Modified Approach to Unknowns

Resource Type: Curriculum: Laboratory

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Abstract

This exercise has students apply skills and techniques learned in the lab to identify assigned, but unnamed, bacteria in a defined time frame with emphasis on wise use of media and supplies. This method reduces student involvement in the actual manipulation of microbes and media but allows the opportunity for the student to grasp the process of just how one approaches the identification of a microbe. Traditionally students are assigned unknown microbes to identify with generous media and other supplies to use and liberal access to the microbiology laboratory. The approach described here represents a substantial savings in supplies and considers availability of the microbiology lab. Some colleges have a number of different kinds of lab classes scheduled for one lab room (not only microbiology, but also general biology, anatomy and physiology, genetics, etc.) thus restricting access to the facility outside designated class hours.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Background.
This exercise takes place after students have done Gram stains and set up and read primary media and biochemicals. Information regarding the organisms, media use, charts, etc., has been presented in exercises specifically dedicated to the *Enterobacteriaceae* and glucose nonfermenters, *Staphylococci*, *Streptococci*, and related organisms. Students have used various identification charts for all these categories of organisms and have been encouraged to keep accurate notes for all media and supplies.

PROCEDURE

Materials.

- Gram stain reagents, microscope slides, hydrogen peroxide for each student.
- Stock cultures of *Staphylococci*, *Streptococci*, *Enterobacteriaceae*, *Psuedomonas*, *Acinetobacter*.
- Tryptic soy agar (TSA) plates and MacConkey agar plates.
- Blood agar plates, biochemicals, disks, and reagents so that there is one full identification set up for each organism used for unknowns.
- Name that Bug! Directions and worksheet for students
- Media Order List (HTML)
- Media Order List (PDF)
- Observation Forms for Day Two (HTML)
- Observation Forms for Day Two (PDF)
- Flow Chart to Identify *Streptococci* (HTML)
- Flow Chart to Identify *Staphylococci* (HTML)
- Chart for Identification of Some *Enterobacteriaceae* and Some Glucose Nonfermenting Gram-negative Bacilli (HTML)

Student Version.
Day 1:
Each student receives four pure cultures of bacteria. A *Streptococcus* and a *Staphylococcus* have been streaked on tryptic soy agar (TSA) plates. A gram-negative bacilli from the *Enterobacteriaceae* and/or a nonglucose fermenter such as *Pseudomonas* or *Acinetobacter* have been streaked on MacConkey agar plates.

Determine Gram stain and, if necessary, catalase results. Since TSA is not a selective medium, organisms on these plates must be Gram stained. Recall the use of MacConkey agar to record the Gram stain result without actually performing the Gram stain. You may perform a Gram stain on the MacConkey microbes if you choose. At this point you are expected to submit a list of media appropriate to identifying each organism. The media list is in chart form with areas to designate whether the medium is a plate, broth, slant, or deep; whether it should be streaked confluently, stabbed, fishtailed on the surface, etc.; and whether any additional reagents such as Kovács reagent, disks, etc., are needed. This concludes lab activities for Day 1.

The organisms used in the unknowns are those that have been introduced in previous labs and can be identified using techniques and procedures from previous labs. This is an open book activity and you may use your lab manuals, notes, and identification charts; however, you may not borrow someone else's materials or involve another student in the process.

Day 2:
Visit only the workstations where each of your four organisms have been set up. Fill out a chart that includes reactions (change in media color, hemolysis on blood agar, zone sizes, etc.), interpretation of the results (positive or negative, susceptible or resistant, etc.), and the scientific name of the organism.

Note: samples of the directions and charts given to the students follow in the Supplementary Materials section.

Instructor Version.
This exercise takes place during two 2-hour labs. Students work independently but may use their notes and lab manual.

The instructor assigns a number to each microbe and streaks two microbes to either TSA or MacConkey agar the day before the exercise begins. Note: the objective of Day 1 is to have the students determine Gram stain results and catalase reactions. It is acknowledged that not all of the *Streptococci* will grow on TSA but to streamline set up time and conserve media the instructor may set up the "Streps" using *Enterococcus* species to simulate all the Streps. *Enterococci* will grow on TSA, produce gram-positive cocci on the Gram stain, and show a negative catalase reaction. If the instructor feels that it is more appropriate, the Streps can be set up on blood agar plates, and students can read hemolysis on Day 1. The grading process would need to be adjusted to reflect this change. Schedule at least an hour to do the set up.

Suggested microorganisms:

Streptococcus pyogenes
Escherichia coli
Streptococcus agalactiae
Klebsiella pneumoniae
Streptococcus viridans
Enterobacter aerogenes
Streptococcus pneumoniae
Serratia marcescens
Enterococcus faecalis
Proteus vulgaris
Staphylococcus aureus
Salmonella species
Staphylococcus epidermidis
Shigella flexneri
Staphylococcus saprophyticus
Providentia startii
Pseudomonas aeruginosa
Acinetobacter anitratus

You may want to use a couple of these more than once.

Work stations are often set up with four students per table. Here is an easy way to be certain that each student at the table receives a unique set of organisms. Number the Streps 1 through 6, the Staphs 7 through 10, and the gram-negative bacilli species 11 through 20. Assign each student four organisms as follows:

Table 1	Table 2	Table 3	Table 4	Table 5
1, 7, 11, 16	5, 7, 15, 20	3, 7, 14, 20	1, 8, 13, 20	5, 8, 12, 20
2, 8, 12, 17	6, 8, 11, 17	4, 8, 15, 16	2, 9, 14, 16	6, 9, 13, 16
3, 9, 13, 18	7, 9, 12, 18	5, 9, 11, 18	3, 10, 15, 17	1, 10, 14, 18
4, 10, 14, 19	2, 10, 13, 19	6, 10, 12, 19	4, 7, 11, 19	2, 7, 15, 19

The instructor sets up appropriate media to identify each organism of the entire set of organisms that were provided to the students (about 20 in all) prior to Day 2. It will take between 2 and 3 hours to label and inoculate the plates and tubes, place disks, etc., provided that the media is available. Before students arrive on Day 2, all media for organism #1 will be set at workstation #1, all media for organism #2 will be set at workstation #2, etc., until all media is at the individual stations. The instructor adds Kovács and ferric chloride reagent to the sulfur-indole motility (SIM) and phenylalanine tubes, respectively, as needed, just prior to the arrival of the students. It is advisable to set up a SIM and phenylalanine for each class period for each of the gram negatives as the colors of the added reagents fade or absorb into the media after awhile.

Safety Issues.
Students should be reminded that they are working with bacteria that are either recognized pathogens or have the potential to be pathogens. Students should observe aseptic technique and safe handling procedures for the microbes, safe use of the

Bunsen burner, and concern for flammable reagents such as the acetone-alcohol decolorizer for Gram stain decolorization.

ASSESSMENT and OUTCOMES

Suggestions for Assessment.
The student can receive a maximum of 25 points for identifying each organism, based on Gram stain and catalase results (5 points), appropriate order for media (10 points), accurate observation and interpretation of media that is set up (10 points), and accurate identification of microbe (5 points). The grade for ordering media is broken down into: media required, proper set-up method, and additional reagents and disks needed. The grade for observation and interpretation is broken down to each biochemical, etc. This method allows for an objective and relatively easy grading approach to unknowns.

If students accurately order appropriate media (including proper notations) for Day 1, they receive all points. An inadequate request results in loss of the points for the missing media. Ordering too much media is penalized by half points.

Field Testing.
This approach to unknowns has been used for both regular and summer sessions for the past 7 years. Class sizes vary from 16 to 24 students. Student comments at the end of the exercise indicate that they finally see the big picture, and a few have said that it was their favorite exercise of the semester.

Student Data.

Example of a student comment:
"Unknowns - Testing my ability to see if I really understood how to identify a bug - putting it altogether....It was good to see that I could critically think a bit and come up with the right bug!"

Sample of Student Outcomes

Individual GIF images of samples of student outcomes:

- Student sample of Name That Bug worksheet
- Student sample 2 of Media Order List worksheet
- Student sample 3 of Media Order List worksheet
- Student sample 4 of Media Order List worksheet
- Student sample of Gram Positive worksheet
- Student sample of Gram Negative worksheet

SUPPLEMENTARY MATERIALS

Answer Keys.

- Key for Gram-negative Bacilli (HTML)
- Key for Gram-positive Cocci - Catalase Negative (HTML)
- Key for Gram-positive Cocci - Catalase Positive(HTML)

Curriculum Resources

Modified Approach to Unknowns - Matthews

Name That Bug!

Organism #	Gram stain report	Catalase (if applicable)

Directions:
This is a test of your ability to identify microorganisms. You may use your notes, handouts, lab manual, etc., but you must work independently. You **may not** consult with your neighbor, use his or her notes, ask his opinion, or ask someone to focus your microscope. Assume that I am the patient and I will become very agitated if you expect me to know what is wrong with me. YOU are the professional, you figure it out.

Day 1:
Today you need to determine the Gram stain of your organism. Remember, let the media do some of the work for you. On **gram positive** organisms, you need to perform a catalase test. If you do a catalase test on **gram negative** organisms, please be prepared to explain how that information is relevant to the identification of the organism. Using the chart provided, please **circle** the agar and/or test you are requesting. **Indicate** whether this agar is a broth, slant, or plate. **Indicate** the tool you will be using to inoculate this medium, and the method of inoculation (isolation, confluent, stab, etc). **Check** all other things that apply such as pre- or post-incubation additives are required. **Describe** test set up on the appropriate line or at the page bottom for all asterisked (*) tests, such as CAMP, coagulase, etc. **Assume that you have 24 hours** to identify your organisms. Your list should be sufficient for identification without undue waste of materials and time. Turn in ALL worksheets with Gram stain results, catalase testing, and the media list **at the end of this class period.**

Day 2:
Next class period. The appropriate work will have been set up in order for you to identify your organisms. Regardless of what you may have requested, you can still identify your organisms to species level (with few exceptions). You can proceed to the workstation that corresponds to your organism number and read and interpret **all** tests or media you find present. Record on your worksheets both the **observations** (what you actually saw) and the interpretations (what you think this means: positive or negative (+/-), susceptible or resistant (S/R), etc.). **Include** all disk measurements. In all final identifications, the organism names should be correctly spelled with genus and species underlined by you.

Scoring:

- 5 pts. Gram stain results and catalase test results
- 5 pts. list of media and tests, etc., to identify organism

- 10 pts. reading and interpretation of media, etc.
- 5 pts. organism name

(25 points per organism x 4 organisms = 100 points)

Name _____ Date ____ Class Day/ Time

Organism # _____ Gram Stain

Medium	broth, plate, slant, deep, other	needle, loop, other	Method of inoculation	Preincubation reagents/ disks	Post incubation reagents

			Stab	fishtail	isolation	confluent	simple	AP@	AA@	Nov.	*Staph aureus*	Mineral Oil	Kovac	Fer chl
BAP														
BEA														
CAMP*														
Coag.*														
DNA														
Lysine														
MSA														
TSA														
Phen Deam														
SIM														
Sim.Cit.														
TSI														
Urea														
MacC														
Oxidase*														

* describe in detail

Name_____ Date _____ Class Day /Time_____

Gram Positive Organisms

Report all reactions at the work station using correct and appropriate format (hemolysis, zone size, color, +/ -, S/R)

Organism #

Blood agar plate hemolysis _____ (Alpha, beta, gamma)

Bacitracin disk ____ (S/ R) Optochin disk ____ (S/ R) Bile esculin agar ____ (+/ -)
Zone size _____ mm Zone size _____ mm Color

CAMP test_____ (+/ -)

Coagulase _____ (+/ -) Mannitol Salt Agar_____ (+/ -) Novobiocin disk _____ (S/ R)
Describe _____ _____ Zone size _____ mm
(Growth on plate/ color of agar)

Organism identification

****

Organism

Blood agar plate hemolysis _____ (Alpha, beta, gamma)

Bacitracin disk ____ (S/ R) Optochin disk ____ (S/ R) Bile esculin agar ____ (+/ -)
Zone size _____ mm Zone size _____ mm Color

CAMP test_____ (+/ -)

Coagulase _____ (+/ -) Mannitol Salt Agar_____ (+/ -) Novobiocin disk _____ (S/ R)
Describe _____ _____ Zone size _____ mm
(Growth on plate/ color of agar)

Organism identification _____

Gram Negative Organisms

Report all reactions at the work station using correct and appropriate format. Refer to your notes for TSI reactions. Use the first line under the biochemical name to record the interpretation of the reactions and the second line to record your observation (color change, turbidity, etc.) on which you based your interpretation.

Organism

	TSI	SIM	Simmon-s Citrate	Lysine Decarb.	Urease	Phenyl. Deam	DNase	Oxidase
Interp.	___	___	___	___	___			
Observ.	___	S___	___	___	___			
		I						
		M						

Organism Identification

****

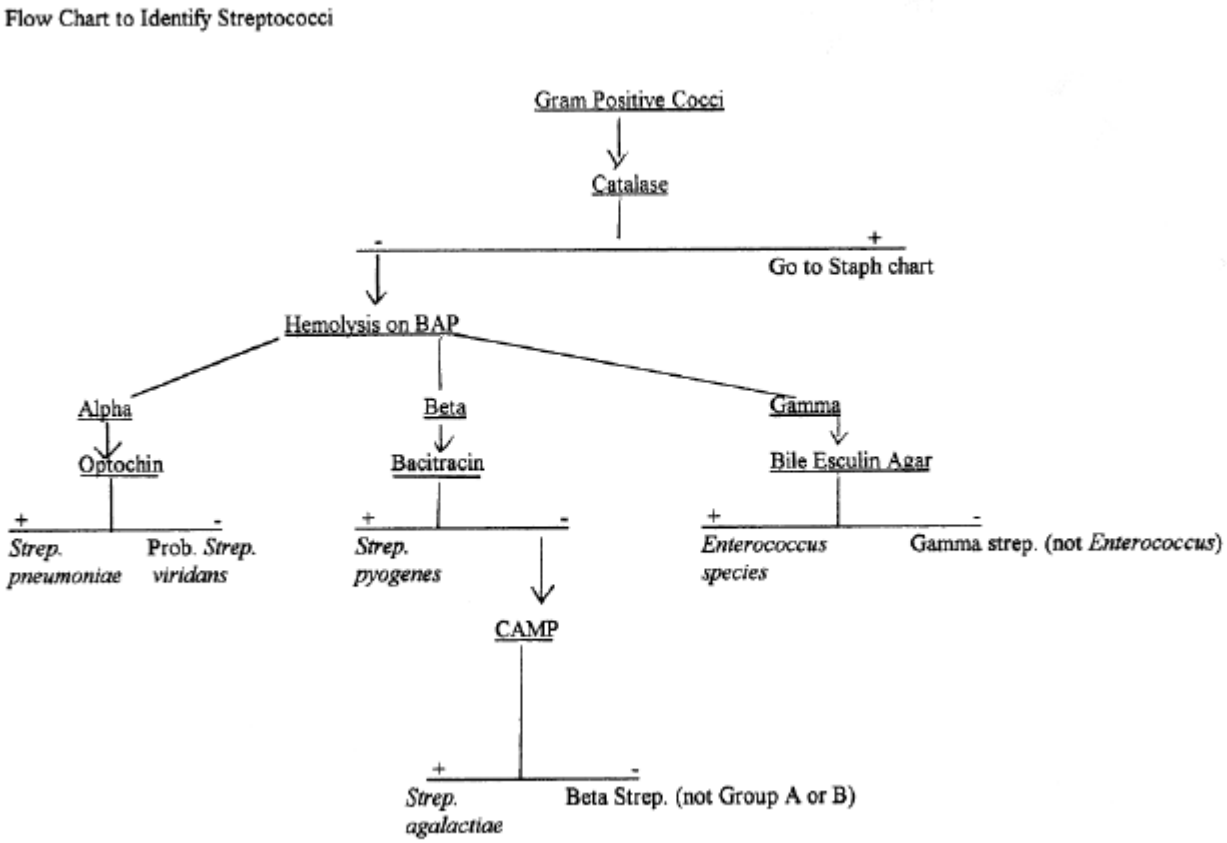
Organism

	TSI	SIM	Simmon-s Citrate	Lysine Decarb.	Urease	Phenyl. Deam	DNase	Oxidase
Interp.	___	___	___	___	___			
Observ.	___	S___	___	___	___			
		I						
		M						

Organism Identification

Curriculum Resources

Modified Approach to Unknowns - Matthews



Curriculum Resources

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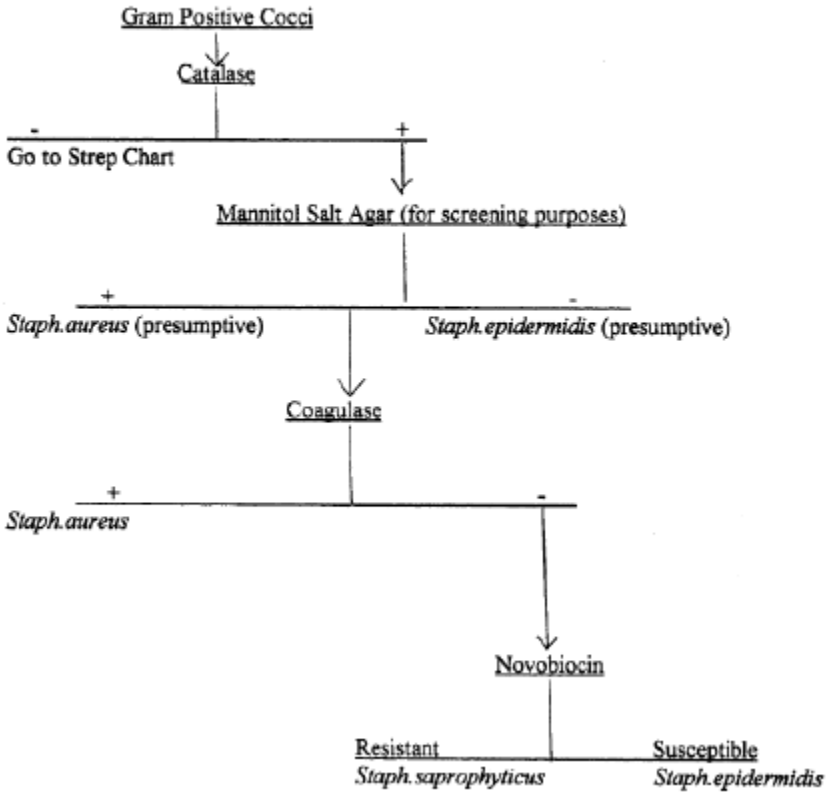
Flow Chart for the Identification of Staphylococci



Curriculum Resources

Modified Approach to Unknowns - Matthews

Chart for Identification of Some *Enterobacteriaceae* and Some Glucose Non-fermenting Gram-negative Bacteria

organism	TSI	SIM	Citrate	Lysine	Urea	PDA	DNA	Oxidase
Citrobacter freundii	K/A H_2S+	+-+	+	-	-	-	-	-
Enterobacter aerogenes	A/A +	-++	+	+	-	-	-	-
Escherichia coli	A/A +	-++	-	±	-	-	-	-
Klebsiella pneumoniae	A/A	+---	+	+	±	-	-	-
Morganella morganii	K/A H_2S+	+++	-	-	+	+	-	-
Proteus vulgaris	K/A H_2S+	+++	-	-	+	+	-	-
Providentia stuartii	K/A	-+±	+	-	-	+	-	-
Salmonella species	K/A H_2S+	+-+	+	+	-	-	-	-
Serratia marcescens	K/A	--+	+	+	-	-	+	-
Shigella flexneri	K/A	---	-	-	-	-	-	-
*Pseudomonas aeruginosa**	K/K	--+	+	-	±	-	-	+
Acinetobacter anitratus **	K/K	---	-	-	±	-	-	-

NOTES

None of the *Enterobacteriaceae* are oxidase positive.

Occasionally some of the *Enterobacteriaceae* that usually produce A/A results on the TSI slants may show a slight amount of alkaline reaction on the slant. Read as A/A.

Glucose nonfermenters:

* *Pseudomonas aeruginosa* is beta-hemolytic on blood agar plates, oxidase positive, and often produces a green pigment. Motility is difficult to read as this organism is aerophilic.

** *Acinetobacter anitratus* is nonhemolytic on blood agar plates, oxidase negative, and does not produce

pigment.

Abbreviations: TSI=triple sugar iron, SIM=sulfur-indole motility, Citrate=Simmons citrate, Lysine= lysine decarboxylase, Urea=Christensen=s urea, PDA= phenylalanine deaminase, DNA= DNase.

Note to Instructors

Biochemical reactions of some of these organisms could actually be designated as v or + according to references such as *Bailey and Scott's Diagnostic Microbiology*, however, the strains in use here at Midland College are giving the reactions as designated in the above chart. The goal of the unknowns exercise is to get the student to use an identification chart with a limited number of biochemical reactions. Also having a number of organisms included in the chart, even though not all are used in the assigned unknowns, is a way of expanding the critical thinking skills of the student.

Curriculum Resources

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Sample of Student Outcomes

Name [redacted] 97

NAME THAT BUG!

Organism #	Gram Stain Reaction	Catalase (if applicable)
20	gram ~~positive cocci~~ negative bacilli	____
12	gram ~~positive cocci~~ negative bacilli	____
2	gram positive cocci	—
8	Gram Positive Cocci	+

20 @ 1.2 ea = 37.2
60 = 97.2

OK Em

Directions:

This is a test of your ability to identify micoorganisms. You may use your notes, handouts, lab book, etc... but you must work independantly. You **MAY NOT** consult with your neighbor, use his/her notes, ask their opinion or ask someone to focus your microscope. Assume that I am the patient and I will become very agitated if you expect me to know what is wrong with me. YOU are the professional, you figure it out.

DAY 1: Today. You will need to determine the gram stain of your organism. Remember, let the media do some of the work for you. On **gram positive** organisms you will need to perform a catalase test. If you do a catalase test on **gram negative** organisms please be prepared to **explain how that information is relevant to the identification of the organism.** Using the chart provided, please circle the agar/test you are requesting. **Write in** whether this agar is a broth, slant, or plate. **Write in** what tool you will be using to inoculate this media. **Check** the method of inoculation, isolate, confluent, etc... **Check ALL** other things that apply, if pre or post incubation additives are required. **Describe test set-up** in the appropriate line or at the bottom for all asterick (*) tests, such as CAMP, Coagulase, etc... **Assume you have 24 hours** to identify your organisms. Your list should be sufficient without undue waste of materials and time. Turn in ALL worksheets with gram stain results, catalase testing, and media list **at the end of this class period.**

DAY 2: Next class period. The appropriate work will have been set up in order for you to identify your organisms. Regardless of what you may have requested, you can still identify your organsims to species level, with rare exceptions. You can proceed to the work station that corresponds to your organism number and read/ interpret **all** tests or media you find present. **Record** on your worksheets both the **observations** (what you actually saw) and the **interpretation** (what you think this means, +/ -, S/R, etc...). **Include** all disk measurements. All final identifications/ organism names should be correctly spelled with genus and species underlined by you.

Scoring: 5 pts -- gram stain reaction / catalase
5 pts -- list of media / tests, etc... to identify organism
10 pts -- reading & interpretation of media/ biochemical,etc..
5 pts -- organism name
(25 points per organism X 4 = 100 points)____

NAME [redacted] DATE 10/31/01 CLASS DAY/TIME MW8:00

ORGANISM # 2 GRAM STAIN gram positive cocci

Agar	Method of Inoculation							Pre-Incubation					Post-Incubation REAGENTS	
	Broth, plate Or slant	Needle Or Loop	Stab	Isolation	STREAK confluent	Simple / Fishtail	"P"	DISKS "A"	Nov.	Staph. aureus	Mineral oil	Ferric Chloride	Kovac
BAP	plate	loop			confluent	[redacted]	P	A					
BEA													
CAMP * on BAP	plate	loop			Streak straight confluent growth					Staph aureus			
COAGULASE													
DNA													
LYSINE													
MSA													
TSA													
Phen. Dea.													
SIM													
Simon's Citrate													
TSI													
UREA													
MAC													
OXIDASE*													

+6

14

streak colonies at 90°

stab

* Describe set-up in detail.

on BAP streak Staph aureus
streak group A Streptococci at 90°

10/14 = .714 = 3.6

NAME [redacted] DATE 10/31/01 CLASS DAY/TIME MW 8:00

ORGANISM # 8 GRAM STAIN gram positive Cocci

Agar	Broth, plate Or slant	Needle Or Loop	Method of Inoculation: Stab	Isolation	STREAK confluent	Simple / Fishtail	Pre-incubation: "P"	DISKS "A"	Nov.	Staph. aureus	Mineral oil	Post-Incubation REAGENTS: Ferric Chloride	Kovac
BAP	plate	loop			Confluent		[redacted]	[redacted]	Nov.				
BEA													
CAMP *													
COAGULASE	brain plasma	heated loop											
DNA													
LYSINE													
MSA	plate	loop				Fishtail							
TSA													
Phen. Dea.													
SIM													
Simon's Citrate													
TSI													
UREA													
MAC													
OXIDASE*													

* Describe set-up in detail.

NA [redacted] DATE 10/31/01 CLASS DAY/TIME MW 8:00

ORGANISM # 12 GRAM STAIN gram negative bacilli

Agar	Method of Inoculation							Pre-Incubation				Post-Incubation	
	Broth, plate	Needle Or			STREAK			DISKS				REAGENTS	
	Or slant	Loop	Stab	Isolation	confluent	Simple / Fishtail	"P"	"A"	Nov.	Staph. aureus	Mineral oil	Ferric Chloride	Kovac
BAP													
BEA													
CAMP *													
COAGULASE													
4 DNA	plate	needle				Simple							
4 LYSINE	broth	needle				(Simple)					✓		
MSA													
TSA													
4 Phen. Dea.	slant	needle				Fishtail						(+)	
5 SIM	deep	needle	✓										✓
4 Simon's Citrate	slant	needle				Simple							
5 TSI	slant	needle	✓			Fishtail							
4 UREA	slant	needle				Fishtail							
MAC	~~plate~~	~~loop~~				Fishtail							
5 OXIDASE*	on cotton swab												

* Describe set-up in detail.

[redacted] This student had #12 + #20 for gram negative bacilli - both tests were identical

place bacteria on swab drop oxidase reagent purple(+) positive NCC (-) negative

35/37 = 4.7

[redacted] Date 11/5 Class day/time 8:00Am

GRAM POSITIVE ORGANISMS

Report all reactions at the work station using correct and appropriate Format. (zone size, color, positive (+), negative (-), susceptible, resistant, etc...

ORGANISM # 2

Blood plate agar hemolysis beta (alpha, beta, or gamma)

Bacitracin disk S (S/R) Zone size 13 mm

Optochin disk R (S/R) Zone size 0 mm

Bile Esculin Agar — (+/-) ~~Zone~~ size NCC mm color of media

CAMP Test ____(+/-)

Coagulase ____(+/-) ~~(Accu-staph)~~

Mannitol Salt Agar ____ (growth on plate? Color of agar?)

Novobiocin disk ___(S/R) Zone size _____mm

15

Organism identification group A Beta Streptococcus

****

ORGANISM # 8

Blood plate agar hemolysis beta (alpha, beta, or gamma)

Bacitracin disk ___(S/R) Zone size _____mm

Optochin disk ___(S/R) Zone size _____mm

Bile Esculin Agar ___(+/-) color ____

CAMP Test ____(+/-)

Coagulase + (+/-)

Mannitol Salt Agar + yes yellow (growth on plate? Color of agar?)

Novobiocin disk S (S/R) Zone size 18 mm

15

Organism identification Staphylococcus aureus

GRAM NEGATIVE ORGANISMS

Report all reactions at the work station using correct and appropriate format, such as for TSI reactions (refer to your notes). Use the first line under the biochemical name to record the interpretation of your reactions and the second line to record your observation (color change, turbidity, etc...) on which you based your interpretation.

ORGANISM # 20

	TSI	SIM	Simmons Citrate	Lysine Decarb	Urease	Phenyl. Deam.	Dnase	Oxidase
Intrep.	K/A H_2S^+	+-+	+	+	-	-	-	—
Observ.	S black		blue	purple	NCC	NCC	no zone of clearing	—
	I NCC							
	M turbid							

15 **ORGANISM IDENTIFICATION** Salmonella species

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ORGANISM # 12

	TSI	SIM	Simmons Citrate	Lysine Decarb	Urease	Phenyl. Deam.	Dnase	Oxidase
Intrep.	K/A	-+-	+	+	-	+	-	—
Observ.	S NCC		blue	yellow	NCC	green	no zone of clearing	—
	I red							
	M turbid							

15 **ORGANISM IDENTIFICATION** Providencia stuartii

Curriculum Resources

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Media Order Key for Gram-negative Bacilli

NAME Key DATE _____ CLASS DAY/TIME _____

ORGANISM # _____ **GRAM STAIN** gram negative bacilli

	Agar			Method of Inoculation					Pre-Incubation				Post-Incubation	
		Broth, plate	Needle Or			STREAK			DISKS				REAGENTS	
		Or slant	Loop	Stab	Isolation	confluent	Simple / Fishtail	"P"	"A"	Nov.	Staph. aureus	Mineral oil	Ferric Chloride	Kovac
	BAP													
	BEA													
	CAMP *													
	COAGULASE Accu-staph*													
4	DNA	plate	needle				simple					5-6 drops		
5	LYSINE	both	needle				simple					5-6 drops		
	MSA													
	TSA													
5	Phen. Dea	slant	needle				fish tail						5% drops	
5	SIM	deep	needle	stab										8-10 drops
4	Simon's Citrate	slant	needle				simple							
5	TSI	slant	needle	stab			fish tail							
4	UREA	slant	needle				fish tail							
	MAC													
5	OXIDASE*	reagent + swab	1. touch swab on colony 2. drop 2 drops reagent				light on swab							

* Describe set-up in detail.

Curriculum Resources

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Media Order Key for Gram-positive Cocci - Catalase Negative

NAME Key DATE CLASS DAY/TIME

ORGANISM # GRAM STAIN gram positive cocci (catalase negative)

Agar			Method of Inoculation					Pre-Incubation			Post-Incubation	
	Broth, plate	Needle Or			STREAK			DISKS			REAGENTS	
	Or slant	Loop	Stab	Isolation	confluent	Simple / Fishtail	"P"	"A"	Nov.	Staph. aureus	Mineral oil	Ferric Chloride	Kovac
BAP	plate	loop			✓		✓	✓					
BEA	slant	needle				simple							
CAMP*		loop								✓			
COAGULASE Accu-staph*													
DNA													
LYSINE													
MSA													
TSA													
Phen. Dea.													
SIM													
Simon's Citrate													
TSI													
UREA													
MAC													
OXIDASE*													

BAP

Unknown

Staph aureus

* Describe set-up in detail.

Curriculum Resources

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Media Order Key for Gram-positive Cocci - Catalase Positive

NAME Key DATE _____ CLASS DAY/TIME _____

ORGANISM # _____ GRAM STAIN gram positive cocci (catalase positive)

	Agar	Broth, plate Or slant	Needle Or Loop	Method of Inoculation: Stab	STREAK: Isolation	STREAK: confluent	Simple / Fishtail	"P"	"A"	Pre-Incubation: DISKS: Nov.	Staph. aureus	Mineral oil	Post-Incubation: REAGENTS: Ferric Chloride	Kovac
5	BAP	plate	loop			✓				✓				
	BEA													
	CAMP *													
5	COAGULASE Accu-staph*	nurse	loop		glove, drop of Accu-Staph rgt on well									
	DNA				mix organism in drop Rgt & loop									
	LYSINE				rock, Hold up to 1 min.									
4	MSA	plate	loop		✓									
	TSA													
	Phen. Dea.													
	SIM													
	Simon's Citrate													
	TSI													
	UREA													
	MAC													
	OXIDASE*													

* Describe set-up in detail.