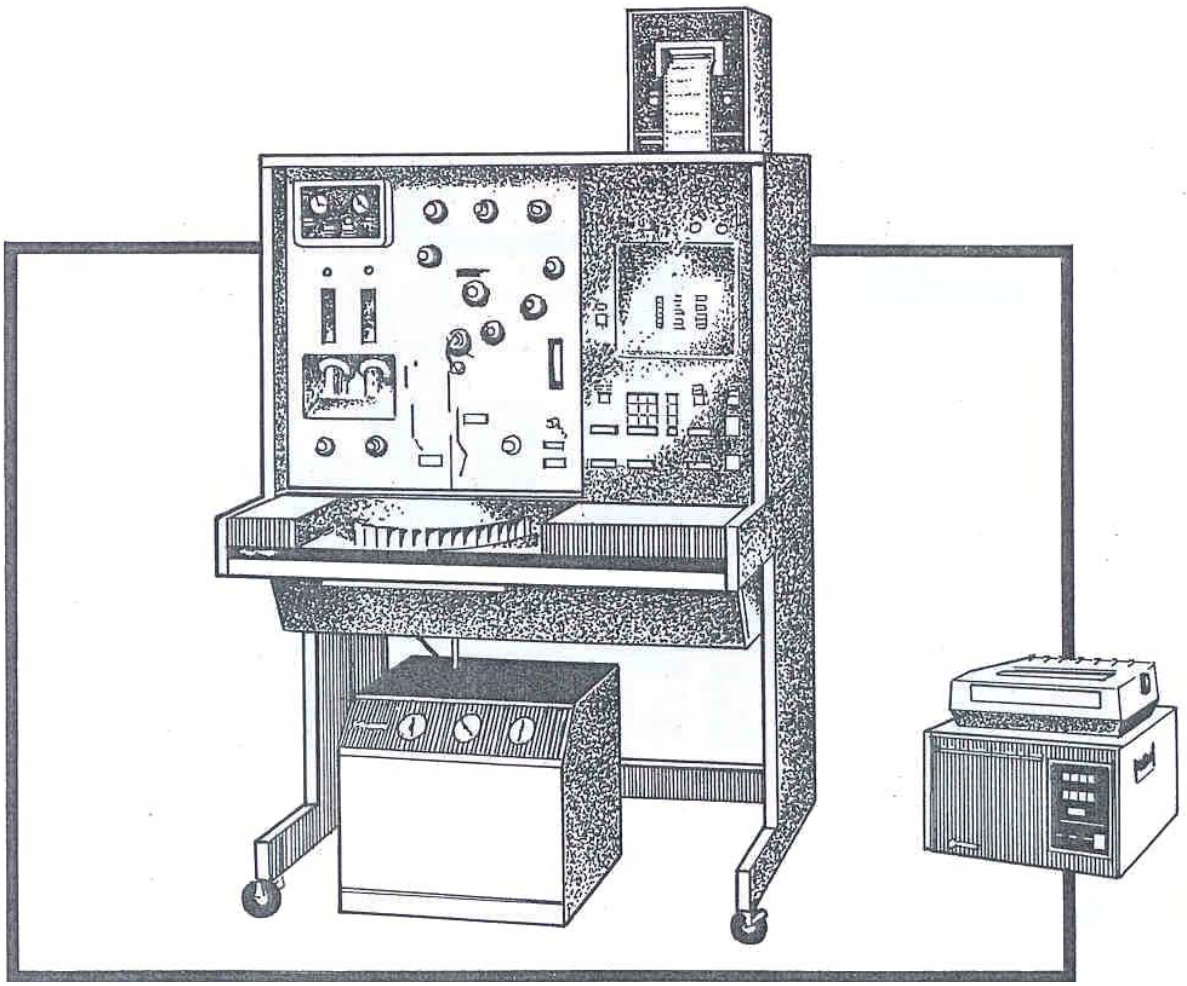


Figure 3-27. Sysmex Model CC-800.

3-29. SYSMEX MODEL E-5000 HEMATOLOGY ANALYZER

The E-5000 is a fully automated hematology analyzer which provides up to 18 parameters of results per specimen. These include: WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, PLT, and histograms for WBC, RBC, and PLT. The WBC Tri-Modal Histogram provides three cell subpopulation analyses, the RBC histogram, and provides RDW information. The PLT histogram, provides additional data such as PDW, MPV, and P-LCR. (PDW and P-LCR, are for research purposes only.) Sample size is 200 µl of whole blood and throughput is at 120 samples per hour. The cassette rack for the autotray system that the instrument uses can hold up to 100 samples. The built in QC program allows two variants of the Levey-Jennings plots. Memory can store up to 300 samples including all parameters and histograms. Twin CPU's allow the operator to simultaneously display, view, edit, or print stored data. Optionally, a bar code reader can be added for sample identification. A comprehensive system self-monitoring program, verifies total system operations (see figure 3-28).

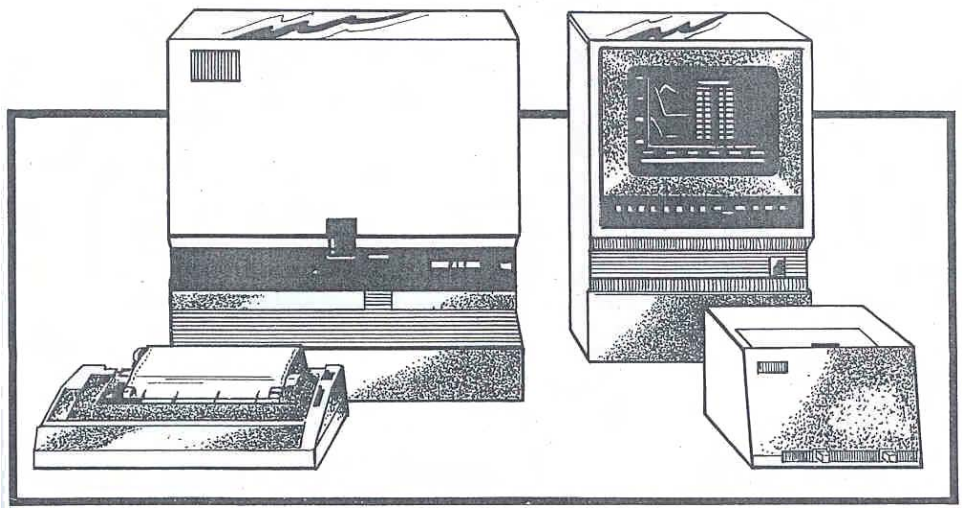


Figure 3-28. Sysmex Model E-5000.

3-30. AMES HEMA-TEK I, 1000, & II AUTOMATIC SLIDE STAINERS

a. The Hema-Tek I and 1000 performed all operations required for staining peripheral blood smears with the exception of the initial production of the blood smear itself (see figure 3-29). The instrument uses disposable, prepackaged stain packs, which contain stain, buffer, and rinse solutions. The process is continuous, for as long as the stainer is on. Specimen throughput is at the rate of 60 per hour but it may be used for staining individual slides. The only difference between the 1 and 1000 was an improved platen. The Hema-Tek II performs the same operations as the 1 and 1000 but uses an intermittent slide progression, where the slide moves then stops at a station. The Hema-Tek II system provides a variable staining rate, microprocessor control, and adjustable stain intensity.

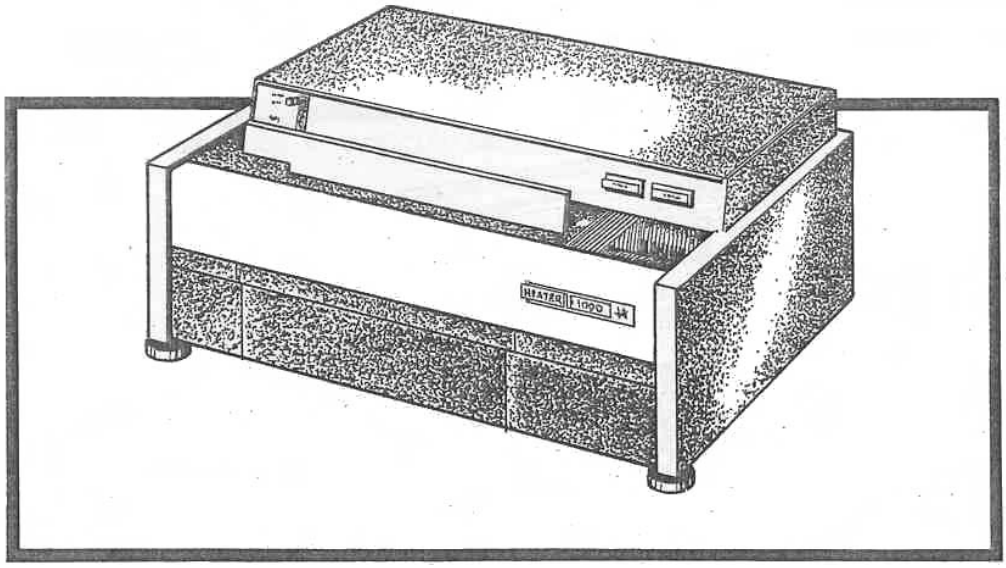


Figure 3-29. Ames HEMA-TEK I 1000 slide strainer.

b. The instrument uses spiral conveyors to drop and move the slide along the platen for staining. The slide is supported just above the platen to allow reagent to flow between the slide's surface and the platen. As the slide progresses, first stain is supplied filling the space between the slide and the platen by capillary action. The length of time that the stain remains in contact with the blood smear is determined by the speed of the conveyor and the length of the platen. When the slide reaches the designated area of the platen, buffer is added to the slide and the slide continues to progress. When the slide reaches the end of the platen, the stain and buffer are drained off. The slide is then raised by the conveyor and rinsed off with the rinse solution. The slide is allowed to drain and then passes over a warm air heater to dry the slide. Following this air drying, the stained slide is dropped into the collection draw, where it may be removed. The collection draw will hold up to 100 stained slides. Excess stain, buffer, and rinse solution drain into a waste collector, which will signal by lighting a pilot light if full, but should be drained regularly.

NOTE: The stain pack contains enough reagent for 1000 slides, but rarely does the stain last that long with required priming upon to be stained, the stain and buffer drain out of the lines, requiring the use of blank slides to be placed ahead of actual slides or having to prime the reagents, thereby decreasing the number of actual slides the reagent pack can handle.

3-31. MIDAS II AUTOMATED STAINER

The Midas II stainer employs a microprocessor designed for use in both microbiology and hematolgy staining. The instrument uses the classical dip-staining technique (see figure 3-30). This methodology eliminates precipitates and artifacts from forming on the slide and results in a 50 decrease in the amount of stain consumption compared to the standard flood technique. The stainer consists of four staining stations, one rinse station, and one heat-forced air drying station. The instrument can be programmed for 6 separate procedures, with up to 25 processing steps for each program. Up to 20 slides may be stained at one time within the slide carrier. Depending upon programming, the stainer can provide up to 240 stained slides per hour.

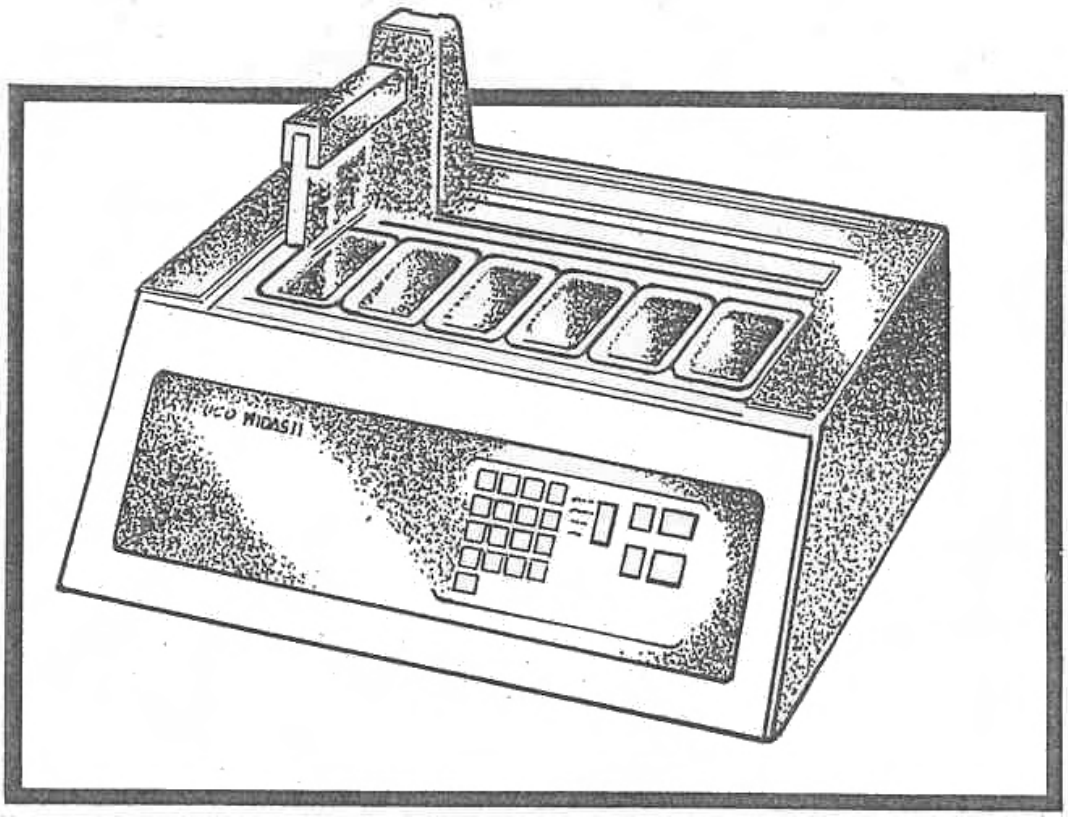


Figure 3-30. Midas II automated stainer.

3-32. CLAY ADAMS QBC II SYSTEM

a. **Introduction.**

(1) It has been known for many years that the thin, grayish-white, buffy coat in the hematocrit tube consists of packed leukocytes and platelets; and that platelets, being less dense, settle in a separate layer above the leukocytes (see figure 3-31). In the 1930s Wintrobe and Olef described methods for estimating elevated white cell and platelet populations based on the milky appearance and thickness of the buffy coat. Quantitative measurements, however, proved difficult because of the very small size and nonhomogeneity of the cell layers. In later studies of cell density gradients, further subdivision or layering was found to occur between two subpopulations of leukocytes by virtue of their different specific gravities. The upper layer was reported to contain predominantly lymphocytes and monocytes; the lower, predominantly the granulocytes, e.g., neutrophils (juvenile, segmented, band), eosinophils, and basophils.

(2) Utilizing mechanical expansion and optical magnification, augmented by supravital cell staining, the QBC II System derives a platelet count, white cell count, and counts of the two white-cell subgroups from linear measurements of the packed cell layers in the buffy coat. As in conventional microcentrifugation procedures, a hematocrit of PCV is also obtained.

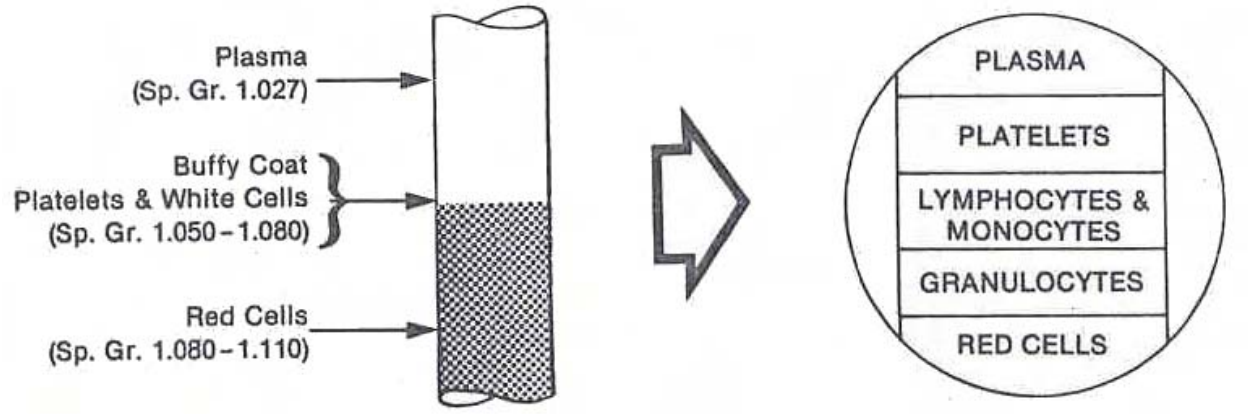


Figure 3-31. Section of typical spun microhematocrit tube showing relative densities and layering of the formed elements.

b. **System Use.**

(1) The Clay Adams QBC II System (see figure 3-32) is a seven-parameter hematology screening device which yields the following quantitative values from a centrifuged blood tube:

(a) Hematocrit.

(b) Platelet count.

(c) White blood cell count.

(d) Granulocyte count (Lymphocyte-monocyte count).

(2) The QBC II platelet count, white blood cell counts, and counts of the granulocyte and lymphocyte/monocyte white cell subpopulations are estimates derived from electro-optical measurements of the packed cell volumes in a specially-designed QBC blood tube. Some disease states are characterized by the presence of abnormal white cell types, but may yield normal quantitative relationships of granulocytes to lymphocytes/monocytes. The QBC II System cannot discriminate between normal and abnormal cell types.

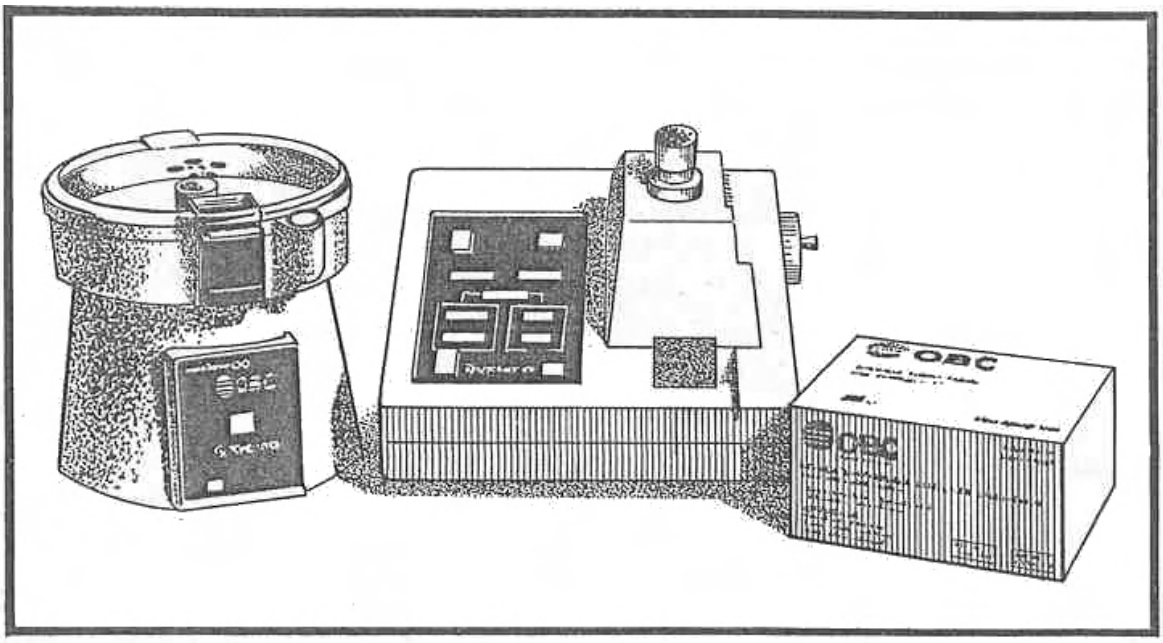


Figure 3-32. The QBC II Centrifugal Hematology System.

(3) The QBC blood tube is internally coated with the supravital fluorochrome acridine orange (AO). Diamidines, such as AO, have long been employed in cytologic labeling and clinical diagnostics because of their uptake by cellular nucleoproteins and by glycosamines in the granulocytic series. Under excitation by blue-violet light the cells differentially fluoresce. The granulocytic cells fluoresce orange-yellow, lymphocytes and monocytes, a brilliant green; and platelets, a pale yellow. Erythrocytes, however, are unaffected by AO and exhibit their normal dark red appearance.

c. **Principles of the Procedure.**

(1) Two different types of precision bore, 75 mm glass tubes are utilized for QBC II tests: one type for venous blood and a different type for capillary blood. The venous-blood tube (figure 3-33) incorporates a black calibration line and is normally filled by means of the semiautomatic QBC pipette supplied with the system. Filled volume is 111.1 µl.

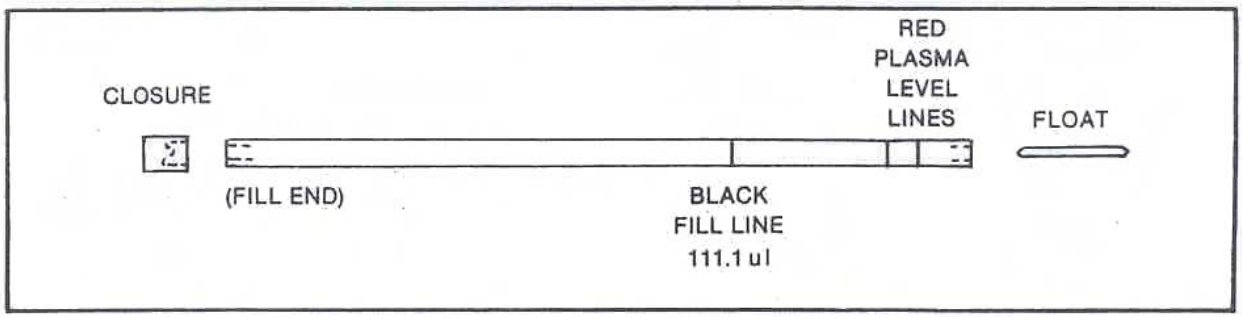


Figure 3-33. QBC tube for venous blood.

(2) To simplify blood collection from a finger puncture, the QBC capillary-blood tube (see figure 3-34) is provided with 55 µl and 65 µl black fill lines. The tube is filled by capillary action to any level between the two lines. As subsequently described, the exact fill volume is measured during the tube reading procedure.

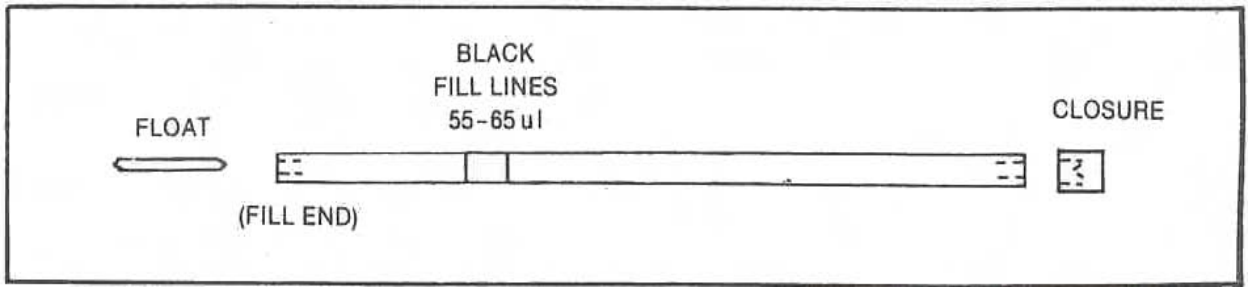


Figure 3-34. QBC tube for capillary blood.

(3) Both types of QBC tubes are internally coated with AO and potassium oxalate. The former, a supravital fluorochrome, stains the white cells and platelets as previously described. The potassium oxalate osmotically removes water from the erythrocytes, causing their density to increase and their volume to shrink. This tends to prevent commingling of certain equal-density erythrocytes and leukocytes at the interfacing boundary between these cell layers. QBC capillary-blood tubes additionally contain a coating of sodium heparin and dipotassium EDTA to inhibit the clotting of finger puncture blood.

(4) Each QBC tube is supplied with a plastic closure and float. The float, whose density approximates that of the white cells and platelets, is inserted into the sealed, filled tube immediately prior to high speed centrifugation. Under centrifugal force, the float settles into the buffy coat where it axially expands the formed cell layers by a factor of 10. Part of the float also descends into the red cells (to a variable depth), similarly expanding the upper portion of the packed erythrocyte column and creating a clearly visible lighter band of red cells surrounding the bottom portion of the float.

(5) Two red plasma level lines are provided on QBC venous-blood tubes (see figure 3-35) for checking fill volume in the prepared blood tube prior to testing. Because of the volume of specimen displaced by the float, the plasma level must be between the two red lines after centrifugation of the blood tube. If the plasma is above or below the red lines, the blood tube must be discarded and a new tube prepared. Failure of the plasma level to fall between the red lines may result from a pipette malfunction or loss of blood through evaporation or leakage.

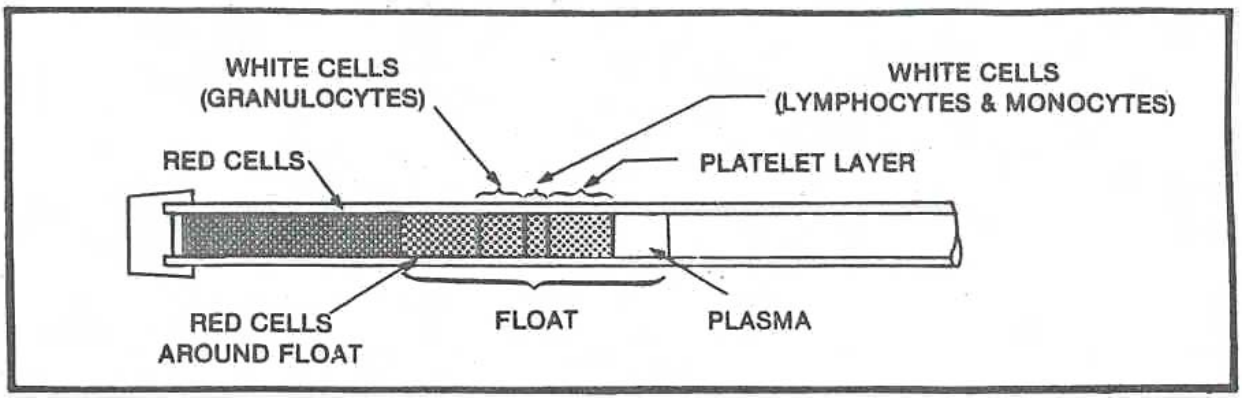


Figure 3-35. Cell layers in the spun QBC blood tube.

(6) Layer lengths (packed cell volumes) are measured in the QBC II Reader (see figure 3-36). A "MODE" button is provided to program the instrument for reading venous-blood tubes or capillary-blood tubes. By means of an external knob on the side of the Reader, the operator can control the axial position of the tube under a viewing microscope. The tube is "read" by sequentially aligning each interface (starting at the bottom of the red cell column or zero position) with a stationary reticle arrow within the optical system. The length or thickness of each cell layer is fed into the Reader's microcomputer by pressing an "ENTER" button each time an interface is aligned with the arrow. Values for HCT, PLT, WBC, granulocyte, and lymphocyte-monocyte counts are displayed on the front panel of the Reader after location of the last interface has been entered.

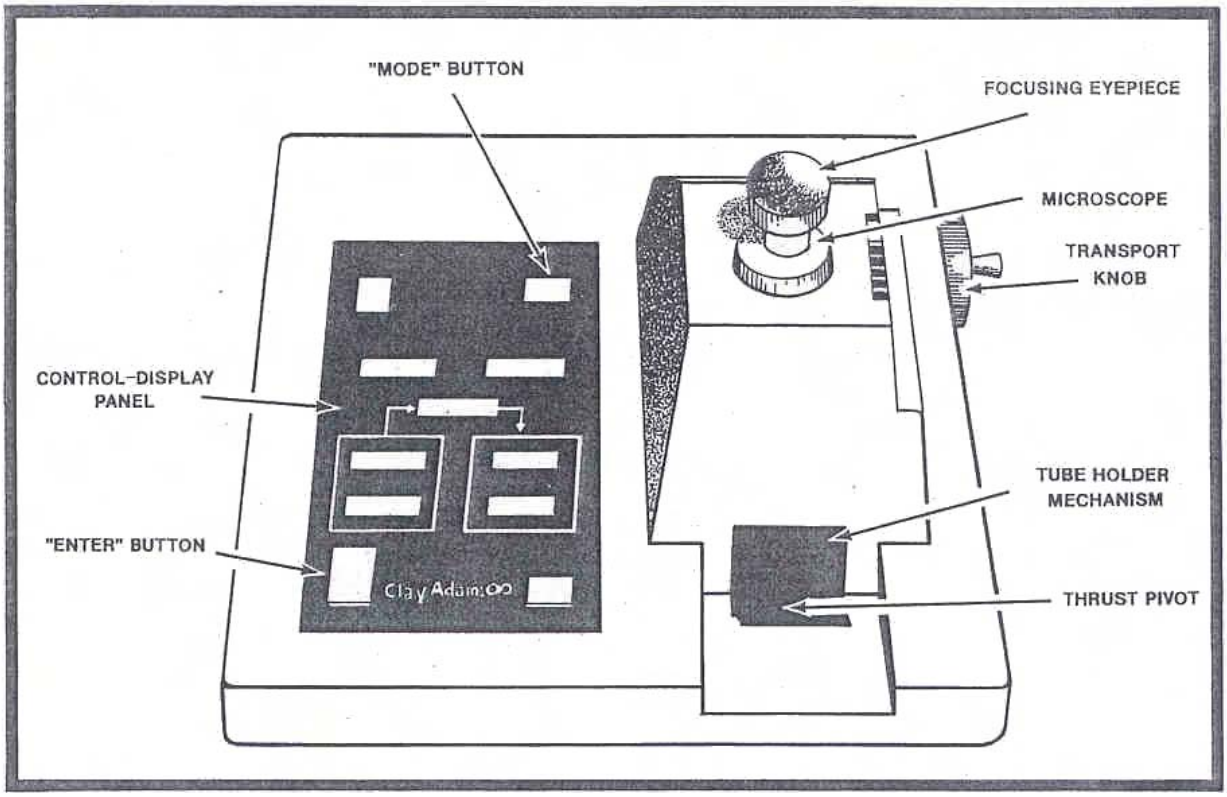


Figure 3-36. QBC II Reader.

(7) A total of six interfaces in venous-blood tubes and seven interfaces in capillary-blood tubes must be located and entered. In capillary-blood tubes, the length of the plasma column or 7th interface reading is measured in order to determine total fill volume. The latter measurement is utilized by the Reader in computing the capillary-blood hematology parameters.

3-33. BBL FIBROMETER COAGULATION TIMER AND HEATING BLOCK

a. The Fibrometer is a semi-automated device for testing most coagulation determinations. The timer and heating blocks maintain a constant temperature of 37.2° ± 0.5° C (see figure 3-37). A plastic cup beneath the probe on the fibrometer is charged with reagents and specimen. The probe contains two platinum wires, one of which moves through the test specimen once dropped, checking for a fibrin fiber. A fibrin fiber will conduct an electrical current. Once a fibrin fiber is sensed, by the transmission of electrical current between the probe tips, the timer stops immediately as does the probe. The elapsed time in seconds is read directly from the digital readout register on the timing device. Two separate type probes are available, one for 0.3 ml volumes and one for 0.4 ml volumes. The 0.4 ml probe is used in fator assays.

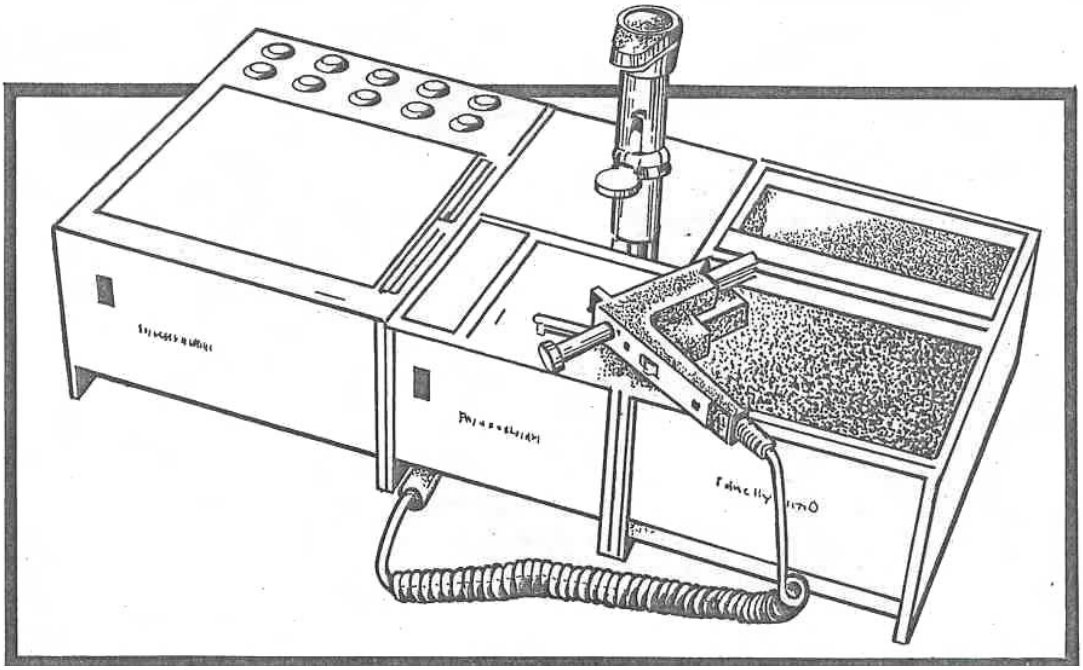


Figure 3-37. Fibrometer, heating block, organizer, and automatic pipette.

b. The Fibrometer comes with a precision pipette that will dispense 0.1 or 0.2 ml volumes, as selected. To change volumes, turn the plunger 180 degrees, clockwise or counterclockwise. When using the manual pipette, the green timer bar must be pressed exactly at the same time as dispensing the reagent or sample with the pipette. The Fibrometer timer then continues until a clot is detected.

c. Optionally, an automatic, electric pipette can be used. This pipette appears exactly like the manual pipette, except for a coiled cord that is connected from the bottom of the handle of the pipette to the Fibrometer timer block. There is also an On/Off switch on the back surface of the handle. With the automatic pipette, when the switch is "On," once the plunger is depressed, the probe on the Fibrometer block drops down and starts the timer.

d. In between samples, the probes platinum wire tips must be wiped clean of the previous specimen. Extreme care must be taken in wiping these tips, in that excess force used in either pulling or pushing, during wiping, will cause the tips to break off at the worst. At the least, it will alter its ability to test for clot formation, causing erroneous times for results. In addition, not cleaning the tips well will leave contamination on the probes, also causing erroneous results.

3-34. MLA ELECTRA 750 SEMIAUTOMATIC COAGULATION TIMER

The Electra 750 is for a low volume laboratory that wants broad capabilities. The instrument offers a wide range of coagulation tests including: PT, APTT, thrombin time (TT), and factor assays (FA) including saline dilutions (see figure 3-38). Clot detection is by photometric means, with its optical range permitting accurate results with dense icteric, hemolyzed, or chlylous specimens. Results are displayed on a large LED digital readout. The unit comes with a built-in incubator and thermometer, incubation timer, temperature verification lamp, heated pipette tip tray, off-scale optical indicator light, and covered reagent well with magnetic stirrers.

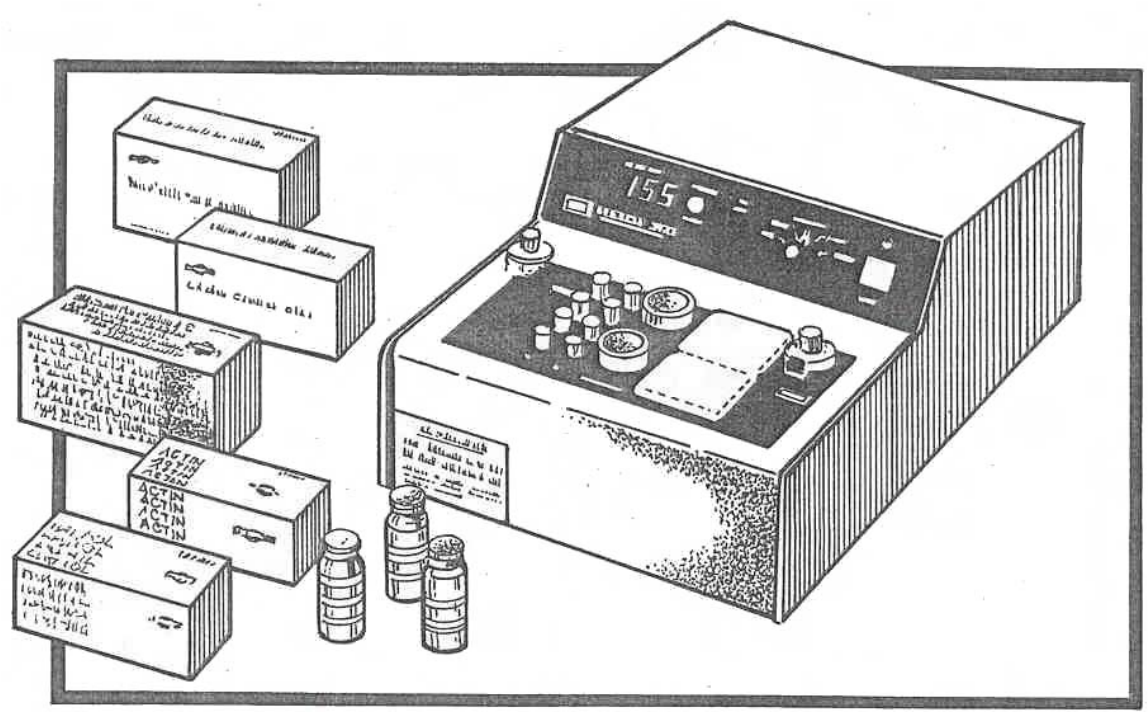


Figure 3-38. MLA Electra 750 coagulation timer.

3-35. ASTRAL MEDICAL SYSTEMS COATRON II COAGULATION ANALYZER

The Coatron II is a fully automated system for analyzing all coagulation testing. Its repetoire includes PT, APTT, thrombin time, fibrinogen, the extrinsic coagulation factors (II, V, VII, and X), and the intrinsic coagulation factors (VIII, X, XI, XII). The Coatron II can perform up to 44 samples in duplicate per hour with the first patient's results reported in just 3 minutes. A 32 sample turntable automatically moves the samples under pipetting stations and through a heating block (see figure 3-39). PT and APTT reagents are kept ready at all times in respective reagent wells. The coagulation reaction is detected by an infrared beam passing through the sample cup. Duplicate results are reported out individually as well as averaged. The instrument will incubate at preprogrammed times, as determined by test selection button, or can be set manually by selection on the control panel.

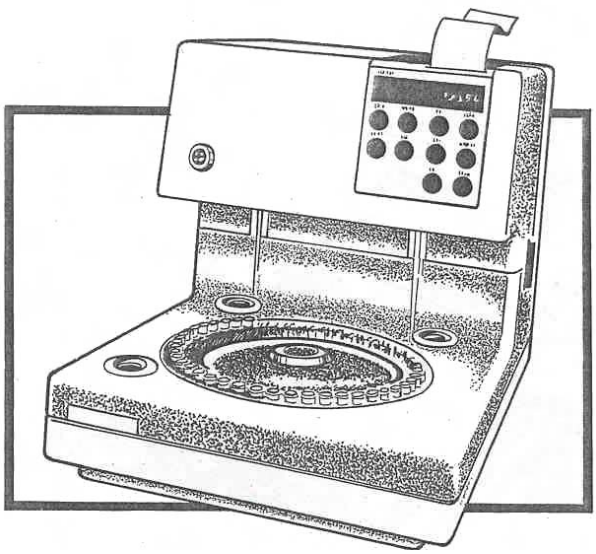


Figure 3-39. Astral Coatron II Coagulation Analyzer.

3-36. ASTRAL COATRON JR COAGULATION ANALYZER

The Coatron Jr* is a dual channel analyzer that performs most of the features that the Coatron II does, only semi-automatically. Its procedures include: PT, APTT, fibrinogen, and factor assays. The instrument is microprocessor controlled, with easy to select test options. The instrument automatically zeros the specimen, is capable of detecting when reagent is added (manually) to the sample, and begins timing immediately. Samples may be run simultaneously. The instrument provides a 20-sample incubation area (see figure 3-40). An audible signal indicates the beginning and ending of the test. Results are printed out on a LCD digital timer which monitors incubation time and infrared optics. Stored reference curves for factor assays are kept in its memory. Results are not affected by stray light, and are reported out in seconds, percent activity, mg/dl or ratio.

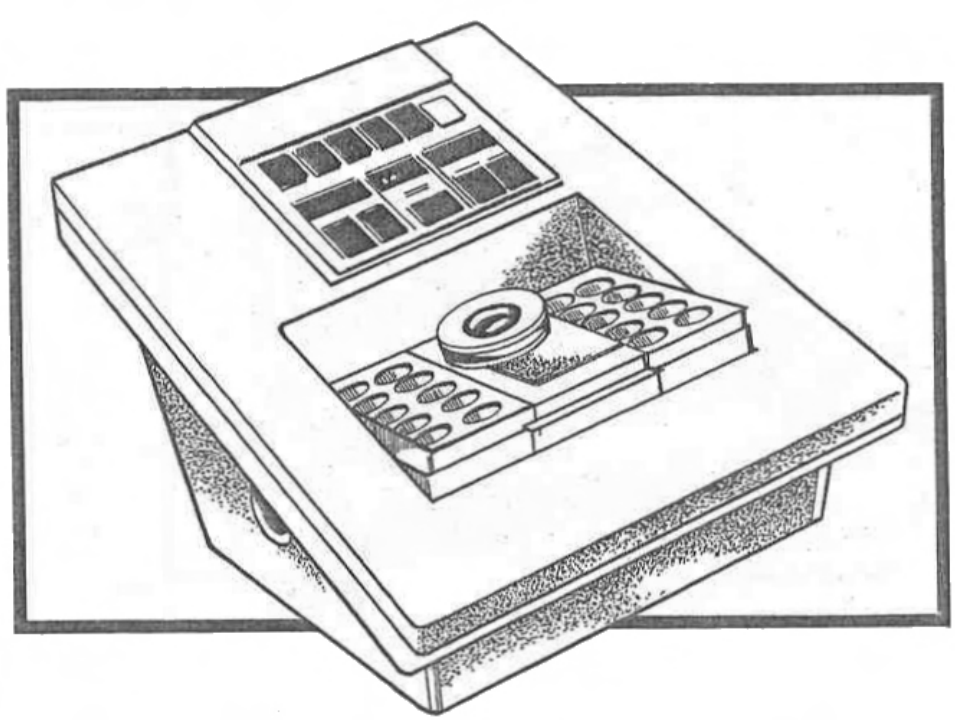


Figure 3-40. Astral Coatron Jr Coagulation Analyzer.

3-37. MLA ELECTRA 800 AUTOMATIC COAGULATION TIMER

a. The Electra 800 is a photometric instrument which allows the testing of PT, APTT, fibrinogen, factor assays, and saline dilutions from a preset program (see figure 3-41). Intelligent software allows modification of existing test parameters for special testing situations. A precise reagent delivery system cuts down on waste. Four individual photocells analyze a pair of twin-well cuvettes (four separate plasmas) simultaneously, at a rate of 360 PT's or 136 APTT's per hour. Six cuvettes can be loaded initially, and additional cuvettes added as the instrument moves the carosel into the incubation area. Individual results are displayed on a LCD panel in groups of four results and each pair is averaged and printed out also. A permanent record is printed on a thermal printer located at the left rear corner. The main control panel indicates instrument status and displays messages, leading the operator through all testing functions. With the added data management accessory, manual plotting of standard curves is eliminated. The internal processor automatically converts patient results to reportable units such as mg/dl for fibrinogen and percent activity for factor assays. Printouts provide curve with data points and patient results in units or percentage activity. The instrument also checks between duplicate measurements, flagging results according to the setup range selected.

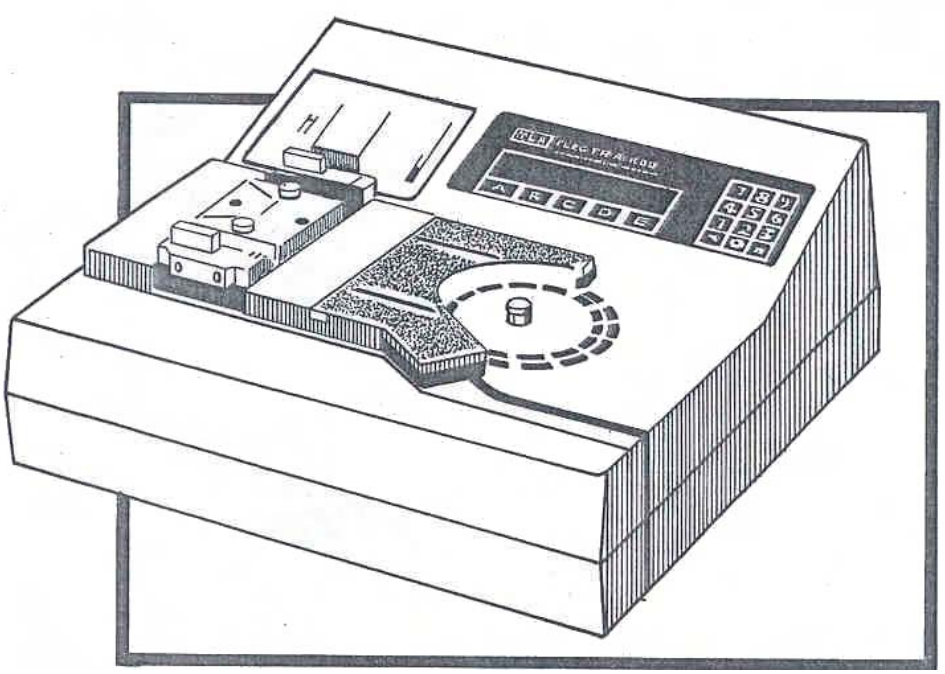


Figure 3-41. MLA Electra 800 Automatic Coagulation Timer.

b. Reagents are kept cool using frozen, cooling blocks. Each block lasts at room temperature approximately 1 to 2 hours before it needs to be replaced by another frozen block. Thawed blocks take approximately 2 hours to refreeze. Reagents are pumped using a peristaltic method and are sent through a color coded heat exchanger. The heat exchanger for the PT test is red and will contain 0.2 ml of reagent, so that the entire amount of reagent pumped into the cuvette will be heated to 37^{o} C. The heat exchangers for the APTT test are blue and will contain only 0.1 ml of reagent.

c. Only one test type can be run at a time (batch testing). To run another type of test, requires the reservoirs to be changed and primed prior to being used. Should the instrument stand unused for any length of time, the individual lines should be primed back into the reservoirs, or have the instrument automatically deprime following the end of each run. This then requires forward priming prior to running the next specimen or batch of specimens. The LCD display provides constant monitoring of heating block temperatures, method selected, date, and time. The instrument will sound a tone when beginning and ending a run; runs can be cancelled at any time. The LCD display varies according to the angle it is viewed. This can be adjusted to appear more intense, by a control knob at the back, right side. The date and time feature can only be changed by turning the instrument off, then on again, where it will reload its programming and request the date and time to be entered. Maintenance is little and easy to do. The instrument comes with a training video which explains operation and maintenance.

3-38. MLA ELECTRA 700 AUTOMATIC COAGULATION TIMER

a. The Electra 700 is a fully automated coagulation instrument. It performs PT's, APTT's, factor assays, saline dilutions, and fibrinogen assays. It has a number of advantages over the 800 model discussed above. As the 800, it is based upon photometric methodology. The Electra 700 at figure 3-42 has room for all three major reagents. Reagents and samples are kept refrigerated by the instrument itself.

b. Each test is run in a twin-well, color coded specimen cuvette. The color coding is used to program the computer controlled instrument. A red cuvette instructs the computer to perform a PT test; a blue cuvette programs the instrument to run an APTT test. The internal computer directs the sample incubation and activation time, reagent dispensing, and clot timing. Results are reported out on both paper and a digital LED display. The instrument accepts test cuvettes in any order. Mixing of PT and APTT test cuvettes, including statistics, is possible without changing the instrument setup. Up to 60 samples may be loaded into the refrigerated turntable. After each test is run, the used cuvette is automatically dropped into a convenient, disposable catch bin. A "Confidence Test," checks instrument function automatically. The instrument may be interfaced with a computer. The instrument comes with a training video which explains operation and maintenance.

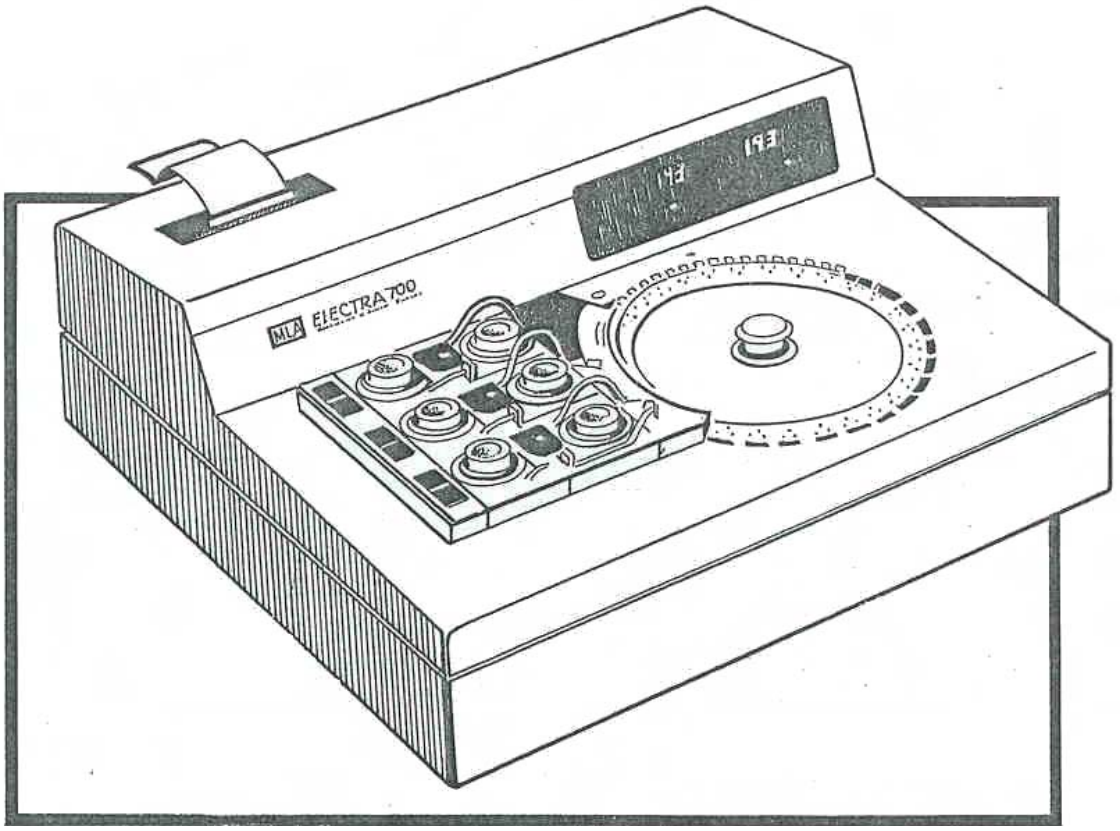


Figure 3-42. MLA Electra 700 Automatic Coagulation Timer.

3-39. HELENA CASCADE 480 HEMOSTASIS ANALYZER

a. The Cascade 480 is a rapid throughput coagulation timer. The instrument performs PT's, APTT's, fibrinogens, thrombin times, and factor assays (see figure 3-43). It can process 360 PT's or 144 APTT's per hour. The instrument can process the tests in any order. The Cascade 480 has five reagent pumps, so all reagents are available, ready for use, with no need to stop and change reagents. Continuous specimen loading allows easy addition of statistic samples. With the optional Cascade Auto Sample Processor (CASP), the instrument automatically aspirates plasma directly from blood collection tubes, transfers the specimen to cuvettes, and dilutes the specimens for fibrinogens and factor assays if requested. The instrument has available an auto-prime and deprime mode. All reagents are stored in a cooling area and a half-volume mode can reduce reagent costs. The instrument comes with a stand alone companion analyzer which lets you do over 17 different chromogenic substrate assays without interrupting routine coagulation testing.

b. The CASP system can use an optional bar-code reader to eliminate keystroking to enter patient demographics or selecting tests. Results are printed out on 8 ½ x 11 inch sheets for direct entering into patient records. Multiple options are available for reporting results from single test results to full profile studies, to cumulative summaries, and graphing of factor assay curves. Using the comprehensive data management program, storage and retrieval of data from daily worklists, reviews of patient's cumulative hemostasis history, and quality control results are all very easy. Reference curves stored for fibrinogen and factor assays can be displayed and printed. Incubation times can be set for optimal sensitivity. Quality control data is automatically archived for easy statistical analysis and evaluation to give the mean, SD, and CV. Lot numbers, ISI, and expiration dates for reagents, controls, and standards are stored and retrieved from its internal computer. Free QAR service provides evaluation of your QC data with that of peer laboratories.

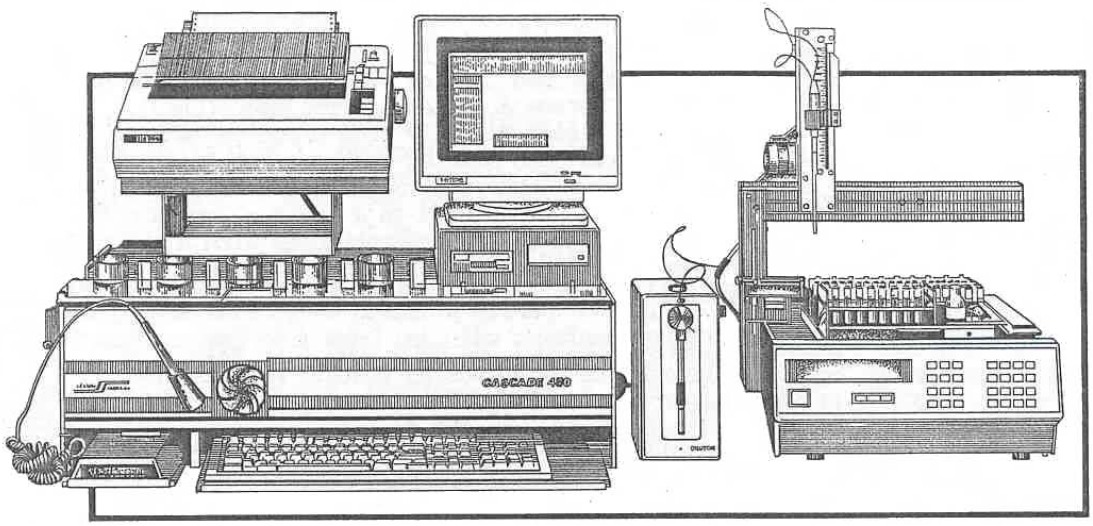


Figure 3-43. Helena Cascade 480 Hemostasis Analyzer.

3-40. HELENA PACKS-4 PLATELET AGGREGATION AND CHROMOGENIC ASSAY SYSTEM

a. The Platelet Aggregation Chromogenic Kinetics System-4 (PACKS-4), runs platelet aggregation, ristocetin cofactor, and chromogenic testing (see figure 3-44. During platelet aggregation studies, the full-size color monitor can be used to observe curve formation as the reaction occurs. Each channel can be viewed separately or all four at once, with each channel displayed in a different color.

b. In the chromogenic assay mode, kinetic or endpoint readings and calculations can be selected. There are over 17 assays available, with reduced volume testing for economical savings of reagents.

c. While testing for ristocetin cofactor, the instrument will show curve calculations with slope and percent activity.

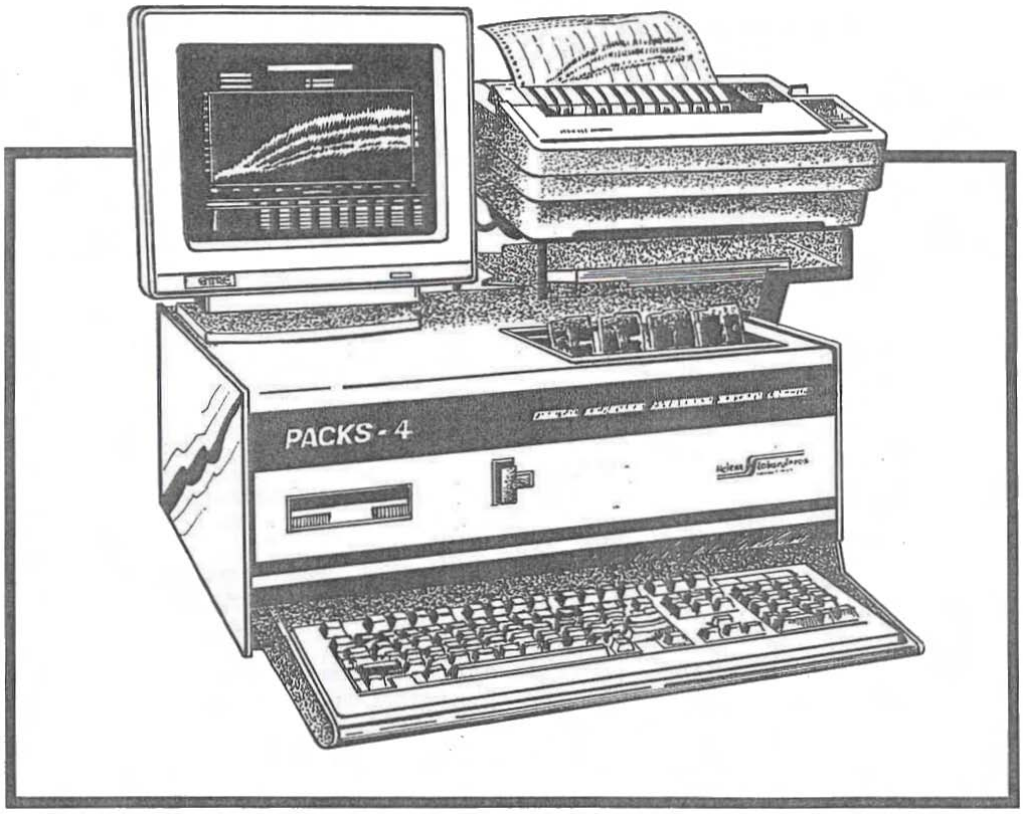


Figure 3-44. Helena Packs-4 Platelet Aggregation and Chrogenic Substrate Analyzer.

Continue with Exercises

EXERCISES, LESSON 3

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Two syringes operated systematically to measure and draw samples and diluent are the functional equivalent of a/an:

   a. Dilutor.

   b. Pipetter.

   c. Hemacytometer.

   d. Electronic cell counter.

2. Identify the instrument that fixes, stains, buffers, rinses, and dries differential slides at a rate of 1 per minute.

   a. Dilulter.

   b. Electronic cell counter.

   c. Automatic slide stainer.

   d. Model S counter.

3. What property of a blood cell is utilized by the Coulter Model FN Counter to count blood cells?

   a. Light dispersion.

   b. Electrical resistance.

   c. Opacity.

   d. Absorbance.

4. What are the advantages of using an electronic cell counter?

    a. It can count up to 30 to 50 counts per day.

    b. No training is necessary.

    c. Its count is equivalent to that of 120 chamber counts per specimen.

    d. It counts an average of 30,000 to 50,000 cells per specimen.

5. How many individual counts can an electric cell counter handle easily per day?

    a. 200.

    b. 400.

    c. 600.

    d. 800.

6. What are other advantages of an electric cell counter?

    a. Error rates are reduced one-tenth to that of visual methods; allowance for accuracy and reproducibility factors are about 2 needed is reduced.

    b. Error rates are reduced one-fifth to that of visual methods; allowance for accuracy and reproducibility factors are about 2 needed is reduced.

    c. Error rates are reduces one-tenth to that of visual methods; allowance for accuracy and reproducibility factors are about 8 needed is reduced.

    d. Error rates are reduces one-tenth to that of visual methods; allowance for accuracy and reproducibility factors are about 2 needed is increased.

7. When using the Coulter Model FN Counter, what is the end product once mass cells change the resistance between electrodes and alter the current flow and electronic pulses?

    a. The number that represents the number of blood cells and their volume in the sample being counted.

    b. The distance between the electrodes and the alteration of the current flow.

8. Which tests performed by the Coulter Model FN Counter must be corrected for coincidence passage?

    a. All WBCs tests over 10,000/cu mm.

    b. WBCs above 3.4 million/cu mm.

    c. WBCs above 5.4 million/cu mm.

    d. WBCs below 10,000/cu mm.

9. The incidence of Coulter Counter instrument failure can be reduced by:

    a. Cleaning the orifice with detergent monthly.

    b. Oiling the vacuum pump weekly.

    c. Weekly observance of the mercury travel time on the manometer.

    d. Dilution of high cell counts with saline.

10. What is the sequence for cleaning Coulter Model FN Counter so as to reduce the incidence of instrument failure?

    a. Flush orifice with dilute sodium hypochlorite (bleach). Flush the system thoroughly with saline and then distilled water.

    b. Flush orifice with distilled water. Flush the system with sodium hypochlorite (bleach) and then thoroughly with saline.

    c. Flush orifice with dilute sodium hypochlorite (bleach). Flush the system thoroughly with distilled water and then saline.

    d. Flush orifice with saline and the system with dilute sodium hypochlorite (bleach) and distilled water.

11. Which of the following is **NOT** a significant source of error with electronic particle counters?

    a. Contamination.

    b. Mathematical mistakes.

    c. Dilution inaccuracies.

    d. Electronic interference.

12. What is a problem on an electronic counter?

    a. Qualitive measurements are difficult to prove because of very large sized cell layers.

    b. Positive impulses result from electronic problems such as interference from other machines can cause erroneous results.

    c. Contaminating particles in apparently clear diluent are a source of impulse. Background counts must be made daily on all diluents.

    d. White blood cell counts are not accurate over 8,000 per cu mm and must be diluted with saline.

13. The complete hematological systems developed encompass which hematology parameters:

    a. RBC count, WBC count; hemoglobin concentration; hematocrit; and mean corpuscular volume, hemoglobin, MCH, MCHC, and hemoglobin concentration.

    b. RBC distribution width, mean platelet volume, and a five-part differential.

    c. Reporting neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

    d. All of the above.

14. The Coulter Model S Counter differs from the Coulter Model FN because it:

    a. Operates on different principles.

    b. Performs all counts in duplicate.

    c. Runs the WBC and RBC counts simultaneously.

    d. Requires only minimal maintenance.

15. If the microcapillary samples for a complete workup and unopettes are accomplished at one time, this will:

    a. Increase the amount of sample necessary but no more testing is necessary.

    b. Reduce the amount of sample necessary for this and other related procedures and enables testing for pediatric patients.

    c. Cause the sample dosage to remain the same.

    d. None of the above.

16. The Coulter Model S counts RBC and WBC counts in triplicate. If there is any disagreement due to debris or other error, how will the technician be alerted?

    a. A printout.

    b. An alarm system.

    c. Another technician.

    d. By trial and error.

17. On what piece of hardware would the CRT display all results, operator alerts, and histograms?

    a. CASH.

    b. Auto-reporter printer.

    c. S Plus III.

    d. Data Terminal.

18. In what form are Data Terminal control results displayed on the Coulter Model S Plus III Counter?

    a. Tabular.

    b. Levey-Jennings graphs.

    c. Both a and b.

    d. None of the above.

19. What is the process called when differentials are performed using the Coulter Model S Plus IV Counter?

    a. Coded slip.

    b. Volumetric cytochemistry.

    c. Cytochemical raction.

    d. Data storage.

20. A cytochemical reaction:

    a. Alters white cell volumes in predictable ways. The resulting volume-distribution histogram is then analyzed by the computer's program to determine numerical differential results and the detection of abnormalities.

    b. Sometimes alters white cell volumes in predictable ways. The resulting volume-distribution histogram is then analyzed by the computer's program to determine numerical differential results and the detection of abnormalities.

    c. Is quite intricate and takes approximately two hours total time.

    d. Assists in interpreting suspected abnormal differential results by flagging significant abnormalities with less than 1

21. The Coulter Model S Plus V Counter differs from the Coulter Model S Plus V Model main sample aspiratior collects 125 µl samples from an automatic cap piercer. This automatic piercer:

    a. Speeds up the process.

    b. Increases the hazard of bacterial growth inside the tubes.

    c. Eliminates the hazard of opening and closing collection tubes.

    d. Collects in exact-fill.

22. Which Coulter Counter, with addition of the Data Terminal option, has the ability to handle statistical analyses at the touch of a button?

    a. FN.

    b. S.

    c. S Plus V.

    d. S Plus VI.

23. Which Coulter Model Counter is designed for small labs doing only a few tests per day?

    a. S Plus IV.

    b. CBC5.

    c. JR.

    d. STKR.

24. Which Coulter Model Counter, with the addition of an optional printer/plotter, can profile a full 16-parameters profiles?

    a. S.

    b. S Plus IV.

    c. S Plus VI.

    d. CBC5.

25. The Coulter Model M430 Counter is:

    a. A compact semi-automated system for small labs doing 30 to 50 samples per day.

    b. For the large laboratory.

    c. For small labortories doing only a few tests per day.

    d. Inclusive of volume referenced histograms.

26. The Coulter Model M430:

    a. Provides 10 parameters-WBC, RBC, Hgb, Hct, MCV, MCH, and MCHC.

    b. Includes aperture viewing, audible counts, automatic coincidence correction, low reagent indicators, electronic aperture cleaning, and operator alerts.

    c. Provides for each sample to take only 61 seconds to analyze and print results.

    d. Provides for the following parameters: WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, MPV, Plt, and a complete WBC differential.

27. Which model of Coulter Counter is capable of running 80 samples per hour?

    a. M430.

    b. JR.

    c. STKR.

    d. CBC5.

28. Which Coulter Model Counter possesses the following features: closed-tube auto sampling at 80 samples per hour, automatic differentials, detection, and flagging of significant abnormalities with less than 1 negatives?

    a. S.

    b. S Plus II.

    c. JS.

    d. T-series.

29. Which model of Coulter Counter has a data management portion that is able to store 270 patient results or 37 composite histograms and patient results?

    a. S.

    b. JS.

    c. S Plus II.

    d. T-series.

30. The Coulter JS Model has:

    a. A complete Coulter Histogram Differential with interpretive report.

    b. The ability to read low regeant indicators.

    c. Premeasured regeants in color coded vials.

    d. The capability for XB analysis.

31. Which Coulter Counter features bar code identification of patient specimens; a worklist generation and positive sample identification; and reports of red cell morphology and suspected presence of eosinophilia and basophilia?

    a. JR.

    b. JS.

    c. STKR.

    d. T-series.

32. Autoprocessing can be interrupted for statistics at any time. This is indicative of which Coulter Counter?

    a. JS.

    b. JR.

    c. STKR.

    d. T-series.

33. How many samples per hour and parameters can the T540 basic model provide?

    a. 40; 2.

    b. 20; 6.

    c. 40; 7.

    d. 15; 12.

34. With what are the JT-Series counters equipped and what is its _____ capability?

    a. Data terminals; controls the cap-piercing needle.

    b. Data terminals; performs differentials using the CHD method.

    c. Push button controls; controls autosampling up to 330 µl.

    d. Data terminals and bar-code storage are standard; counts 50 to 80 samples per hour.

35. The Coulter MAXM and MAXM AL are:

    a. Moderate volume throughput instruments (75 samples per hour) that work on a new Coulter WBC differential methodology, called VCS.

    b. A group of instruments developed to handle moderate volume facilities with throughput of 50 samples per hour.

    c. Counters with many valuable options, of which auto-sampling from sealed collection tubes, makes this instrument totally automated.

    d. Operated mainly with individual samples, so they are semi but not a fully automated.

36. What is the next event for the closed-vial samples after they are rocked and moved the sampling mechanism using the Coulter MAXM and MAXM AL?

    a. The samples are measured.

    b. They are pierced and bar coded labels are read simultaneously for positive sample ID.

    c. They are wiped.

    d. The samples are manually coded and read individually.

37. Over how many WBCs does the STKS study?

    a. 600 WBC for each differential.

    b. 6,000 WBC for each differential.

    c. 7,000 WBC for each differential.

    d. 8,000 WBC for each differential.

38. How many reagents does the Coulter STKS require?

    a. 2.

    b. 3.

    c. 4.

    d. 5.

39. The Sysmex CC-130 Hematology Analyzer:

    a. Has many valuable options. The autosampling from sealed collection tubes, makes this instrument totally automated.

    b. Operates mainly with individual samples, so that it is a semiautomated device, not a fully automated instrument.

    c. Is a small, compact, desktop, semiautomated instrument that provides WBC, RBC, and Hgb analysis.

    d. Is a compact unit that provides the most frequently asked for parameters such as: WBC, RBC, Hgb, Hct and MCV. It comes with premeasured reagents in color-coded vials.

40. With the Sysmex Model CC-150, the WBC and Hgb are analyzed:

    a. Separately.

    b. Together.

    c. With other cells.

    d. None of the above.

41. What happens when abnormal results are detected using the Sysmex Model CC-180?

    a. Automatic recounts are made for values that are outside of established ranges. Data from the last 10 samples may be displayed on the CRT screen, simultaneously.

    b. Flow system rinses the entire hydraulic system between samples.

    c. None of the above.

42. What length of time does it take to perform the complete process on a sample using the Sysmex Model CC-780 Hematology Analyzer?

    a. 15 sec.

    b. 20 sec.

    c. 45 sec.

    d. 1 min.

43. Using the Sysmex Model CC-780 Hematology Analyzer, approximately how many samples can be run in 1 hour?

    a. 42.

    b. 47.

    c. 60.

    d. 80.

44. Which statement is correct for the Sysmex Model E-5000 Hematology Analyzer?

    a. The E-5000 is a semiautomated hematology analyzer which provides up to 18 parameters of results per specimen.

    b. The WBC Tri-Model Histogram provides three-cell subpopulation analyses, the RBC histogram, and provides RDW information.

    c. The PLT histogram provides built in data such as PDW, MPV, and P-LCR.

    d. Sample size is 300 µl of whole blood and throughput is at 160 samples per hour.

45. Which quantitative values can be obtained using the QBC II System?

    a. Granulocyte count.

    b. RBC's present.

    c. Total RBC count.

    d. MCV.

46. The Clay Adams QBC II System operates on the principles of:

    a. Electrical resistance and optical magnification.

    b. Mechanical expansion and optical magnification.

    c. Mechanical expansion and electrical conductivity.

    d. Electrical resistance and electrical conductivity.

47. The filled volume of the QBC II venous-blood tube is:

    a. 56 µl.

    b. 67 µl.

    c. 100.1 µl.

    d. 111.1 µl.

48. The filled volume of the QBC II capillary blood tube is:

    a. Always 55 µl.

    b. Always 65 µl.

    c. Between 55 and 65 µl.

    d. Between 65 and 111.1 µl.

49. The purpose of coating QBC II tubes with potassium oxalate is to:

    a. Inhibit clotting of finger-stick blood.

    b. Stain white cells orange.

    c. Increase the volume of RBCs.

    d. Increase the density of RBCs.

50. The float, which is supplied with each QBC tube,

    a. Is inserted immediately prior to centrifugation.

    b. Axially expands the formed cell layers.

    c. Settles into the buffy coat.

    d. All of the above.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 3

1. a (para 3-2)
2. c (para 3-3)
3. b (para 3-5a)
4. d (para 3-4b)
5. d (para 3-4b)
6. a (para 3-4b)
7. a (para 3-5b)
8. a (para 3-5a)
9. b (para 3-5d(1))
10. c (para 3-5c(4))
11. b (para 3-5g)
12. c (para 3-5g)
13. a (para 3-6)
14. c (para 3-7a)
15. b (para 3-7a)
16. b (par 3-7c)
17. d (para 3-9)
18. c (para 3-10)
19. b (para 3-11)
20. a (para 3-11)
21. c (para 3-12)

22. d (para 3-13b)

23. b (para 3-14)

24. c (para 3-13a)

25. a (para 3-15)

26. b (para 3-15)

27. b (para 3-16)

28. c (para 3-17)

29. b (para 3-17)

30. a (para 3-17)

31. c (para 3-18)

32. c (para 3-18)

33. c (para 3-19)

34. b (para 3-20)

35. a (para 3-21a)

36. b (para 3-21a)

37. d (para 3-22b)

38. d (para 3-22b)

39. c (para 3-23)

40. b (para 3-24)

41. a (para 3-26)

42. c (para 3-27)

43. d (para 3-27)

44. c (para 3-29)

45. a (para 3-32b(1))

46. b (para 3-32a(2))

47. a (para 3-32c(2))

48. c (para 3-32c(2))

49. d (para 3-32c(3))

50. d (para 3-32c(4))

End of Lesson 3

GLOSSARY OF TERMS

A

Agranulocyte: A leukocyte without definite cytoplasmic granules.

A**granulocytosis:** Complete or nearly complete absence of the granular leukocytes from the blood and bone marrow.

Aleukemic Leukemia: A fatal condition of the blood-forming tissues, characterized by marked proliferation of immature cells in the bone marrow, without their presence, in any great numbers, in the blood steam.

Anemia: A condition in which the blood is deficient in quantity or quality of erythrocytes.

Anisocytosis: Variation in size of the erythrocytes.

Anomaly: Abnormality.

Anoxemia: Lack of normal proportion of oxygen in the blood.

Antecubital Space: The area on the forearm frontal to the elbow.

Anticoagulant: A substance that prevents the coagulation of blood. Commonly used ones are potassium oxalate, sodium oxalate, sodium citrate, EDTA, and heparin.

Aplasia: Incomplete or defective blood development; cessation of blood cell formation.

Aplastic Anemia: Anemia characterized by incomplete or defective blood development.

Asynchronous: Uncoordinated development.

Azurophilic Granule: Rounded, discrete, reddish-purple granule, smaller than the granules of neutrophils; 0-10 are common in lymphocytes and they are very numerous and smaller in the cytoplasm of monocytes.

B

Band Form: In the Schilling classification, a neutrophil with the nucleus unsegmented and ribbonlike; also stab, staff, nonfilamented.

Basket Cell: A degenerated, primitive cell which has ruptured and in which the cell nucleus appears as a pale staining smear without prescribed form or shape.

Basopenia: An abnormal decrease in the number of basophils.

Basophil: A granular leukocyte, the granules of which have an affinity for the basic dye of Wright stain (methylene blue). The granules are large, irregular and blue-black in color.

Basophilia: An abnormal increase in the number of basophils.

Basophilic: Staining readily with basic dyes, for example, blue with Romanovsky type stains.

Binary Fission: Simple cell division.

Bleeding Time: The time required for a small standardized wound, made in the capillary bed of the finger or ear lobe, to stop bleeding.

Blood Dyscrasia: A disease of the blood or blood-forming organs.

Buffy Coat: The layer of leukocytes that collects immediately above the erythrocytes in sedimented or centrifuged whole blood.

C

Cabot's Rings: Lines in the form of loops or figures-of-eight seen in erythrocytes in severe anemias.

Centriole: A minute cell organoid within the centrosome.

Centrosome: An area of condensed cytoplasm active in mitosis.

Chemotaxis: The phenomenon of movement of leukocytes caused by a chemical influence.

Chromatin: The more stainable portion of the cell nucleus contains genetic materials.

Clot Retraction: The rate and degree of contraction of the blood clot.

Coagulation Time: The time required for venous blood, in the absence of all tissue factors, to clot in glass tubes under controlled conditions.

Cocatalyst: A substance that works in tandem with another group of chemicals to accelerate a reaction velocity without being used up in the reaction.

Color Index: The ratio between the amount of hemoglobin and the number of red blood cells.

Complete Blood Count: A hematology study which consists of a red cell count, white cell count, hematocrit, hemoglobin, and blood smear study including differential white cell count.

Congenital: Born with a person; existing at or before birth.

Cooley's Anemia (Mediterranean Disease or Thalassemia): A chronic, progressive anemia commencing early in life and characterized by many normoblasts in the blood, unusual facies, splenomegaly and familial, and racial incidence. Target type red blood cells are often present in the peripheral blood.

Crenation: The scalloped or notched appearance of the periphery of erythrocytes found when the cells are suspended in a hypertonic solution. Also found in smears caused by dirty glassware, slow drying, and poor smearing technique.

Cytoplasm: Protoplasm of a cell excluding the nucleus.

D

DNA: Deoxyribonucleic acid.

Differential Count: An enumeration of the types of white blood cells seen on a stained blood smear.

Discrete: Separate.

Dyscrasia: Abnormality.

E

Ecchymosis: Subcutaneous extravastion of blood covering a large area.

Endothelial Leukocyte: Monocyte.

Eosinopenia: An abnormal decrease in eosinophils.

Eosinophil: A granular leukocyte, the granules of which have an affinity for the acid dye of Wright's stain (eosin). The granules are large, round, uniform in size, red-orange in color, and are shiny and refractile.

Eosinophilia: A relative or absolute leukocytosis in which the main increase is in eosinophils.

Eosinophilic: Readily stained with eosin; red-orange stain.

Epigastric: Pertaining to the upper middle portion of the abdomen.

Erythremia: A disease marked by persistent polycythemia and increased blood volume; also polychythemia vera.

Erythrocyte: Red blood cell.

Erythrocytosis: An increase in the total number of erythrocytes.

Erythrogenic: Producing erythrocytes.

Erythroleukemia: An abnormal condition characterized by proliferation of erythroblastic and myeloblastic cells.

Erythropenia: A decrease in the number of red cells in the blood.

Erythropoiesis: The production of erythrocytes.

Etiology: The theory of the causation of a disease.

Extravascular: Occurring outside of the blood vessels.

Extrinsic: Originating outside of the particular area.

F

Fibril: A microscopic filament often composed of fibrin.

Fibrin: The end product of the clotting mechanism which forms a network of fibers that enmesh the formed elements of blood.

Fibrinogen: The precursor of fibrin which is present normally in the plasma and produced by the liver.

Fragility Test (Osmotic): A test devised to measure the resistance of the erythrocytes to break down (hemolyze) when subjected to varying concentrations of hypotonic salt solutions.

Fulminating: Sudden and severe.

G

Golgi Apparatus: A meshwork of lipid containing fibrils within the cytoplasmic portion of a cell.

Granulocyte: A white blood cell that contains specific cytoplasmic granules (neutrophils, eosinophils, and basophils); these granules are peroxidase positive.

Granulocytosis: The presence of increased numbers of granulocytes in the blood.

Granulocytopenia (Granulopenia): A decrease in the number of granulocytes in the blood.

Granulopoiesis: The production of granulocytes.

H

Hemacytometer: A calibrated chamber in which blood cells are counted.

Hematin: A brown or blue-black amorphous iron substance which unites with globin and forms hemoglobin.

Hematocrit: The packed cell volume (PCV) of red blood cells obtained by centrifugation of a blood specimen in a hematocrit tube.

Hematology: The branch of medicine that deals with the study of blood cells, blood-producing organs and the manner in which these cells and organs are affected in disease.

Hematoma: Subcutaneous effusion of blood with resulting swelling, pain, and discoloration forming a tumorlike mass.

Hematopoietic (Hemopoietic): Blood forming.

Hemoglobin: The coloring matter of the red blood cells. A complex iron-bearing pigment which carries oxygen and carbon dioxide.

Hemoglobinuria: The presence of free hemoglobin in the urine.

Hemogram: The blood picture.

Hemolysis: The dissolution or dissolving of the erythrocytes.

Hemolytic Anemia: That type of anemia characterized by excessive intra-vascular destruction of red cells.

Hemophilia: A hereditary disease characterized by a prolonged coagulation time and repeated hemorrhages, occurring only in males and transmitted only by females and affected males. The cause is a deficiency in a plasma factor (antihemophilic globulin or thromboplastinogen) resulting in a defect in thromboplastic activity.

Hemoptysis: The spitting of blood; coughing up blood.

Hemostasis: The checking of the flow of blood, especially from a vessel.

Hepatic: Originating from the liver.

Heterozygous: Derived from germ cells unlike in respect to one or more factors.

Homeostasis: Stability in normal body states.

Homozygous: Derived from germ cells which are alike.

Howell-Jolly Bodies: Small basophilic particles sometimes found in erythrocytes, remnants of nuclear material.

Hygroscopic: Readily taking up and retaining water.

Hyperplasia: An increase in cell formation.

Hypertonic: Greater than isotonic concentration.

Hypertrophy: Enlargement of an organ or part due to increase in the size of the constituent cells.

Hypochromia: A decrease in color of the erythrocytes, hence a decrease in their hemoglobin content.

Hypoplasia: A decrease in cell formation.

Hypotonic: Less than isotonic concentration.

I

Idiopathic: Disease of unknown cause.

Inclusion: Usually lifeless, an accumulation of fats, proteins, crystals, pigments or secretory granules within a cell cytoplasm.

Inhibitor: A substance directed against a coagulation factor or factors which interferes with the coagulation process.

Intravascular: Occurring within the blood vessels.

Intrinsic: Situated within the particular part.

In Vitro: Within a test tube (glass, etc).

In Vivo: Within the living organism, as in life.

Isotonic: Solutions with the same osmotic pressure.

J

Jaundice: Yellowness of the skin and eyes characterized by hyperbilrubinemia and disposition of bile pigment in the skin; follows excessive destruction of the blood, obstruction of the bile passage, diffuse liver disease, certain infections, toxic chemical agents, and drugs.

Juvenile Cell: In the Schilling classification, the cell between the myelocyte and band forms; also metamyelocyte.

K

Karyolysis: Apparent destruction of the nucleus of a cell.

Karyorrhexis: Fragmentation of the nucleus; a degenerative process usually followed by karyolysis.

L

L.E. Cell: A large segmented neutrophil or eosinophil that contains ingested autolyzed nuclear fragments in its cytoplasm.

Leukemia: An ultimately fatal disease of the blood-forming organs characterized by increased numbers of leukocytes and associated anemia.

Leukemoid Crisis or Reaction: A temporary appearance of immature leukocytes in the blood stream, with a marked increase in the total white count. In the laboratory sometimes temporarily indistinguishable from leukemia.

Leukocyte: White blood cell.

Leukocytosis: An increase in leukocytes in the blood.

Leukopenia: A reduction in the number of leukocytes in the blood.

Leukopoiesis: Leukocyte formation.

Lymphoblast: The parent cell of the lymphocytic series.

Lymphocyte: A white blood cell having a round or oval nucleus and sky blue cytoplasm. The nuclear chromatin is densely clumped but separated by many clear areas giving a "hill and valley" effect. A few red-purple (azurophilic) granules may be present in the cytoplasm.

Lymphocytosis: A relative or absolute increase in the number of circulating lymphocytes.

Lymphopenia: An abnormal decrease in the number of lymphocytes.

Lysis: Destruction by a specific agent.

M

Macrocyte: An erythrocyte larger than normal.

Macrocytosis: An increase in the number of macrocytes.

Mast Cell: A basophil or a true tissue cell.

Maturation Factor: A substance which will cause cells to ripen and come to maturity.

Mean Corpuscular Hemoglobin (MCH): The average amount of hemoglobin in the red blood cell.

Mean Corpuscular Hemoglobin Concentration (MCHC): The average percent hemoglobin saturation in the red blood cell.

Mean Corpuscular Volume (MCV): The volume of the average red blood cell.

Megakaryoblast: The parent cell of the megakaryocytic series.

Megakaryocyte: An extremely large cell with an irregularly lobed, ring- or doughnut-shaped nucleus which stains blue-purple. The cytoplasm is abundant, light blue, and is packed with fine azurophilic granules. This cell gives rise to thrombocytes.

Megaloblast: The type of red cell precursor found in pernicious anemia. This differs from the normal erythrocyte precursor (normoblast) in that the megaloblast is larger and the nuclear chromatin has a fine meshwork.

M:E Ratio: The ratio of myeloid to erythroid cells in the bone marrow.

Mesentery: The fold of peritoneum which attaches the intestine to the posterior abdominal wall.

Metamyelocyte: Juvenile cell of Schilling.

Metarubricyte: An erythrocyte with a pyknotic, contracted nucleus. Also called orthochromatophilic normoblast.

Methemoglobin: A spectroscopically detected compound of hemoglobin found in nitrobenzol and other poisonings. The blood is a chocolate brown color

Microcyte: An erythrocyte smaller than normal.

Microcytosis: An increase in the number of microcytes.

Micron: One-thousandth of a millimeter, the common unit of microscopic measure.

Mitochondria: Granular components of a cell cytoplasm active in oxidative processes.

Mitosis: A series of changes through which the nucleus passes in indirect cell division. A tissue showing many cells in mitosis indicates rapid growth of that tissue.

Monoblast: The parent cell of the monocytic series.

Monocyte: A large white blood cell with a pale blue-gray cytoplasm containing fine azurophilic granules. The nucleus is spongy and lobulated.

Monocytosis: A relative or absolute increase in the number of circulating monocytes.

Mucosa: Mucous membrane.

Myeloblast: The parent cell of the granulocytic or myelocytic series.

Myelocyte: The stage in development of the granulocytic series which is characterized by the first appearance of specific granules (eosinophilic, neutrophilic, or basophilic) and a round nucleus.

Myeloid Cells: The granular leukocytes and their stem cells.

Myelopoiesis: Formation of bone marrow and the blood cells that originate in the bone marrow.

Myeloproliferative: Rapid production of bone marrow constituents.

Necrosis: The death of a circumscribed portion of tissue. Simple necrosisgeneration of the cytoplasm and nucleus without change in the gross appearance of the tissue.

N

Neutropenia: A decrease in the number of neutrophils in the blood.

Neutrophil (Polymorphonuclear Neutrophil or Segmented Neutrophil): A granulocyte having fine neutrophilic (pink-violet) granules in the cytoplasm. The nucleus is divided into two or more lobes; each lobe is usually connected by a filament.

Neutophilia: An increase in neutrophils.

Normoblast: The nucleated precursor of the normal red blood cell. Also called a rubriblast.

Normocyte (Erythrocyte): A red blood cell of normal size.

NRBC: Nucleated red blood cell, usually a metarubricyte when seen in the peripheral blood smear.

Nucleolus: An intranuclear pale blue body surrounded by a dense condensation of chromatin.

O

Occult Blood: The presence of blood which cannot be detected except by special chemical tests.

Oligochromemia: A decrease in hemoglobin.

Oligocythemia: A decrease in the number of erythrocytes.

Organoid: Structures present in cells resembling organs.

Ovalocyte: An elliptical erythrocyte.

Oxyhemoglobin: The bright red hemoglobin that is loosely combined with oxygen and found in arterial blood.

P

Pancytopenia: A reduction in all three formed elements of the blood, namely, the erythrocytes, leukocytes, and thrombocytes.

Pathologic Increase (Or Decrease): Caused by abnormal function or disease as contrasted to physiological (caused by normal body function).

Pernicious Anemia: A chronic, macrocytic anemia caused by a defect in production of "intrinsic factor" by the stomach. There is accompanying megaloblastic erythropoiesis, poikilocytosis, granulocytic hypersegmentation, achlorhydria, an neurological disturbances.

Petechiae: Small spots on the skin formed by subcutaneous effusion of blood (also purpura and ecchymoses).

Phagocytosis: The destruction of organisms and extraneous matter by a process of envelopment and absorption.

Plasma: The fluid portion of the blood composed of serum and fibrinogen obtained when an anticoagulant is used.

Plasma Cell: A lymphocyte-like cell with an eccentrically placed deep-staining nucleus. The nuclear chromatin is distributed in a "wheel-spoke" fashion. The cytoplasm is deep blue with a lighter halo about the nucleus.

Platelet: Thrombocyte.

Poikilocyte: A red blood cell having abnormal shape (pear-shape, sickle-shape, etc.).

Poikilocytosis: Increased number of abnormally shaped erythrocytes.

Polychromasia: Diffuse basophilia of the erythrocytes.

Polychromatophilia: The presence in the stained blood smear of immature, nonnucleated, bluish-staining red blood cells.

Polycythemia: An increase in the total number of erythrocytes. (See erythremia.)

Precursor: A substance from which another substance is formed.

Promyelocyte: The precursor of the myelocyte having nonspecific azurophilic (red-purple) cytoplasmic granules.

Prorubricyte: The second stage of development of the red cell.

Prothrombin: The inactive precursor of thrombin which is formed in the liver and present normally in the plasma. Its formation depends upon adequate vitamin K.

Punctate Basophilia: Small basophilic aggregates in the erythrocytes that stain blue with the basic dye of Wright's stain; also basophilic stippling.

Purpura: Small spots on the skin formed by subcutaneous effusion of blood.

Pyknosis: A condensation and reduction in size of the cell and its nucleus.

Q

R

Reduced Hemoglobin: A combination of hemoglobin and carbon dioxide which is found in venous blood.

Reticulocyte: A red blood cell showing a reticulum or network when stained with vital dyes (for example, brilliant cresyl blue). The stage between the nucleated red cell and the mature erythrocyte.

Reticulocytosis: An increase above normal values of reticulocytes in peripheral blood.

RNA: Ribonucleic acid.

Rouleaux Formation: The arrangement of red cells with their flat surfaces facing, in which they appear as figures resembling stacks of coins.

Rubricyte: Polychromatophilic normoblast.

S

Sedimentation Rate, Erythrocyte (ESR): The rate at which red cells will settle out in their own plasma in a given time under controlled conditions.

Serum: The fluid portion of the blood, after clot formation.

Shift to the Left: A term used to designate that condition in which the immature forms of the neutrophils are increased above their normal number.

Shift to the Right: Increase in mature, pyknotic, and hypersegmented neutrophils.

Sickle Cell: A sickle- or crescent-shaped erythrocyte.

Sickle Cell Anemia: A hereditary and familial form of chronic, hemolytic anemia essentially peculiar to Blacks. It is characterized clinically by symptoms of anemia, joint pains, leg ulcers, acute attacks of abdominal pain, and is distinguished hematologically by the presence of distinct hemoglobin, peculiar sickle-shaped and oat-shaped red corpuscles, and signs of excessive blood destruction and active blood formation.

Smudge Cell: A ruptured white cell; also basket cell, or degenerated cell.

Spherocyte: A red blood cell which is more spherical, smaller, darker, and more fragile than normal.

Stasis: A stoppage of blood flow.

Supravital Stain: A stain of low toxicity which will not cause death to living cells or tissues.

Synchronous: Occuring at the same time and in a regular pattern.

T

Target Cell (Leptocyte): An abnormal, thin erythrocyte characteristic of Cooley's or Mediterranean anemia.

Triturate: To grind together.

Thrombin: This is an enzyme formed from prothrombin which converts fibrinogen to fibrin. This is not present in circulating blood.

Thrombocyte: A blood platelet.

Thrombocytopenia: A decrease in blood platelets; also thrombopenia.

Thrombocytosis: An increase in blood platelets.

Thromboplastin: The substance that initiates the process of blood clotting. It is released from injured tissue and/or formed by the disintegration of platelets in combination with several plasma factors.

Thrombopoiesis: The production of thrombocytes.

Thrombosis: Formation of a thrombus or blood clot.

U

V

Vacuole: A space or cavity formed in the protoplasm of a cell.

Venipuncture: The act of puncturing a vein in order to remove a sample of blood.

Viscous Metamorphosis: Friction between molecules resulting in a structural change.

Vitamin K: A vitamin constituent of the normal diet requiring bile salts for absorption. This vitamin is utilized by the liver in the production of prothrombin.

W

X

Xanthochromia: A yellowish discoloration, usually associated with spinal fluid.

Y

Z

End of Glossary