Date: April, 1957

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Institution: Oklahoma A. & M. College

Location: Stillwater, Oklahoma

Title of Study: Bacteriology Experiments Designed For Use in a Course of High School Biology

Number of Pages in Study: 43

Under Direction of What Department: Natural Science

Nature of Study: This report deals with the neglect of intergrating bacteriology in high school biology. Certification requirements for high school biology teachers in the public schools of the various states are presented in tabular form. Also included are experiments which should give a biology student the basic understanding of this science, and the fundamental manipulative skills in the handling of the bacteriologists laboratory tools.

Findings and Conclusions: The certification requirements for biology teachers in secondary schools are vague and inadequate in most states. There is no specific requirement of preparation in bacteriology for certification in any state. The lack of teacher preparation reflects the neglect of the presentation of bacteriology in high school courses.

Use of the Study: The experiments are offered as an aid to biology teachers with little or no preparation in bacteriology.

Adviser's Approval James H. Zant

BACTERIOLOGY EXPERIMENTS DESIGNED FOR

USE IN A COURSE OF HIGH SCHOOL BIOLOGY

1956-1957

By

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Bachelor of Science

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1951

Submitted to the faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
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for the degree of
MASTER OF SCIENCE
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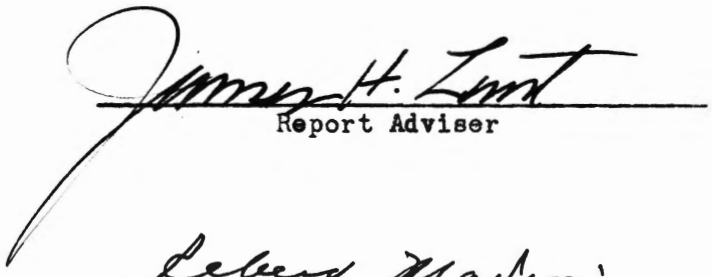
Report Approved:



Report Adviser

Dean of the Graduate School

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Special acknowledgement is due my wife, Betty Martin, who rendered invaluable aid as typist.

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CHAPTER I

INTRODUCTION

Statement of the Problem. There is a tendency among high school biology teachers to neglect the subject of bacteriology in their teaching. This study was made in hope of finding an answer for this neglect, and to offer suggested bacteriology experiments as an aid in partial elimination of this neglect.

Industrial bacteriology since World War II, has become increasingly more important. It is the writer's contention that we, as educators, are not making our students aware of the great possibilities of this field of science. In the opinion of most educators, biology on the high school level, should create an awareness and some understanding of the students environment. Certainly in his study of living things, should be included a study, or an introduction to the study, of the most abundant of all living organisms. Few factors influence our environment more than do the microrganisms. Again we, as educators, are failing to meet our student's needs.

Methods Used in Partial Solution of This Problem. 1. In trying to arrive at an answer for the neglect, a study was made on the certification requirements for high school biology teachers in the various states. A teacher tends to teach only those things he has been taught, and in the manner he was taught. A course in high school biology will reflect the teacher's college biology preparation. 2. It was the writer's be-

lief that a collection of laboratory exercises in bacteriology on the high school level might prove useful to biology teachers. However, a search for such exercises proved fruitless. Therefore, there are included in this report, experiments written by the author based on ideas and opinions received from college laboratory manuels and practicing high school teachers.

Use of the Study. It is the hope of the writer that these experiments will be of value to the teacher who has had little or no training in bacteriology. Through the use of these experiments a high school student should become familiar with the field of bacteriology and some of the methods used in this field.

CHAPTER II

CERTIFICATION OF HIGH SCHOOL BIOLOGY TEACHERS

Certification as Pertinent to Study. A request was made to all of the Departments of Education, for the requirements for certification of biology teachers. All states responded with the exception of Arizona, California, and New York.

The requirements are wide and varied in this field. The tables on the following pages indicate each state's requirements for certification. In most cases those listed are the minimum requirements.

As the tables indicate, the average number of college semester hours of biology required, is about seventeen. The greatest number for any state was Indiana with forty. However, such courses as conservation are included in the forty hours. In many colleges conservation is taught as a social science.

The lowest number of semester hours of biology required, was six in West Virginia. Nebraska requires only eight; while Texas requires a preparation of twenty-four semester hours in the field of science. A teacher in Texas may teach biology in high school without one semester hour of biology.

Certification requirements may then be part of the answer to the question of why there is neglect in the presentation of a study of bacteriology in high school biology.

In the following tables "S.H." refers to semester hours.

Tables of Certification by State.

STATE	REQUIREMENTS FOR CERTIFICATION
Alabama	12 S.H. must include full year course in a Biological Science and a Physical Science.
Arizona	no response
Arkansas	24 S.H. (8 each in biology, chemistry, & Physics)
California	no response
Colorado	12 S.H. in science and 5 S.H. in biology.
Connecticut	15 S.H. in biology.
Delaware	18 S.H. in biology and at least 3 S.H. in each of the two related science fields.
Florida	15 S.H. in biology and if possible, one course in bacteriology.
Georgia	Major in biology.
Idaho	12 S.H. in biology.
Illinois	32 S.H. of science. (no mention of the number of hours of biology.)
Indiana	40 S.H.; (biology 3; botany 3; zoology 3; health and safty 3; physiology 3; conservation 3; and a physical science.)
Iowa	15 S.H. in biology. (zoology and botany)
Kansas	24 S.H. in science field with 6 S.H. in subject taught.
Kentucky	24 S.H. in biology.
Louisiana	12 S.H. of biology, 6 S.H. chemistry, and 6 S.H. physics.

STATE	REQUIREMENTS FOR CERTIFICATION
Maine	24 S.H. with major in biology or 15 S.H. with a minor in biology.
Maryland	18 S.H. in biology.
Massachusetts	18 S.H. in biology.
Michigan	24 S.H. in biology.
Minnesota	24 S.H. of biology.
Mississippi	24 S.H. in science with 12 S.H. in biology (including botany).
Missouri	24 S.H. in science with 15 in biology. (botany, zoology, anatomy, and bacteriology).
Montana	30 S.H. in biology.
Nebraska	15 S.H. in science field with 8 S.H. in biology.
Nevada	B.S. from approved college with a major in biology.
New Hampshire	18 S.H. in science field with 6 S.H. in biology.
New Jersey	18 S.H. (including biology, botany, & zoology).
New Mexico	24 S.H. in science.
New York	no response
North Carolina	12 S.H. in biology.
North Dakota	Major or Minor in biology from an approved school.

STATE	REQUIREMENTS FOR CERTIFICATION
Ohio	15 S.H. (basic courses in general biology, or zoology, and botany)
Oklahoma	12 S.H. of biology with work in two of the following: botany, zoology, and physiology.
Oregan	15 S.H. of biology.
Pennsylvania	Certification for biology is valid if certified in general science. (includes 12 S.H. in biology)
Rhode Island	15 S.H. of biology.
South Carolina	12 S.H. of biology.
South Dakota	B.S. with major in biology from an approved school.
Tennessee	18 S.H. in biology.
Texas	24 S.H. in science field.
Utah	12 S.H. in biology.
Vermont	24 S.H. in science including biology.
Virginia	12 S.H. in biology.
Washington	Major or Minor in biology.
West Virginia	6 S.H. in biology.
Wisconsin	15 S.H. in biology.
Wyoming	24 S.H. in biology.

Bibliography on Certification Data

Alabama Dept. of Education.
1953. Certification of Alabama Teachers. Bulletin no. 7. Outlook Publishing Company, Alexander City, Alabama, p 26.

Arkansas Dept. of Education.
1955. Summary of Certification Rules and Regulations. Unpublished Bulletin, p 3.

Colorado Dept. of Education.
1957. The Law Governing the Issuance of Certificates. Unpublished Bulletin.

Connecticut Dept. of Education.
1955. Teaching Certificates-Secondary. Unpublished Bulletin.

Delaware Dept. of Public Instruction.
1955. Certification Rules and Regulations. Bulletin no. 7-55. Unpublished Bulletin, p 9.

Florida Dept. of Education.
1951. Excerpts from Florida Requirements for Teacher Education and Certification. Unpublished Bulletin.

Georgia Dept. of Education.
1952. Minimum Requirements for Teacher's Professional Certificates. Unpublished Bulletin, p 2.

Idaho Dept. of Education.
1955. Certification of Teachers and Administrators. State of Idaho, Boise, Idaho, p 14.

Illinois State Teacher Certification Board.
1956. Circular Series A, no. 100 as Compiled by Luther J. Black. State of Illinois, p 34.

Indiana Dept. of Public Instruction.
No date. Indiana Teacher Certification Requirements. Bulletin no. 192, p 3.

Iowa Dept. of Public Instruction.
1954. Certification and Approval of School Personnel. Bulletin no. 31, State of Iowa, Des Moines, Iowa, p 23.

Kansas State Board of Education.
1955. Certificate Handbook. State of Kansas, Topeka, Kansas, p 26.

Kentucky Dept. of Education.
1956. High School Certification Circular. Unpublished Circular, p 3.

Louisiana Dept. of Education.
1956. Louisiana Standards for State Certification of School Personnel. Bulletin no. 746. State of Louisiana, Baton Rouge, Louisiana, pp 9-12.

Maine Dept. of Education.
1953. State Certification of Teachers. Unpublished Circular, p 1.

Maryland Dept. of Education.
1950. Requirements for Certification. Maryland School Bulletin, vol. XXI, no. 2. Maryland State Dept. of Education, Baltimore, Maryland, p 14.

Massachusetts Dept. of Education.
1956. Teacher Certification Requirements. Unpublished Circular.

Michigan Dept. of Public Instruction.
No date. Requirements-State Elementary and Secondary Certificates. Unpublished Bulletin.

Minnesota Dept. of Education.
1951. Regulations for the Certification of Teachers. Unpublished Circular.

Mississippi Dept. of Education.
1956. Requirements for Teacher's Certificates. Unpublished Circular.

Missouri Dept. of Education.
1950. Standards for Certification Teachers in Secondary Schools. Unpublished Bulletin, p 3.

Montana Dept. of Public Instruction.
1954. Secondary Certification. Form letter from the office of Harriet E. Miller, State Superintendent of Public Instruction.

Nebraska Dept. of Education.
1956. Approval and Accreditation of Nebraska Public Schools. Nebraska Dept. of Education, Lincoln, Nebraska, pp 16-17.

Nevada Dept. of Public Instruction.
1956-1957. Teacher's Certification Requirements. Nevada State Dept. of Education, Carson City, Nevada, p 5.

New Hampshire Dept. State Board of Education.
1948. Regulations for the Certification of Secondary School Teachers. Unpublished Circular no. 2853.

New Jersey Dept. of Education.
No date. Unpublished Bulletin on Secondary Certification.

New Mexico Dept. of Education.
1956. Rules and Regulations Governing Certification. State Dept. of Education, Santa Fe, New Mexico. p 8.

North Carolina Dept. of Public Instruction.
1956. Teaching in North Carolina. Publication no. 306. State Superintendent of Public Instruction, Raleigh, North Carolina, p 21.

North Dakota Dept. of Public Instruction.
No date. Unpublished Circular on secondary certification.

Ohio Dept. of Education.
1955. Laws and Regulations Governing the Certification of Teachers. State of Ohio, Columbus, Ohio, p 22.

Oklahoma Dept. of Education.
1955. Annual High School Bulletin. Bulletin no. 113-D. State of Oklahoma. Dept. of Education, Oklahoma City, Oklahoma, p 13.

Oregan Dept. of Education.
1956. Secondary Teachers Certificates. Unpublished Pamphlet, p 8.

Pennsylvania Dept. of Public Instruction.
1956. Certification of Secondary School Teachers of Academic Subjects. Unpublished Circular.

Rhode Island Dept. of Education.
No date. Requirements for the Secondary Certificate. Unpublished Circular.

South Carolina Dept. of Education.
No date. Circular on certification.

South Dakota Dept. of Public Instruction.
1954. Secondary School Standards. Bulletin no. 21B, State of South Dakota. Dept. of Public Instruction, Pierre, South Dakota, pp 30-31.

Tennessee State Board of Education.
1955. Regulations for Certification of Teachers. Unpublished Phamphlet, p 15.

Texas Education Agency.
No date. Requirements of Teacher Education Program for Permanent Provisional Certificate. Unpublished Circular.

Utah Dept. of Public Instruction.
1951. Certification Requirements. Utah State Dept. of Public Instruction, Salt Lake City, Utah, p 18.

Vermont Dept. of Education.
1957. Circular on certification requirements. Unpublished.

Virginia State Board of Education.
1955. Certification Regulations for Teacher. State Board of Education, Richmond, Virginia, pp 13-21.

Washington State Board of Education.
1956. Teacher Education. Unpublished Pamphlet.

West Virginia Dept. of Education.
1956. Minimum Standards and Requirements for the Preparation and Certification of Public School Personnel. Division of Teacher Preparation and Professional Standards, Charleston, West Virginia, pp 8-9.

Wisconsin Dept. of Public Instruction.
1957. Certification Requirements, Mimeographed, Unpublished Circular.

Wyoming Dept. of Education.
1956. Certification Regulations. Unpublished Bulletin.

CHAPTER III

OTHER POSSIBLE FACTORS FOR THE NEGLECT OF BACTERIOLOGY IN HIGH SCHOOL BIOLOGY

It is well known that a great majority of our high schools do not provide the science departments with adequate equipment to carry on a science program to meet the demands of the students of today. However, with a little ingenuity, the high school teacher could do a better job with the materials at hand.

Our colleges, year after year, send science teachers into the public schools, "spoiled" by the use of almost a limitless supply of equipment. When these teachers start the laboratory work of their own classes, they are sometimes at a loss because of the lack of material on hand and the lack of funds with which to secure more equipment. The colleges do not, and possibly can not, teach the student to improvise. As a result, until the high school teacher learns to improvise, his classes suffer from the lack of proper laboratory equipment.

Lack of the knowledge of improvision and the lack of equipment are possibly the two main reasons why bacteriology is not intergrated in most courses of high school biology.

CHAPTER IV

BACTERIOLOGY EXPERIMENTS WRITTEN ON THE HIGH SCHOOL LEVEL

Foreword on the Experiments. These following experiments are by no means, the only ones to be used in high school biology. It is the writer's wish only to give suggestions as to what might be done.

The lack of equipment has been kept in mind at all times. Helpful suggestions for improvision have been included. In most cases ordinary household articles, or at least the basic necessities of any high school laboratory, are employed.

List of Materials Needed

1 One lb. Nutrient Broth from any supply house.

2 One lb. Brilliant Green Bile 2 % from any supply house.

3 ¼ lb. Lactose U.S.P. from any drug store.

4 One lb. Agar Agar U.S.P. No. 1 Powder.

5 Petri dishes (not less than 12)

6 Test Tubes, 6 inch, Pyrex.

7 Test Tubes (or very small bottles) to be used as fermentation tubes.

8 Sterile Cotton, 1 lb.

9 Prescription Bottles VI oz. to be used for water blanks. (Obtained from any drug store)

10 Common laboratory equipment such as a balance, graduates, beakers, flasks, etc. (These are not necessary as one might substitute household articles.)

Preparation of Culture Media. Probably the most desirable method for purchasing media in high school would be the plan of buying the dehydrated media and powdered agar separately. This should add more flexibility to the course.

A Preparation of Nutrient Broth.

1 This formula makes 500 ml. of broth. If a larger or smaller amount is desired, the weights of the nutrient and volume of water may be changed proportionally.

2 Weigh four grams of nutrient broth into a large flask. Mix in slowly, 500 ml. of water. (Distilled water is preferred.)

3 Place flask over burner and dissolve the nutrient.

4 After contents have gone into solution, cool and pour into clean test tubes.

5 Stopper broth tubes with cotton plugs and sterilize.

B Preparation of Nutrient Agar.

1 Exactly the same procedure is followed in the preparation of nutrient agar as in the preparation of the broth. However, here we add 1.5 - 2.0% agar to nutrient broth. Care must be taken in dissolving this mixture as agar is present. Heating should be done in a water bath or double boiler.

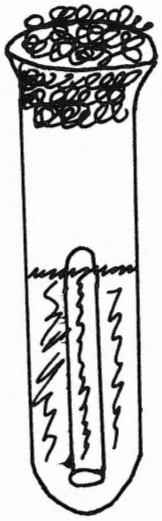
C Preparation of Lactose Broth and Fermentation Tubes.

1 Weigh 4 grams of nutrient broth into a flask. Add 2.5 grams of lactose and 500 ml. of distilled water.

2 This mixture should be brought into solution by heating.

3 Place fermentation tubes in large test tubes. The solution should be placed in large test tubes and heated. After sterilization, tubes should resemble those pictured on next page.

4 The large test tubes are then stoppered with cotton plugs and sterilized.



(Smaller tube completely filled)

D Preparation of Brilliant Green Bile 2%.

1 This is to be used in fermentation tubes as was the lactose broth. The following chart [1] shows the desired relationship between the amount of inoculum used, the volume of medium used, and the weight of bacto-brilliant green bile 2% used per liter of water. (Formula (a) is used in the experiment in this report.)

Inoculum	Volume of Medium	Volume of Medium and Inoculum	Bacto-Brilliant Green Bile 2% used per 1000 ml.
(a) 1 ml. or less	10 ml.	10 ml.	40 grams
(b) 10 ml.	20 ml.	30 ml.	60 grams
(c) 10 ml.	30 ml.	40 ml.	53 grams

E If it is Desirable to Make Your Own Nutrient Agar Follow This Formula.

(a) 5 grams of peptone per liter.

(b) 3 grams of beef extract per liter.

(c) 15 to 20 grams of agar per liter.

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1 No Name, Difco Manual of Dehydrated Culture Media and Reagents, Detroit, Michigan: Difco Laboratories Inc., 1953, p 38.

Methods of Sterilization of Media:

A Place tubes in a deep enough sauce pan so that when the tubes are in an upright position a lid may be placed on the pan. Pour approximately one-half cup of water into the pan. Place lid on the pan and bring the water to a boil. Keep boiling for twenty minures. Sterilization should be accomplished by repeating this procedure for three successive days for twenty minutes each day. Hold at room temperature between heatings. Check for sterility by incubation for 48 hours after last heat treatment and discard any showing turbidness.

B Place test tubes containing media in a pressure cooker. Pour one-half cup water into cooker. Sterilization should be accomplished by heating cooker until the pressure is fifteen pounds and holding this pressure for twenty minutes.

C Either method may be used.

Methods of Sterilization of Petri Dishes and Other Pyrex Glass Dishes:

A The glass ware may be sterilized in the pressure cooker as above or by heating in an oven at 170°C. or 340°F. for two hours.

Methods of Keeping Material Sterile:

A Test tubes of nutrient broth, nutrient agar, etc. may be kept for an indefinite period of time if the cotton stoppers are placed in the tubes properly and securely and if the cotton is kept dry. Petri dishes can be sterilized in coffee cans and kept sterile indefinitely.

Preparation of Agar "Slants":

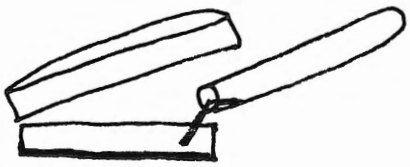
A Place sterilized agar tubes in a position so that when the agar solidifies it will be slanted as pictured below.

EXPERIMENT NUMBER ONE

OBJECT: To determine the ommipresence of bacteria and learn something of the conditions most favorable for their growth.

APPARATUS: Seven test tubes of previously sterilized nutrient agar (pH 6.0 - 7.4) Note the pH of the agar need not be adjusted for if instructions are followed the pH will fall in this range. Six sterile petri dishes and one unsterile dish.

PROCEDURE: Liquify the agar by heating in boiling water. Then cool the agar to 52°C. Remove the cotton stopper, flame the top of the test tube over a bunsen burner and pour the tube contents into a sterile plate. (The dish covers are lifted and the solution poured with the covers over the test tubes and dishes as to keep bacteria from coming in from the air.)



Replace lid as quickly as possible and allow agar to cool and solidify.

Keep one petri dish as a sterile control. Also pour sterile agar into non-sterile plate as a control.

Have one student uncover a petri dish in the class room for five minutes. Have two students uncover petri dishes

in the hallway as classes are changing. Have a student uncover a dish in a filing cabinet, being very careful not to stir the air and dust. Have one exposed in the cafeteria. If extra dishes are available, these may be placed in various places.

Plates should be properly labeled, inverted (as to keep condensation water off of the agar), and placed in a dark, warm, and humid place with the exception of one of the plates from the hallway. This plate should be placed in direct sunlight from an open window for thirty minutes with the cover removed. (A cardboard box kept at 90°F. by means of a light bulb may be used as an incubator. However, plates may also be inverted and incubated in the class room since the organisms are not pathogenic and will grow at 25°- 30°C. very nicely. Also, pigmentation and mold growth occure best at these temperatures.)

At the end of the first day record observations. Again after three days. Note the number of bacterial and/or mold colonies developing. Keep one plate with a large isolated colony for a later experiment.

Observe that mold colonies will appear "hairy". Color may be confined to the bacterial colony if the pigment is not water soluble, or diffuse into the agar medium if the pigment is water soluble.

RECORD OF OBSERVATIONS

Petri Dish	Colonies after 1 day			Colonies after 3 days		
	Bact.	Mold	Color	Bact.	Mold	Color
1						
2						
3						
4						
5						
6						
7						

QUESTIONS AND DISCUSSION

1 As shown by this experiment, what is one chief method of bacterial dispersion?

2 What are some of the conditions which favor the growth of bacterial colonies?

3 If bacteria are microscopic, how is it possible to see the bacteria on the petri dishes?

4 In what type of environment are bacteria most numerous?

5 Why were two petri plates used as controls? What is a control?

6 If a microscope is available, transfer portions of some colonies to a glass slide containing a drop of water using a needle that has been sterilized in a flame. Cover the water suspension with a cover slip. Examine under low, high dry and/or oil immersion lens.

EXPERIMENT NUMBER TWO

OBJECT: To give students some idea of personal health and cleanliness.

APPARATUS: Six petri dishes with nutrient agar as the medium. (Melt and pour agar as in experiment number one.)

PROCEDURE:

A Place a hair from a boy's head in an agar plate.

B Have a student streak one dish with his pencil. (one who habitually chews pencils)

C Have a student touch an agar surface with one finger. Have the student wash his hands with soap and water and touch the same finger to the other side of the agar surface. (Label carefully.)

D Have a student touch a finger to an agar surface. This time wash with water only and touch the other side of the agar surface. (Label carefully.)

E Allow a fly, ant, or roach to walk across one petri dish.

F Invert the plates and incubate in a warm, dark place. (Perferably at, or near 37°C.) Observe daily.

QUESTIONS AND DISCUSSION

1 Is it possible to be completely "germ free" during daily living?

2 Why is one not continually sick because of bacteria?

3 How could bacteria contribute to the presence of body odor?

4 Would it be just as good to wash without soap? Why?

EXPERIMENT NUMBER THREE

OBJECT: To see one means of control of bacteria.

APPARATUS: Four different kinds of antiseptics. Five agar slants. Petri dish with active colonies from demonstration number one or two. Piece of nichrome wire, from any electrical heating element, sealed in the end of a glass tube or melted into the end of an aluminum rod.

PROCEDURE: Sterilize the piece of nichrome wire by holding it in a flame. Touch one of the colonies in the petri dish from experiment number one or two with the wire. Very carefully tilt the agar slant and remove the cotton plug. Flame the top of the tube then streak the slant and replace the cotton plug. Streak each of the other slants in the same manner using the same colony if at all possible. Into four of the slants pour a little of a different antiseptic. Allow to stand for a minute. Pour off the antiseptic, replace the cotton plug and set in a warm, dark place for several days. Keep one as a control. After two or three days examine the results and record.

QUESTIONS AND DISCUSSION

1 Why streak all the slants from one colony?

2 Are all antiseptics equally powerful?

3 How is the strength of an antiseptic determined?

4 What is sterilization?

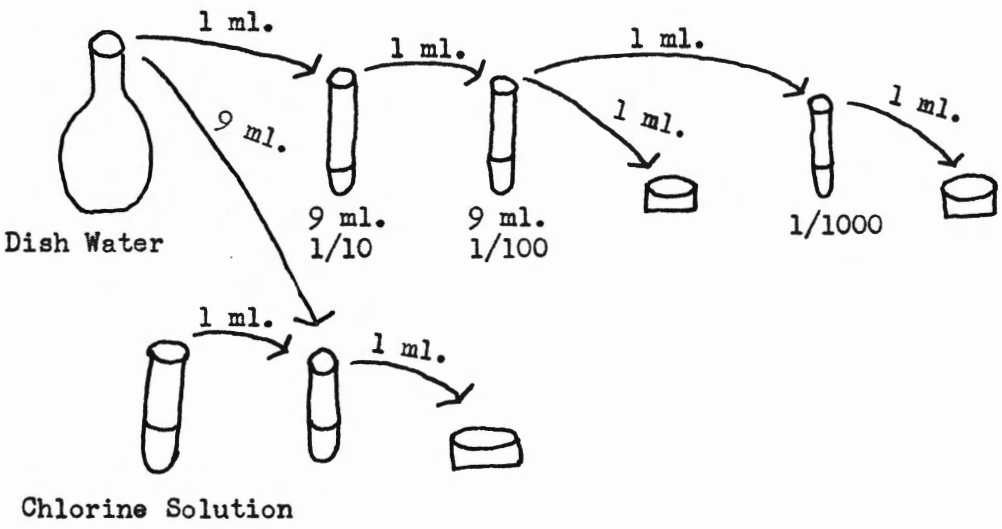
5 Why should any small cut be treated with an antiseptic?

EXPERIMENT NUMBER FOUR

OBJECT: To study the effects of a disinfectant used in dishwater.

APPARATUS: Samples of dish or rince water collected in sterile bottles from homes of students. Sterile water blanks. Bottles of previously sterilized distilled water. Three sterile petri dishes with nutrient agar. Disinfectant obtained from local resturant. 5 sterile test tubes. Sterile medicine droppers.

PROCEDURE: Mix sample of dish water thoroughly by shaking. Dilute dish water by scheme outlined on page 24. Transfer aseptically, 9 ml. of dish water to a sterile test tube. Add 1 ml. of the hypochlorite solution. (Chlorine solution should be mixed according to methods used by the restaurant.) Shake and allow to stand for 5 minutes. Transfer 1 ml. of the above to a sterile petri dish by means of a sterile medicine dropper, and pour in melted agar. Twenty drops from a medicine dropper equals 1 ml. (Agar should be at 50°C.) Rotate as to assure an even distribution. Place petri dishes in a dark, warm place for two to three days. Examine and record results.



QUESTIONS AND DISCUSSION

1 How should dishes and silverware in public eating places be washed and treated? Are there laws to this affect?

2 Name some diseases most likely transmitted through the medium of poorly washed dishes.

3 How should dishes be washed at home?

EXPERIMENT NUMBER FIVE

OBJECT: To learn something of the official procedure for testing water for human consumption.

APPARATUS: Water samples from various places, including water fountain, streams, pumps, and wells. One fermentation tube of lactose broth and one fermentation tube of brilliant green bile 2% for each water sample to be tested.

PROCEDURE:

A Shake the water sample and place one ml. of each sample in a 10 ml. lactose broth fermentation tube. Label each tube.

B Incubate the tubes at about 37°C. for 24 hours. If 10% or more gas is formed, as measured by the inner inverted tube, the water may be presumed contaminated and recorded as "Positive Presumptive".

C Inoculate one tube of brilliant green bile broth from each positive presumptive lactose tube by placing one ml. or less in the brilliant green bile tubes.

D Incubate these tubes in a dark place at about 37°C. Inspect after 19 to 24 hours. If fermentation has occurred, it is confirmed that colon bacteria are present in the water samples.

To confirm that E coli and not the soil bacteria, A aerogenes, is present or absent in the brilliant green bile, a confirmation test may be run using E.M.B. and Endo. agar plates. These media may be purchased at any biological supply house. A book, giving many selective media and tests to run using these media, may be had for the asking from Difco Laboratories, Detroit, Michigan.

QUESTIONS AND DISCUSSION

1 What is meant by a selective medium?

2 What precautions should be observed in collecting water samples?

3 What is one of the chief water borne diseases?

4 Why should water supplies be checked periodically? How are they checked?

5 What are indicator groups?

ALTERNATE EXPERIMENT NUMBER FIVE

OBJECT: To learn the importance of approved water systems by running bacteria counts on water samples from wells and other water supplies of the community.

APPARATUS: Water samples taken in sterile bottles from various places such as pumps, wells, streams, and from the school fountain. Nutrient agar plates. Sterile water blanks size 9 ml.

PROCEDURE: Place one ml. (20 drops) of one water sample in a 9 ml. water blank. Mix thoroughly by shaking. Take 1 ml. of this solution and place it in a 9 ml. water blank. This makes the dilution 1/100. One ml. of the 1/100 diluted solution should be placed in the petri dish. The agar should be melted in a water bath at 50°C. and poured carefully into a petri dish. Mix with a circular motion.

This procedure is followed for the other water samples. The plated petri dishes are then placed in a warm, dark place to incubate. All dishes should be carefully marked.

After a day or so count the colonies in each petri dish and record results. Pour one sterile plate for a control.

Petri Dishes	Colonies per plate	Bacteria per ml.
1 H_2O from well		
2 H_2O from stream		
3 H_2O from pump		
4 H_2O from fountain		
From other sourses		

QUESTIONS AND DISCUSSION

1 Why is it so important to be sure your water supply is checked periodically?

2 Consult your public health officer or family doctor and find what diseases may be contracted by drinking poluted water?

3 What measures are taken to safe guard the city water supply?

4 What measures could be taken to safe guard the water supply on a farm?

5 Does the presence of bacteria make the water unfit to drink? Explain.

6 What are indicator groups?

7 What tests does a Sanitation Engineer run on water samples?

EXPERIMENT NUMBER SIX

OBJECT: To show growth factors of molds and to acquaint the students with the structure of molds.

APPARATUS: Four sterile fruit jars with lids. Four slices of stale bread. One slice of moldy bread. One slice of fresh bread.

PROCEDURE:

A Moisten one piece of stale bread and place on it a small amount of the mold from the moldy bread. Then place the bread in a sterile jar and number it "1".

B Moisten another piece of stale bread in the same manner and number this jar "2".

C Innoculate a third and forth slice of stale bread, placing these in jars numbered "3" and "4". Do not moisten these!

D Place "1" and "3" in a refrigerator. Place "2" and "4" in a warm, dark place. Be sure that all lids are on tightly.

E Place the slice of fresh bread somewhere in the laboratory.

F In a few days compare the results.

QUESTIONS AND DISCUSSION

1 What factors are necessary for the growth of mold as shown by this experiment?

2 Where did the mold come from on the bread left exposed in the laboratory?

3 Examine some mold from a slice of bread under a microscope.

(a) Become familiar with these terms: spores, mycelium, spore case.

(b) What causes the black color of bread mold?

4 Are molds pathogenic? Name some diseases caused by molds.

5 How are molds helpful to man?

6 What are antibiotics?

EXPERIMENT NUMBER SEVEN

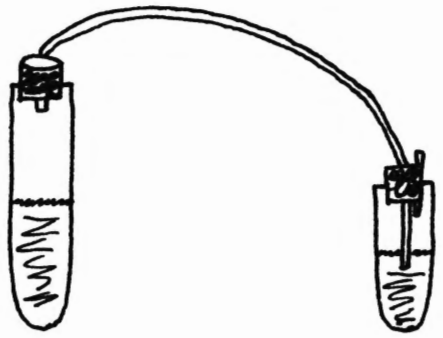
OBJECT: To become familiar with the process of fermentation and yeasts.

APPARATUS: Yeast cake, four test tubes, sugar solutions, lime water, glass tubing, and rubber stoppers.

PROCEDURE: Pour two test tubes half full of sugar solution (about 15% solutions). Label them "A" and "B". Inoculate "A" with a small piece of yeast cake. Arrange tubes as in diagram below and leave in a warm place.

Examine after twenty-four hours. If distillation apparatus is supplied or can be improvised, try distilling alcohol off of the residue in tube "A".

A.



sugar solution and yeast lime water

QUESTIONS AND DISCUSSION

1 What does the cloudy reaction in the lime water indicate?

2 What is formed in test tube "A"?

3 What makes bread rise?

4 What narcotic poison is formed by the action of yeasts?

5 Observe yeast cells from a smear on a slide under high-power of a microscope.

(a) What is the shape of the cells?

(b) How is reproduction accomplished in yeasts?

OPTIONAL EXPERIMENT

OBJECT: To become acquainted with one method of classifying bacteria and to view bacteria under a microscope.

APPARATUS: Microscope with high and low power (and oil immersion lens if possible). Grams staining solutions, slides, and cultures of bacteria from previous experiments.

PROCEDURE:

A With a sterile needle make a smear from an isolated colony on a petri plate. (Smear bacteria with a small drop of water on a slide.)

B Fix smear by passing slide through the flame of a burner.

C Stain with gentian violet for 15 seconds, then wash slide gently with water.

D Add Gram's iodine solution for 15 seconds and wash with water.

E Flood the slide with the decolorizing agent for about 20 seconds, then wash with water to stop the action of the alcohol.

F Counterstain with safranin for 20 to 30 seconds.

G Wash slide with water and allow to drain dry.

H Locate subject under low power of the microscope, and then switch to high power to observe. Always focus up. Why? (Observe under oil immersion if possible.) Prepare stains of other colonies.

QUESTIONS AND DISCUSSIONS

1 How is this procedure used by a bacteriologist?

2 What various shapes of bacteria did you observe?

SUGGESTIONS FOR OTHER EXPERIMENTS

1 Write your name on the surface of some sterile nutrient agar with a needle dipped in a colony from a previous experiment.

2 Run a bacteria count on milk. The general outline for dilution procedures may be taken from the experiment on dish water counts or water counts. However, the dilution should be more on the order of 1/1000.

3 Run a bacteria count on soil samples. The general outline for procedure and dilution techniques may be taken from previous "plate count" experiments. Dilution should be on an order of 1/10000, but these may be adjusted for desired results. This will give an idea of the countless soil forming saprophytes.

4 After carefully flaming the top of a can of preserved food, open it and run a count on some of the juices. The juices need not be diluted. This should show how canning eliminates bacteria.

5 Isolate a pure colony from a culture plate. Inoculate a sterile agar slant on plate by streaking with a small amount of a colony with a needle. Check for the purity of the colony before and after transfer with gram's stain.

6 The effect of chilling of milk might be shown by running counts on two samples of milk from the same containers. Allow one sample to sit out in the room for several hours prior to the laboratory period. Leave the other sample in a refrigerator until time for experimentation.

BIOLOGICAL PRINCIPLES INVOLVED IN EACH EXPERIMENT

EXPERIMENT NUMBER ONE:

1 Bacteria are everpresent in countless numbers.

2 These bacteria are carried from place to place by means of air currents.

3 Bacteria, as well as all forms of life, must have food, warmth, and moisture in order to grow and reproduce.

4 Bacteria are killed by exposure to ultra violet light.

5 Not all bacteria are harmful. In fact only a small percentage are pathogenic.

EXPERIMENT NUMBER TWO:

1 Bacteria are everpresent. Even though only a small percentage are pathogenic, frequent bathing with soap is highly desirable for one's own comfort and health.

2 Soap immulsifies oils allowing the oil and water to mix and carry off organic materials from the surface of the body.

3 Insects are capable of carrying bacteria from place to place and are the vectors of some diseases.

4 The conditions for the growth and reproduction of bacteria are again reviewed.

EXPERIMENT NUMBER THREE:

1 The skin is the primary barrier to disease germs.

2 Antiseptics are specific for bacteria or bacterial types.

3 There is a standard for the measurement of strength of an antiseptic just as there are standards of measurement for most things.

EXPERIMENT NUMBER FOUR:

1 Chemical agents, such as chlorine, are used to kill bacteria.

2 There are laws which are inforced to protect the health of the citizens of this country. Some of the laws concern the handling of eating untensils and foods in public eating places.

3 Disease germs may be transmitted by means of eating utensils.

EXPERIMENT NUMBER FIVE:

1 Many diseases producing bacteria are transmitted by means of water.

2 The presence of coliform bacteria is an indication of the possible presence of disease producing bacteria, such as those that cause thyphoid fever.

3 Bacteria display an ecological relationship with their surroundings. The coliform bacteria are the only ones able to servive in an environment containing bile.

4 This experiment, if used properly, should show the use of the "scientific method".

5 The student is introduced to selective media and ecological relationships.

ALTERNATE EXPERIMENT NUMBER FIVE:

1 Not all bacteria are harmful. (Pathogenic, etc.)

2 Bacteria may be water borne.

3 Bacteria may enter the body orally.

EXPERIMENT NUMBER SIX:

1 Molds require warmth, moisture, and the absence of ultra violet light if they are to live and reproduce.

2 The structure of the mold is more complex than that of a bacterium. In the molds we see an advance in the specialization of cells such as spores, the cells of the sporangium, the sporophore, etc.

EXPERIMENT NUMBER SEVEN:

1 The enzymatic reaction of yeast on sugar is called fermentation. In this reaction the sugar is converted to alcohol with the liberation of carbon dioxide.

2 The test for the presence of carbon dioxide is made by bubbling the gas through lime water.

3 Yeast cells are larger than bacterial cells and reproduce chiefly by means of budding.

4 Alcohol is classified as a narcotic poison because of its effects on the body.

OPTIONAL EXPERIMENT:

1 Bacteria are classified according to their staining properties with the gram stain.

2 Bacteria are classified according to their various shapes.

ORDER OF EXPERIMENTS

Experiment Number One will give the student the basic concept of the bacteriological procedures. It should acquaint him with the need of sterile techniques and give him practice in the manipulation of the agar and petri dishes. The student will also learn of the omnipresence of bacteria and their requirements for growth and reproduction. By proper pre-teaching and post-teaching, the student should learn that only a small percentage of the bacteria are pathogenic. He should see some means of controlling bacteria through the knowledge of their growth requirements.

By using the knowledge acquired in the first experiment, the student should be better fitted to accept the fact that bacteria will be found on practically everything upon which he comes in contact. He should have acquired some techniques in pouring sterile plates and better manipulative skill in handling the equipment in a slightly more difficult experiment.

In Experiment Number Two, the student should see the value in the use of soap in body care, and should see the need for bathing regularly for health's sake as well as that of his friends.

In the second experiment, insects are shown to be capable of carrying many bacteria. And although all bacteria are not pathogenic, insects could be the vectors of disease germs.

Experiment Number Three should follow number two. In the second experiment the student learned of the omnipresence of bacteria on his body and the control of these bacteria by proper washing. In the third experiment, the control of bacteria by means of antiseptics is seen. Here

also is shown the skins role in the protection of the body against disease. Then too, the third experiment acquaints the student with agar slant as a bacteriological device. He is able to further his techniques in handling of these slants while learning basic concepts in the study of bacteriology.

Experiment Number Four is designed to give the student a practical application of bacteriology while giving him still another technique in this study. Dilution procedures are common in this field and in a high school biology course the most common procedures should be included.

Following the general scheme of the control of bacteria, the fourth experiment shows the control on a community basis. The student may be acquainted for the first time with the fact that laws have been passed to protect his health.

Experiment Number Five gives a procedure used by professional bacteriologist in the problem of community health. In this way it gives the student a practical application of bacteriology. He is introduced to the use of broth as a medium and is acquainted with the uses of selective media. Here also, is the opportunity to learn the names of some common, specific bacteria and their habitants. The student should learn the control measures of what used to be one of the greatest epidemic killers and how its threat has been lessened.

The Alternate Experiment is included for those who wish to use only nutrient agar as a medium. This experiment will not give the standard method of water analysis, but will show the presence of bacteria in water and how the number of bacteria is reduced by the proper treatment at the local processing plant. The student is better able to use the dilution

techniques since becoming acquainted with them in the fourth experiment.

Experiment Number Six and Experiment Number Seven are included to show that the subject of Bacteriology might well be called the study of microbiology. The study of yeasts and molds is usually included in a basic study of bacteriology and it is only fitting that it should be included here. The growth and reproduction requirements are shown for both organisms as it has been for bacteria in previous experiments. These last two experiments can be done in any order and could be done before those on bacteria.

The Optional Experiment is optional in the sense that it may or may not be done without interrupting the sequence of the other experiments. Performing this experiment depends upon the staining solutions. The writer would recommend this experiment for the opportunity it gives to view the bacteria and thus get an idea of their size and shapes. It also shows one means of the difficult task in the classification of such minute organism.

These seven experiments should be enough to acquaint a student in high school biology with the study of bacteriology. They should show the omnipresence of bacteria without causing undue concern of this omnipresence. They should at the same time, give the student a need for the control of these bacteria as a measure in personal and public health.

If more experiments are desired, a few suggestions are included. It is the writers belief, however, that these seven will give the basic requirements for a high school student in biology. It might well be, that these experiments will serve their purpose in suggesting better experiments.

A Word of Caution in the Use of Any of These Experiments and the Use of the Suggestions. Pre-teaching and post-teaching in high school biology is a must. In the use of bacteriology this teaching is of greater need. If the experiment on the bacterial count in milk is used, do not let the student know the brand used as there might be a high count for some reason and much damage might be done if he were left to draw his own conclusion.

APPENDICES

BIBLIOGRAPHY

1 Buchanan, Robert E., Buchanan, Estelle D., 1951. Bacteriology. The MacMillan Company, New York.

2 Mackie, T.J., McCartney, J.E., 1953. Handbook of Practical Bacteriology. E. and S. Livingstone, Edinburgh and London.

3 Melson, Casper I., 1946. Intimate Bacteriology. Burgess Publishing Company, Minneapolis, Minnesota.

4 Nungester, Walter J., Williams, Phebe G., 1950. An Illustrated Study Aid for Medical Bacteriology. Cushing-Malloy, Inc., Ann Arbor, Michigan.

5 Peltier, George L., Georgi, Carl E., Lindgren, Lawrence F., 1946. Laboratory Manual for General Bacteriology. John Weley and Sons, Inc., New York.

6 Witton, Catherine Jones, 1952. Laboratory Manual for Microbiology. McGraw-Hill Book Company, Inc., New York.

7 No Name. 1953. Difco Manual of Dehydrated Culture Media and Reagents. Difco Laboratories, Inc., Detroit, Michigan.

January 29, 1957

Director
Teacher Education
and Certification

Dear Sir:

I am attending a science program at Oklahoma A and M College under a grant from the National Science Foundation. At the present time, I am working on a problem concerning certification of high school science teachers.

I would like a copy of the requirements for certification for high school biology teaching in your state. If you publish a booklet listing requirements for all phases of science, I would appreciate receiving a copy.

Yours truly,

Kaye H. Martin
311 Garfield Street
Stillwater, Oklahoma

KHM:bm

VITA

Kaye Hutchinson Martin

Candidate for the Degree of

Master of Science

Report: BACTERIOLOGY EXPERIMENTS DESIGNED FOR USE IN A COURSE OF HIGH SCHOOL BIOLOGY

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