Birzeit University
Department of Biology and Biochemistry
Microbiology Lab (BIOL243)
[redacted] Technique

Name and ID: [redacted]

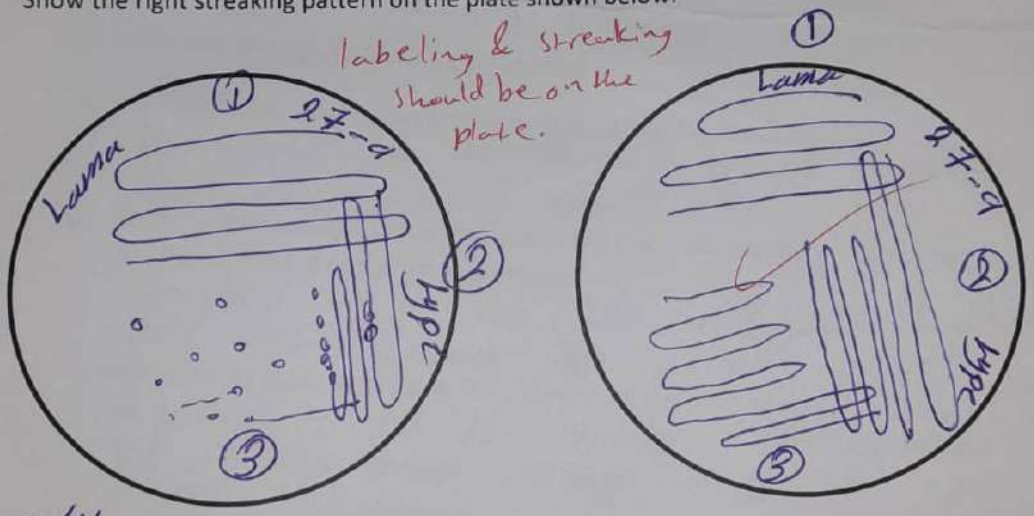
Choose the correct answer:

a) The correct species for the genus *Pseudomonas*:
   a. *Aeruginosa*
   b. *aeruginosa*
   c. *mirabilis*
   d. *Mirabilis*

b) A solid medium to grow bacterial culture:
   a. Broth
   b. Agar
   c. Gelatin

Answer the following questions:

c) Show the right streaking pattern on the plate shown below:



labeling & streaking should be on the plate.

empty ← Lid ??

Plate

d) Why it's important to cool the loop before taking the inoculum?
Because the temprature (high) will kill the microbes and the inoculumation will not occur (after waiting no culture will occur)

e) What is the purpose of using the oil with the oil immersion objective lens?
to increase the resolution so we can see the objects better (collect the light and focuses it better than without oil) its better to use it while seeing microbs. it decrease the wavelength

of Aspirin, β-Naphthol, naphthalene.

synthesis of Nerolin.

Second year →

4/5 nice

Birzeit University

Department of Biology and Biochemistry

Microbiology Lab (BIOL243)

Quiz#2: Simple staining, Gram staining, and bacterial shapes

Name and [redacted]

Answer with TRUE or FALSE:

a) I (acidic) Basic dyes are not attracted to most types of bacteria because of the dye's (-ev) ions are repelled by the (-ev) charged bacterial surfaces.

b) F Streptococcus bacteria are considered to be multicellular bacteria.

c) T Gram staining is considered to be a differential staining technique.

-0.5

Answer the following questions:

d) Mention the three common shapes of bacteria, and draw each one of them:

coccus → ○○

Bacillus → ○ spirilla.

Staphylococcus → ○○ & spirilla ε

-0.5

e) Why is it important that the smear should be well fixed on the slide before staining?

~~So when we put~~ because if not it will be wash up with the staining procedure

f) What is the main purpose of a simple stain?

to visualize the microb shapes and basic structures.

t and distillation methods.

3.5/5

Birzeit University

Department of Biology and Biochemistry

Microbiology Lab (BIOL243)

Quiz#3: Endospore staining and special staining + Media preparation and evaluation

Name: [redacted]

Fill in the blank:

a) Bacterial capsules are usually composed of ~~Poly saccharide, polypeptide~~ polypeptide, polysaccharide or both. (-1)

→ Negative staining

b) The technique that we use to demonstrate the presence of capsules is ~~endospore staining, which we use green stain and put it above boiling water~~ (2)

c) Mannitol-salt agar is considered to be selective and ~~differential~~ medium.

Answer the following questions:

d) What is the main purpose of special stains? ~~to visualize the endospores, to study bacterial structures and its boundary~~ to determine the presence of some bacterial structures like endospores and the presence of capsules (0.5)

e) Give two examples of environmental conditions where some bacteria may differentiate into the endospore state. higher concentration of salt, higher pH, lack of nutrients ~~it will be colorless if we don't use it~~

f) Can we consider MacConkey agar as a selective medium? Explain your answer.

yes, because only the gram - bacteria live in it ~~so~~ and it inhibit the gram + bacteria ↓ the growth.

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Department of Biology and Biochemistry

Microbiology Lab (BIOL243)

Quiz#4: Effects of physical agents on bacterial growth + Biochemical activities of bacteria

9/10

Name and ID: [redacted]

Q.1: Choose the correct answer:

1) Which of the following falls under the dry heat dependent methods of microbial growth control?
   a. Autoclave X
   b. Boiling water X
   c. Incineration
   d. Pasteurization

2) Both ionizing and nonionizing radiation tend to effect what?
   a. DNA
   b. Proteins
   c. Cell wall
   d. Cell membrane

3) Which of the following solutions is preferred to be used in food preservation?
   a. 0.1% NaCl
   b. 0.9% NaCl
   c. 10% NaCl
   d. None of the above

Q.2: Answer with **true** or **false**:

1. (F) Indole is a chemical group that results from the catalytic break down of citrate.
2. (F) The most cidal wavelength of UV light is 270 - 400 nm.
3. (T) Freezing at -10°C is considered to be a useful method to kill microorganisms and to keep food fresh for several months. inhibit growth of Microorganisms

C1

7/10

Department of Biology and Biochemistry
Microbiology Lab (BIOL230)
Sheet#1: Microscopes & streaking technique +
Environmental plate for gr[redacted]

Name and [redacted]

Fill in the blanks:

a) The scientific name of the bacteria that you have used for streaking is: Entrococus coli (-0.5) (α α)

b) The type of media that you have used for streaking was agar.

c) The method of preventing unwanted microorganisms from gaining access is termed ~~[illegible]~~ autoclave (α) (-1)

d) We use the loop to transfer an wire (α) ~~loop~~ loop of growing organisms from a pure culture to a sterile medium. (-1)

e) Microorganisms that can be found living inside or on the surfaces of our bodies are known to be called Microb flora.

Answer the following questions: (Short Answers)

f) What is the purpose of performing such a pattern for streaking?
to increase the number of specific culture and إلخ to study it (pure culture)

g) What was the purpose of keeping one of the plates closed all the time during the "microbial content of the air" experiment?
control → to see the differand between the pure media and the microbs media gained from air

h) What was the purpose of using "six different plates" for the microbial content of the air experiment? to see the increasing of number of cultures when leaving it for a differand times.

i) What are the expected sources of microorganisms that could be found in the air?
sneezing, coughing, air population from smoking and cars.

j) Why do we use the saline solution during the environmental plate experiment?
to help us take the microbs that planted in a solid agar (help the microbs to connect to (cotton swap) loop to plant it in plate

k) Mention two types of microorganisms that may grow on your plates during the environmental plate experiment:
S. aureus, M. Luteus] Bacteria (-0.5) Can other organisms (other than bacteria) grow?
microb flora

Birzeit University

Department of Biology and Biochemistry

Microbiology Lab (BIOL243)

Sheet#2: Media preparation and evaluation

Name a[redacted]

19.5/20 V.good

Q.1: According to these two tables, answer the following questions: (4 points)

Medium for *Escherichia coli*	Amount (g/liter)
Glucose	1.0
Na_2HPO_4	16.4
KH_2PO_4	1.5
$(NH_4)_2SO_4$	2.0
$MgSO_4 \cdot 7H_2O$	200.0 mg
$CaCl_2$	10.0 mg
$FeSO_4 \cdot 7H_2O$	0.5 mg
Final pH 6.8–7.0	

Medium (A)

MacConkey Agar	Amount (g/liter)
Pancreatic digest of gelatin	17.0
Pancreatic digest of casein	1.5
Peptic digest of animal tissue	1.5
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

Medium (B)

a) Which of these two media is considered to be a **liquid medium**?

~~Medium~~ Medium A (for Escherichia coli)

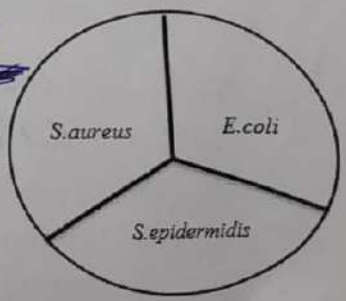
b) What is the function of **bile salt** and **crystal violet** in Medium (B)?

It inhibits the growth of Gram+ bacteria so we can use the medium as a differentiated medium

c) Which medium is considered **undefined**? **Explain your answer.**

Medium B because it employ digests from animal or plant products such as ~~casin~~ casein (derived from milk) and peptic digic digist of animal tissue.

Q.2: On MSA agar you have streaked three genera of bacteria as shown in the figure below, answer the following question: (4 points)



a) Which of the three genera is expected to grow on this media? Why?

E.coli will not grow (it will be inhibited)

S.aureus will grow and do fermentation

~~for [illegible]~~ S.epidermidis will grow but won't do fermentation, ~~for its~~ contains high conc. of salt 75%.

b) What is the source of carbohydrates in this media?

carbohidrates mannitol

c) What is the purpose of the pH indicator in this media?

indicates the production of acid after fermentation takes place (shows which colonies can ferment mannitol)

Q.3: Answer the following about Blood Agar (BA) media: (4 points)

a) You have streaked the following types of bacteria on a (BA) media, according to your results complete the table below:

Type of bacteria	Type of hemolysis	Appearance on (BA) media
B. subtilis	Alpha hemolysis	green
S. aureus	Beta "	colorless
E. faecalis	Gamma "	red

b) Can we consider Blood Agar (BA) as an enriched media? Why?

yes, because it is an environment incubated that allows the cultivation of fastidious microbes (rich of nutrients)

Q.4: On EMB agar you have streaked four types of bacteria as shown in the figure below, answer the following question: (8 points)

a) T/F EMB is a selective medium?

True

b) Explain your answer in (a).

based on the fact that the medium contains blue that inhibites the growth of Gram+ while allowe the detection of Gram- lactose fermentation.

c) Which of these genera's growth is inhibited by this medium?

A. *K. pneumoniae*

B. *E. coli*

C. *S. aureus* inhibited

D. *P. mirabilis*

E. None of them

d) T/F EMB is a deferential medium?

T

e) Explain your answer in (d).

distinguish between enteric bacteria that can fermented lactose

f) One of these organisms is known to produce a metallic green color on this media. According to your results, what is this organism? And what is the explanation of this appearance? E. coli (Escherichia coli) it appear blue-black which do fermentation to lactose change eosin from colorless to black in acid

0.5

g) If you are working in a microbiology lab and you need to use an EMB media. However, this media were not available in the lab, what is the alternative media that can be used for the same purpose in this case?

Mac

Birzeit University

Department of Biology and Biochemistry

Microbiology Lab (BIOL243)

Sheet#3: Chemical effects on bacterial growth

Name and [redacted]

7/10

Q.1: You have tested the effects of different disinfectants and antiseptics (Dettol, Listerine, 95% ethanol, and 70% ethanol) on the growth of two bacterial genera (*E. coil* and *S. aureus*), answer the following question: antiseptic

a) Listerine is considered a disinfectant. True or False? Explain your answer. (-1)

yes, because it's used to disinfect inanimate objects but generally toxic to use on human tissue.

b) What are the expected differences between using 95% ethanol and 70% ethanol as antimicrobial agents? 70% kill the bacteria better than 95% because it has a high conc. of H_2O

c) What is the mechanism of action that most of these agents use to kill or inhibit the growth of microorganisms? (-1)

Antimicrobial chemotherapy → selectively toxic (kill)

X d) According to your results fill the following table:

Antimicrobial agent	Zone of Inhibition (mm)	
	E. coil	*S. aureus*
Dettol		
Listerine		
95% ethanol		
70% ethanol		

X e) According to the table that you have filled above, what is the most effective antimicrobial agent against each of the two bacterial genera that you have used?

- *E. coli:* Gram −
- *S. aureus:* Gram +

Q.2: You have tested the effects of different chemotherapeutic agents (Different antibiotics) on the growth of two bacterial genera (*E. coli* and *S. aureus*), answer the following questions:

a) What are chemotherapeutic agents?

are synthetic chemicals that can be used therapeutically it inhibit or kill microorganisms in or on the host

b) What is the purpose of using two bacterial genera in these tests?

to test selective toxicity, it interact with some microbial function or microbial structure that is either not present or is substantially different from that of the host (to determine the type of Gram + or −)

c) In this experiment we used (0.5) McFarland standard for a certain reason. Can you explain its role? we want the minimum inhibitory conc. of each antimicrobial agent

d) When do we say that a bacterium is: **(In general)**

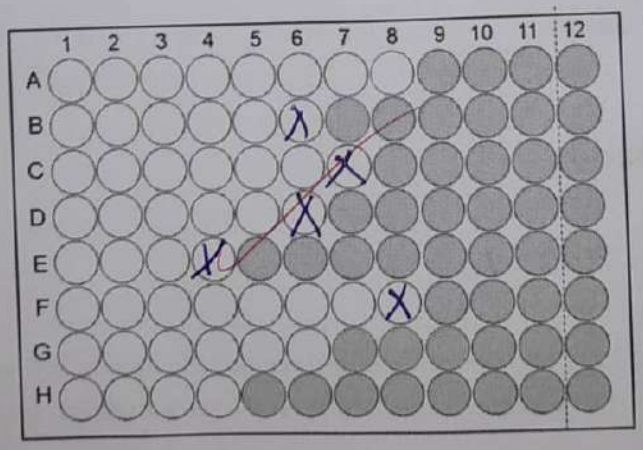
- Resistant: has a low zone of growth inhibition
- Intermediate: the result inconclusive for that drug-organism combination
- Susceptible: has a high zone of growth inhibition.

Q.3: A 96 wells plate was used to determine the effects of the antibiotics erythromycin and ciprofloxacin on to genera of bacteria (*E. coli* and *S. aureus*), answer the following questions:

a) A serial dilution was done for both antibiotics to determine the MIC and MBC values for each antibiotic, what is the meaning of MIC and MBC? They stand for what?

- MIC (Minimum inhibitory conc.
- MBC (Minimum bacterial conc.

b) According to the figure below, add (X) mark over the wells that are expected to be MIC?



c) If the above wells plate was used to determine the MBC value and it was found that for one of the antibiotics the MIC is (7.8 µg/ml) and the MBC is (15.62 µg/ml). Is this antibiotic bacteriostatic or is it bactericidal? =2 so its cidal

oiling point and distillation methods.

23.5/25 V. good

Birzeit University
Department of Biology and Biochemistry
Microbiology Lab (BIOL243)
Sheet#4: Biochemical activities of bacteria

Name and ID: [redacted]

Q.1: In TSI test you got the following results, according to them answer the questions below:

- *E. coli*: A/A → fermentation of glucose only
- *P. mirabilis*: K/A
- *P. aeruginosa*: K/K → no fermentation for any sugars

a. Explain what each result means with respect to fermentation.
E.coli: acid/acid → gas positive (yellow/yellow) the bacteria can ferment the 3 sugars (Lactose, sucrose, glucose)
P.mirabilis:

b. How could you indicate the positive result for H_2S production, and which bacteria gave this result?
P. mirabilis +
When peptone is utilized aerobically it produce ammonia gas
H_2S reach with iron and form ferric sulfide (black ppt)
E.coli -
P. aeruginosa -

c. Which bacteria indicated a positive result for gas production?
E.coli
Ferrous ammonium sulfate and sodium thiosulfate serve as indicator for H_2S

Q.2: Answer the following regarding the catalase test:

a. In catalase test, H_2O_2 was added directly to the bacterial culture on a microscope slide.

b. A positive result produced by *S. aureus* is indicated by bubbles.

c. Why should we perform this test on a blood-free medium?
there's nothing look like the catalase so it give false result. ??

Q.3: Answer the following regarding gelatin liquefaction test:

a. Complete the following table:

Bacteria Used	Tube Result	Explanation
E. coli	Negative	solid in ice bath or refrigerator after 5 min, don't break the peptide bond between amino acids
P. aeruginosa	positive	Liquide in ice bath or refrigerator after 5 min break peptide bond between amino acids.

to see which of the bacteria breaks the peptide bond that will prevent it from turning to solid in low temp.

b. In order to take the results, the tube must be placed in the refrigerator for 5-10 minutes. Explain, because gelatin is semi solid at room temp and melt at higher temp ~~when it was put in refrigerator it will protect and do the breaking for the Gelatin positive~~.

Q.4: Regarding starch and lipid hydrolysis tests complete the following table:

Test	Starch hydrolysis	Lipid hydrolysis
Enzyme	exoenzymes. amylase	Lipase
Indicator	I+I	neutral red pH indicator
How could you indicate the positive result?	if there was no change in medium color or a clear zone appear	turns to bright red at low pH
Bacteria that gave positive result	B. subtilis.	S. aureus.

Q.5: Answer the following regarding the SIM test:

a. Give an example for the following:

1. Positive for Indole, negative for H_2S production and motile: E. coli
2. Negative for Indole, negative for H_2S and non-motile: K. pneumonia
3. Negative for Indole, positive for H_2S and motile: P. mirabilis

b. The medium contains Sulfer (less agar), providing a semi-solid structure that allows for the detection of bacterial motility.

c. The reagent used to identify indole production is Kovac's reagent, and it indicates for the presence of Tryptophanase. enzyme.

Q.6: Answer the following regarding citrate utilization test:

a. Why is a chemically defined media necessary for detection of citrate utilization by bacteria? Semmon's citrate agar, it has citrate as the only carbon source and pH indicator bromothymol blue to know which bacteria produce citrase

b. The positive result like K. pneumonia bacteria was taken by the development of blue color, and the pH indicator in the media is bromothymol blue (acidic).

Q.7: Answer the following regarding the oxidase test:

a. Why the bacterial sample should be taken using wood stick?

خوفاً من إنه يكون في صدأ فيأثر على النتيجة وعشان (iron oxidase). تكون صحيحة النتيجة ما عرفت أحطها بالانجليزي)

b. Ali performed the oxidase test; he took the results after 2 minutes of completing the procedure. All the samples tested positive. What errors could've happened? the use of metal loops to transfer the colonies for the test gives false positive. the test must be interpreted within 10 to 20 sec, many organisms in this family can give delayed false positive.

Q.8: How could urinary tract infection be diagnosed?

some bacteria produce urease, an enzyme capable of breaking down urea and produce alkaline end product Inoculate the media with a loop and incubate at 37°C for 24 hours (urea utilization)

Q.9: :Answer the following regarding the nitrate test

a. Complete the following table, and for each result describe if it is positive or negative:

Bacteria used	The observation after the addition of α-naphthylamine and sulfanilic acid	The observation after the addition of zinc dust
E.coli	+ red	+ red.
p. aeruginosa	+ no color	+ red
E. faecalis	−	− no color

b. What is the meaning of a positive result after the addition of α-naphthylamine and sulfanilic acid? Explain.

Detection of nitrate reduction which form diazonium compound (red color.

c. What is the meaning of a positive result after the addition of zinc dust? Explain.

To check the gas production if it red it indicates that nitrate reduction didnt take place