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LABORATORY OUTLINE

— FOR —

General Bacteriology

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ANCIENT HISTORY OF THE

GENERAL BACTERIOLOGY

LABORATORY OUTLINE

===== FOR =====

GENERAL BACTERIOLOGY

UNIV. OF
CALIFORNIA

LABORATORY OUTLINE

===== FOR =====

GENERAL BACTERIOLOGY

BY

CONNECTICUT AGRICULTURAL
COLLEGE

BACTERIOLOGICAL DEPARTMENT

SEPTEMBER 1922

U.S. DEPT. OF AGRICULTURE
BUREAU OF PLANT INDUSTRY

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PREFACE

The exercises and descriptions in this laboratory manual are intended primarily for students in General Bacteriology. The work outlined can be covered in an eighteen week period having two two-hour laboratory periods a week, preferably at intervals of two and five days. The course offered at this institution includes two one-hour lectures a week. The lectures and laboratory work are planned as far as possible to parallel and supplement each other.

Students in this course do not prepare their culture media or sterilize their glassware for we feel that the beginner in bacteriology can use the limited time more advantageously in other directions.

The description of bacterial species includes some pathogens but only non-pathogenic organisms are used in this course.

The glossary of bacteriological terms appended will be found helpful to the student beginning the study of bacteriology.

Storrs, Conn.,
September, 1922.

The Authors.

CONTENTS

PART I.

The microscope
Measurement of bacteria
Sterilization
Lockers and equipment
Examination of unstained bacteria
Gelatin
Agar
Melting and solidifying points of agar and gelatin
Air analysis
Cautions and directions for identification of unknown cultures
Descriptive chart
Plate 1. Types of growth in stab and streak cultures
Age for examination of various cultures
Stains
Morphology
Descriptions of culture media
Descriptions of various species of bacteria

PART II.

Water testing
Bacteria in milk. Plating method
Direct microscopic examination of milk
Effect of temperature on keeping quality of milk
Bacteria from various sources
Distilled water experiment
Bacteria in meats
Testing for presence of spores
Conversion factors
Comparison of metric and English systems
Glossary of laboratory bacteriological terms

Part I.

THE MICROSCOPE

The microscope is a delicately adjusted instrument and great care should be exercised in using it to keep it in good condition. The following rules should be observed:

1. When not in use keep in the case. Dust causes unusual wear if allowed to work into the mechanism.
2. Alcohol should never be used on the lacquered parts. If oily material is to be removed use xylol with gentle rubbing.
3. Should the stage or objectives become gummed with immersion oil or balsam it can be removed with lens paper moistened with xylol.
4. Use the plane mirror for daylight and the concave for artificial light.
5. Regulate the amount of light by raising or lowering the Abbe condenser. This is done with a screw under left of stage. The higher the condenser the greater the amount of light. Dust in the eyepiece makes the field appear covered with specks. Wipe with lens paper.
6. Do not touch the surface of the lens with the fingers.
7. Objectives of one microscope should not be changed to another even of the same make.
8. To focus with the two highest powered objectives place object to be examined in center of stage. With eyes at the side at the level of the stage lower the objective with the coarse adjustment until it nearly touches the cover glass. Adjust mirror and condenser to give desired amount of light. **Focus slowly upward** with the fine adjustment until the object becomes clear. With the 1/12 or oil immersion objective a drop of cedar oil between the lens and the cover glass is necessary to prevent dispersion of light.
9. The fine adjustment is used for bringing out details in very small objects consequently has a limited range and is mechanically delicate. When the fine adjustment screw stops **do not force it**.
10. Never use oil with any but the oil immersion objectives. With Leitz microscopes the 1/12 objectives (oil immersion) are copper colored with nickel end. With Bausch & Lomb microscopes the oil immersions are nickered on the lower half.
11. For measuring bacteria a finely ruled scale is used. This is placed ruled side down in top part of eyepiece. The eyepiece may be swung around to make the scale lie in any direction desired. There are ten microns between each of the numbers on the scale.

All apparent measurements have to be corrected by multiplying by a microscope constant. This varies for the different microscopes.

12. Clean the oil immersion objective with lens paper immediately after using.

13. With the Bausch & Lomb microscope using the 10X eyepiece and the 1/6 objective the magnification is 430 and the working distance is .3 mm. With the 1/12 objective the magnification is 950 and the working distance is .15 mm.

MEASUREMENT OF BACTERIA

For measuring bacteria use the highest power objective—the 1/12 oil immersion. When an object is magnified 1000 times light is diminished at the same rate and the Abbe condenser is necessary in order to increase the light sufficiently to see the object.

Raise the condenser as high as possible. It is regulated by a screw at the left side under the stage.

Place a drop of cedar oil on the preparation to be examined. The oil has the same refractive index as the glass and prevents dispersion of the rays of light.

Lower the objective into the oil while looking at it from the side.

Adjust the mirror to give the desired amount of light. Usually the full amount is best unless the light is exceptionally bright. Use the plane mirror for daylight and the concave for artificial light.

With the eye at the microscope focus up slowly with the fine adjustment until the object shows clearly.

In the eyepiece is a ruled scale by which bacteria are measured. The eyepiece is ruled to 1/10 millimeter. The space between numbers on the scale is one millimeter. A micron is 1/1000 of a millimeter or 1/1,000,000 of a meter, which magnified by the microscope covers about .7 of the space between two lines on the eyepiece micrometer. The magnification of different microscopes varies slightly so all have been tested with a stage micrometer and the factor or microscope constant determined. This microscope constant is marked on the stage of the microscope. All apparent measurements have to be multiplied by this factor to get the actual measurements.

The following four stained preparations are furnished:

- No. 19 *B. mesentericus*
- “ 32 *Micrococcus agilis*
- “ 114 *Bact. aerogenes*
- “ 84 *Sarcina flava*

Note the shape and arrangement of the bacteria and measure their size. Bacteria of the same kind vary somewhat in size so

measurements of the smallest and largest are required in addition to the size of the majority. Measure at least ten of the medium sized ones and average them in finding the size of the majority. If bacteria are spherical only one dimension—diameter—is required; if rod shaped two dimensions—length and width are required. Measure length and width of the same organisms.

To be one micron in length the bacterium has to extend from one line to the corresponding point on the next line, that is, across one space and one line. The lines have width and on most of the scales the width of the line is about .3 micron and the width of the space .7 micron. Now if a bacterium is just covered by a line we know the measurement is .3. If it projects beyond the line it is possible to make an accurate estimate of the proportion of the .7 covered which added to the .3 gives the size; or if it just fills the space between two lines it is .7 micron. The scale may be moved in any direction by turning the eyepiece around.

Multiply apparent measurements by the microscope constant and record results in the following form:

For Rods		
Smallest	Largest	Average
Length x width	Length x width	Length x width
Arrangement		
For Cocci		
Smallest	Largest	Average
Diameter	Diameter	Diameter
Arrangement		

When through with the microscope carefully wipe cedar oil from lens with lens paper furnished. Also remove oil from slides.

Laboratory note books about 6¾ X 8¼ inches are preferred. These are to be left on the table at your place. They are not to be taken from the laboratory.

STERILIZATION

Sterilization is the destruction of all forms of life. It may be accomplished in various ways. Only the common methods of sterilization by heat will be considered here.

Sterilization in a naked flame. The simplest means of sterilizing a metal instrument is to heat it in the flame. This method is usually

recommended for sterilizing platinum needles, spatulas, forceps, etc. When sterilizing a needle heat the entire length of it to redness in the flame. When sterilizing hold needle vertically in flame. Pass the lower part of the handle three or four times through the flame.

Before sterilization a platinum needle should be dried by holding it near the flame. This prevents sputtering which scatters organisms, especially when materials such as fat and protein are thrust into the flame. This drying is of special importance when working with pathogenic material.

Cool the sterilized needle before using.

Do not let the sterilized needle touch anything except the culture from which the inoculation is being made and the medium to be inoculated.

Sterilize needle after using before laying it down.

By passing instruments like forceps and spatulas several times through a hot flame they may be sterilized.

Sterilization by hot air. The usual method of sterilizing all glassware, instruments with metal handles, etc., is by exposure to hot air. A temperature of 160° C. should be maintained for one hour. Heating and cooling is done slowly to avoid breaking the glassware.

Sterilization by steam. Under ten pounds pressure for fifteen minutes in an autoclav all life is destroyed unless the material is in large bulk. For flasks and larger volumes of liquid the period of heating is prolonged to twenty minutes. The temperature thus obtained is from 114° to 115° C.

Steam without pressure at 100° C. is used. Materials are heated for twenty to thirty minutes on three successive days. On the first day the vegetative cells are killed. The spores remaining develop in time to be killed by the heating on the second day. Heating on the third day is to make certain that all spores have had time to change to the vegetative stage and be killed.

LOCKERS AND EQUIPMENT

Lockers fitted out with apparatus needed in the work of the course are assigned to students. A deposit of 25c is required for the key. This is refunded at the end of the course when the key is returned. The following materials are in each locker:

- 1 counting plate
- 1 hand lens
- 1 platinum needle

Laboratory Outline for General Bacteriology

- 1 platinum loop
- 1 test tube rack
- 2 wire baskets
- 1 slide box
- 1 ruler
- 1 thermometer
- 2 pipette cases
- 50 Petri dishes
- 25 1cc pipettes
- 6 1cc pipettes graduated to 1/10 cc.
- 2 2cc pipettes

The above list together with the prices is on the inside of the locker door. Check up carefully and if anything is missing report at once. **Absolutely no allowance is made for any shortage unless reported at this time.** Each student is held responsible for these materials and will be charged at the end of the semester for anything broken or missing. Do not leave materials out on the tables or lying around the laboratory. They are returned when the ownership is known but in most cases it is not known and the student loses them.

The Petri dishes or plates in the lockers are sterile so do not open them. When counting pipettes count from the end without taking them from the case, as they are sterilized ready for use.

Bring a towel or cloth for drying glassware.

Students are required to wash their Petri dishes and pipettes after using them. Other glassware is cared for by the department. Care must be used to wash the glassware perfectly clean. If any particles of media remain, sterilizing burns them on and leaves dark brown patches which can only be removed by scraping or by a strong acid cleaning mixture. Petri dishes are to be wiped dry. Mark each one with your locker number. All glassware is marked with red glass marking pencils. These pencils are sold in the laboratory for 10c each. After pipettes are cleaned they are returned to their case with points down. Cases are also marked with the locker number. Place cases of pipettes and Petri dishes which you wish sterilized on the long table in north end of laboratory. In the book there write your name, locker number, number of dishes, number of cases of pipettes left to be sterilized, and the date. They will be sterilized and returned to your locker. Plates and pipettes must be washed promptly when you are through using them.

Forms for recording notes will be given out on the board. Use the forms given. Leave one page in the note book for each experiment.

EXAMINATION OF UNSTAINED BACTERIA

The purpose is to study bacteria in a living condition; to demonstrate their form, arrangement, and motility.

Bacteria have two kinds of motion, the so-called Brownian or molecular movement, and true motility. Brownian movement is shown more or less by all small particles of insoluble matter (including living non-motile or dead bacteria) in suspension. It is characterized by a vibratory movement; the relative positions of the particles or bacteria are not changed. This type of movement may be distinguished from true motility which is characterized by progressive movement, more or less rapid, of an organism across the field of a microscope, and which changes its position in the field independently of and in a direction contrary to other organisms present. If large numbers of bacteria are moving in one direction it is an indication that they are being carried by currents in the liquid.

Method

Place a drop of water on a cover glass held in cover-glass forceps. Sterilize platinum loop by heating to redness in gas flame. When cool transfer a small amount of the culture to be examined to the drop of water. Use only enough to cloud the water slightly. Take the culture from the edge of the growth in the lower part of the tube. The bacteria are on the surface of the agar, so do not allow the loop to cut down into the agar. In opening the tube remove the cotton plug and hold it so that the inner part will not touch anything. Never lay it on the table. This is necessary to prevent the culture in the tube from becoming contaminated with other kinds of bacteria or molds. After removing loopful of culture replace cotton plug. Invert the preparation onto a slide and examine with the 1/6 objective. No oil is used with this objective. With the coarse adjustment lower the objective until it nearly touches the cover glass, being careful not to touch it. Then with the eye at the ocular, focus up with the fine adjustment until the bacteria come into view. This focal point will be passed without noticing it if passed too quickly, **if the light is too intense**, or if the light is too dim. Regulate the amount of light by raising or lowering the condenser and by adjusting the mirror. Unstained bacteria are nearly colorless and appear as faint shadows.

Bacteria lose their motility very quickly so if there is a delay of two or three minutes in getting the focus a wrong determination may be made. In such a case it is better to make a new preparation. Do not make but one preparation at a time.

Bacteria move by means of flagella. These are slender, hair-like processes usually several times as long as the bacterial cell. Some species have but one on the end, monotrichiate; some have a tuft on one end, lophotrichiate; some have them distributed all over the organism, peritrichiate. The arrangement of the flagella causes a difference in the kind of motion. Special staining methods are necessary in order to see the flagella.

Examine No.	19	<i>B. mesentericus</i>
"	32	<i>M. agilis</i>
"	129	<i>M. lactis varians</i>
"	20	<i>B. proteus</i>
"	48	<i>B. pullorum</i>
"	23	<i>B. mycoides</i>

Examine preparation from a cake of yeast. Make drawings showing appearance of yeasts, starch grains, and bacteria.

GELATIN

Gelatin serves two purposes. As a solid culture medium, it is a technical device by which the isolation of a single species of microorganism is made possible. To those organisms which secrete proteolytic enzymes it serves as a nitrogenous food material. Beef extract and peptone are added as food for bacteria.

Gelatin was the first substance used for a solid culture medium. This medium was originated in 1882 by Koch and has since revolutionized the science of microbiology. Milk sugar and litmus are also added when acid production is to be determined.

Gelatin media will melt at a temperature of about 24° C to 26° C solidifying again at 20° C to a clear transparent jelly.

Gelatin plates do not have to be inverted during incubation, as do the agar plates, because no water of condensation ordinarily collects on the cover of the Petri dish in case of the gelatin.

AGAR

Agar is a seaweed product used in preparing various kinds of solid culture media. Ordinarily agar is composed of beef extract, peptone and agar. It becomes fluid at about 85° C and solidifies below 40° C, becoming a very solid opaque jelly, which retains its shape well in slants and plates.

Agar is commonly used in determining the number of bacteria per cc. in milk, water, and various other substances. A definite

amount of the material to be tested is mixed with the fluid agar and allowed to cool. Each bacterium is held in a certain spot on the agar. Bacterial colonies develop upon several days incubation and each colony represents one bacterium or clump of bacteria originally present.

Agar itself is not a food for microorganisms. Its special value is in hardening media used for cultivating organisms which grow at a temperature above melting point of gelatin. This feature has made possible the great strides that have been taken in medical bacteriology.

TO DETERMINE THE MELTING AND SOLIDIFYING POINTS OF AGAR AND GELATIN

Place tubes of agar and gelatin in wire basket.

Set in pan with water above level of media in tubes.

Insert thermometer in gelatin.

Place pan over gas flame and watch thermometer.

When liquefied record temperature.

Change thermometer to agar tube and record melting temperature.

Place tubes in cold water and watch for solidifying point first of agar, then of gelatin.

	Melting point	Solidifying point
Agar	°C.	°C.
Gelatin	°C.	°C.

AIR ANALYSIS

Sterile melted agar tubes will be furnished. Pour seven into Petri dishes using one tube to a dish. In doing this remove cotton stopper without touching end of tube. Raise one side of the cover of the dish just enough to allow the pouring of the agar. This is done to prevent bacteria dropping in from the air. When agar is solid expose plates with cover removed for five minutes in the places designated below. Keep cover right side up while off from plate. The one out of doors should be taken away from roads or buildings. Face the wind in exposing plate so that bacteria will not blow from you onto the plate. With red glass marking pencil mark the plates with your name, the date, and place of exposure. After exposing plates place in locker **inverted** to incubate.

Laboratory Outline for General Bacteriology

	No. Bact.	No. Molds	Area of Plates	Molds per liter	Bact. per liter
1 Bacteriology laboratory					
2 Out of doors					
3 Bottling room of dairy					
4 Cow barn					
5 Dining room (at meal time)					
6 Study					
7 Control—not exposed					

Counting Bacteria and Molds on Air Plates

It has been shown that the bacteria from ten liters of air will fall on one hundred square centimeters in five minutes. From this is derived the formula

$$\frac{10 \times \text{no. bact. or molds on plate}}{\text{area of plate}} = \text{no. bact. or molds per liter of air.}$$

Measure diameter of plate and from the following table determine the area.

Diam. plate	Area plate
86 mm	58 sq. cm.
87	59
88	61
89	62
90	63.5
91	65
92	66.5
93	68
94	69.5
95	71
96	72.5
97	74
98	75.5
99	77
100	78.5

In counting a plate place it on the glass plate ruled in squares. Remove the cover of the dish. Use the lens holding it near the eye. Bring the plate near enough to focus clearly. Beginning at the upper part of the plate follow along the lines working back and forth until the whole plate is counted.

From air plates select seven bacterial colonies of different appearances and write descriptions of them in note book. Then make transfers from them to agar slants. Mark tubes so they can be identified with descriptions. To transfer colony sterilize platinum loop by heating entire length of wire to redness in the gas flame. Pass the lower part of the handle three or four times through the flame. When cool touch loop to the colony to be transferred. Remove cotton plug and hold it so the lower part will not become contaminated by touching anything. Touch the loop to the surface of the agar at the lower part of the tube and make a straight streak up to the top. Care is necessary not to cut down into the agar. Replace cotton stopper. Place tubes in locker to incubate.

Glossary on pages 57, 58, 59 is to be learned. These terms will be constantly used so it is necessary to become familiar with them now. There will be a written on it soon, probably at the next laboratory period.

CAUTIONS AND DIRECTIONS FOR IDENTIFICATION OF UNKNOWN CULTURES

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1. Do keep cultures pure so correct results may be obtained.

Agar Slants

2. Do touch only the tops of cotton plugs when removing them from and replacing them in tubes. While out hold between fingers with lower parts away from hand so they will not touch anything.
3. Do burn off cotton adhering to mouth of tube instead of pulling it off with the fingers, rods, etc.
4. Do sterilize the needle or loop by heating entire length of wire to redness and pass lower part of holder through flame three or four times before making a transfer.
5. Do allow needle to cool before using.
6. Do hold needle so nothing touches it while cooling.
7. Do avoid opening tubes in currents of air. Bacteria may be blown in.
8. Do hold tube nearly horizontal while making transfers so bacteria cannot fall in.
9. Do push plugs in until they are firm.
10. Do use care to keep all cultures pure even when you think it is the last time they will be used.
11. Do keep all cultures until advised to discard them.
12. Do make records complete.
13. Do record all negative as well as positive results.
14. Do remember that "chromogenesis" refers to the color of the bacterial growth itself, not to the color of the medium on which the bacteria are growing.
15. Do touch surface of agar slants so lightly that needle or loop never breaks into the medium. Bacteria are usually only on the surface.

(Continued on Page 24)

CAUTIONS AND DIRECTIONS FOR IDENTIFICATION OF UNKNOWN CULTURES

1. Don't allow cultures to become contaminated. Later work with contaminated cultures gives incorrect results. The following notes give some hints about avoiding contaminations and obtaining satisfactory records.

Agar Slants

2. Don't lay cotton plug on the table or let it fall there, or hold the inner part of the plug next the palm of the hand, or allow it to touch anything. If it accidentally touches anything burn off a layer of the cotton before replacing plug in tube.
3. Don't pull off cotton adhering to the mouth of the tube with the fingers, rods, matches, etc. Burn it off.
4. Don't use the needle or loop without first thoroughly sterilizing it by heating the **entire length** to redness and passing the lower part of the holder three or four times through the flame.
5. Don't use the needle or loop when hot enough to kill the bacteria.
6. Don't feel of the needle with the fingers or touch it against something not sterile to see if it is cool enough to use.
7. Don't open tubes in currents of air. Bacteria are blown in.
8. Don't hold tubes vertical when open thus allowing bacteria to fall in.
9. Don't insert plugs so slightly that they fall out when tubes are handled.
10. Don't be careless with cultures when you think it may be the last time you are going to use them. You frequently find they are needed for later work.
11. Don't discard any cultures until you are advised to do so.
12. Don't make partial records of cultures when complete ones are possible.
13. Don't leave record spaces blank when you find that no change has taken place or when you obtain a negative reaction.
14. Don't record the color of the medium under "chromogenesis" or leave the space blank if the bacterial growth is white or colorless.
15. Don't break the surface of the agar slant with the needle or loop. The bacteria are usually entirely on the surface of the agar and mixing them with the agar greatly increases the difficulty of making later inoculations.

(Continued on Page 25)

Laboratory Outline for General Bacteriology

16. Do take small amounts of bacteria for inoculation. Only a few bacteria are necessary.
17. Do keep bacterial cultures out of the sunlight. ✓

Liquid Media

18. Do keep cultures upright enough so that plugs will not become wet.
19. Same as 1 to 13 inclusive for agar slants.
20. Do keep fermentation tubes so no air enters inner tube.
21. Do sterilize loop after touching it to litmus paper in testing for reaction before putting it back into the tube for another loopful.
22. Do handle incubated cultures gently before taking the observations. This is particularly important with nutrient broth as the appearance may be greatly changed by jarring.
23. Do use uninoculated tubes of media for comparison in making observations and tests. .

Plate Cultures

24. Do lift covers only high enough to insert the pipette or needle when adding inoculation material.
25. Do have medium at right temperature when pouring—agar about 40° C., and gelatin between 25° C. and 40° C.
26. Do remove cotton plugs from tubes or flasks without touching lips of tubes or flasks and burn off any adhering cotton that would interfere with pouring.
27. Do lift cover when pouring just high enough so tube or flask will not scrape on edges of dish.
28. Do mix medium well with inoculation so bacteria will be evenly distributed.
29. Do keep medium from getting on edges of plate when pouring or mixing as this glues them together and frequently leads to breakage and contamination.
30. Do pour plates where air is quiet.
31. Do keep plates closed while looking to see if medium has hardened.
32. Do place plates near center of table to harden as edges of table are not level.
33. Do always carry gelatin plates level and right side up.
34. Do keep agar plates inverted after hardening and while incubating.

(Continued on Page 26)

Laboratory Outline for General Bacteriology

16. Don't take all the growth possible for the first inoculation as you will have about nineteen more to make from the same culture. Simply touching the cool sterilized needle to the growth is usually sufficient.
17. Don't allow the sun to shine on cultures. Sunlight destroys certain species of bacteria very quickly.

Liquid Media

18. Don't lay tubes of liquid media on table or hold them so that plugs become wet. Contaminations usually follow wet plugs.
19. Same as 1 to 13 inclusive for agar slants.
20. Don't allow air to get into the small inner tube of the fermentation tubes.
21. Don't put loop back a second or third time without sterilization after touching it to litmus paper in testing the reaction.
22. Don't shake or handle tubes roughly before taking the observations.
23. Don't make observations or tests of cultures without using uninoculated tubes for controls.

Plate Cultures

24. Don't lift covers clear off when adding inoculating material with pipette or loop.
25. Don't pour agar or gelatin when too hot or too cold.
26. Don't touch the lip of the tube or flask when removing cotton stopper.
27. Don't scrape outside of tube or flask on edge of plate when pouring.
28. Don't leave plates without mixing inoculation with medium.
29. Don't slop medium onto edges of plates when pouring or mixing.
30. Don't pour plates in a current of air.
31. Don't remove or lift cover of plate to see if medium has hardened.
32. Don't leave plates anywhere except near the center of table to harden as tables are crowning. This makes the layer of medium uneven.
33. Don't ever carry gelatin plates tipped or invert them to incubate.
34. Don't incubate agar plates right side up as condensation water causes trouble by dropping back from the cover.

(Continued on Page 27)

Laboratory Outline for General Bacteriology

35. Do allow agar to harden in plates without tipping them around and breaking the medium.

Gelatin Stab

36. Do have needle straight before puncturing.
37. Do puncture clear to bottom of tube.
38. Do remove needle in line of puncture.
39. Do puncture in center of tube and only once.
40. Do hold tube above medium so warmth of the hand will not melt the gelatin.
41. Do watch cultures carefully to see when liquefaction begins and when it is complete as well as for the form of growth in both liquefying and non-liquefying cultures.

Morphology

42. Do come at right times to make agar slants to have them at the required age for studying at laboratory periods.
43. Do prepare films with only enough bacteria to show slight cloudiness.
44. Do spread films over the entire cover glass.
45. Do leave stains on the exact time called for in the directions.
46. Do measure at least ten bacteria to find the size of the majority.
47. Do measure both length and breadth of rod shaped bacteria and put both measurements down.
48. Do use care to fill out all points called for under "Morphology."
49. Do examine preparations for motility as soon as possible after making, as many bacteria lose their motility very quickly; and do shut down on the light enough so bacteria can be seen.
50. Do handle cover glasses with forceps.

Laboratory Outline for General Bacteriology

35. Don't tip agar plates constantly while they are hardening. It breaks the agar so it will not form a solid mass which can be satisfactorily inverted.

Gelatin Stab

36. Don't puncture medium with a crooked needle or with the loop.
37. Don't stop puncturing until the needle touches the bottom of the tube.
38. Don't split the medium when removing the needle.
39. Don't puncture near the side of the tube or more than once.
40. Don't hold the tube in the hand until the warmth of the hand melts the gelatin.
41. Don't fail to record the form of growth, when liquefaction begins, and when it is complete.

Morphology

42. Don't forget to come extra times when necessary to make cultures so as to have them the right age to use at laboratory periods.
43. Don't use too much or too little material in preparing films.
44. Don't leave all the material of the film in one small thick mass which can never be seen through. Spread.
45. Don't leave stains on for longer or shorter times than called for in the directions.
46. Don't measure less than ten different bacteria before filling in measurements called for.
47. Don't think the size of a rod simply means its length, or that you multiply the length by the width.
48. Don't think that most of the information called for under "Morphology" does not amount to much and needs not to be filled out.
49. Don't make preparations for motility examination until you are ready to examine them, or leave on the full amount of light when trying to find them.
50. Don't ever touch the surface of a cover glass with the fingers. It leaves enough greasiness to prevent the proper spreading of films.

THE CHART

The purpose of the descriptive chart is to provide a uniform and concise method of recording nearly all the ordinary and some of the unusual observations concerning the morphology and activities of bacteria. It should familiarize students with bacterial characteristics and facilitate in the grouping and identification of species.

For preliminary practice in handling bacteria five pure cultures will be given each student. This work includes the Gram's stain, fuchsin stain for measurement, inoculations into and observations on agar slants, gelatin stabs, nutrient broth, milk, litmus milk, potato, and the nitrite test.

After this six unknown cultures are given each student. All data called for on the Descriptive Chart is worked out and filled in. When complete the cultures are named by comparing descriptions with those on pages 43, 44, 45, 46. For work on unknown cultures 12-14 laboratory periods are given.

No bacteria pathogenic to man are given out to students in this course.

Laboratory Outline for General Bacteriology

DESCRIPTIVE CHART, ENDORSED BY SOCIETY OF AMERICAN BACTERIOLOGISTS AT THE ANNUAL MEETING DEC. 30, 1920.

Prepared by H. J. Conn, K. N. Atkins, I. J. Kligler, J. F. Norton, G. E. Harmon, Committee on bacteriological technic.

MORPHOLOGY

Note. Underscore required terms.

- Vegetative Cells**, Medium used.....
temp age.....days.
Form, spheres, short rods, long rods, filaments, commas, short spirals, long spirals, curved.
Arrangements, single, pairs, chains, fours, clusters, cubical packets.
Limits of SizeSize of Majority
Ends, rounded, truncate, concave.
- Capsules**, present on
How stained
- Sporangia**, present, absent. Medium used.....
temp age.....days.
Form, elliptical, short rods, spindled, clavate, drumsticks.
Limits of SizeSize of Majority
- Endospores**, present, absent.
Location of endospores, central, polar.
Form, spherical, elliptical, elongated.
Limits of SizeSize of Majority
Wall, thick, thin.
Sporangium wall, adherent, not adherent.
- Motility**
In broth On agar.....
- Flagella**. No. Attachment, polar, bipolar peritrichiate.
How stained
- Irregular Forms**.
Present on in.....days at°C.
Form spindled, cuneate, filamentous, branched,
or
- Staining Reactions**
Gram **Acid fast**
Special Stains

CULTURAL CHARACTERISTICS

Underscore required terms.

- Agar Stroke**. Incubation temp.....°C, Age.....days
Growth, scanty, moderate, abundant, none.
Form of growth, filiform, echinulate, beaded, spreading, arborescent, rhizoid.
Elevation of growth, flat, effuse, raised, convex.
Lustre, glistening, dull.
Topography, smooth, contoured, rugose.
Optical Characters, opaque, translucent, opalescent, iridescent.
Chromogenesis Photogenic. Fluorescent.

Laboratory Outline for General Bacteriology

- Odor, absent, decided, resembling
Consistency, butyrous, viscid, membranous, brittle.
Medium, grayed, browned, reddened, blued, greened.
- Gelatin Stab**, Incubation temp.....°C, Agedays
Growth, uniform, best at top, best at bottom.
Line of puncture, filiform, beaded, papillate, villous, arborescent.
Liquefaction, none, crateriform, napiform, infundibuliform, saccate, strati-
form, begins in..... d complete in.....d.
Depth of liquefaction in tube of 10 mm. diameter evenly inoculated at
20°C for 30 daysmm.
Medium, fluorescent, browned.
- Potato**, Incubation temp°C, Agedays
Terms as in Agar stroke.
- Nutrient Broth**, Temp°C, agedays
Surface growth, ring, pellicle, flocculent membranous, none.
Clouding, slight, moderate, strong, transient, persistent, none, fluid turbid.
Sediment, compact, flocculent, granular, flaky, viscid on agitation, abun-
dant, scant, none.
- Agar Colonies**, temp°C, Agedays
Growth, slow, rapid.
Form, punctiform, circular, irregular, mycelioid, filamentous, rhizoid.
Surface, smooth, rough, concentrically ringed, radiate.
Elevation, flat, effuse, raised, convex, pulvinate, umbonate.
Edge, entire, undulate, lobate, erose, filamentous, curled.
Internal structure, amorphous, finely-, coarsely-, granular, filamentous,
curled, concentric.
- Gelatin Colonies**, temp°C, Agedays
Growth, slow, rapid.
Form, punctiform, circular, irregular, mycelioid, filamentous.
Elevation, flat, raised, convex, pulvinate, crateriform, (liquefying)
Edge, entire, undulate, lobate, erose, filamentous, floccose, curled.
Liquefaction, cup, saucer, spreading.
Internal structure, amorphous, finely-, coarsely-, granular, filamentous,
curled, concentric.

PHYSIOLOGY

TEMPERATURE RELATIONS

- Optimum temperature for growth.....°C.
Maximum temperature for growth.....°C
Minimum temperature for growth.....°C.

CHROMOGENESIS

- Nutrient broth.....
Nutrient gelatin.....
Nutrient agar.....
Potato.....

PRODUCTION OF INDOL

- Medium:.....
Indol absent, present in.....days

PRODUCTION OF HYDROGEN SULFIDE

- Medium:.....
H₂S absent, present in.....days

Laboratory Outline for General Bacteriology

FERMENTATION

Temperature.....°C.

Medium containing	LACTOSE	DEXTRROSE	SACCHAROSE	GLYCERIN		
.....						
.....						
.....and:						
Gas						
First appearance of acid						
First appearance of alkali						
Reaction after days						
Reaction after days						
Max. H-ion Conc.						

RELATION TO OXYGEN

Method used.....
 MediumTemperature.....°C.
 Aerobic growth; absent present, better than anaerobic growth.
 Anaerobic growth: absent, occurs in presence of dextrose, of sucrose, of lactose, of nitrate;
 better than aerobic growth.

DIASTATIC ACTION

Breadth of clear zone on starch agar plates.
 in.....days:.....

MILK

Temperature.....°C.

Reaction: 1 day.....2 days.....4 days.....7 days.....10 days.....
 Acid curd: 1 day.....2 days.....4 days.....7 days.....10 days.....
 Rennet curd: 1 day.....2 days.....4 days.....7 days.....10 days.....
 Peptonization: 1 day.....2 days.....4 days.....7 days.....10 days.....
 Reduction of litmus in.....days; of methylene blue in.....days.
 Influence of indicator on growth.....

NITRATE REDUCTION

Medium.....Temperature.....°C.

Nitrite 1 day.....2 days.....4 days.....7 days.....10 days.....
 Gas: 1 day.....2 days.....4 days.....7 days.....10 days.....
 Medium.....Temperature.....°C.
 Gas: 1 day.....2 days.....4 days.....7 days.....10 days.....
 Nitrite 1 day.....2 days.....4 days.....7 days.....10 days.....

Index No.*.....
BRIEF CHARACTERIZATION

As each of the following characteristics is determined, indicate in proper marginal square by means of figure, as designated below:

PRIMARY CHARACTERISTICS	Microscopic Features	Form: 1, streptococci; 2, diplococci; 3, micrococci; 4, sarcinae; 5, rods; 6, commas; 7, spirals; 8, branched rods; 9, filamentous Spores: 1, central; 2, polar; 3, absent Flagella: 1, peritrichic; 2, polar; 3, absent Gram stain: 1, positive; 2, negative
Miscellaneous Biochemical Reactions	Pathogenicity, etc.: 1, for man; 2, for animals; 3, for plants; 4, parasitic but not pathogenic; 5, saprophytic; 6, autotrophic Relation to oxygen: 1, strict aerobe; 2, facultative anaerobe; 3, strict aerobe Gelatin liquefaction: 1, positive; 2, negative In nitrate media: 1, nitrite and gas; 2, nitrite but no gas; 3, neither nitrite nor gas Chromogenesis: 1, fluorescent; 2, violet; 3, blue; 4, green; 5, yellow; 6, orange; 7, red; 8, brown; 9, pink; 0, none	
Carbohydrate Reactions	Diastatic action: 1, positive; 2, negative From dextrose: 1, acid and gas; 2, acid without gas; 3, no acid From lactose: 1, acid and gas; 2, acid without gas; 3, no acid From sucrose: 1, acid and gas; 2, acid without gas; 3, no acid	
SECONDARY CHARACTERISTICS	Vegetative Cells	Diameter: 1, under 0.5μ ; 2, between 0.5μ and 1μ ; 3, over 1μ Length: 1, less than 2 diameters; 2, more than 2 diameters Chains (4 or more cells): 1, present; 2, absent Capsules: 1, present; 2, absent
	Spores	Shape: 1, round; 2, oval to cylindrical Diameter: 1, less than diameter of rod; 2, greater than diameter of rod
	Cultural Features	<div style="display: flex;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg); text-align: center; padding: 5px; width: 10%;">Agar Stroke</div> <div style="padding: 5px; flex-grow: 1;"> Abundance: 1, abundant; 2, moderate; 3, slight; 4, absent Lustre: 1, glistening; 2, dull Surface: 1, smooth; 2, contoured; 3, rugose </div> </div> <div style="display: flex;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg); text-align: center; padding: 5px; width: 10%;">Milk</div> <div style="padding: 5px; flex-grow: 1;"> Agar colonies: 1, punctiform; 2, round (over 1 mm. diameter); 3, rhizoid; 4, filamentous; 5, curled Gelatin colonies: 1, punctiform; 2, round (over 1 mm.); 3, irregular; 4, filamentous Acid: 1, sufficient for curdling; 2, insufficient for curdling; 3, no acid Rennet curd: 1, present; 2, absent Peptonization: 1, present; 2, absent </div> </div>

* Recording the "Index Number" here is optional; but its use will be found convenient if the charts are to be filed according to the salient characteristics of the organisms. The Index Number consists of the first thirteen figures from the margin (primary characteristics) copied down in the order of their occurrence in the margin, placing a dash wherever a heavy rule occurs in the margin. Thus, *B. coli* belongs to the group 5312-41220-1111.

PLATE 1.

A.

B.

C.

A. Stab Cultures

1. Filiform
2. Beaded
3. Villous
4. Arborescent
5. Papillate

B. Type of Liquefaction

1. Stratiform
2. Napiform
3. Infundibuliform
4. Crateriform
5. Saccate

C. Streak Cultures

1. Arborescent
2. Spreading
3. Filiform
4. Beaded
5. Echinulate

AGE OF CULTURES, TO DETERMINE

Motility	24 hours
Flagella	24 "
Spores and sporangia	3 days to 1 week.
Stains for measurement of vegetative cells	24 hours
Gram's stain	24 "

AGE OF VARIOUS CULTURES WHEN EXAMINED

Agar slants	described after 7 days, checked up at 14 days
Potato slants	same as agar slants
Nutrient broth	same as agar slants
Gelatin stabs	examine every laboratory period until 30 days old, recording any changes
Agar plates	examine after 1 week
Gelatin plates	from 2 to 7 days
Dunham's peptone for Indol test	4 days
Lead acetate agar for H ₂ S test.	7 days
Milk	1, 2, 4, 7, and 10 days
Litmus milk	1, 2, 4, 7, and 10 days
Nitrate broth	1, 2, 4, 7, and 10 days
Nitrate agar	1, 2, 4, 7, and 10 days
Starch agar for diastatic action	7 days, unless the growth is very rapid, then the test is made sooner.
Fermentation tubes of dextrose lactose, saccharose, glycerine	examined 2, 7 and 14 days.

TEMPERATURE OF INCUBATION

All cultures are to be grown at locker temperature, which is about 20° C. except the one set of agar slants made for testing growth at 37° C.

STAINS

Their Composition and Methods of Use

Standard Alcoholic Solutions. Standard alcoholic solutions of anilin colors, especially fuchsin, gentian violet, and methylene blue, are made by dissolving 10 grams of dry color in 100 cc of 95% alcohol. These are used as stock solutions from which the various stains are made.

Watery Fuchsin. Made by diluting one part of standard alcoholic solution of fuchsin with nine parts of distilled water.

Use. Leave on 1 minute, wash with water.

Watery Gentian Violet. Made by diluting one part of standard alcoholic solution of gentian violet with nine parts of distilled water.

Use. Leave on 1 minute, wash with water.

Carbol Fuchsin. Made by diluting one part of standard alcoholic solution of fuchsin with nine parts of a five per cent. solution of carbolic acid.

Use. Leave on $\frac{1}{2}$ minute, wash with water.

Methylene blue, Loeffler's. Made by adding thirty cc of standard alcoholic solution of methylene blue to seventy cc of potassium hydroxide solution (1-10,000).

Use. Leave on 10 minutes, wash with water.

Gram's stain (Chester). Made by shaking 1 cc of aniline oil with 10-15 cc of distilled water vigorously for several minutes and filtering. To 10 cc of the filtrate add 1 cc of standard alcoholic solution of gentian violet and filter again. This is the stain. The Gram's solution has the following composition:

Metallic iodine	1 gram
Potassium iodide	2 grams
Water, distilled	300 cc
Use. Anilin gentian violet	$\frac{1}{2}$ minute, wash with water
Gram's mixture (iodine)	$\frac{1}{2}$ " " " "
Alcohol 95%	about 1 " " " "

Flagella stain (Duckwall). Mordant is prepared as follows:

Desiccated tannic acid	2 grams
Water, distilled	15 cc
Saturated alcoholic solution of fuchsin	1 cc
Normal NaOH solution	2-4 drops

When ready to use mix with

Ferrous sulphate, saturated
aqueous solution 5 cc

Filter.

Make stain by adding 2 cc of saturated alcoholic solution of fuchsin to 8 cc of a 5% solution of carbolic acid.

Use Mordant $\frac{1}{2}$ —1 minute, wash with water.

Carbol fuchsin $\frac{1}{2}$ —1 minute, wash with water.

MORPHOLOGY

The size, form, arrangement, etc., of vegetative cells is determined from twenty-four hour old agar slant cultures. Watery fuchsin stain is used as it has been found satisfactory with most bacteria.

The Gram stain is made at the same time. This stain is for differential purposes only. Some bacteria keep the stain and some do not. When the bacteria remain **dark blue** they are recorded as Gram positive, otherwise as Gram negative.

Preparation of films. The method of preparing films for these two stains is as follows:

Place cover glasses in forceps without touching them with the fingers.

With sterile loop place a drop of water on each of two cover glasses.

With a cool sterile needle or loop transfer from edge of culture in lower part of tube to the drops of water on the cover glasses. Use only enough to cloud the water slightly. Burn any excess of bacteria from the loop and **spread the drops** over the surface of the cover glasses. If a drop will not remain spread but rolls together again it shows that the cover glass is greasy and should be discarded.

Air dry without heat.

Fix film to the cover glass by passing it three times through the gas flame. Keep the film side up. Do not hold in flame longer than **you could** hold your finger in it without burning.

They are now ready to be stained. For staining methods see page 35.

After staining drying may be hastened by blotting with filter paper. When dry mount on slide film side down in drop of cedar oil.

Label slides carefully.

Never pile slides together. Place them in slide boxes.

Motility and flagella staining. Motility is determined from day-old nutrient broth or agar cultures. Motile cultures are stained for flagella. Preparations are made as follows: With a pipette place about 4 drops of water together in a watch glass. Inoculate center of water with culture taken from edge of growth in lower part of agar tube. Be sure the needle is cool before starting and avoid any rapid vigorous movements as the flagella are easily broken off. Use enough bacteria to cloud the center of the water slightly. Let the dilution stand for five minutes, then remove from edge of dilution several loopfuls onto clean cover glasses. Do not spread the loopfuls but let them dry on the several spots where they were placed. The best flagella are usually found near the edges of the drops. Air dry, and pass **once** through the flame. Cover with freshly mixed mordant $\frac{1}{2}$ to 1 minute. Wash and stain with carbol fuchsin $\frac{1}{2}$ to 1 minute. Wash, dry, and mount.

Sporangia and endospores. Determinations on these are usually made from watery fuchsin stains from week-old cultures. Sometimes, however, it is necessary to use younger cultures in order to find sporangia.

AGAR STROKE

Agar medium is placed in test tubes and cooled in a slanting position. This leaves a large surface. Inoculations are made in one streak the entire length of the slanted surface, care being exercised not to break down into the medium. For comparison of growth at different temperatures two tubes are inoculated from each culture. These are kept at 20°C. and 37°C. respectively. Descriptions are made at 7 and 14 days.

GELATIN STAB

Gelatin is used in tubes of about 10 mm. diameter. The depth is about 4 cm. Inoculation is made with a straight needle stabbing once in the center to the bottom of the tube. They are watched to determine when liquefaction begins and is complete as well as for the form of both liquefiers and non-liquefiers. Cultures are kept 30 days.

POTATO CULTURES

Potatoes cut in cylindrical pieces with a slanted surface are sterilized in tubes having moistened cotton on the bottom to prevent drying out of the potato. Inoculation is made in a streak up the slanted surface. After incubation description is made in the same manner and using the same terms as for agar slants. Records

are made on the chart in the blank space with heading "Medium solid."

NUTRIENT BROTH

Nutrient broth is made from beef extract, peptone, and distilled water. After inoculation the nutrient broth cultures must be handled carefully to avoid shaking down any pellicle or surface growth that may be present. After recording surface growth and clouding, which may be distinguished better by comparison with an uninoculated tube, the character of the sediment may be determined by giving the tube a whirling motion and observing.

AGAR PLATES FOR COLONY DESCRIPTIONS AND SUNLIGHT TEST

Inoculate a water blank with a small amount of culture taken from the original agar slants. Shake, and with a sterile loop transfer once from water blank to a tube of melted agar. Shake agar without having medium touch the cotton plug. Sterilize loop and make two transfers from first agar tube to a second. Pour both agar tubes into plates marking them "a" and "b" in addition to the culture number. When cool paste a thick opaque paper over one-half the bottom of each plate. This is to shut off the light. Expose plates to the sunlight for fifteen minutes, watching to see no shadows creep over them. Place in locker to incubate. After seven days make descriptions of both surface and deep colonies from the plate that shows the most favorable number of bacteria.

To determine the percentage of bacteria killed by sunlight count an equal area on both the shaded and sun exposed portion of the Petri dish. Subtract the number of bacteria in sun exposed area from the number in the shaded area and divide by the number of bacteria in the shaded area.

Example. Number of bacteria in shaded area 28, number in sun exposed are 12. $28 - 12 = 16$ $16 \div 28 = 57.1$ The result is approximately 57 per cent.

GELATIN PLATES

These are made in the same manner as the agar plates but are not exposed to sunlight. Incubate in the locker **right side up**. Liquefying bacteria must be watched carefully to get descriptions before the gelatin is entirely liquefied.

NITRATE BROTH

Nitrate broth is a medium made by using small amounts of peptone and potassium nitrate in tap water. After cultures have

grown for the required length of time tests are made as follows: With sterile pipette remove 2 cc. of culture to a clean test tube. Add 1 cc. of test solution which is made up of naphthylamin, acetic acid, sulphanilic acid and water. A pink color shows the presence of nitrite. Test an uninoculated tube in the same manner for a control. Observations are made for gas bubbles rising in the nitrate broth cultures.

NITRATE AGAR

Nitrate agar is the same as standard agar with .1 per cent KNO_3 added. It is used in slants, which are inoculated by making both a streak on the surface and a stab to the bottom of tube. The test solution is poured over the surface of the medium. Gas production is shown by cracks in the agar.

HYDROGEN SULPHIDE

A beef extract agar very rich in peptone and containing lead acetate is used in tubes. This is inoculated by stabbing with a straight needle. A darkening of the medium within a few days shows that hydrogen sulfide has been produced.

INDOL PRODUCTION

Cultures are grown in Dunham's peptone solution. It is composed of peptone, sodium chloride and distilled water. After four days the Ehrlich test is made as follows: Add to the culture 1 cc. of a 2% solution of paradimethylaminobenzaldehyde in 95% alcohol. Then add drop by drop $\frac{1}{2}$ cc. of concentrated HCl. If indol is present a pink zone appears which deepens and widens on standing. The test may be confirmed by shaking the culture with chloroform to see if the pigment dissolves. If it proves soluble the test is considered positive.

FERMENTATION TUBES

The medium is nutrient broth with one per cent of the different sugars added. It is put into tubes in which are small inverted inner tubes which are also filled with the medium. If gas is formed some of it will collect in the inner tube and the percentage can be measured by the gasometer chart shown on page 41. If the bacteria grow only within the inner tube (this is shown by cloudiness or sediment) it shows that they can grow only in the absence of oxygen and are anaerobic. When growth occurs only outside the small tube it shows that the bacteria require oxygen and are aerobic. When growth occurs both inside and outside the inner

tube it is called facultative anaerobic. Observations on growth and tests for alkalinity or acidity are made usually at 2, 7, and 14 days.

STARCH AGAR

Starch agar is like the ordinary agar with .2% of soluble starch added. It may be poured hot into plates. When cool it is inoculated by making **one** streak across the center. Do not let the loop cut down into the agar. Invert to incubate.

After the culture has grown the required length of time, usually 7 days, it is covered with a solution of iodine. If the starch is unchanged by the bacteria the entire plate will turn blue. When diastase is present a clear zone will appear along the line of inoculation. The width of the clear zone shows the intensity of the diastatic action. This is measured in millimeters from the edge of the growth to the edge of the blue. Records should be made at once as the blue reaction disappears within a few minutes.

MILK AND LITMUS MILK

Fresh skimmed milk is tubed and sterilized. For the litmus milk enough litmus solution is added to give it a distinctly blue color.

In testing reaction of milk sterilize loop and take a loopful out onto litmus paper. If it is necessary to take a second loopful sterilize loop before taking it to avoid contaminating the culture. Test an uninoculated tube at the same time for comparison. Fresh milk has what is called an amphoteric reaction, that is, it turns red litmus slightly blue and blue litmus slightly red. If bacteria have produced a change of reaction litmus paper will be turned redder or bluer than the control. Milk and litmus milk tubes from the same culture should show the same reaction.

Coagulation is when milk becomes thick like sour milk. Peptonization is shown by a clearing or whey like appearance due to destruction of the casein. It generally begins at the surface and works downward. The milk may or may not be curdled. Sometimes when a hard curd is formed whey is pressed out. This is called "extrusion of whey" and can usually be distinguished from peptonization by the clearness of the whey. Reduction refers to the destruction of litmus color. When complete the litmus milk looks like the plain milk. Reduction usually begins at the bottom of the tube and works upward. Sometimes the color becomes pale throughout the entire tube. A change of color to red or blue indicates change of reaction not reduction.

THERMAL DEATH POINT

The thermal death point of an organism is customarily defined as the least temperature that will destroy it in ten minutes under known conditions. Several factors influence the thermal death point. Old cultures are less resistant than younger ones. An acid medium renders heat much more effective. Moist heat is much more efficient than dry heat. For comparative work it is necessary to use media of uniform reaction and composition. The presence of spores indicates that the organism has two thermal death points; one for the spores and another lower one for the vegetative cells. The medium recommended by the Committee on Identification of Species of the Society of American Bacteriologists, is nutrient broth. Several tubes are inoculated then heated in water baths at different temperatures ranging usually from 50°C to 70°C for 10 minutes. They are then incubated to see if growth takes place.

Fermentation Tube Chart

PLATE 2.

DESCRIPTIONS OF VARIOUS SPECIES OF BACTERIA

The need has long been felt of having for student use a variety of bacterial cultures that have been so carefully worked through that their characteristics are known. With this in view named cultures have been obtained from the American Museum of Natural History in New York and from various other laboratories as well as from our own. These have been carefully worked through, in most cases several times, and by different persons. Work on doubtful points has been repeated many times.

Anyone familiar with similar work will understand the difficulties encountered, such as changes in the cultures due to prolonged cultivation on artificial media, differences in appearances of cultures and in reactions due to differences in materials, especially peptone, from which the culture media are made, differences due to personal equation, etc.

We have retained the names of bacteria as they were sent to us even when in a few cases it is believed they were named incorrectly.

In the condensed form in which the descriptions are tabulated we have not found it practical to always give all the characteristic points and details in regard to a culture. The main points, however, are given in the majority of cases.

The fifth figure in the Index Number, which refers to pathogenicity, has been omitted from these charts.

Group Number	Limits of size	MORPHOLOGY		AGAR SLANT										GELATIN STAB				POTATO				NUTRIENT BROTH				GELATIN PLATE				AGAR PLATE				MILK				NAME AND NUMBER
		Flagella	Capsules	*Growth	Elevation	Glistering	Topography	Consistency	20° discolored	37° discolored	Grows at 37° C	Surface growth	Line of puncture	Liquefaction begins days	Form	*Growth	Elevation	Glistering	Topography	Chromogenesis	Discolored	Ring	Pellicle	*Clouding	*Sediment	Form	†Liquefaction	Internal structure	Form	Internal structure	% Killed by sunlight	Reaction	Curd, acid or rennet	*Peptonization	Indol	*Hydrogen sulfide		
1331-2230-2222	.7 x .6 - .9 x .7	0	0	1 F	F	+	S	Bu	0	0	+	+	F	0	0	0	0	0	0	0	0	0	0	1	1 C	P	0 A	P A	61	+	A	3	2	0	Streptococcus lactis 90			
1331-2230-2222	.7 x .5 - .9 x .8	0	0	1 Bea	F	+	S	Bu	0	0	+	+	Bea	0	0	0	0	0	0	0	0	0	0	1	1 C	P	0 A	P A	53	+	0	3	1	0	Streptococcus lactis 100			
1331-2230-2222	.5 -1.1	0	0	1 FB	F	+	S	Bu	0	0	+	+	F	0	0	1 F	+	S	0	0	0	0	0	1	1 C	P	0 A	P A	43	+	0	1	3	0	Streptococcus rheumatis 152			
1332-2230-2222	.4-.7	0	0	1 B	F	+	S	Bu	0	0	+	+	F	0	0	0	0	0	0	0	0	0	0	0	1 C	P	0 A	P A	20	+	0	0	0	0	Streptococcus hemolyticus 161			
3331-1120-2222	.8-1.4	0	0	2 F	F	+	S	V	0	+	+	+	1	1	3 F	+	S	Cr	+	+	0	0	2	3 V	C	2 Fgr	C A	60	+	R	3	3	0	0	Micrococcus lactis varians 130			
3331-2120-2333	.8-1.4	0	0	2 F	F	+	S	V	0	+	+	+	1	1	2 R	+	S	Cr	+	+	0	+	2	2 V	C	2 Cgr	C A	73	+	0	3	2	0	1	Micrococcus lactis varians 129			
3331-2136-2333	.7-1.6	0	0	2 F	R	+	S	Bu	+	+	+	+	F	2	1	2 F	+	S	Y	+	+	0	0	1	1 F	C	2 Fgr	C A	60	+	0	0	0	0	0	Micrococcus lactis varians 162		
3322-2135-1333	.4-.7	1	0	3 S	R	+	S	Bu	+	+	+	+	F	13 St	1 F	+	S	Y	+	+	0	0	3	3 V	P	0 A	C A	70	0	0	0	2	1	0	Micrococcus agilis 32			
3331-1120-2232	.9-.5	0	0	2 F	F	+	S	V	0	+	+	+	+	1 Sac	3 R	+	C	0	+	+	+	+	0	2	1 V	C	3 A	C A	70	0	0	0	0	0	0	Staphylococcus albus 127		
3331-1129-1333	.84-.56	0	0	2 Ech	R	+	C	Bu	0	0	+	+	F	12 Crat	1 F	+	S	Pink	+	+	0	0	1	2 gr	P	0 A	C A	90	0	0	0	0	3	0	Rhodococcus roseus 124			
4331-1136-1222	.7-1.1	0	0	3 S	R	+	S	Bu	0	0	+	+	+	2 Sac	2 R	+	S	Orange	+	+	+	+	0	1	3 gr	C	2 A	C A	100	0	0	0	0	2	1	Sarcina aurantiaca 102		
4331-1136-1233	1.2-.6	0	0	2 F	R	+	C	Bu	0	0	+	+	+	1 Sac	3 R	+	S	Orange	+	+	0	0	1	2 V	C	2 A	C A	100	0	0	0	0	0	0	1	Sarcina aurantiaca 5		
4331-2135-2333	1.7-8	0	0	3 S	R	+	S	Bu	0	+	+	+	+	1 Nap	1 F	+	S	Y	+	+	0	0	0	2 C	C	3 Cgr	C A	50	—	0	1	2	1	1	Sarcina lutea 101			
4332-1135-1333	1.5-.7	0	0	3 S	R	+	C	Bu	+	+	+	+	+	16 St	3 R	0 C	Y	+	+	+	+	0	0	0	2 C	C	0 A	C A	0	—	0	0	1	0	1	Sarcina flava 84		
4332-1239-2233	1.1-.56	0	0	2 F	F	+	C	Bu	0	0	+	+	F	0	0	3 F	+	S	Pink	+	+	0	0	0	3 gr	C	0 A	C A	0	0	0	0	0	0	0	Sarcina Slaneii 128		

1 Scanty slight 2 Moderate 3 Abundant †Liquefaction 1 Cup 2 Saucer 3 Spreading

*1 Scanty slight 2 Moderate 3 Abundant †Liquefaction 1 Cup 2 Saucer 3 Spreading

Group Number	Morphology	Agar Slant				Gelatin Stab		Potato				Nutrient Broth				Gelatin Plate		Agar Plate		Milk		Name and Number																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
		Flagella	Capsules	*Growth	Form of Growth	Elevation	Topography	Consistency	20° discolor	37° discolor	Grows at 37°	Surface growth	Line of puncture	Liquefaction begins days	Form	*Growth	Elevation	Clotting	Topography	Chromogens	Discolored		Ring	*Clouding	*Sediment	*Liquefaction	Form	Internal structure	% Killed by sunlight	Reaction	Curd, acid or rennet	*Peptonization	*Litmus reduced	Indol	*Hydrogen sulfide																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
5311-1130-2232	1.9 x 5 - 2.3 x 6	Peri 6-8	+	2F	E	+S	Bu	0	0	+	+	2St	1F	3R	+S	+C	Buff	+	+	+	+	+	2	1V	C	3Frr	C	0	0	1	3	0	0	0	0	B. cohaerens 71																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
5311-1135-1333	1.9 x 3 - 2.9 x 5	Peri 4-6	0	3F	R	+S	Bu	0	0	+	+	1Nap	3R	3F	+S	Y	Y	+	+	+	+	+	0	3	2V	C	2Frr	C	0	0	0	0	0	0	0	0	B. aquatilis 38																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
5311-2127-2232	5 x 3 - 1.8 x 5	Peri 6-10	+	3F	R	+S	Bu	+	+	+	+	11	3F	3F	+S	Red	Red	+	+	+	+	+	0	3	3C	C	3A	C	0	0	R	2	3	0	0	0	0	B. prodigiosus 3																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
5311-2127-2232	7 x 5 - 2.5 x 6	Peri 6-10	+	3F	R	+S	Bu	+	+	+	+	11	3R	3F	+S	Red	Red	+	+	+	+	+	0	3	3C	C	3Frr	C	0	0	R	2	3	0	0	0	0	0	0	B. prodigiosus 10778																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
5311-2220-2233	8 x 6 - 2.7 x 7	Peri 5	+	3Sp	F	+C	Bu	0	0	+	+	0	2F	3F	+S	Buff	Buff	+	+	+	+	+	0	2	2V	C	0A	C	0	0	0	0	0	0	0	0	0	0	0	0	B. icteroides 21																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
5312-1230-2232	6 x 4 - 1.9 x 6	Peri 6	0	1F	F	+S	Bu	0	0	+	+	0	3F	3F	+S	Buff	Buff	+	+	+	+	+	0	2	2V	C	0A	C	0	0	+	0	0	0	0	0	0	0	0	0	0	0	B. amylovorus 40																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
5312-2120-2232	6 x 4 - 1.9 x 6	Peri 4-5	0	2F	F	+S	Bu	0	0	+	+	2Sac	2F	2F	+S	Buff	Buff	+	+	+	+	+	0	2	2C	C	3A	C	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	B. enteritidis 143																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
5312-2120-2111	4 x 3 - 1.5 x 9	Peri 4-8	+	3F	F	+S	Bu	0	0	+	+	7Crat	2F	2F	+S	0	0	+	+	+	+	+	0	3	3Frr	C	0A	C	0	0	A	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B. cloacae 6																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
5312-2120-2111	9 x 4 - 2.1 x 6	Peri 6	+	2F	F	+S	Bu	0	0	+	+	1St	2F	2F	+S	0	0	+	+	+	+	+	0	3	3V	C	3A	C	0	0	A	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0</

*1 Scanty slight 2 Moderate

**Liquefaction 1 Cup 2 Sauces

3 Spreadings

1Mannite agar

GROUP NUMBER	MORPHOLOGY		AGAR SLANT				GELATIN STAB		POTATO				NUTRIENT BROTH				GELATIN PLATE		AGAR PLATE		MILK				NAME AND NUMBER																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
	Limits of size	Size of Mamority	Flagella	Capsules	*Growth	Form of growth	Elevation	Clistering	Topography	Consistency	20° discolored	37° discolored	Grows at 37°	Surface growth	Line of puncture	Liquefaction begins days	Form	*Growth	Elevation	Clistering	Topography	Chromogenensis	Discolored	Ring		Pellicle	*Clouding	Sediment	Form	*Liquefaction	Internal structure	% killed by sunlight	Reaction	Curd, acid or rennet	*Peptonization	*Litmus reduced	Indol	*Hydrogen sulfide																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
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5332-1230-2333	.6 x .4 - 1.8 x .4	1.3 x .6	0	0	3	F	R	R	S	Bu	0	0	+	+	F	0	0	0	3	R	+	S	Buff	+	+	+	+	3	3	V	C	0	A	C	A	40	+	0	0	0	1	0	B. 41 91																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
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5332-2220-2111	.9 x .6 - 3.5 x .9	2.7 x .9	0	+	3	F	R	R	S	Bu	0	0	+	+	+	0	0	0	3	R	+	S	Buff	+	+	+	+	+	3	V	C	0	A	C	A	0	+	0	0	0	3	1	B. coscoroba 54																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
5332-2220-2133	.7 x .5 - 9.1 x .4	1.7 x .5	0	+	2	F	F	F	C	Bu	0	0	+	+	+	0	0	0	2	F	+	S	Buff	0	0	+	0	2	2	C	C	0	A	C	A	64	+	0	0	0	0	3	B. pulorum 48																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
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		2 Scanty, slight	2 Moderate	3 Abundant			** Liquefaction		1 Cup	2 Saucer	3 Spreading																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										

*1 Scanty, slight 2 Moderate 3 Abundant ** Liquefaction 1 Cup 2 Sauter 3 Spreading † Mannite Agar

WATER TESTING

Part II.

WATER TESTING

The samples of water to be tested are from wells, springs, reservoir, and swimming pool. Two kinds of media are used—agar for plate cultures to give the bacterial count, and a bile salts medium in fermentation tubes to show gas production. The bile salts inhibit the growth of nearly all bacteria other than the intestinal forms. More than 10% of gas in the tubes after incubating two days at 37° C. is regarded as a positive presumptive test for *B. coli* and the water is considered unsafe for drinking purposes. When confirmation is desired further tests are necessary.

No absolute standard can be given to determine the potable quality of water from the number of bacteria in it. Water containing thousands of germs to the cubic centimeter may be far less dangerous than one containing but two germs if one of these two be a typhoid bacillus. It is not the number that proves dangerous, it is the kind. The agar count at 37° C. eliminates the water flora but not the soil bacteria. Harrison states that for deep waters the agar count should generally not exceed 10 per cc. and for surface water not over 100 per cc.

Four agar plates are made from each sample using 1 cc., .5cc., .2 cc., and .1 cc. respectively from the sample of water which has been shaken vigorously twenty-five times. Five bile salts fermentation tubes are inoculated with 1 cc. each.

The work should be done in the following order.

Make records in note book giving each sample an experiment number and one page.

Mark Petri dishes and tubes with locker number, sample number, and amount of water added.

With pipettes graduated to tenths of a cubic centimeter add the required amounts of water to the plates and fermentation tubes. These pipettes have eleven marks or graduations. They are marked beginning at the top 0, .1, .2, .3, .4, .5, .6, .7, .8, .9, and 1. Use the forefinger, never the thumb, on the top of the pipette to control the flow of water from the pipette. When 1 cc. is desired draw water up to the top mark and let out into the plate or tube until the lowest graduation marked 1 cc. is reached. This gives 1 cc. of water. When fractional parts of a cubic centimeter are desired draw water to the top mark and let out till it reaches the graduation marked with the number of tenths required. Each of the divisions represents .1 cc. One pipette is required for each sample. In handling pipettes never let the lower part touch anything but the sample to be tested. **Keep the fingers off from it.** Let the case of pipettes lie on the table—never stand it on end.

Pour into the plates melted agar cooled to 40° C. and mix well with the water.

When hard place plates and fermentation tubes in 37° C. incubator.

Incubate two days. They are then ready to be studied. Measure the percentage of gas by the gasometer chart on page 41.

Count the numbers of bacteria on the plates using the ruled glass counting plate and a hand lens. Make an average of the four plates before stating the number of bacteria per cubic centimeter. To make this average multiply the .1 cc. plate count by 10, the .2 cc. by 5, and the .5 cc. by 2 before adding to the 1 cc. plate count. Divide by four.

Gas percentages are not averaged. Results are stated in number of tubes showing more than 10% of gas.

BACTERIA IN MILK

Samples are taken from well mixed milk into sterile bottles and tested as soon as possible. Samples that cannot be tested at once should be kept cold—below 40° F. Standard agar is used for plating.

Milk cannot be added directly to the plates as is done in plating ordinary water samples because it usually contains so many bacteria that the colonies would not have room to develop properly or be readily counted. Also the milk would cloud the agar so the colonies could not be easily seen. By diluting the milk with sterile water clear plates having between 25 and 300 colonies can usually be obtained. Plates having numbers of colonies outside these limits do not give as reliable results. Four plates are made using at least two different dilutions. For miscellaneous milk samples the character of which is not known dilutions may be made ranging from 1-100 to 1-10000. When something is known as to the quality of the milk, dilutions are made so that the highest one will give about 100 colonies to the plate. For a sample of milk which may contain 20,000 bacteria per cc. dilutions of 200 and 100 would be best for giving the desired numbers of colonies on the plates. The method of plating is as follows: Shake the milk sample vigorously 25 times. With a sterile pipette transfer 1 cc. to dilution bottle containing 199 cc. of sterile water. As this pipette is to be used once later it is laid aside so that the lower half will touch nothing. Shake dilution 25 times and with another sterile pipette transfer 1 cc. to each of two plates. This gives the 200 dilution. Lay aside this pipette also so it will not become con-

taminated. With the first pipette used transfer another cubic centimeter of milk to the dilution bottle. This doubles the amount of milk and decreases the dilution one-half, thus giving the 100 dilution. Discard the pipette. Shake sample and make transfers from it to the remaining two plates, using the same pipette as was used for the first plates but first rinsing out any of the first dilution remaining in it by drawing up some of the second dilution and letting it out. Transfers are always made using 1 cc. quantities in dilution work. Melted agar is poured into the plates and mixed with the dilution. Plates are inverted after hardening and incubated at 37° C. for two days.

Now if the sample of milk is older or of poorer quality and may contain 500,000 bacteria, allowing 100 bacteria per plate would make the required dilution 5000. This is too large a quantity of water to be handled in one container. The same result is secured by using two bottles in succession having 50 cc. and 100 cc. of water respectively. Multiplying amounts of water in the bottles gives the dilution. When bottles of different sizes are used always use the smaller first. In this case 1 cc. is taken from milk sample to 50 cc. bottle and pipette discarded. This 50 cc. dilution is used exactly the same as the milk sample was in the preceding case. Laboratory dilution bottles may be had marked for 10 cc., 20 cc., 40 cc., 50., 60., 100., 200., 400, and 500 cc.

After incubation counts are made from the plate cultures. To find the number of bacteria per cc. of milk, multiply the plate counts by their respective dilutions, add together and divide by four. If some plates are covered entirely by spreaders or otherwise spoiled they are left out of the average.

When it is desired to find the number of acid producing bacteria and liquefying bacteria in milk, litmus lactose gelatin plates are made in the same manner as the agar plates except that 2 cc. of litmus solution is added to the gelatin just before pouring plates. The plates are incubated right side up at 20° C. for seven days unless liquefying bacteria make it necessary to count earlier. Acid bacteria turn blue litmus red.

The interpretation of the results must depend upon the history of the milk.

1. When analysis is made immediately after milking conclusions may be drawn as to the cleanliness and care in the dairy, the thoroughness in cleaning and sterilizing of the utensils, and sometimes as to the presence of cows with infected udders. With properly cleaned and sterilized milk vessels and care in milking the numbers of bacteria should not exceed 10,000, and may easily be brought lower. Larger numbers indicate unclean methods, unsterile utensils or infected udders.

2. If milk is properly cooled with ice the numbers should increase only very slowly. 50,000 is a high count for milk delivered the next day even in warm weather.

3. For milk which must be a longer time in transportation higher numbers may be expected. Milk with more than 1,000,000 bacteria has not been properly cared for.

DIRECT MICROSCOPIC EXAMINATION OF MILK

The advantages of this method are that it is very rapid, results being obtained within a few minutes and before the milk from which the sample is taken is used. It is simple and requires little apparatus. It also gives some idea as to the kinds of bacteria. It does not, however, distinguish between the living and the dead bacteria. With milk of fairly good quality the numbers cannot be easily determined as no bacteria can be found. Its chief advantage is in the quick detection of milk of very poor quality.

The milk samples are taken and shaken as for plating. With pipette calibrated to deliver 1-100 cc., transfer this amount of milk to a glass slide. Spread over just 1 sq. cm. The slide may have the square centimeter ruled on it or it may be placed over glass or paper ruled in centimeter squares to serve as a guide. Duplicate smears should be made on the same slide. Milk on the exterior of the pipette should be wiped off before making the smear. Pipettes do not need to be sterilized. They are rinsed out with water between samples. The water remaining is rinsed out in the next milk sample. Dry the smears without heat. Cover smears with xylol for 1 minute. Drain and dry. Cover with 95% alcohol for 1 minute or more to fix the film to the slide. Drain and stain with a fresh aqueous solution of methylene blue for 10 minutes. Wash. If film is too dark decolorize slightly with alcohol. Slides may be examined at once or kept for later reference. They are examined under the microscope using the oil immersion objective. The bacteria in at least 30 fields in a square centimeter should be counted. With the Leitz microscope there are about 4800 fields in a square centimeter; with the Bausch & Lomb 5,400. Since only 1-100 cc. of milk is used the number of bacteria is found by multiplying the average number of bacteria per field by the number of fields in 1 sq. cm. and this by 100.

EFFECT OF TEMPERATURE ON KEEPING QUALITY OF MILK

Absolutely fresh milk of good quality is taken at 5 P. M. and after mixing well is divided into three parts. These are kept at 5° C., 20° C., and 37° C. respectively for twenty hours.

Make four litmus lactose gelatin and four agar plates from each sample using the following dilutions for each medium:

5° C.

200 200 100 100

20° C.

100,000 100,000 50,000 50,000

37° C.

1,000,000 1,000,000 500,000 500,000

Incubate agar plates at 37° C. for two days. Count and find the average number of bacteria per cubic centimeter for the three samples.

Incubate the litmus lactose gelatin plates at 20° C. for seven days unless liquefiers threaten to destroy the plates when they should be counted sooner. Count total number of bacteria, acid bacteria (those that turn litmus around them pink) and liquefying bacteria. Calculate the average number per cubic centimeter for total bacteria, acid bacteria, and liquefying bacteria.

How do the total numbers on agar and gelatin compare?

BACTERIA FROM VARIOUS SOURCES

The purpose of these experiments is to show the abundance of bacteria in their relation to our everyday life.

1. Wash hands in pan containing 1000 cc. of sterile water. Plate using

1 cc. .5 cc. .2 cc. .1 cc.

2. Wash hands carefully with soap under tap then without drying them or touching anything wash again in a second pan containing 1000 cc. of sterile water. Plate using

1 cc. .5 cc. .2 cc. .1 cc.

3. An apparently clean wash bowl is washed with a sterile brush and 1000 cc. of sterile water. Plate using

1 cc. .5 cc. .2 cc. .1 cc.

4. Place a pencil in test tube with 10 cc. of sterile water. Wash pencil well and plate using

1 cc. .5 cc. .2 cc. .1 cc.

Laboratory Outline for General Bacteriology

5. Pour three plates with agar. When cool sneeze into one, cough into another and place a hair in the third.

6. Catch two flies and place them in bottle with 100 cc. of sterile water. Shake 5 minutes and plate using
1 cc. .5 cc. .2 cc. .1 cc.

7. Shake a dishcloth, which has been used twice in ordinary way since being thoroughly boiled, in 1000 cc. of sterile water. Plate using

1 cc. .5 cc. .2 cc. .1 cc.

Pour agar in plates and incubate 2 days at 37° C. and study.

8. Flame a clean glass slide, cool, and touch lip to it as much as you would touch it to a glass in drinking. Dry, and pass three times through flame. Stain 1 minute with watery fuchsin. Wash, dry and examine under the microscope using the oil immersion objective. Note the forms and arrangement of the bacteria present and the approximate number of bacteria per field.

9. Sterilize platinum loop, cool, scrape along the teeth near the gums. Mix with drop of water on a slide. Spread, dry, flame three times, stain 1 minute with watery fuchsin, wash, dry, and examine under the microscope using the oil immersion objective. Note the forms, arrangement, and abundance of the bacteria.

DISTILLED WATER EXPERIMENT

To demonstrate the small amount of food needed by bacteria, and that distilled water is not the same as sterile water.

Draw off 100 cc. of distilled water into each of two sterile flasks. Mark them "A" and "B".

Sterilize flask "A" in autoclav for 15 minutes at 10 pounds pressure. When cool make an agar plate from each flask using 1 cc. for inoculation.

Inoculate flask A with a **very small amount** of B coli. Shake and plate 1 cc. in agar.

Keep flasks and plates in locker.

After five days count plates and make others from flasks using dilutions of 1-10, 1-100, and 1-1000.

After 5 days count and find whether there has been an increase or decrease in the numbers of bacteria.

BACTERIA IN MEATS

Meats are brought directly from the market to the laboratory. Those not already ground are passed through sterilized meat grinders. Weigh out 5 grams on sterile paper. Place in warm mortar. Add enough sterilized quartz sand to grind well. After grinding well add little by little 95 cc. of warm (about 38° C.) sterilized water. This makes a dilution of 20. Use bottles for the further dilutions required. For Hamburg steak and sausage use dilutions of 200,000 and 100,000; for mutton, veal, and pork use 100,000 and 50,000. Make four agar plates from each sample. Incubate at 37° C. for two days. Count plates and find the number of bacteria per gram of meat.

TESTING FOR PRESENCE OF SPORES

Inoculate tubes of sterile nutrient broth with the following:

1. Potato parings
2. Hay
3. Corn meal
4. Hamburg steak
5. Oatmeal
6. Wheat meal
7. Beans
8. Old milk
9. Mornings milk
10. Sweet corn
11. Raisins

After inoculating place in steam sterilizer in flowing steam for 15 minutes. This kills all vegetative forms. Incubate. After seven days examine tubes and make records of presence or absence of growth.

CONVERSION FACTORS

Grams to grains	multiply by	15.432
Grams to ounces avoirdupois	" "	0.03527
Kilograms to pounds	" "	2.2046
Cubic centimeters to fluid ounces imperial	" "	0.0352
Liters into fluid ounces imperial	" "	35.2
Meters into inches	" "	39.37

Laboratory Outline for General Bacteriology

Grains into grams	"	"	0.0648
Avoirdupois ounces into grams	"	"	28.35
Troy ounces into grams	"	"	31.104
Fluid ounces into cubic centimeters	"	"	28.42
Pints into liters	"	"	0.568
Inches into meters	"	"	0.0254

Degrees C. $\times 1.8 + 32 =$ degrees F.

Degrees F. $- 32 \div 1.8 =$ degrees C.

COMPARISON OF METRIC AND ENGLISH SYSTEMS

Meter = 39.3704 inches.

Millimeter = 0.03937 inches (0.04 approximately)

Inch = 25.3997 mm. (25.4 approximately)

Liter = 2.11 pints (1 quart approximately)

Cubic centimeters = 16.23 minims.

Fluid ounce = 29.578 cubic centimeters (30 cc. approximately)

Gram = 15.432 grains

Kilogram = 2.204 avoirdupois pounds

Ounce avoirdupois = 28.349 grains

Pound avoirdupois = 453.584 grams

Ounce troy = 31.103 grams

Square centimeter = 0.1548 square inches

Square inch = 6 square centimeters (approximately)

Cubic centimeter = 0.0609 cubic inches

Cubic inch = 16 cubic centimeters (approximately)

Appendix

Glossary of Laboratory Bacteriological Terms

Acid curd,	a curd produced by an acid.
Adherent,	applied to the sporangium wall, when it continues to enclose the developed spore.
Aerobic	(Strictly), growing only in the presence of free oxygen.
Amorphous	without visible differentiation in structure.
Anaerobic,	growing in the absence of free oxygen; cannot grow in presence of oxygen.
Anaerobic,	(facultative), growing both in the presence and absence of free oxygen.
Anaerobic,	(strictly), growing only in the absence of free oxygen.
Arborescent,	branched, tree-like growth.
Autotrophic	obtaining their atomic elements carbon, oxygen, hydrogen, nitrogen, sulphur, and iron from inorganic compounds or molecules.
Beaded,	in stab or stroke culture, disjointed or semi-confluent colonies along the line of inoculation.
Bipolar,	at both poles or ends of the bacterial cell.
Brittle,	growth dry, friable under the platinum needle.
Butyrous,	growth of butter-like consistency.
Capsule,	a gelatinous envelope surrounding bacteria (not easily stained.)
Chains,	short chains contain from 2 to 8 elements, Long chains more than 8 elements.
Chromogenesis,	the production of color.
Chromogenic,	relating to or having chromogens.
Chromogen,	any organic coloring matter.
Ciliate,	having fine, hair like extensions, resembling cilia, sometimes not visible to the naked eye.
Clavate,	club-shaped. (Usually an involution form.)
Coagulate,	to curdle or clot by chemical action or fermentation.
Commas,	shaped like a comma.
Concave,	curving in.
Concentric,	having a common center, as spheres or circles; said loosely of any curves that are parallel or nearly so.
Contoured,	an irregular, smoothly undulating surface, like that of a relief map.
Convex,	curving out.
Coriaceous,	growth tough, leathery, not yielding to the platinum needle.
Crateriform,	the crater-shaped liquefaction of a medium.
Cretaceous,	growth opaque, and white, chalky.
Cuneate,	wedge-shaped.
Dextrose,	grape sugar, glucose.

Laboratory Outline for General Bacteriology

Diastatic action,	conversion of starch into simple carbohydrates, such as dextrins and sugar by means of diastase.
Drum sticks,	bacteria in the shape of a club.
Echinulate,	a growth along the line of inoculation with toothed or pointed margins.
Effuse,	growth thin, veilly, unusually spreading.
Elongated,	drawn out, lengthened or extended.
Endospore,	thick walled spores formed within the bacterial cell; i. e. typical bacterial spores like those in <i>B. anthracis</i> or <i>B. subtilis</i> .
Entire,	smooth, having a margin destitute of teeth or notches.
Filamentous,	growth composed of long, irregularly placed or interwoven threads.
Filaments,	applied to the morphology of bacteria, it refers to thread-like forms, generally unsegmented; if unsegmented, to be distinguished from chains (q. v.) by the absence of constrictions between the segments.
Filiform,	in stroke or stab cultures, a uniform growth along the line of inoculation.
Flagella,	fine hair-like extensions that propel certain kinds of bacteria through liquids.
Flaky,	resembling or consisting of flakes or separable into flakes.
Flocculent,	containing small adherent masses of bacteria of various shapes floating in the culture fluid.
Fluorescent,	having one color by transmitted light and another by reflected light.
Granular,	composed of small granules.
Gram's stain,	a method of differential bleaching after gentian violet, methyl violet, etc. The + mark is to be given only when the bacteria are deep blue or remain blue after counterstaining.
Grumosc,	clotted.
Heterotrophic,	obtaining their elemental atoms of carbon, oxygen, hydrogen, nitrogen, sulphur and iron from organic compounds.
Infundibuliform,	form of a funnel or inverted cone.
Iridescent,	exhibiting changing rainbow colors in reflected light.
Lactose,	milk sugar.
Litmus,	red indicates acid, blue indicates alkaline.
Liquefaction,	change of a solid into a liquid.
Lobate,	having the margin deeply undulate, producing lobes. (see undulate.)
Long rod,	more than two diameters in length.
Luminous,	glowing in the dark, phosphorescent.
Maximum temperature,	the temperature above which growth does not take place.
Membranous,	growth thin, like a membrane.
Minimum temperature	temperature below which growth does not take place.
Motile,	having power of motion.
Myceloid,	colonies having the radiately filamentous appearance of mold colonies.
Napiform,	liquefaction in the form of a turnip.
Non-chromogenic,	not having chromogens or coloring matter.
Opaque,	impervious to light.

Laboratory Outline for General Bacteriology

Opalescent,	resembling the color of an opal.
Optimum temperature.	the temperature at which growth is most rapid.
Papillate.	growth beset with small nipple like processes.
Pathogen,	a kind of bacteria causing disease.
Pellicle,	bacterial growth forming either a continuous or interrupted sheet over the culture fluid.
Peritrichiate,	covered with flagella over the entire surface.
Peptonization,	rendering albumen of milk soluble by the action of trypsin.
Persistent,	lasting many weeks or months.
Photogenic,	producing phosphorescence.
Plumose,	a fleecy or feathery growth.
Polar,	at the end of pole of the bacterial cell.
Prototrophic,	prototrophic bacteria are those that can use elemental atoms of carbon, oxygen, hydrogen, nitrogen, sulphur and iron.
Pulvinate,	decidedly convex, in the form of a cushion.
Punctiform,	very small, but visible to the naked eye; under one mm. in diameter.
Pure culture	culture containing only one species of bacteria.
Rapid,	developing in 24 to 48 hours.
Raised,	growth thick, with abrupt or terraced edges.
Reaction,	refers to the production of acid or alkali in culture media by bacteria. Use + to indicate acid, - to indicate alkaline, and O no change.
Reduction,	removing oxygen from a chemical compound. Refers to the conversion of nitrate to nitrite, ammonia, or free nitrogen, and to the decolorization of litmus.
Rennet curd,	a curd produced by a lab ferment, not by an acid.
Rhizoid,	growth of an irregular branched or root-like character, as in <i>B. mycoides</i> .
Ring,	growth at the upper margin of a liquid culture, adhering more or less closely to the glass.
Rods,	long, more than two diameters in length.
Rugose,	wrinkled.
Saccharose	cane sugar.
Saccate,	liquefaction in the form of an elongated sac, tubular cylindrical.
Saprophyte,	an organism living on dead organic matter.
Short rods,	less than two diameters in length.
Slow,	requiring 5 or 6 days for development.
Spirals,	bacteria growing in the form of a spiral.
Spreading,	growth extending much beyond the line of inoculation, i. e. several millimeters or more.
Sporangia,	cells containing endospores.
Sporangium wall,	the wall of a cell within which a spore has been produced.
Spore,	the resting stage of bacteria.
Stratiform,	liquifying to the walls of the tube at the top and then proceeding downwards horizontally.
Sucrose,	same as saccharose.
Transient,	lasting a few days.
Translucent,	semi-transparent.
Truncate,	ends abrupt, square.
Turbid,	cloudy with flocculent particles; i. e. cloudy plus flocculence.
Umbonate,	having a button-like, raised center.
Undulate,	border wavy, with shallow sinuses.
Villous,	having hair-like extensions.
Viscid	growth follows the needle when touched and withdrawn; sediment on shaking rises as a coherent swirl.

Index

A

Acid, production of.....	31, 40, 51, 53
Adjustment, coarse.....	11
fine	11
Aerobic bacteria, strict	31, 32, 39, 57
Agar	
composition of.....	17
melting point of.....	17, 18
nitrate.....	31, 34, 39
slant.....	20, 29, 32, 34, 37
solidification of.....	18
source of.....	17
starch.....	31, 34, 40
types of growth on...	29, 30, 32, 33
use of 17, 18, 22, 23, 38, 49, 51, 54,	55
Air, bacterial analysis of.....	18-20
Alcohol, cleaning of microscope....	11
for decolorization.....	35, 52
for stains.....	35, 52
Anaerobic, facultative	32, 39, 57
strict.....	31, 32, 39, 57
Anilin gentian violet,	
preparation of	35
use of.....	35
Apparatus, sterilization of....	13, 14
Area of Petri dish,	
determination of.....	19
Autoclav	14
Azotobacter chroococcum.....	46

B

Bacteria (B. and Bact. listed together)

abortus	46
acidophilus	45
aerogenes	45, 46
albolactus	44
arborescens	44
amylovorus	45
aterrimus	44
aquatis	45
buccalis	44
butyricus	44
campestris	45
cereus	44
cohaerens	45
coli	45
coscoroba	46
cyaneus	44
cyanogenes	45
enteritidis	45

Bacteria (continued)

erythrogenes	45
fluorescens	45
fluorescens liquefaciens	45
fluorescens non liquefaciens	45
fluorescens tenuis.....	45
fusiformis	44
gasiformans	45
graveolens	44
Grunthal	45
Hartlebii	45
icteroides	45
lactis	44
lactis acidi	43
lactis erythrogenes	45
megatherium	44
mesentericus	44
mirabilis	45
moelleri	44
mucosus	52
mycoides	44
niger	44
niger lactis	44
petasites	44
prodigiosus	45
proteus	45
proteus vulgaris	45
proteus Zopfii	44
pullorum	46
radicicola	45
simplex	44
subtilis	44
subtilis ruber	44
terminalis	44
tumefaciens	45
vulgatus	44
Bacteria, effect of moist	
and dry heat	13, 14
flagella of	17
from various sources	53
Gram negative	36, 58
Gram positive	36, 58
identification of 22, 23, 24, 25, 26,	27, 28, 29, 30, 31, 32
in air	18, 19, 20
in meats	55
on pencils	53
on hands	53
spores of	14, 55
unstained, examination of ...	16, 17
vegetative forms, destruction	
of, by heat	14
Broth, nitrate	38, 39
nutrient	38

Laboratory Outline for General Bacteriology

C

Calculations	
for microscopic counts	52
for plate counts	19
Capsules	57
Carbol fuchsin	35
Cautions for bacteriological work	22, 23, 24, 25, 26, 27
Chart, descriptive (Soc. Am. Bact)	28, 29, 30, 31, 32
Coagulation	40, 57
Condensation water	17
Condenser, microscope	11
Conversion, Metric to English	55
English to Metric	56
Cough, bacteria disseminated by	54
Counting,	
direct microscopic	52
lens	19
plates	19
Cover-glass	16, 36
Culture media, descriptions of	17, 18, 37, 38, 39, 40
Cultures,	
age for examination	34
plate	24, 25
pure	59
slant	33
stab	33
streak	33
Curd, acid	31, 57
rennet	31, 59

D

Death-point, thermal	41
Descriptions of bacteria	42, 43, 44, 45, 46
Descriptive chart	43, 44, 45, 46
Dextrose fermentation tubes	34
Diastatic action	58
Dilution methods of	50, 51
Dishcloth, bacteria on	54
Dish, Petri	15, 38, 49
Distilled water experiment	54
Do's in identification of unknown cultures	22, 24, 26
Don'ts in identification of unknown cultures	23, 25, 27

E

Ehrlich's test for indol	39
Endospores	29, 37, 58
Equipment of lockers	14, 15
Examination of cultures, age for	34

F

Facultative anaerobic	32, 39, 57
Fermentation tubes	39
chart for measuring gas	41
Films, preparation of	36
Flies, bacteria on	54
Flagella, method of staining	37
Flame, gas for sterilization	13
Focusing	11
Form, for writing up exercises	15
Formulae, for changing °C. to °F.	56
Metric to English, vice versa	55, 56
Fuchsin	
alcoholic solution of	35
alcohol	56
watery	35

G

Gelatin, history of	17
constituents of	17
litmus lactose	51, 53
liquefaction of	32, 33
plating	24, 25
solidifying points	17
stabs, types of growth	33
Gentian violet	
alcoholic solution of	35
anilin water	35
watery	35
Glassware, sterilization of	14
Glossary	57, 58, 59
Glycerine fermentation tubes	31
Gram negative	36, 58
positive	36, 58
Gram's stain	35
mixture	35

H

Hair, bacteria on	54
Hands, bacteria on	53
Heat, dry for sterilizing	14
moist, for sterilizing	14
Hot air sterilization	14
Hydrogen sulphide, test for	34, 39

I

Illumination for microscope	11
Incubation, temperatures of	34
Indicator	40
Indol	39
Ehrlich's test for	39
Inoculation of media	22, 23, 24, 25, 26, 27

Laboratory Outline for General Bacteriology

L

Lactose fermentation tubes	31
Lead acetate agar	34, 39
Light, effect on bacteria	25, 38
for microscopic work	11
Lips, bacteria on	54
Liquefaction of gelatin	32, 33
types of	33
Litmus milk	40
reduction of	40
Lockers, equipment of	14, 15
Loeffler's methylene blue	35

M

Magnification	12
Meats, bacteria in	55
Media, liquid	24, 25
nutrient	38
solid	37
Method for determining the per cent of bacteria killed by sunlight	38
Methylene blue	35
Micrometer scale	11
Measurement of bacteria	12
Micrococcus agilis	12, 43
Micrococcus lacticus varians	43
Microscope	11
Abbe condenser	11
cleaning of	11
how to use	11
lighting for	11
magnification	12
mirror of	11
Milk, dilutions for plating	50, 51
direct microscopic examination	52
effect of temperature on	53
peptonization of	32, 40
reaction of	40
staining of	52
Mirror, concave	11
plane	11
Molds	19
Mordant for flagella staining	35
Morphology	36
Motility	16, 17
Mouths of culture tubes, flasks, sterilization of	22, 23

N

Nitrate agar	38
Nitrate broth	38
test solution for	39
Nitrites, test solution for	39
Nutrient broth	38

O

Objectives, oil immersion	11
cleaning of	12
Oil, cedar	11
immersion	11

P

Paradimethylaminobenzaldehyde	39
Pencils, bacteria on	53
Peptone	39
Peptonization of milk	40
Pipette	15, 49
Plates, agar	38
gelatin	38
Plate cultures, examination of	50, 51
Plating	24, 25, 26, 27
Platinum needle, sterilization of	14
Potato	
preparation of	37
types of growth on	33
Pressure, of steam in sterilization	14
Prototrophic	59

R

Reaction	59
Reduction of litmus	40
Rennet curd	31, 59
Resistance of spores	55
Results, interpretation of	51
Rhodococcus roseus	43
Rules for use of microscope	11

S

Saccharose fermentation tubes	31
Sarcina	
aurantiaca	43
flava	43
lutea	43
Slanecii	43
Sneeze, bacteria disseminated by	54
Spores, testing for presence of	55
Staining	
flagella	37
Gram's method	35
Stains, preparation of	35
Staphylococcus albus	43
Starch agar	
test for diastatic action of bacteria on	40
Steam, sterilization by	14
Sterilization	
by dry heat	14
by gas flame	14
by steam under pressure	14

Laboratory Outline for General Bacteriology

Streptococcus hemolyticus	43
lactis	43
rheumaticus	43
Sunlight, effect on bacteria	38

T

Teeth, bacteria on	54
Temperature, conversion of de- grees of, effect on bacteria	53
Thermal death point	41
Testing for spores in various foods	55
Time for sterilization	14
requirements of cultures	34
requirements of stains	35, 36
Tubes, fermentation	31, 39

W

Washbowl, bacteria on	53
Water, bacterial analysis of	49
distilled	54
Whey, extrusion of	40

X

Xylol, use of	11
---------------------	----

Y

Yeasts, examination of	17
------------------------------	----

Z

Zone, clear, produced by diastatic enzyme	40
--	----

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