PRINT YOUR NAME

TA's NAME

Please sign below if you wish to have your grades posted by the last five digits of your SSN

Signature

INSTRUCTIONS:

MIC 129K EXAM II has 7 pages, and 40 questions.
There are a total of 200 points, accounting for 20% of your final grade.
Place your name at the top of each page and check that your exam is complete.
ANSWER ALL QUESTIONS.
Be brief and precise in your answers. DO NOT RAMBLE!
You must show your calculations where asked.
Copying and all other forms of cheating will be met with the appropriate disciplinary action.

In case you dispute the answer that is deemed correct, you MUST first submit to your TA your question and the reason that you think your answer is the best (not just right, but the best of the answer choices) along with references that support your viewpoint. This must be done within 1 week after the exams are returned.

YOU MUST HAND OVER YOUR COMPLETED EXAM TO YOUR TA WHEN LEAVING THE ROOM AND MAKE SURE THAT YOUR NAME HAS BEEN WRITTEN DOWN

GRADES WILL BE POSTED BY MONDAY MAY 15 ON THE LAB DOORS
1. (4 points) Which is the first thing and the last thing you should do every time you work in microbiology lab? (Besides washing your hands with soap and water)

2. (4 points) What should you do if you spilled your mixture of Hfr and F culture on the lab floor? (Even if it was spilled after you had completed your plating)

3. (2 points) Where should you discard the slide after you have looked at the organism after Gram staining?

4. (6 points) 100 n m = ________ µ m.
   
   10 m m = ________ µ m

5. (6 points) *E. coli* is approximately 5000 m m / n m / µ m in length and can / can not be seen by light microscope.

6. (4 points) Why must you incubate agar plates upside-down?

7. (4 points) True / False Immersion oil has approximately the same refractive index as that of glass allowing a greater fraction of light rays to pass through the specimen into the objective lens.

8. (4 points) You used a soil sample for the enrichment lab. The soil sample had:
   
   a. a single type of bacteria
   b. only Gram - negative bacteria
   c. only Gram - positive bacteria
   d. a mixture of different kinds of bacteria

9. (5 points) Mention the composition of the medium you will use to isolate an enteric from a soil sample.
10. (4 points) What is a prototroph?

11. (18 points) In the following biochemical pathway:

\[
\begin{array}{cccc}
\text{Gene} & \alpha & \beta & \gamma & \delta \\
\text{Enzyme} & a & b & c_i & d \\
I \longrightarrow & II \longrightarrow & III \longrightarrow & IV \longrightarrow & V \\
\end{array}
\]

a. If an organism does not have functional enzyme c, what product would build up in the cell? 

b. Which product(s) would be required for crossfeeding in order for this organism to return to a wild type phenotype? 

c. Would a mutant organism with a nonfunctional enzyme c be able to crossfeed the following mutants back to a wild-type appearance? (Circle the correct answer)

\[
\begin{array}{ccc}
\text{Gene} & \alpha & \text{knockout} \\
\text{Gene} & \beta & \text{knockout} \\
\text{Gene} & \gamma & \text{knockout} \\
\text{Gene} & \delta & \text{knock out} \\
\end{array}
\]

12. (4 points) An organism when incubated at 37°C gave rise to pale yellow colonies, where as at room temp the colonies were red. This is an example of a **phenotypic** / **genetic** change.

13. (4 points) On a blood agar plate you inoculated an unknown organism to determine what kind of hemolysis it produces. On examining the plate after 24 hours of incubation you saw no change in the blood agar surrounding the isolated colonies. What kind of hemolysis did this organism show?

14. (6 points) Which two components of the CNA blood agar make it selective and which one makes it differential?

Selective: 

Differential: 
15. (15 points) An organism that produces acidic byproducts (but no hydrogen sulfide) both in the slant and in the stab part of a KIA slant will turn the agar _____________ (write down the color). This organism is a glucose / lactose / both glucose and lactose fermenter.

Another organism that also produces hydrogen sulfide would have turned the KIA slant _____________ (write down the color). Hydrogen sulfide reacts with ______________________ (write down the name of the chemical in the medium) to give this color. The pH of the agar after the reaction would be acidic / alkaline / neutral.

16. (4 points) Why should you not allow a carbohydrate fermentation test to run longer than 36 hours? Hint: Discuss how the pH changes during this incubation time, say at 24 hours and 48 hours.

17. (8 points) How would you interpret the following symbols for a carbohydrate fermentation test?

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Interpretation</th>
<th>Color of the medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR²</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

18. (4 points) Which of the following tests does not use a pH indicator to show change in color for a positive reaction?

a. phenyl alanine  b. Simmon's Citrate  c. KIA slant  d. lysine decarboxylase

19. (4 points) Fermentation products generated by various organisms include:

a. acid  b. alcohol  c. gas  d. all of these

20. (4 points) Decarboxylases are induced in the presence of the substrate and the following environmental conditions:

a. aerobic and acidic  b. aerobic and alkaline

c. anaerobic and acidic  d. anaerobic and alkaline
21. (8 points) If an organism is unable to grow on Simmon’s Citrate agar (but grows well on nutrient agar), you can safely assume that it can not use ___________________________ (name the chemical) as the sole source of carbon. Growth on Simmon’s Citrate agar is indicated by change in color from ______________ to ______________ indicative of the pH of the medium becoming more acidic/alkaline.

22. (4 points) Name the enzyme responsible for degrading hydrogen peroxide in the cell before toxic levels accumulate.

__________________________

23. (4 points) In the bacteriophage assay, we had:
   a. equal numbers of phage particles and bacteria
   b. phage greatly outnumber the bacteria
   c. bacteria greatly outnumber the phage
   d. no bacteria

24. (4 points) Define a plaque.

25. (4 points) True/False All phage particles in a phage sample are capable of forming plaques.

26. (10 points) How many plaques would you expect to have on the plate if the stock solution is at a concentration of $1 \times 10^5$ PFU / ml and you used 100 μl from a $10^2$ dilution and 2 ml of soft agar? (Show your calculations)

   ANSWER: ____________________
27. (4 points) True / False During gel electrophoresis large fragments of DNA migrate faster as compared to the small fragments of DNA.

28. (4 points) Which of the following represents a palindromic sequence of DNA which can be cleaved by a restriction endonuclease (the kind you used in the lab). Note: ONLY one strand of the duplex is shown.

   a. GAGAGA    b. AAGCTT    c. CCTTCC    d. ACGTGT

29. (4 points) Name the dye that was used to stain the DNA fragments before taking its photograph.

30. (2 points) DNA fragments are negatively/positively charged at pH 7.

31. (2 points) The dial of the P20 micropipettor is set at 0-2-5 (from top to bottom). What volume of water will this setting correspond to?

   ____________________ milliliters  ____________________ microliters

32. (4 points) Conjugation, transformation and transduction are mechanisms by which bacteria acquire new genetic information. Which of these mechanisms requires a cell to cell contact?

33. (8 points) List 2 differences between an F* and an Hfr cell.

34. (4 points) Which of the following cells will be the recipient in a conjugation?


35. (4 points) When you did the lab on gene transfer, neither the recipient nor the donor strain grew on minimal agar. Why did the donor strain NOT grow on minimal agar?
36. (4 points) Define "Serology".

37. (4 points) What is a cross-reacting antigen?

38. (4 points) In which way is an agglutination reaction different from a flocculation?

39. (4 points) You got a positive agglutination reaction using a 10 fold dilution of an antibody and a negative reaction with a 20 fold dilution. What is the titer of this antibody? (Remember to write down the appropriate units)

   Titer of the antibody: ______________________

40. Bonus (6 points)

List the genus and species names of three bacterial species that were used in MIC 129K this semester. Be sure to spell and format them correctly. Hint: *Saccharomyces cerevisiae* is not a bacterial species. (No abbreviations)