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MANUAL OF CLINICAL LABORATORY METHODS

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Preface

This book is the outgrowth of an outline of laboratory methods prepared for the use in the teaching of medical students and laboratory technicians. It is not designed to be a textbook of Clinical Pathology in that it does not discuss, except incidentally, the clinical significance of the results of the tests. In most instances only one method is included for each determination. The procedures are given in outline form so that they may be easily followed step by step in the laboratory.

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OPAL E. HEPLER

Foreword

The clinical laboratory is an essential part of current medical practice. Through its use, modern medicine is approaching the status of an exact science. The extent and manner of use of the clinical laboratory is a measure of the quality of medical service rendered by the staff of any hospital. Since the actual work of such laboratories is usually done by technicians with varying degrees of training and experience, the more detailed and explicit the available instructions for the performance of the various tests, the more accurate and dependable the results of such tests are likely to be. This *Manual of Laboratory Procedures* is intended to be just such a guide.

As stated in the Preface, this Manual is the outgrowth of an outline prepared several years ago for use in the laboratory by medical students in the course in Clinical Pathology in Northwestern University Medical School. By gradual accretion it has grown to its present size. The method of presentation of the subject matter is the result of the author's extensive experience in teaching medical students and student technicians and in directing the clinical laboratories of the Montgomery Ward Clinics and of Passavant Memorial Hospital. This method of presentation is based upon the principle that explicit directions are necessary, particularly for beginners, if laboratory tests are to be done with expedition and accuracy. Each test is, therefore, treated as a unit; that is, the principle of the test, the various steps in the order of their performance, the calculation of results and their interpretation and significance, the sources of error and the formulae of the solutions used, are all given in systematic order and in simple language. This step by step method is a unique feature of this Manual.

This Manual has gone through several previous editions in planograph form. Of the last edition there were four reprintings. Without advertising of any kind and without review in any medical journal, the demand for it became so great that its publication in book form seemed desirable. It has been used in many civilian hospitals throughout the United States. During World War II requests for it came from numerous military hospitals in South America, North Africa, Italy, France and the Western and Southwest Pacific areas. It has, therefore, proved its value under the most varied working conditions. The present edition is much larger and more extensively illustrated than any previous one and its usefulness should be proportionately enhanced. The illustrations and diagrams of different types of complicated apparatus—such as the electrocardiograph and the Van Slyke machine—should enable any technician to understand the principle upon which the particular apparatus is based and to use it with increasing assurance and accuracy.

This is not a textbook of Clinical Pathology. Its sole purpose is to improve the work of laboratory technicians and medical students by furnishing them with explicit, step-by-step directions for the performance of the different tests. References to the practical application and clinical significance of a particular test are inserted for the purpose of stimulating the interest of the technician in her work. Intelligent employees do better work if they understand the use that will be made of the product of their efforts.

JAMES P. SIMONDS

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MANUAL OF CLINICAL LABORATORY METHODS

Urinalysis

TABLE 1. RECORDING ROUTINE URINALYSIS

COLOR: Colorless, pale straw, straw, light amber, amber, reddish amber.
SP. GR.: 1.001-1.060.
REACTION: pH determined with nitrazine paper.
APPEARANCE: Clear, white or orange flocculent sediment, or shreds.
CLOUDINESS: Amount present; + (slight), + + (moderate), + + + (cloudy), + + + + (very cloudy).

ALBUMIN: Ring test.
Neg. = no cloudiness appears at zone of contact.
Tr. = ring is just perceptible against a black background (less than 0.05 gm.%).
+ = ring is distinct against a black background and can barely be seen when held up to the light (0.05 to 0.2 gm.%).
+ + = ring is very definite against light, faintly visible when viewed from above (0.2 to 0.5 gm.%).
+ + + = ring is heavy against light, distinct cloudiness when viewed from above (0.5 to 2 gm.%).
+ + + + = ring is thick and dense against light, opaque when viewed from above (2 gm.% and over).

Heat test.
Neg. = no cloudiness is perceptible.
Tr. = cloudiness is just perceptible against a black background (less than 0.01 gm.%).
+ = cloudiness is distinct but not granular against a black background and can barely be seen when held up to the light (0.01 to 0.05 gm.%).
+ + = cloud is distinct and granular against light (0.05 to 0.2 gm.%).
+ + + = cloud is heavy with distinct flocculi (0.2 to 0.5 gm.%).
+ + + + = cloud is dense with large flocculi, may solidify (0.5 gm.% or higher, 3 gm.% albumin becomes solid on boiling).

SUGAR: Benedict's test (read before precipitate settles).
Neg. = clear blue to marked cloudy green.
+ = yellowish green (0.5 to 1 gm.%).
+ + = greenish yellow (1 to 1.5 gm.%).
+ + + = yellow (1.5 to 2.5 gm.%).
+ + + + = orange (2.5 to 4 gm.%), red (4 gm.% and over).

ACETONE: Specimens positive for sugar and all specimens from prenatal patients and patients in acidosis must be examined routinely for acetone.

DIACETIC ACID: Test only specimens positive for acetone.

MICROSCOPIC EXAMINATION (sediment of centrifuged specimen):
CASTS: Identify type—hyaline, finely granular, coarsely granular, etc. Report number on slide or average per low power field.
ERYTHROCYTES and LEUKOCYTES: Average range per high power field.
EPITHELIUM (low power): + (occasional), + + (few), + + + (many), + + + + (great many). Identify type—squamous, transitional, renal, etc.
CRYSTALS (low power): + (occasional), + + (few), + + + (many), + + + + (great many). Identify type—calcium oxalate, uric acid, etc.
AMORPHOUS (low power): + (occasional), + + (few), + + + (many), + + + + (great many). Urates in acid, phosphates in neutral or alkaline urine.
BACTERIA (high power) and MUCOUS THREADS (low power): + (occasional), + + (few), + + + (many), + + + + (great many).

General Considerations

The primary function of the kidney is to maintain the chemical and physical qualities of the blood plasma within normal limits by excreting metabolic wastes, especially those of nitrogenous composition. The fluid filtered from the blood plasma through the glomeruli contains all the substances present in the blood except protein and the cellular elements. As this filtrate passes down the tubule, the water is almost completely reabsorbed and the solid elements in solution (glucose, bicarbonate, phosphates, urea, sodium, and potassium) are selectively reabsorbed in varying amounts according to each individual threshold value. In this manner about 150 liters of plasma are purified each day by the process of glomerular filtration and tubular absorption. It is estimated

that each kidney has a million nephrons (glomeruli with tubules) only a part of which act at one time; therefore in normal kidneys there is a reserve of 60 to 75 per cent.

Collection of Urine Specimen

I. Single Specimen.

A. *Qualitative Tests.*

1. When it is desired to make only qualitative tests, a specimen voided at random is satisfactory. It should be examined within 1 to 2 hours after voiding.
2. Urine passed about 3 hours after a meal is most likely to contain pathological substances.
3. First urine voided in the morning is least likely to contain them.
4. To diagnose cyclic albuminuria, samples obtained at various intervals during the 24 hours must be examined.

B. *Bacteriological Examinations.*

1. The urine must be a fresh, catheterized specimen obtained under aseptic conditions.
2. If cultures cannot be made immediately, place specimen in the refrigerator.

II. Twenty-four Hour Specimen.

A. *Quantitative tests* are of value only on 24 hour samples.

B. *Collection.*

1. The patient should be instructed to empty the bladder at the beginning of the period (8 A.M.) and discard the urine.
2. Save all urine passed until 8 o'clock the next morning, emptying the bladder at that time and adding this urine to the 24 hour specimen.
3. It is sometimes desirable to have the day and night specimens examined separately.
   a. *Day specimen*—obtain as for the 24 hour specimen, including the specimen voided 3 hours after the evening meal.
   b. *Night specimen*—save all urine voided during the night and empty bladder at 8 A.M., adding this to the night specimen.
4. If the amount is important, the patient should bring the pooled specimens to the laboratory; if not, 8 ounces of the specimens, well mixed, are sufficient.
5. The urine should be kept in a clean receptacle and in a cool place.

Physical Properties

I. Quantity.

A. *Normal.*

1. The 24 hour specimen for adults contains 800 to 1600 cc. (varies greatly with the liquid intake, perspiration, etc.).
2. Specimens from children, 6 to 12 years, contain 500 to 1500 cc.
3. Specimens from children, 1 to 6 years, contain 300 to 1000 cc.
4. The day volume is usually 3 to 4 times the night volume.

B. *Abnormal.*

1. *Increased quantity (polyuria)* is found in diabetes mellitus and insipidus, chronic nephritis, certain nervous diseases, during disappearance of an edema, and during convalescence from an acute febrile disease.
2. *Decreased quantity (oliguria)* is found in uremia, acute nephritis, eclampsia, severe diarrhea, excessive vomiting, profuse sweating in fevers, cardiac decompensation, calculus or tumor of the kidney, nephrosis with edema, atrophic hepatic cirrhosis, and acute yellow atrophy of the liver.
3. *Total suppression (anuria)* occurs in "collapse" with systolic blood pressure below 70 mm. of mercury, severe acute nephritis, and in poisoning with bichloride of mercury.
4. *Residual urine* is that obtained by catheter immediately after the patient has emptied the bladder voluntarily.

II. Color.

A. *Normal.*

1. *Straw to amber.*
2. *Colorless to straw* indicates low specific gravity and large quantity (except in diabetes mellitus).
3. *Amber* indicates high specific gravity and small quantity.

B. *Pathological Coloration.*

1. *Reddish amber* may indicate an increase in urobilinogen or porphyrin.
2. *Brownish yellow or green* with a yellow foam when shaken may indicate bile pigments.
3. *Red to smoky brown* may be due to blood and blood pigments.
4. *Milky* may be due to large amounts of pus, bacteria, fat, or chyle.
5. *Brownish black* may indicate melanin which may appear only after the urine stands and the chromogen melanogen is converted into melanin. If present a gray precipitate, which blackens on standing, forms when a few drops of 10% ferric chloride is added to 10 cc. of urine.
6. *Black* may indicate homogentisic acid which occurs in alkaptonuria. Urine becomes black after standing or after it is alkalinized. Homo-

gentisic acid reduces Benedict's solution.

C. ***Nonpathological Coloration***—following the ingestion of various drugs and foods.

1. *Red*—beets, mercurochrome or prontosil instillation, phenolphthalein, selenium.
2. *Blue or green*—methylene blue.
3. *Brown*—rhubarb, senna, cascara, argyrol instillation.
4. *Yellow*—carotene, santonin, or pyridium.
5. *Green*—acriflavine.

III. Specific Gravity.

A. ***Method.***

1. Fill the container three-fourths full of well mixed urine, remove all foam with filter paper.
2. Float the urinometer in the urine by rotating it rapidly to prevent its touching the bottom or the sides of the container.
3. The specific gravity is obtained by reading the gradation on the stem of the instrument at the level of the lower part of the meniscus.
4. Each urinometer is calibrated to give readings at a definite temperature, usually 25°C. If the temperature of the urine is above or below this, a correction of 0.001 must be added for each 3°C. above or deducted for each 3°C. below.
5. If the quantity of urine is small and the specific gravity is important, the urine may be diluted with distilled water and the specific gravity read; to obtain the correct number, multiply the last two figures of the specific gravity number by the amount of dilution. *This diluted urine cannot be used for qualitative or quantitative tests.*

B. ***Normal.***

1. For a 24 hour specimen—1.015 to 1.025. It varies inversely with the volume and directly with the amount of salt, urea, and protein in solution. For each gram of albumin per 100 cc. of urine, the specific gravity is increased 0.003.
2. Single specimens may range from 1.002 to 1.030.

C. ***Pathological Significance.***

1. Varies from 1.001 to 1.060.
2. Low in chronic nephritis and diabetes insipidus.
3. High in diabetes mellitus, fevers, and acute nephritis.
4. See Renal Function Tests, page 27.

IV. Reaction.

A. ***Nitrazine Paper.***

1. To determine pH of urine, use one of the following methods:
   a. With a clean glass rod transfer a drop of urine to the surface of a piece of nitrazine paper and spread evenly by stroking or leave a small drop as such on the paper.
   b. Dip paper into urine three consecutive times and then shake off excess liquid.
2. One minute after placing urine on paper, compare color with color chart.

B. ***Litmus Paper.***

1. Test urine with neutral litmus paper dipping a strip of the paper into the urine.
   a. Pink color—acid.
   b. No change in color—neutral.
   c. Blue—alkaline.
2. When the test is alkaline, dry the litmus paper with heat. If it loses its blue color, the "alkalinity" is due to a volatile substance, ammonia; if a nonvolatile alkali the paper remains blue.

C. ***Normal Values.***

1. Freshly voided urine is usually acid in reaction, the pH ranging from 4.8 to 7.5 with an average of 6.
2. It may be alkaline after a full meal, after taking large quantities of citrus fruits, or as a result of taking alkalies.
3. Twenty-four hour specimens are less acid than freshly passed specimens and may become alkaline after standing due to the decomposition of urea by bacteria and the liberation of ammonia.

D. ***Pathological Significance.***

1. Acidity is increased in acidosis, fevers, and a diet with an excess of protein.
2. May be alkaline in chronic cystitis and urine retention due to decomposition of the urine in the bladder. Same reason as in C3.
3. Fixed alkaline urines are associated with anemia, rapid absorption of transudates, some nervous diseases, obstructing gastric ulcer, severe vomiting, and alkaline therapy.

V. Cloudiness.

A. **Report** as +, ++, +++, or ++++ after suspending the sediment in the urine by shaking. See Table 1 on page 3.

B. **Freshly voided urine** is usually clear; but is sometimes cloudy due to the following sediments which are identified with the microscope.

1. *Amorphous phosphates* form a white cloud or precipitate in neutral or alkaline urine. This cloud disappears upon addition of dilute acetic acid.
2. *Amorphous urates* form a white or pink cloud of sediment (brick dust deposit) in acid urine which disappears on heating.

3. *Epithelial cells and mucus* give cloudiness to urine when present in large amounts.
4. *Blood* gives urine a red or brown smoky color.
5. *Pus* makes urine turbid, but clears up on filtering or centrifuging.
6. *Bacteria* produce a uniform cloudiness which does not settle out and cannot be filtered out.
7. *Fat and chyle* may render urine turbid.
8. *Shreds* are often present in chronic gonorrhea.

VI. Odor.

A. ***Normal.***

1. Aromatic, due to volatile acids. More marked in concentrated urine.
2. Various articles of diet and drugs impart peculiar odors, especially asparagus and turpentine.

B. ***Abnormal.***

1. It is ammoniacal after decomposition; important only in fresh urine and found in cystitis and urine retention.
2. It is fruity in diabetes if ketone bodies are present.

Chemical Tests

I. Protein.

A. ***Albumin*** in the urine is derived from a number of sources.

1. *Physiologic albuminuria* appears after excessive muscular exertion, prolonged cold baths, excessive ingestion of proteins, etc.
2. *Orthostatic or postural albuminuria* appears after a person has been in an erect position and disappears with rest in bed.
3. *Accidental or False Albuminuria.*
   a. Due to pus, blood, and vaginal discharge.
   b. Found in pyelitis, cystitis, and chronic vaginitis.
4. *Pathologic Albuminuria.*
   a. Albumin in kidney disease is derived from the blood plasma and indicates increased permeability of the glomerular filter. Albumin, because of its smaller molecule, is excreted in larger amounts than globulin or fibrin, markedly decreasing the albumin-globulin ratio in the blood plasma when albuminuria is of severe grade. See Table 4 on page 31.
   b. Also present in febrile diseases, toxemia of pregnancy, passive congestion of the kidneys, and anemias.

B. ***Principle of the Tests:*** All methods depend upon the precipitation of protein by chemical agents or coagulation by heat.

C. ***Qualitative Tests.***

1. *General Considerations.*
   a. Urine must be clear; if not filter or centrifuge.
   b. If the urine is alkaline, add acetic acid drop by drop until slightly acid.
2. *Robert's Test.*
   a. Place 3 to 5 cc. of clear urine in a test tube three-fourths to an inch in diameter.
   b. Place the tip of a 5 or 10 cc. volumetric pipette containing Robert's reagent to the bottom of the tube and allow about 3 cc. of the reagent to layer beneath the urine.
   c. If several tests are being done, wipe off the tip of the pipette before inserting it into the next tube.
   d. A positive test is indicated by a white ring at the zone of contact.
   e. The ring must be read within 3 minutes after adding the reagent and with the eyes on the level of the contact ring. In order to observe a faint ring create a dark background by placing a finger between the tube and source of light.
   f. Rings that are 1 to 2 mm. above the zone of contact are due to mucin and nucleoalbumin; rings 1 to 2 cm. above the zone of contact are due to urates, uric acid, urea, and bile acids. These are not to be reported positive for albumin.
   g. The test may be performed by holding a test tube containing a few cc. of Robert's reagent in an inclined position and allowing filtered urine to run slowly down the side of the tube from a pipette or medicine dropper. It is difficult to get a good contact ring this way.
   h. Record the result according to Table 1 on page 3 (ring test).
   i. *Robert's reagent:*

Saturated magnesium sulfate (U. S. P. $MgSO_4 \cdot 7H_2O$) sol. (add 1 liter of water to 800 gm.)	5 parts
Nitric acid (conc.)	1 part

3. *Heller's Nitric Acid Test.*
   a. Perform the test as directed under Robert's test using conc. nitric acid instead of Robert's reagent and read the white ring at the zone of contact in the same manner.
   b. A red or reddish-violet ring which tends to extend downward into the acid is sometimes obtained with normal urine, but this ring is due to the reaction of the urinary pigments with nitric acid and is below the white one produced by albumin.
   c. If bile is present, a play of colors (red, violet, blue, and green) will be found at the line of contact.

d. Interfering rings outlined under Robert's test also apply to the nitric acid test.
e. A less accurate but rapid method of performing a series of tests is as follows:
1) Use a piece of glass tubing with an inside diameter of about 5 mm.
2) Immerse this tube in the urine to about an inch, wipe off the outside, and immerse in a test tube containing 2 inches of conc. nitric acid.
3) By holding the finger over the top until the upper level of the urine in the tube is just below the surface of the acid in the test tube and then removing the finger and lowering the tube to the bottom of the test tube, the nitric acid rises in the tube and forms a sharp line of demarcation between the two.
4) Place the finger over the upper end of the tube while removing it, hold up to the light, and read the width of the white ring in terms of +, ++, etc.
5) Rinse out the tube and fill with another urine, using the same test tube of nitric acid for a number of tests.

4. *Sulfosalicylic Acid Test* (Exton's Method).
a. Mix equal volumes of clear urine and Exton's reagent in a test tube.
b. If no cloudiness develops, albumin is absent.
c. If cloudiness appears, warm gently but do not boil.
d. If the cloudiness persists or increases on heating, albumin is present.
e. Read while warm and record result according to Table 1 on page 3 (heat test).
f. Proteoses will cause a cloud on cooling.
g. Bence-Jones protein causes a heavy precipitate which clears partially or wholly upon boiling.
h. *Exton's qualitative reagent.*

Sodium sulfate, anhydrous ..... 88 gm.
Sulfosalicylic acid ..... 50 gm.
Distilled water to make 1 liter.

1) Dissolve the sodium sulfate in 800 cc. of water with the aid of heat.
2) Cool, add the sulfosalicylic acid, and make up to volume with water.

5. *Heat and Acetic Acid Test.*
a. Fill a test tube three-fourths full of clear urine and gently heat the upper portion to boiling; boil for 1 or 2 minutes being careful not to shake the tube more than necessary. Rotate the tube to prevent cracking.
b. A turbidity is due either to phosphates, carbonates, or albumin.
c. Add 3 drops of 10% acetic acid drop by drop, boiling between each drop.
d. A white cloud now disappearing is due to earthy phosphates or carbonates.
e. A faint trace of albumin may appear only upon the addition of the acid. Larger traces appear upon boiling and may become heavier upon addition of the acid.
f. The addition of too much acid may dissolve faint traces of albumin and give a falsely negative reaction.
g. In order to detect slight traces, the tube must be held against a black background.
h. See Table 1 on page 3 (heat test) for recording amount present.

6. *Purdy's Test Modified.*
a. Fill a test tube half full of filtered urine.
b. Add about one-fifth of its volume of saturated aqueous solution of sodium chloride to raise the specific gravity (high specific gravity prevents the precipitation of mucin).
c. Add 2 to 5 drops of glacial acetic acid.
d. Mix well and boil the upper portion gently. Rotate tube to prevent cracking.
e. A cloud denotes the presence of albumin.
f. Record amount present according to Table 1 on page 3 (heat test).

D. *Quantitative Tests.*

1. *Esbach's Test* (Tsuchiya's Modification).
a. The urine must be filtered until clear.
b. If the qualitative test for albumin is 3 plus, make a 1-5 dilution; if 4 plus, make a 1-10 dilution with distilled water.
c. If not acid, add 10% acetic acid drop by drop to give a pH of 5.0.
d. Add enough powdered pumice or barium sulfate to an Esbach's tube to just cover the bottom of the tube. This increases the rate of sedimentation.
e. Fill tube with urine to mark U and add Tsuchiya's reagent (or Esbach's) to mark R.
f. Close tube with a rubber stopper and invert slowly 10 times.
g. Place in a test tube rack and keep in a cool place for sedimentation.
h. The height of the sediment must be read at the end of 30 minutes.
i. If pumice is not added, read at 24 hours.
j. The readings on the tube indicate grams of albumin per liter of urine. To change to per cent divide by 10.
k. If the urine has been diluted, multiply the final reading by the dilution.