A Laboratory Guide in

VIROLOGY

FIFTH EDITION

CHARLES H. CUNNINGHAM

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by

CHARLES H. CUNNINGHAM
B.S., M.S., D.V.M., Ph.D.
Professor of Microbiology and Public Health
Michigan State University
East Lansing, Michigan

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PREFACE

A formal course in virology was first offered in the Department of Microbiology and Public Health, Michigan State University, in 1947. The present course is designed for a term of 10 weeks as a complete unit of 4 term credits. The weekly schedule consists of three lecture periods and two laboratory periods.

When the course was started, there was no published guide or manual suitable for student laboratory instruction and mimeographed outlines were prepared. Interest and encouragement by many friends and colleagues faced with the same situation of establishing courses at other institutions led to the first edition of "A Laboratory Guide in Virology" in 1948. Prior to revision for subsequent editions, assistance with the content of the guide has been sought from those who have used it for courses in virology. The recommendations offered have been utilized in so far as possible. The guide represents the experiences of the author and other instructors as to several avenues of approach for introduction of students to some of the technics and procedures employed in virology. The exercises are suitable for advanced undergraduate and graduate students in the medical and biological sciences. No pretense is made at an exhaustive review of all possible procedures. It is not intended that the exercises are standardized to the satisfaction of all instructors. The exercises have merit and are flexible enough for utilization under a variety of circumstances.

Fundamental virology is applied in studies of infectivity, pathogenesis, and serologic and immunologic responses to afford a foundation for advanced study and to supplement lecture material. Further characterization on morphologic and biophysical bases, and the effect of environmental influences, is afforded by appropriate exercises.

The primary purpose of the guide is student instruction. It is not designed to be a handbook of virology. Each section has a text for orientation with the subject. Review questions are planned to stimulate the student to seek further information and to become acquainted with some of the literature pertaining to virology. A number of tables are included. Some tables are for data from the exercises. Others are to be filled out from specific assignments.

If the objectives of the guide and of the courses are accomplished, the student should have a valuable source of information on virology. The procedures can be readily adapted to the diagnosis and investigation of viral infections and to the formulation and pursuit of research problems.

ACKNOWLEDGEMENTS

During the preparation of "A Laboratory Guide in Virology", information was freely sought and permission was given to reproduce some previously published material. The American Instrument Co., Inc. granted permission to use Figure 1. The Spinco Division of Beckman Instruments, Inc. granted permission to reproduce Figures 6 and 7 and the text. Figures 8 and 9 and the text were provided by the Perkin-Elmer Corporation. G. T. Dimopoullos supplied Figure 10. The Radio Corporation of America provided Figure 11 and the text. Figures 12, 13, 14 and Table 35 and much of accompanying text were reproduced through the courtesy of W. R. Bryan and The New York Academy of Sciences. Table 25 was reproduced through the courtesy of H. R. Cox. The Williams and Wilkins Company granted permission to use Order Rickettsiales. C. E. Van Rooyen provided Order Virales, Suborder III. Table 41 was reproduced through the courtesy of J. F. Crow and the Burgess Publishing Company. Table 43 by D. J. Finney et al. was reproduced through permission granted by The Journal of General Microbiology. The National Research Council of Canada gave permission to use Tables 44, 45, 46, and 47 by W. R. Lockhart.

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INSTRUCTIONAL INFORMATION

Newcastle disease virus has been selected as the model for the majority of the exercises because of the wide variety of responses and means by which many properties of viruses can be studied in the laboratory. This virus has received attention by many investigators and it is widely reported in the literature. Students can enrich their findings in the laboratory with the results obtained by others in advanced studies of virology.

Instructors may wish to substitute viruses maintained in their individual laboratory. Several different viruses could be used equally well without significantly changing the procedure for the exercises which are offered as a guide for laboratory work.

Instructors may wish to select only certain exercises or to modify somewhat the procedures. For convenience when completely different exercises may be desired, several sheets for supplemental exercises are included. The student can use these and have all exercises in one bound book.

Some exercises can be used to good advantage either individually or collectively as demonstrations by the instructor. Others afford individual or group participation by students with assignments of certain exercises, or portions of an exercise. Visual aids will be found helpful for introduction to and interpretation of cytology, pathology, and other areas. Advanced planning is necessary to have all materials and reagents available at the specific time to integrate the exercises.

Current research projects, and clinical material in medical and veterinary schools, can be used to good advantage for supplementary instruction. These are excellent sources for stimulation of student interest and to introduce some of the technics, procedures, analyses, and interpretations employed in virology.

EXERCISES 2, 3 and 4 are designed to teach the student some of the procedures used for collection and processing of specimens for isolation of viruses from the infected animal and to emphasize the relationship between certain diseases and clincial signs. Too often, this very important biological relationship is not given its due consideration in beginning courses in virology. EXERCISES 6 - 11 are designed to show that the virus contained in the specimen collected from the infected host can be transmitted to a susceptible host which in several instances is the natural host. Chickens have been selected for most of these exercises because of their ready availability and, more importantly, the viruses offer a wide range of responses that are basically interpretable as principles for many viruses and are not human pathogens with the possible exception of Newcastle disease virus. To demonstrate the infectivity of viruses for various hosts, it would not be feasible to use viruses that could be a source of infection for students.

EXERCISES 2, 3 and 4 readily can be used for demonstrations by the instructor if the facilities available and the size of the class do not warrant individual participation by the students. In this case, stock cultures of the viruses could be used for subsequent exercises in which reference is made to EXERCISES 2, 3 and 4 for source material.

REQUIREMENTS

1. Students will be expected to be familiar with the procedures for each exercise at the beginning of the laboratory period. A schedule for the exercises will be posted.
2. Students will work independently unless other assignments are made.
3. In some exercises the laboratory work will consist of parts assigned to either individuals or groups, but each student will be responsible for complete knowledge of the entire exercise.
4. Students will be expected to work carefully and to observe the proper technics for prevention of personal infection, transmission of infection to animals and contamination of sterile equipment and materials. Vaccination against certain virus diseases will be required of all students as an added precaution and for use of serum in serologic tests.
5. The tables are to be completed with observations and data from the respective exercises and information from specific assignments.
6. All review questions are to be answered as laboratory assignments. It is recommended that the questions be typed and placed in a suitable folder.
7. The guide and the review questions will be collected at certain intervals during the term and graded.

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EQUIPMENT

The proper selection and use of laboratory equipment is an integral part of the successful performance of an experiment regardless of whether the experiment is an exercise in the student laboratory or a portion of a research program. A good quality piece of laboratory equipment is a precision instrument, a working tool of the laboratorian.

Efficiency in technical operations demands an adequate supply of equipment. To expedite laboratory work, all equipment should be kept in good repair, properly prepared and identified, and stored in an orderly manner for immediate use at all times. Each procedure should be carefully planned and all required equipment should be assembled and arranged for the convenience of the operator.

SELECTION, USE AND STERILIZATION OF EQUIPMENT

Each student will be supplied at the beginning of the term with chemically clean equipment sufficient for individual work outlined in the exercise. Other equipment and accessory materials will be available as the occasion demands. Each student will be responsible for the proper care of the equipment assigned to him.

Since much of the work in the exercises requires the use of sterile equipment, the student must observe aseptic precautions in the manipulation of equipment for avoidance of personal infection. All equipment must be sterilized immediately following use so that it may be handled without danger of personal infection when it is cleaned and prepared for future use.

INDIVIDUAL EQUIPMENT

2 - 1 cc. tuberculin syringes, graduation interval, 0.01 cc.
1 - 5 cc. Luer syringe, graduation interval, 0.2 cc.
2 - 27 gauge, 1/2 inch hypodermic needles
1 - 20 gauge, 1 inch hypodermic needle
4 - 1 ml. serological pipettes, graduation interval, 0.01 ml.
4 - 2 ml. serological pipettes, graduation interval, 0.1 ml.
1 - 5 ml. measuring pipette, graduation interval, 0.1 ml.
2 forceps, curved sharp points, length 115 mm.
2 forceps, eye
2 Bard-Parker knives
2 scissors, fine point
1 teasing needle
1 mortar and pestle, porcelain, outside diameter, 80 mm.

Cover glasses, capillary pipettes, culture tubes, cotton, gauze and other miscellaneous equipment, media and reagents will be supplied at the appropriate time for the exercise.

SELECTION

The student should be familiar not only with the mechanics of operation of a piece of equipment but should also be cognizant of the reasons for selection of the equipment for a particular purpose. While the use of calibrated glassware in the exercises of this

course is limited generally to pipettes and syringes, there are certain fundamentals which must be considered when selecting these instruments for a particular procedure. Pipettes and syringes are similar in that both are glass tubing calibrated for volumetric measurement of liquids, and they should be selected for accuracy of total capacity and increments of the fundamental graduation intervals.

PIPETTES

Pipettes are designated as "volumetric" or "transfer", "measuring" and "serological". Volumetric or transfer pipettes have but one graduation interval, the nominal capacity, and can be used only for transfer or measurement of one volume. Pipettes with subdivisions of the nominal capacity are designated as measuring pipettes or serologic pipettes, and the main lines or marks of the larger units are long and partially or completely encircle the pipette. The fundamental graduation interval lines are short to facilitate rapid reading of the meniscus.

Pipettes are calibrated at 20 C on the basis of the true or metric liter and upon the one-thousandth part of the liter or milliliter (ml.).

Glass volumetric apparatus conforming to certain high requirements of the National Bureau of Standards specifications for accuracy is described as CLASS A and is marked with "A". Certified CLASS A apparatus is individually tested at the Bureau and bears its stamp which consists of the letters "NBS" and the year of the test, etched on the apparatus.

The tolerances for CLASS A pipettes and for those not designated CLASS A are listed in Table 1.

Pipettes not designated CLASS A are calibrated to an allowable tolerance twice that specified by the Bureau for CLASS A pipettes and are recommended for routine and general laboratory work where the accuracy of CLASS A pipettes is not required. See Table 1.

For measurement of a given volume of liquid it is more desirable and accurate to select a pipette with a capacity equal to that volume than it is to select a pipette with a capacity less than that volume and make multiple measurements, i.e., 10 ml. could be measured more accurately with a 10 ml. pipette than with a 5 ml. pipette used twice or with a 2 ml. pipette used 5 times, etc.

Likewise, there are certain practical limitations of minimum volumes to be measured with a pipette of a given capacity. Table 2 is offered as a guide.

Table 1 Tolerances for pipettes CLASS A

Volumetric or Transfer			Measuring		
Nominal Capacity, ml.	Tolerance ± ml.	Per cent Tolerance ±	Nominal Capacity, ml.	Tolerance ± ml.	Per cent Tolerance ±
1	0.006	0.6	1	0.01	1.0
2	.006	.3	2	.01	.5
3	.01	.33	5	.02	.4
4	.01	.25	10	.03	.3
5	.01	.2	25	.05	.2
10	.02	.2			
15	.03	.2			
20	.03	.15			
25	.03	.12			
50	.05	.1			
100	.08	.08			
200	.10	.05			

Table 1 (continued) Not designated CLASS A

Volumetric or Transfer			Measuring		
Nominal Capacity, ml.	Tolerance ± ml.	Per cent Tolerance ±	Nominal Capacity, ml.	Tolerance ± ml.	Per cent Tolerance ±
1	0.012	1.2	0.1	0.005	5.0
2	.012	.6	0.2	.008	4.0
3	.02	.66	1	.02	2.0
4	.02	.5	2	.02	1.0
5	.02	.4	5	.04	0.8
10	.04	.4	10	.06	.6
15	.06	.4	25	.10	.4
20	.06	.3			
25	.06	.24			
50	.10	.2			
100	.16	.16			
200	.20	.1			

Table 2 Selection of measuring and serologic pipettes

Total Capacity, ml.	Graduation Interval, ml.	Amounts to be Measured, ml.	Minimum Amounts, ml.
0.2	0.01	0.01 - 0.2	0.01
1	.01	.20 - 1.0	.05
2	.10	1.0 - 2.0	.10
5	.10	2.0 - 5.0	.20
10	.10	5.0 - 10.0	.50
25	.10	10.0 - 25.0	1.00

SYRINGES

Syringes are calibrated on the basis of the cubic centimeter (cc.) and they do not have National Bureau of Standards specifications as do pipettes. The ml. of pipettes and the cc. of syringes are used synonymously in general practice but they are not equal, although the difference is insignificant for practical purposes. At 20 C 1 ml. is equivalent to 1.000027 cc.

On syringes the main lines or marks of the large units are long and the fundamental graduation interval lines are short.

For measurement of a given volume of liquid with a syringe, the same general principles as those for pipettes apply. Table 3 is offered as a guide.

Hypodermic needles for syringes are available in different gauges and lengths. Chromium plated and stainless steel needles are used most frequently. The most satisfactory and useful needles for this course are 27 gauge, 1/2 inch, and 20 gauge, 1 inch. The gauge or bore of the needle varies inversely as the number, i.e., the larger the number the smaller the bore. The 27 gauge needle is particularly useful for inoculating avian embryos and for parenteral injections as the small size of the needle minimizes traumatic injury. The 20 gauge needle is useful for collection of extra-embryonic fluids as well as for certain parenteral injections and bleeding.

Table 3 Selection of syringes

Total Capacity, cc.	Graduation Interval, cc.	Amounts to be Measured, cc.	Minimum Amounts, cc.
Tuberculin Syringe			
0.25	0.01	0.01 - 0.25	0.01
0.50	0.01	0.25 - 0.50	0.05
1.00	0.01	0.50 - 1.00	0.05
2.00	0.05	1.00 - 2.00	0.25
Luer Syringe			
2	0.1	1 - 2	0.5
5	0.2	2 - 5	1.0
10	0.2	5 - 10	2.0
50	1.0	10 - 20	5.0
50	1.0	20 - 50	5.0

MORTARS AND PESTLES, FORCEPS

The 80 mm. diameter mortar has been selected because it is of a convenient size for grinding small pieces of tissue. Larger mortars are too unwieldly for small amounts of material.

Curved, sharp point forceps 115 mm. long are a convenient size and shape for manipulation,and dissection of avian embryos and organs and tissues. Straight, sharp point forceps and cover glass forceps with straight tips are also useful.

USE

PIPETTES

Pipettes should be carefully filled without any bubbles in the column of liquid. The bottom of the meniscus should be exactly on the graduation interval line before delivering the liquid from the pipette.

Volumetric or transfer pipettes should be held in the vertical position and the outflow should be unrestricted until the liquid reaches the upper end of the delivery tube. The tip should then be touched to the wet surface of the receiving vessel and kept in contact with it until emptying is complete. Measuring and serologic pipettes should be held in the vertical position, and after the outflow has ceased the tip should be touched to the wet surface of the receiving vessel to complete emptying. No drainage period is allowed. Pipettes which deliver their total capacities when the small amount of liquid remaining in the tip is to be blown out are marked with a single or double frosted band at the top of the pipette.

Immediately after pipettes are used they should be placed in a "discard" container with a layer of cotton on the bottom. The practice in this course will be to have the container filled with a solution of either hexametaphosphate, Tetra-D or Hemo-Sol as a cleaning compound. The cylinder should then be placed in the steam chamber and sterilized. Two objectives are accomplished by this method: sterilization and cleaning of the pipettes. After the pipettes have cooled the cotton plugs should be removed, the pipettes placed tip up in the pipette washer and thoroughly washed with hot water. They should then be

removed from the washer, drained, thoroughly washed with distilled water, and put aside to drain and dry. Glassware should not be left in any of the cleaning compounds for extended periods of time.

A 5 to 10 percent solution of trisodium phosphate may also be used a cleaning solution in the cylinder, but NBS certified glassware should never be subjected to hot alkali. Non-coagulating disinfectants may be used for chemical sterilization prior to cleaning.

If glassware cannot be cleaned by other means, acid cleaning solution may be used. The solution may be prepared as follows:

Commercial sodium or potassium dichromate ..... 120 gm.
Tap water..... 60 ml.
Dissolve by heating and stirring. Cool. This constitutes a saturated solution.
Transfer 35 ml. of the saturated solution to the glass container to be used. With constant stirring add
Commercial sulphuric acid..... 1,000 ml.

Stock acid cleaning solution should be stored in glass stoppered bottles. A glass cylinder with cleaning solution is a convenient receptacle for pipettes. Fats and greases should be washed from glassware before putting it in the cleaning solution. All glassware should be thoroughly washed after immersion in the cleaning solution to remove all traces of the acid. Clean glassware drains evenly without drops of water clinging to the glass. When the solution starts to turn green from usage, it should be discarded.

It must be remembered that with pipettes graduated to the tip the slightest damage to the tip will render the pipette useless for the purpose for which it was constructed. Handle all pipettes with extreme care.

SYRINGES

Syringes should be carefully filled without any bubbles in the column of liquid. Should bubbles be present, hold the syringe in a vertical position with the needle uppermost and pull the plunger down until the level of the liquid is below the tip of the syringe. The needle should be wrapped in sterile absorbent cotton or cotton soaked with alcohol and the plunger pushed upward until all the air is expelled from the needle. The cotton should be placed in a "discard" tray for sterilization.

Immediately after using, the needle should be removed from the syringe, the syringe dismantled, and all placed in a "discard" tray containing cleaning compound. The tray should then be placed in the steam chamber and sterilized. An electric instrument sterilizer is convenient for this procedure. After sterilization the syringes and needles should be thoroughly washed with distilled water and put aside to drain and dry. Needles should be examined and sharpened if necessary. The bore of the needle should be cleaned with a stylet each time the needle is used. Syringes may require an occasional immersion in acid cleaning solution.

MORTARS AND PESTLES, FORCEPS

Mortars and pestles are used to grind tissues with an abrasive for preparation of tissue suspensions. It is more desirable to hold the mortar at an angle of about 60° from the table top when grinding tissue than to work with the mortar flat on the table top. With the mortar on a slant there is less possibility of contamination of the tissue from the hands and air than when the operator works directly over the mortar.

Immediately after use, mortars, pestles and forceps should be placed in a "discard" tray and sterilized, washed and dried.