# APPROVED LABORATORY TECHNIC

by

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IN MEMORY OF

WARD BURDICK

Founder of the American Society
of Clinical Pathologists

and

FRED BOERNER

Esteemed Colleague and
Former Co-Author

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*No laboratory examination can be better than the thoroughness and skill with which it is conducted.*

# PREFACE

Once again we are very grateful for the generous reception accorded the previous editions of this book and hope that this one will prove even more helpful to medical students, physicians, teachers, clinical pathologists and medical technologists. As in previous editions, the present book is devoted largely to the principles and technic of laboratory methods and normal values. The changes due to disease and their clinical interpretation are given in a separate book by the senior author, *Clinical Diagnosis by Laboratory Examinations,* also published by Appleton-Century-Crofts, Inc.

In the interests of accuracy all methods have been described with considerable detail. As is true of clinical examinations, no laboratory method can be better than the thoroughness and skill with which it is conducted. In this connection we greatly hope that laboratory technologists will conduct the tests exactly as described. Particular emphasis is to be placed upon the importance of using accurately prepared reagents, especially in serologic and chemical examinations. No matter how carefully a test is conducted the results cannot be accurate or acceptable if the reagents are defective. This is at least one important reason for discrepancies in reports by different laboratories examining portions of the same specimen or specimens. During the past sixteen years progressive improvement in the serologic tests for syphilis has resulted from the annual serologic surveys conducted by the United States Public Health Service in cooperation with the American Society of Clinical Pathologists along with serologic surveys conducted by the Departments of Health of various states and municipalities. Undoubtedly, similar surveys in clinical pathology, bacteriology, and blood chemistry would prove extremely valuable from the standpoint of improving the clinical value of laboratory examinations.

Since the accuracy of laboratory examinations may be impaired by the improper collection of specimens by physicians, regardless of the thoroughness and skill with which they are examined in the laboratory, we have thought it advisable to describe methods for the collection of specimens with considerable detail. In this connection laboratories are advised and urged to refuse to examine improperly collected and preserved specimens when these may yield erroneous and misleading results.

The present edition has been extensively revised, largely rewritten and considerably enlarged by the inclusion of newer methods and new illustrations. Every year new methods are developed and added to the facilities of clinical laboratories, but older methods of proven value have not been deleted unless replaced by newer ones of greater accuracy and diagnostic value. In many instances two or more methods have been given for the same examination when it was thought necessary or advisable to do so. Space does not permit the listing of all the new material included in this edition, but mention may be made of phase microscopy, the nomenclature of cells and diseases of the blood and blood-forming organs proposed by the Committee for the Clarification of Nomenclature, a method for determining blood and plasma volumes, the rapid

system for routine urine analysis by Wolman, Evans and Lasker, the Papanicolaou cytodiagnosis of cancer, the V.D.R.L. method for quantitative estimation of spinal fluid protein, the *Rana pipiens* urine hormone test for pregnancy, Isenberg's method for staining spermatozoa, Alving and Miller's inulin clearance and the thymol turbidity tests for liver function, many new and improved culture media, methods for testing the susceptibility of bacteria to various antibiotic compounds and methods for assaying the body fluids for them, newer methods in the rapidly developing field of virology, the laboratory diagnosis of toxoplasmosis, tests for Rh agglutinins, blocking antibody and types of the Rh factor, the Rein-Bossak slide flocculation and the V.D.R.L. micro- and macroflocculation tests for syphilis, flame photometry and skin tests for histoplasmosis and drug allergies. In this connection we are indebted to Dr. Samuel W. Eisenberg for contributing a method for the sigmoidoscopic collection of material for examinations for amebae using the Fradkin aspirator.

In conclusion we wish to express deep appreciation to the collaborators, separately listed, for their kindness in revising various chapters. We are especially indebted to Dr. Chris J. D. Zarafonetis for revising the chapter on Methods for the Examination of the Blood, Dr. Theodore G. Anderson for revising the chapter on Methods for the Preparation and Sterilization of Glassware, Dr. Morton Klein for the preparation of a new chapter on Diagnostic Virologic Methods, Dr. Edwin S. Gault and Mr. Anthony J. Lamberti for revising the chapters on Methods for Parasitologic Examinations of the Feces and Methods for Parasitologic Examinations of the Blood and Tissues, Dr. Israel Davidsohn for revising the chapter on Methods for Conducting Hemagglutination and Blood Grouping Tests and to Dr. William N. Campbell for rewriting the chapters on Methods for the Microscopic Examination of Tissues and Methods for the Preservation of Gross Tissue Specimens. We are deeply appreciative to the publishers, Appleton-Century-Crofts, Inc., for permission to revise this edition so thoroughly, and for their unvarying courtesy and efficiency. We are also indebted to Mrs. Edna M. Kershaw for secretarial services.

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# APPROVED
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# CONTENTS





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# Part One
# GENERAL LABORATORY METHODS

# 1
# THE MICROSCOPE AND METHODS OF MICROSCOPY

## EQUIPMENT

The microscope is among the most essential and most frequently used instruments in the laboratory. An instrument of recent manufacture is always desirable. There is no choice among microscopes insofar as the optical systems are concerned, but a particular type of standard design may be preferred. Their general construction is shown in Figure 1. They should be handled and carried only by the arm H in order to avoid strain on the coarse and fine adjusting mechanisms. The *monocular* types of microscopes are more commonly used because they are least expensive. The *binocular* microscopes, however, are more desirable because they produce less fatigue when used over longer periods of time (Fig. 2).

The *optical system* of a microscope consists of (1) the objective or lens which is compound, *i.e.* made of a series of lenses, (2) the ocular or eyepiece which further magnifies the image, and (3) the substage condenser which concentrates light on the object as it rests upon the stage, thereby increasing the illumination.

**Objectives.** Objectives are named by their equivalent focal length; also according to their construction or manner of use, as (1) dry objectives, (2) immersion objectives, (3) achromatic objectives, and (4) apochromatic objectives.

Dry Objectives. These are simple with air alone between the front lens and the coverglass of the preparation.

Immersion Objectives. Some are so constructed that water must be placed between the front lens and the coverglass for the objective to function properly. Others require more illumination than can be delivered by the condenser with air space or water between lens and objective. Cedar wood oil, which has the same refractive index as glass, must be placed between the front lens and the coverglass and between the glass slide and the condenser. These are the well-known oil immersion objectives. They will not permit full illumination or clear resolution without being immersed in oil. The common practice of using paraffin oil because of its being less sticky is not recommended.

Achromatic Objectives. These are objectives in which the image is free from the rainbow colors. A simple or single lens does not bring light of different wave lengths (different colors) to a common focal point. The light of shortest wave length (the blue-violet end of the spectrum) is bent or refracted most by a lens and comes to a focus nearer the lens than light of a longer wave length (red end of the spectrum) which rays are bent or refracted less. This means that the image of a colored object will not be in sharp focus for all colors. The phenomenon is called *chromatic aberra-*

*tion.* It may be corrected by placing a second, concave, flint glass lens behind the primary convex, crown glass lens. The use of fluorite or fluorspar in the second lens permits a higher degree of correction. Achromatic objectives are corrected for two colors. They are also corrected at the same time for *spherical aberration.*

Apochromatic Objectives. These are corrected for spherical aberration and for three colors and are, therefore, more desirable for photographic purposes.

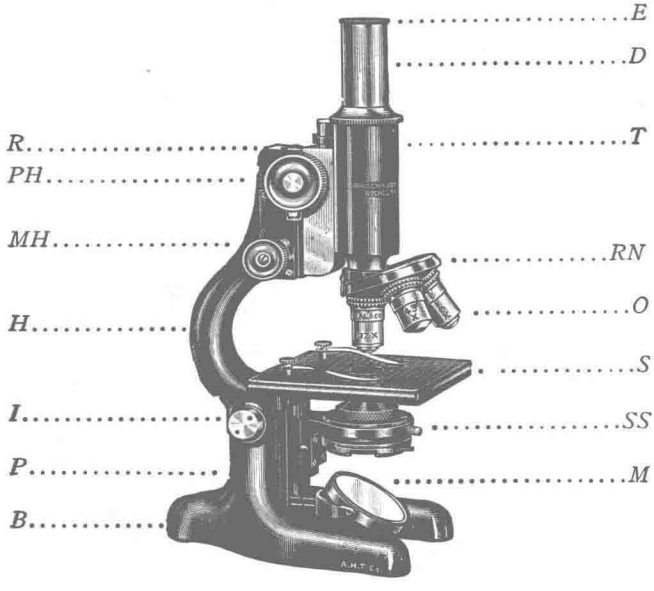


Fig. 1. A satisfactory type of microscope.

*E* is the eyepiece, of which two or three are usually furnished for varying degrees of magnification.
*D* is the draw tube, which is calibrated and should always be drawn to 160 or any other length recommended by the manufacturer.
*T* is the body tube.
*RN* is the revolving or triple nosepiece carrying the objectives.
*O* is one of the usual three objectives.
*R* is the rack upon which the tube is raised or lowered.
*PH* is the pinion screw for coarse adjustment.
*MH* is the micrometer screw for fine adjustment.
*H* is the handle.
*S* is the stage.
*SS* is the substage carrying the Abbe condenser with diaphragm.
*M* is the mirror with plane and concave surfaces.
*I* is the inclination joint for using the microscope in an inclined position.
*P* is the pillar.
*B* is the base, which should be large and solid.

As a general rule there should be three objectives on a triple, revolving nosepiece, namely, a 16 mm., a 4 mm. and a 1.9 mm. (oil immersion).

**Oculars.** Oculars are usually designated by their magnifying power, as 5X, 7.5X, or 10X. It is their function to pick up the image formed by the objective and enlarge it further. Thus the degree of magnification of an object visualized may be determined by multiplying the magnification power of the ocular by the magnification number of the objective. (Tube length must be properly adjusted.) Oculars are also designated according to their construction. *Huygenian oculars* are the least expensive and those

most commonly used. They do not possess the correction of better forms for color or flatness of field. *Compensating oculars* are overcorrected so as to further reduce chromatic and spherical aberration of an objective. They should always be used in conjunction with apochromatic objectives.

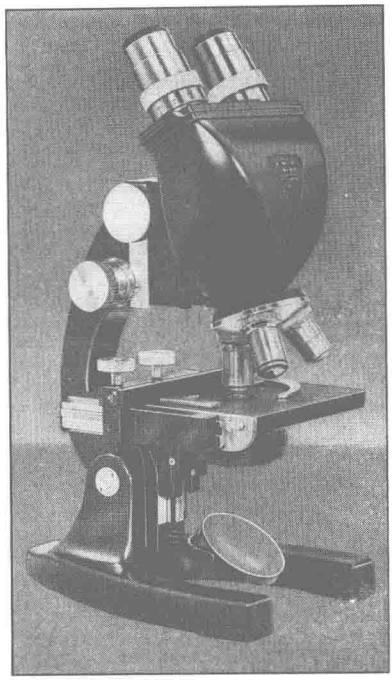

Fig. 2. Binocular microscope.
(Courtesy of Bausch and Lomb Optical Company.)

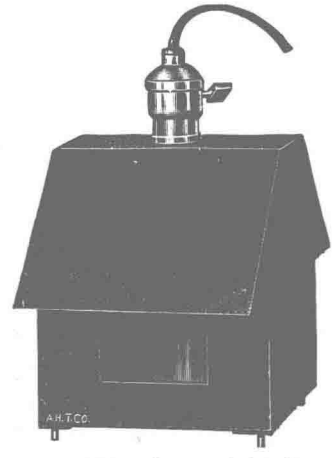


Fig. 3. Micro lamp, chalet form.

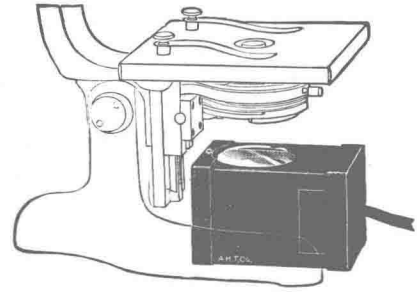

Fig. 4. Micro lamp, substage form.

**Magnification.** The magnification of any combination of objectives and oculars may be obtained by multiplying the magnification of the objective by that of the ocular. The magnification given by different combinations of objectives and oculars is as follows:

| Objectives | Oculars | |
|---|---|---|
| | 6.4 × | 10 × |
| 16 mm. (10 ×) | × 64 | × 100 |
| 4 mm. (43 ×) | × 275 | × 430 |
| 1.9 mm. (95 ×) | × 610 | × 950 |

**Substage Condensers.** These are constructed usually of two lenses for the purpose of concentrating light upon the object as it rests upon the stage of the microscope.

They thereby increase the illumination. They are commonly of the nonachromatic type named after their designer Abbe. Aplanatic, achromatic condensers are available on the more expensive microscopes.

**Illumination.** Daylight from a north window is the ideal source of illumination. However, it is so often unavailable that a more constant and dependable source is desired. Many forms of microscopic lamps are offered by manufacturers (Figs. 3, 4 and 5). The substage lamp or any lamp utilizing a 100 watt nitrogen filled tungsten bulb and a "daylight" glass filter provides a satisfactory source of light. A powerful source, such as the carbon arc or 6 volt ribbon filament bulb is required for darkfield illumination.

**Accessory Equipment.** A mechanical stage (Fig. 6) is advisable for blood counting or wherever a systematic search of an object is required. It is also well to be provided

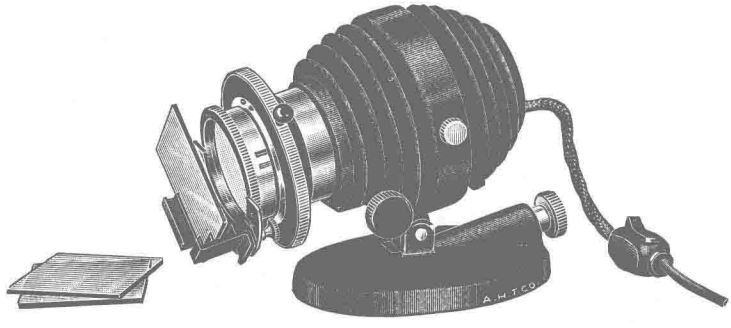

Fig. 5. Micro lamp (Spencer).
(Courtesy of Arthur H. Thomas Company.)

with a *hand lens* for the study of tissues (stained slides), sputa and feces prior to microscopic study. It gives an idea of the composition of the whole specimen and enables one to become better oriented when studying the smaller fields seen through the objective. In the selection of material from sputa or feces better samples will be obtained by the aid of such a lens. A pointer in the ocular is a great convenience. If the top of the lens of the ocular is removed, a diaphragm with a circular aperture will be found in the ocular tube. An eyelash or any fine hair may be cemented on the rim of this diaphragm by means of Canada balsam so that the free end of the hair is in the center of the aperture. With the top lens in place the hair should appear as a fine pointer of focus. If it is not in focus, move the diaphragm up or down until the hair is seen in sharp definition. This will serve to locate objects in the field. For the enumeration of small objects, such as blood platelets or reticulocytes, it is helpful to cut down the size of the field. This may be accomplished by cutting a disk of black paper or metal to such a size that it will fit snugly over the diaphragm in the ocular. Cut a small square about 5 or 6 mm. on a side in the center of this disk. It can be removed easily when not needed.

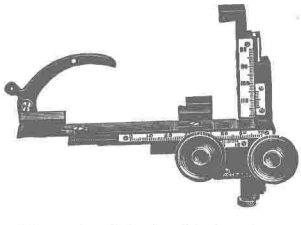

Fig. 6. Mechanical stage (Bausch and Lomb).
(Courtesy of Arthur H. Thomas Company.)

## USE OF THE MICROSCOPE

1. The microscope should rest upon a table or desk of such height that when seated before it one can comfortably look into the ocular without inclining the instrument. If seated before an open window or any other source of light the eyes should be so shaded that no other rays of light enter the eye but those from the microscope. If using a monocular form, both eyes should be kept open and one should learn to relax the accommodation of the unused eye. The ability to do this may be acquired by means of a black card. An opening is cut in a 3 by 6 inch card near the center of one narrow end, large enough to fit snugly over the upper end of the draw tube, after the ocular has been removed. The card then projects into the field of vision of the unused eye. If the surface is blackened it reflects little or no light into the eye. After a time the card may be discarded.

2. *The tube length should be adjusted.* The draw tubes of most microscopes are graduated so that the tube may be pulled out to the proper length for each objective. The tube length for which each objective is corrected is engraved upon it. For most lenses this length is 160 mm. Tube length is extremely important with oil immersion objectives. A variation of 5 mm. will reduce the perfection of the image. Increased or diminished tube length alters the initial magnification of the objective. The tube should be withdrawn with a spiral motion while supporting the coarse adjustment screw lest it be drawn from its bearing. It is returned to its former position with the same care lest the objective be driven forcibly against the stage or object.

3. The microscope lamp should be placed about 10 or 12 inches from the mirror and its rays directed upon the mirror (plane surface). Swing the condenser out of position or rack it down to its lowest point. Look into the tube at the back of the low power (16 mm.) objective and manipulate the mirror with both hands until the tube is evenly illuminated. The ocular is now replaced and the object to be examined is placed upon the stage.

4. Focus the low power objective by first viewing it from the slide and placing it down near (within 1 or 2 millimeters) the object. Then, looking into the ocular, rack the body tube upward by the coarse adjustment screw until the object comes into sharp focus. Illumination is now further adjusted by setting the condenser at the lowest point which gives even illumination yet brilliant sharp definition of the object. It is well to examine the object with the lower powers first as directed above, selecting fields to be studied with the higher magnification. Once the light is centered it need not be changed, but the position of the condenser must be changed for each objective and for each variety of object studied. It must be higher for dense objects and quite low for unstained or transparent objects such as urinary casts. The illumination just described is called *central illumination.* It is the type most frequently used. After a little experience one may place the objective and condenser in their approximate positions and adjust the mirror without removing the ocular. The centering is finally determined by focusing up and down upon the object. If the illumination is correctly centered, the image moves up and down, in and out of focus. If not correctly centered the image will move to one side or another and back again as the objective is raised or lowered. When the sharpest definition is desired, the aperture of the condenser must also be considered for each objective. After correct centering of the light and focusing of the condenser and objective, the ocular is removed and one looks at the back of the rear

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