

Identification of an Unknown Bacterium and Writing Up a Report

A standard part of nearly all lab courses in introductory microbiology is an activity wherein the student must use everything that has been learned in the course to identify and unknown bacterial culture. Your ability to make aseptic transfers, perform the gram stain, identify cell morphology and shape, and conduct metabolic testing are under examination in this process. Your ability to follow the stepwise logic of a dichotomous key will also be tested. Overall, your strategy is to be parsimonious; you should only do needful tests according to your key and should only need to do them once.

Making the Dichotomous Key

The first thing to prepare for an Unknown Identification exercise is to make a dichotomous key. Keys are charts that require decisions at branch points, much like a flow chart in computer logic: If the answer to a question is yes, then do X; if the answer is no, then do Y.

Dichotomous keys are written from known characteristics of the possible organisms you may need to identify. In this Guide you will find a table of characteristics for and the names of the 18-24 bacterial species that you may be given in this exercise. These characteristics have been gathered from various sources, the most important of which is Bergey's Manual of Determinative Bacteriology. The table of characteristics in this guide only list those bacterial features which you have learned how to test for in this class. For this reason, you shouldn't need to consult Bergey's Manual to do this exercise, unless of course you are curious. You certainly don't want to incorporate into your key a test or characteristic which you have no way to examine since we only have the test media which you have previously used.

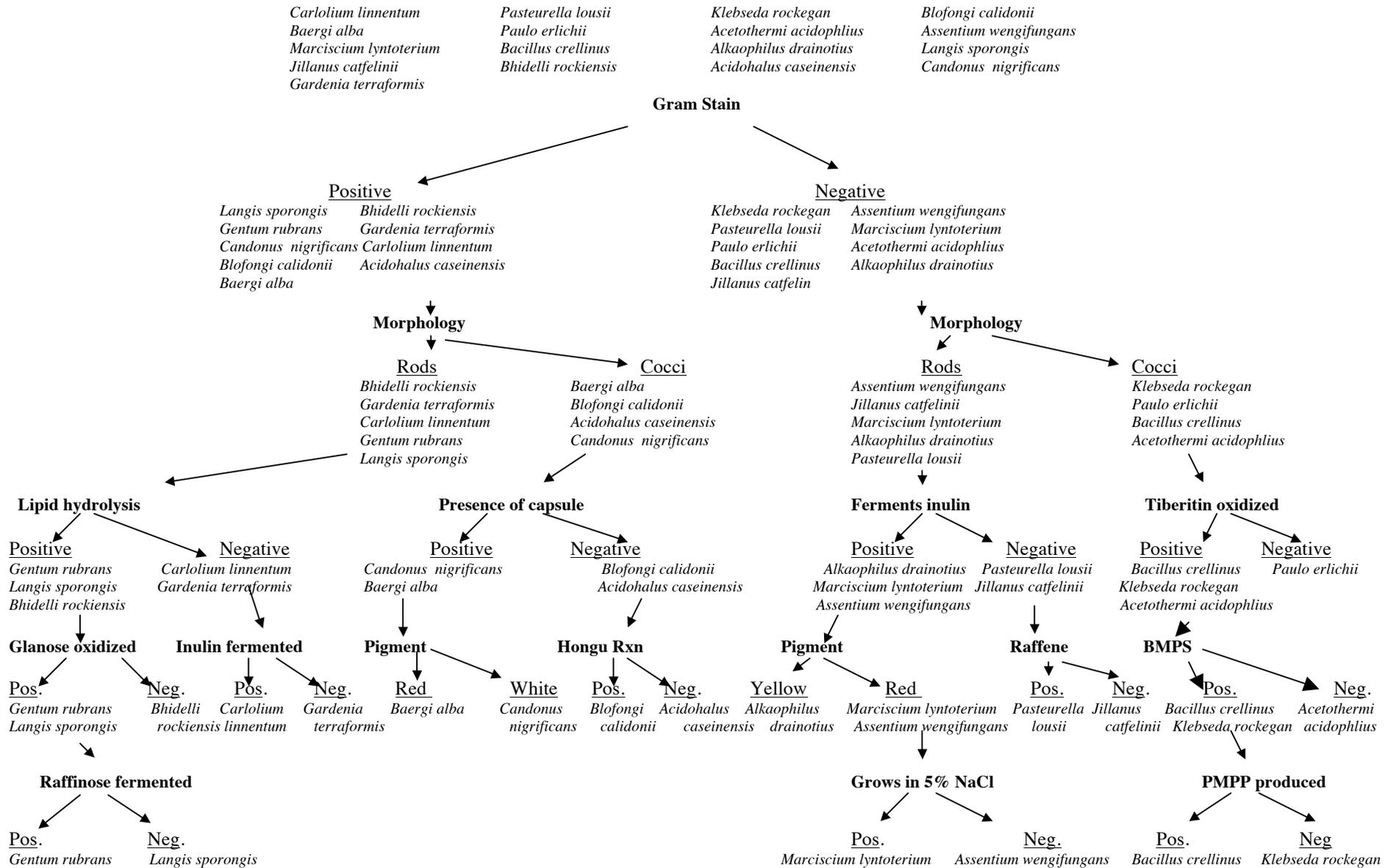
To make a dichotomous key, you first need to sort all the different species into two big groups. Most microbiologists begin with gram stain results, and I encourage you to do the same. Using the Mock Dichotomous Key in this Guide, you will see that you should begin your own key by writing out the names of all the possible bacteria in the test near the top of the page. I recommend using landscape (sideways) mode in laying out a key since the wider the page, the better.

After listing all potential species on your key sheet, you should next draw two arrows downwards that show this group being separated by the gram stain. Write *gram positive* at the end of one arrow and *gram negative* after the other. Follow each result with a list of bacteria still remaining in each group.

The next dichotomy to further separate the gram positives from each other, and the gram negatives from each other, could be one of morphology (bacilli or cocci). Or you can use some other characteristic to separate them into approximately equal subgroups. The important thing is that you are subdividing each group at every dichotomy into two subgroups of nearly equal size. If you separate off only one species at a time at each dichotomy, your key will be far too long and will not be parsimonious.

You will need a completed dichotomous key on the first day of the Unknown ID activity, when you get your assigned culture. The instructor may not give you a culture unless you can demonstrate that you have a completed dichotomous key to work from. Ultimately you will submit a typed copy of your dichotomous key with your final report. Try, if you can, to get it all on one page. Use the Mock Dichotomous Key in this Guide as an example.

Mock Flow Chart Dichotomous Key For Unknown Bacterium ID Exercise



Typical Reactions of Bacterial Unknowns Used at Pierce College (ed. 5/08)

Organism	Gram Stain, Shape	Culture characteristics on agar slant	Hemolysis	Litmus milk	Mannitol fermentation	Endospores	Nitrate reduct.	Glucose ferm.	Sucrose ferm.	Lactose ferm.	Starch hydrolysis	H ₂ S production	Indole production	Motility	Urea	Citrate utiliz.	MR/VP	Gelatin	Oxidase	Catalase
<i>Alcaligenes faecalis</i>	-Bacillus	Thin, white, spreading, viscous growth	Gamma	K	-	-	-	-	-	-	-	-	-	+	-	+/-	-/-	-	+	+
<i>Bacillus cereus</i>	+ Bacillus	Abundant, opaque, white waxy growth	Beta	D	-	+	+	A	A	-	+	-	-	-	-	+	+/-	+	-	+
<i>Bacillus megaterium</i>	+ Bacillus	Abundant, opaque, white waxy growth	Beta	-/-	A	+	-	A	A	-	+	-	-	-	-	+	+/-	+	-	+
<i>Bacillus subtilis</i>	+ Bacillus	Abundant, opaque, white waxy growth	Alpha	A,R	A	+	-	A+/-	A	-	+	-	-	-	-	-	-/-	+	-	+
<i>Citrobacter freundii</i>	-Bacillus	Translucent, moist, medium, round	Gamma	A+/-	A	-	-	A, G	A	A	-	-	-	+/-	-	+	+/-	-	-	+
<i>Corynebacterium xerosis</i>	+Bacillus	Grayish, granular, limited growth	Gamma	K	-	-	+	+	+	-	-	-	-	-	-	-	-/-	-	-	+
<i>Enterobacter aerogenes</i>	-Bacillus	Abundant, thick, white, glistening growth	Alpha	A	-	-	+	A, G	A	A+/-	-	-	-	+/-	-	+	-/+	-	-	+
<i>Enterobacter cloacae</i>	-Bacillus	White, fluffy round, irreg.	Beta	A	-	-	-	AG	AG	AG	-	-	-	+	-	+	-/+	-	-	+
<i>Enterococcus faecalis</i>	+ Cocci	Clear, smooth, small, round	Gamma	-	+	-	-	A	A	A	-	-	-	-	-	-	+/-	-	-	-
<i>Escherichia coli</i>	- Bacillus	White, moist, glistening growth	Alpha	A,C+/- ,G+/- ,R+/-	+	-	+	AG	AG	AG	-	-	+	+	-	-	+/-	-	-	+

Organism	Gram Stain, Shape	Culture characteristics on agar slant	Hemolysis	Litmus milk mannitol fermentation	Endospores	Nitrate reduct.	Glucose ferm.	Sucrose ferm.	Lactose ferm.	Starch hydrolysis	H ₂ S production Inocrite production	Motility	Urea	Citrate utiliz.	MR/V/P	Gelatin	Oxidase	Catalase
<i>Klebsiella pneumoniae</i>	-Bacillus	Translucent-creamy, mucoid, round	Alpha	A, G, C+/-	+	-	+	AG	AG	AG	-	-	-	+	-	-	-	+
<i>Micrococcus luteus</i>	+ Cocci	Soft, smooth, yellow growth	Gamma	K	-	-	+	-	-	-	-	-	+	-	-/-	+	-	+
<i>Proteus mirabilis</i>	-Bacillus	Thin, blue/gray, spreading growth	Gamma	K	-	-	+	AG	-	-	+	+	+	-	+/-	-	-	+
<i>Proteus vulgaris</i>	- Bacillus	Thin, blue/gray, spreading growth	Alpha	K	-	-	+	A	A	-	+	+	+	-	+/-	-	-	+
<i>Pseudomonas aeruginosa</i>	-Bacillus	Abundant, thin, white growth with medium turning green	Beta	K+	-	-	-	-	-	-	-	-	-	-	-/-	++	+	+
<i>Pseudomonas fluorescens</i>	-Bacillus	Transparent-light yellow shiny, smooth, filiform	Beta	K	-	-	-	A	-	-	-	-	+/-	-	-/-	+	+	+
<i>Staphylococcus aureus</i>	+ Coccus	Abundant, opaque, golden growth	Beta	A,R+/-	A	-	-	A	-	A	-	-	-	-	+/-	-	-	+
<i>Staphylococcus epidermidis</i>	+ Coccus	Off white, smooth, small, round	Gamma	A	-	-	+	A	-	A	-	-	-	-	+/-	-	-	+

Reading Chart

C curd, coagulation

R Litmus reduction

+/- Variable

K Alkaline

- No Growth

A Acid

N Neutral

G Gas

H₂S Hydrogen Sulfide

Getting Started on the First Day

Assuming you have prepared and completed your dichotomous key, you will pick up an assigned bacterial culture from the instructor. The culture may be provided as a broth or an agar slant. You should ask whether the culture is < 24 hours old as this will determine whether or not you can perform a reliable gram stain (re-read the Gram Staining test in your lab book if you don't understand why).

Write down in your lab book the number of your assigned culture. It is very important that you do not lose this number, for it is exclusively yours. Perform a gram stain on your culture at your earliest opportunity. You may need to transfer and grow the culture on a TSA plate for the appropriate amount of time before it is ready for gram staining. **In all cases, please do streak a TSA or Nutrient Agar plate (using colony isolating technique) and incubate the culture for at least 24hrs so you have a backup of your culture.** Part of your final score will be based on your ability to correctly streak a plate and get isolated colonies.

All plates, tubes, or other media which are to be placed in an incubator or the refrigerator must minimally have your full name, your culture number, and your lab section (morning, afternoon, evening). You should also write the date of inoculation on all media so you know when you started the test. I'll leave it to you to keep track of what medium is in the tube!

As part of your report, you will be keeping a log or journal of everything you do in regards to your culture, including inoculation and incubation times and dates, reagents added, colors seen, interpretations made, etc. Start writing down what you do on the first day in a notebook that can be kept in a safe place; you cannot lose your journal. **I will be asking for you to turn in both your handwritten journal AND a typed up, edited version of it in your formal report.**

So in summary, on the first day:

- 1.) Show your completed Dichotomous Key to the Instructor.
- 2.) Get your assigned culture from the instructor and write down its number in a safe place, including in your journal.
- 3.) Write your name and section on the culture tube to identify it.
- 4.) Make a subculture of your bacteria by *streaking for isolated colonies* on a TSA or Nutrient Agar plate. Incubate for 24-48 hours at 37°C unless you are told otherwise. Some bacteria are better grown at 30°C – you will be told if that is the case for your culture. Be sure your name, section, and assigned number are on the plate in the appropriate place. Record what you did in your journal. Do not throw away this plate until your instructor tells you to do so; you will be graded on your streaking technique.
- 5.) If your assigned culture is of appropriate age, perform the gram stain reaction. Record what you did in your journal and your results (what they looked like, what color they were, what interpretation you have on this).

- 6.) Based on your gram stain and/or cell morphology results, determine your next step in the identification process according to the logic in your dichotomous key. Show your teacher what you intend to do (bring along your key to show) and order the appropriate medium for your next test. Record your reasoning in your journal and describe the actions you take in setting up the next test.
- 7.) At this point you may be done for the day if your bacteria need to incubate overnight. It is your responsibility to come into the lab and remove your cultures to the refrigerator when they have completed incubation OR to leave Robert or myself a note when you want us to remove the culture to the refrigerator for over a weekend. **You cannot disrupt a class that is in session to move your cultures;** you'll have to wait until our class time or between classes.
- 8.) Be sure you show appropriate actions in cleaning up your lab space and in working safely while in the lab. I will be looking out for correct and safe placement of the Bunsen burners and incinerators, appropriate placement of notes versus cultures, whether you keep your chair pushed in, hair tied back (if appropriate), books and packs underneath, etc.

General Comments on Writing a Report

General Points

1. Unknown reports in microbiology are written in scientific format. Scientific writing is written differently from other types of writing. The results of the exercise or experiment are what are being showcased, not the writing. The purpose of scientific writing is not to entertain, but to inform. The writing should be simple and easy to understand. There is a specific style that must be followed when writing scientific reports.
2. Scientific writing is typically written in the passive voice. The pronouns "I", "We" and "They" are not typically used.. For example, instead of writing "I used a TSA agar plate to isolate my unknown," it is customary to write, "A trypticase soy agar (TSA) plate was used to isolate the unknown." It is also customary to write in the past tense for most of the report. This includes the introduction, the summary, the description of the materials and methods and the results. The present tense is reserved for the conclusions about the results. See the examples given below.
3. The name of the bacterium should be written and spelled correctly, according to scientific convention. The name should be italicized or underlined. Italicized names are preferred (e.g. *Staphylococcus aureus*). The genus is capitalized but the species is not. After the full genus name is given in the paper, it can be written as *S. aureus*, but is still italicized. This is as long as there is no other genera in the paper that starts with the same letter.
4. Your report should be single spaced with 1" margins using a standard font like Times or Helvetica with a size between 10 and 12 pt. Each page should have your name as a footer,

along with the page number, at the bottom. All pages of your report should be neatly stapled together in the upper left-hand corner. Reports submitted in binders or report folders, however nice, will be returned for simple stapling.

5. Your Unknown Identification report is **due within 5 minutes of the day and time according to the syllabus**. You are encouraged to submit your report early to avoid a late penalty. Late reports lose 20% per 24 hr period accrued from the due date and time. It is usually to your advantage to turn in an incomplete report on time than a completed report late. Under no circumstances will reports be accepted later than the last day of instruction for the academic quarter.
6. Look over the **Grading Rubric** at the end of this packet to see exactly which items are being graded in your report.

Specific Instructions for Writing Up the Report

The Unknown Bacterium Identification Report should be divided up into sections just like a scientific paper. All sections are labeled with their title (e.g. RESULTS) except for the title page. Each section will be described below.

TITLE PAGE (don't actually write this on your title page!)

There should always be a title page and should include the following information:

IDENTIFICATION OF UNKNOWN NUMBER #

Title should be centered and at the top or in the middle of the page

The following information should be centered and at the bottom part of the title page:

Your name
Date (the due date)
Lab instructor's name
Course name
Semester / year
Section number

INTRODUCTION

This section introduces the reader to the study and why the study was done. You should entitle this section simply as "INTRODUCTION" in all capital letters without using any underlining (bold emphasis is OK). All further sections of the report are treated the same way.

The introduction should only be a few sentences long. Example: "There are many reasons for establishing the identity of a microorganism. The reasons range from the knowing

the causative agent of a disease in a patient, so as to know how it can be treated, to knowing the correct microorganism to be used for making certain foods or antibiotics. This study was done by applying all of the methods that have been learned so far in the microbiology laboratory class for the identification of an unknown bacterium."

MATERIALS AND METHODS

This is where the details of the study are listed. Where did the specimen come from, and what methods were used to identify it? Be specific, but do not re write the lab manual. One way is to mention the names of the materials used and reference the lab manual for the procedure or method and then continue to elaborate when necessary.

Example:

An unknown labeled #6 was given out by the lab instructor. The methods that have been learned thus far for identifying bacteria have been applied to this unknown. Procedures were followed as stated in the course laboratory manual by LeBoffe (1), unless otherwise noted. The first procedure that needed to be done was to streak the unknown out on a Trypticase Soy Agar plate, using the T streak method described in the lab manual.. This needed to be done in order to test the purity of the unknown. After the plates were incubated and grown, the morphology was observed and recorded and a Gram stain was performed. Quality control bacteria were Gram stained along with the unknown to make sure that the Gram stain reaction was done correctly . After determining the Gram reaction, specific biochemical tests were performed. The biochemical tests were chosen from the unknown identification tables that were in the lab manual. Since unknown #6 was determined to be a Gram negative rod, an oxidase test was performed and the organism was inoculated into a BCP lactose tube. Note all of these tests were performed by the methods listed in the lab manual by LeBoffe (1). Table I lists the test, purpose, reagents and results.

All of the following tests were performed on this unknown:

1. Oxidase test
2. BCP Lactose
3. Indole
4. H₂S
5. Citrate
6. Motility
7. Methyl Red-Voges Proskauer
8. Urea

Another way to complete this section is to write out the methods in detail in either a paragraph form or listed. This way is *not necessary* for this type of paper, since this is lab report for the identification of an unknown bacterium and the methods are explained in detail in the lab manual. If there is a procedure that the instructor added or made changes to, or the student used another procedure not in the course lab manual, then it should be written out and referenced. See some of the examples of papers identifying an unknown from the web sited below

RESULTS

This is where the results are summarized. The method results should be in a table format (see examples below). Tables are enumerated in scientific reports using Roman numerals (I, II, III) followed by a period. A descriptive title should follow the table number.

This is also where the flow chart showing how you arrived at the answer is presented as a Figure. Figures (pictures, charts, or graphs) are enumerated using Arabic numerals (1, 2, 3....)

followed by a period. A descriptive title should follow the figure number. A short paragraph explaining how the results are presented should also be included.

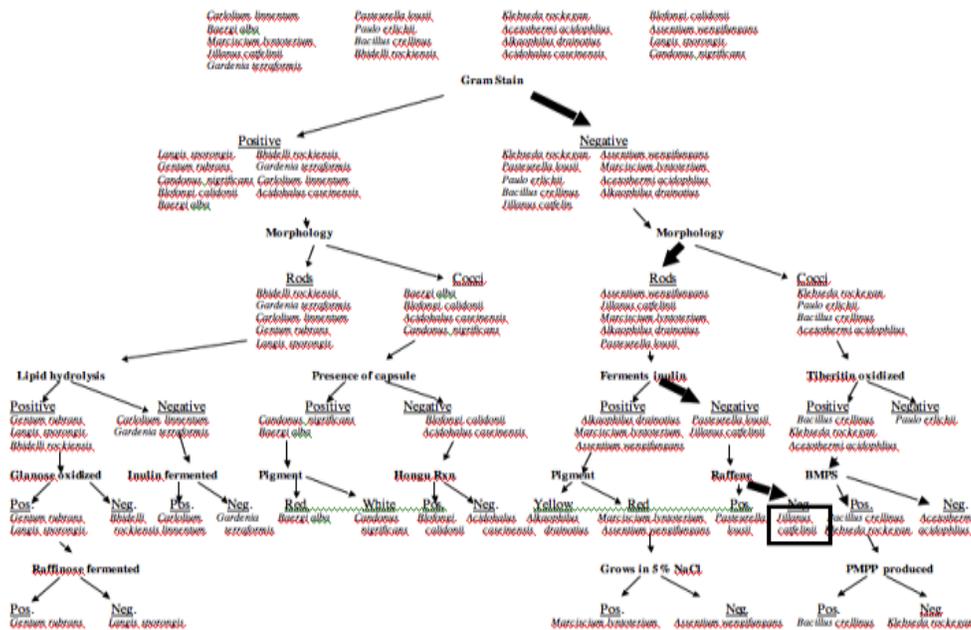
Example:

Unknown #6 had the following morphology on a TSA plate: medium sized opaque cream colored colony. After determining that it was a Gram negative rod, an oxidase test was performed and it was inoculated into a BCP lactose tube and onto a TSA slant. Table I. lists all of the biochemical tests, their purpose and results. The results are also shown in a flow chart form in Figure 1.

Table I. Tests and Results Performed in Sequence in Identifying Unknown #6.

TEST	PURPOSE	REAGENTS	OBSERVATIONS	RESULTS
Gram stain	To determine the Gram reaction of the bacterium	Crystal violet, Iodine, Alcohol, Safranin	Pink rods	Gram negative rods
Oxidase test	To determine the presence of cytochrome c	Oxidase paper	Purple / black color change	Positive oxidase test
BCP Lactose	To determine the ability of a bacterium to ferment a specific carbohydrate	None	Color change from purple to yellow	Positive lactose fermenter
Indole Test	To determine the ability of an organism to split indole from tryptophane	Kovac's added to 1 ml of tryptone broth	Red Ring at top of broth	Positive indole test

Figure 1. The pathway taken in the dichotomous key to identify the unknown bacterium, with bacterial characteristics from (1).



DISCUSSION

This section interprets the meaning of the results. The following questions should be answered here in essay form, using perhaps one or two paragraphs per question.

1. How did the test results lead to identification? Was it the correct identification? If not, why not?
2. What problems were encountered? This is also where the background information on the organism (environment/pathogenicity) that was identified is mentioned.

Examples of testing results:

After several differential tests, it was concluded that unknown #29 was *Escherichia coli*. After performing the Gram stain to determine that the unknown was a Gram negative rod, the organism was grown on a TSA slant for use in inoculating the rest of the biochemical tests. All of the biochemical tests worked well except for the indole test. It gave a false negative result at first. This was determined since it was inconsistent with the rest of the result. The TA suggested that the test be repeated and it was repeated. The repeated test gave a positive result, consistent with the other data. Therefore it was concluded that the unknown was *Escherichia coli*.

[Follow with details of other tests].

Example of background information on the organism:

E. coli is in the Enterobacteriaceae family. It is typically found in the human intestines, as well as other animals. It can cause disease in the right host. [The rest of this information should be researched from the textbook, internet or other microbiology resources.]

REFERENCES

The minimum number of references is three, the lab manual, the textbook and the handout showing bacterial characteristics (which came from Bergey's Manual of Determinative Microbiology, 9th D. Bergey, and John G. Holt, published by Lippinkott, Williams, and Wilkins., 1993). More can be used.

Correct reference format must be used. ***Reference format for scientific papers is different from MLA or ALA format!***

References should be numbered and the works should be cited within the text (called an "in-text" reference) using these numbers. For example, in the section where you present your flow chart based on Bergey's Manual characteristics that was your third reference in the Reference section, you might write "The reactions of the bacteria listed in this flow chart was taken from Bergey's Manual (3)" where the "(3)" is the in-text reference for this citation in references.

The spelling of the authors of the references must be correct.

Example:

1. De Mers, Marlene. Fundamentals of Microbiology Laboratory Manual, 4 th edition. San Diego: Montezuma publishing, 2004

2. Tortora, Funk, Case, etc.....
3. Leboffe, Michael, etc.....

The short guide to proper scientific reference formatting is taken from the Ohio State University Library system (at <http://library.osu.edu/sites/guides/cbegd.html>) and is based on *Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers* 6th edition, 1994.

In general, citation examples are provided in **Name-Year** format. **References section** items are listed alphabetically at the end of the research paper. These items are referred to in the body of the paper using the **In-Text** style.

Book

References section: Voet D, Voet JG. 1990. Biochemistry. New York: J Wiley. 1223 p.

In-Text: (Voet and Voet 1990, or by reference number)

Book Chapter (or other part with different author)

References section: Kuret JA, Murad F. 1990. Adenohypophyseal hormones and related substances. In: Gilman AG, Rall TW, Nies AS, Taylor P, editors. The pharmacological basis of therapeutics. 8th ed. New York: Pergamon. p 1334-60.

In-Text: (Kuret and Murad 1990, or by reference number)

Journal Article

References section: Johnson DL, Lynch WE Jr. 1992. Panfish use of and angler success at evergreen tree, brush, and stake-bed structures. N Am J Fish Manage 12(1):222-9

In-Text: (Johnson and Lynch 1992, or by reference number)

Dissertations and Theses

References section: Dettmers JM. 1995. Assessing the trophic cascade in reservoirs: the role of an introduced predator [dissertation]. Columbus (OH): Ohio State University. 188 p.

In-Text: (Dettmers 1995, or by reference number)

Conference Paper

References section: Meyer B, Hermanns K. 1985. Formaldehyde release from pressed wood products. In: Turoski V. editor. Formaldehyde: analytical chemistry and toxicology. Proceedings of the symposium at the 187th meeting of the American Chemical Society; 1984 Apr 8-13; St. Louis, MO. Washington: American Chemical Society. p 101-6.

In-Text: (Meyer and Hermanns 1985, or by reference number)

Conference Abstract

References section: Mendez MF, Manon-Epaillat R, Lanska DJ, Burstine TH. 1989. Epilepsy and suicide attempts [abstract]. In: American Academy of Neurology 41st annual meeting program; 1989 Apr 13-19; Chicago. Cleveland (OH): Edgell Communications. p 295. Abstract nr PP369.

In-Text: (Mendez and others 1989, or by reference number)

Technical Report

References section: Cowardin LM, Carter V, Golet FC, LaRoe ET. 1979 Dec. Classification of wetlands and deepwater habitats of the United States. Washington: Fish and Wildlife Service. Report nr FWS/OBS/-79/31. 103 p.

In-Text: (Cowardin and others 1979, or by reference number)

Electronic Journals

References section: Slater PJB, Jones AE. Timing of songs and distance call learning in zebra finches. Anim Behav [serial online] 1995; 49(2):123-248. Available from: OhioLINK Electronic Journal Center via the Internet (<http://journals.ohiolink.edu/etext/>).

In-Text: (Slater and Jones 1995, or by reference number)

References section: Wolf BB, Green DR. Suicidal tendencies: apoptotic cell death by caspase family proteinases. J Biol Chem [serial online] 1999; 274 (29): 20049-52. Available from: Journal of Biological Chemistry Website via the Internet (<http://www.jbc.org/>).

In-Text: (Wolf and Green 1999, or by reference number)

Internet or Online Resource (must be from a scholarly source; Wikipedia is not scholarly)

References section: Pauling, Linus. "How to Identify and Unknown in Microbiology", Oregon State University Department of Microbiology, url: www.osu.edu/microbiology/crellin.html, updated 4/06, accessed 5/30/08.

In-Text: (Pauling, 2006 or by reference number)

Putting It All Together- Final Checklist

Bound with a single staple in the upper left-hand corner, you should present the following sections and parts of your paper in the following order:

- Title Page
- Introduction
- Methods
- Results
 - Includes flow chart, presented as a titled figure
 - Includes a table of reactions and their results
- Discussion
- References
- All of your hand-written lab notes, arranged chronologically as you wrote them. Your typed report should mirror what you wrote in these notes. Drawings or diagrams in your notes are welcome!

Have you looked over the grading rubric to make sure you have addressed all the grading criteria?

UNKNOWN REPORT GRADING RUBRIC

Student:

GRADED ITEM	Possible Pts	Your Points
Format Guidelines followed? (margins, font, spacing, title pg, etc.)	2	
Introduction ~ What purpose of study clearly stated?	2	
Materials and Methods ~ Was each technique used described in appropriate detail? ~ Was streak plate isolation described and any problems discussed? ~ Were isolation and testing procedures performed correctly? ~ Was isolation accomplished in a timely manner? (pure cultures requested?) ~ Were tests scheduled in a timely manner to avoid waste and repetition?	16	
Results Tables - Flow Chart: Is path of action indicated for the unknown, including any extra work? Is the chart parsimonious with the fewest steps needed to reach ID? Are all steps true dichotomies and not trichotomies? ~ Is there a complete table of test results showing visual AND interpretive information? ~ Were all tests listing in order of date? ~ Were results correctly interpreted (info in notebook and in discussion section)	12	
Correct ID ~ Was the unknown correctly identified? Verified by instructor? ~ If unknown ID incorrect, was it the result of student error (technique or judgement) or a factor out of the student's control?	6	
Discussion ~ Has student avoided repetition of methods and results in this section? ~ Have rationales for identifications been adequately described? ~ Has student demonstrated he/she can make appropriate conclusions? ~ Has students identified any errors in technique or judgment and suggested alternatives for future work? ~ Is discussion written in a professional manner? ~ Have all changes in original plan been discussed?	15	
References ~ Have the course guide and lab manual been cited (at the very least)? ~ Have all required components been included in proper sci. format? ~ Have all authors been cited, in the order in which they appear?	3	
Neatness, spelling, grammar, nomenclature ~ Is report written in professional, objective manner--no personal pronouns ~ Have all scientific names been written using proper nomenclature? ~ Is grammar and spelling proper and accurate? ~ Is report prepared in a tidy fashion according to requirements?	4	
Report submitted late (-20% per day)		
TOTAL	60	