1) Mannitol-Salt Media is
a. selective and differential for Gram Positives
b. selective and differential for Gram Negatives
c. selective for Gram Positives but not differential
d. selective for Gram Negatives but not differential
e. not selective or differential

2) Aseptic techniques include all EXCEPT
a. thoroughly flaming the wire end of a loop
b. setting test tube caps on the table facing up while transferring samples
c. flaming the lips of all test tubes and flasks between transferring samples
d. using bleach solutions to sterilize benchtops
e. none - all of the above are true

3) Which of the following statements about Gram Staining is TRUE?
a. the treatment order is - safranin, iodine, ethanol, crystal violet
b. following crystal violet treatment, all bacteria appear purple
c. the most crucial and differential step of the procedure is the iodine treatment
d. Gram Positive bacteria appear pink/red
e. Too thick smear preparations of Gram Negative bacteria can erroneously appear pink/red

4) Match the nitrogen-fixing microbe with its correct strategy for controlling oxygen.
a. \textit{Rhizobium} and heterocysts
b. \textit{Azotobacter} and leghemoglobin
c. Methanogens and super-fast metabolic reactions that use oxygen quickly
d. \textit{Anoxygenic phototrophs} and living where there is no oxygen
e. \textit{Anabaena} and combining oxygen with hydrogen to form water quickly

5) In the nitrate reduction tube
a. denitrification was indicated by red color formation following the addition of A/B
b. ammonia was indicated by a black color formation following zinc
c. ammonia was indicated by no change following A/B and zinc
d. nitrite was indicated by the formation of a bubble
e. nitrite was indicated by a red color formation following zinc

6) The ammonia flasks were designed to enrich for
a. \textit{Azotobacter}
b. \textit{Rhizobium}
c. \textit{Nitrosomonas}
d. \textit{Pseudomonas}
e. Enteric Proteobacteria

7) In terms of creating anoxic environments, we used
a. media-filled sealed bottles
b. sealed glove-box apparati
c. special media that contained oxygen-binding compounds
d. soft-agar shake-tubes
e. anaerobic jars with catalysts and H2/CO2 gas generating systems

8) Which of the following statements about phototrophs we studied is FALSE?
a. anoxygenic phototroph enrichments did not fluoresce
b. \textit{Anabaena} is a filamentous phototroph
c. the starting inoculum we used for enrichments was poor soil outside the building
d. chlorophylls and bacteriochlorophylls are hydrophobic, requiring organic solvent extraction
e. the spectrophotometer graph you obtained showed absorbance vs. wavelength

9) Streptomyces
a. are Fungi
b. can be sub-classified based on spore-producing structures
c. all make antibiotics
d. consume monomeric carbon and energy sources
e. all of the above

10) Examples of industrially important Bacillus products include all EXCEPT
a. amylases for making high fructose corn syrup
b. base-tolerant lipases and proteases for detergents
c. antibiotics
d. enzymes for making alcohol from simple sugars
e. acid-tolerant food additives with digestive enzymes

11) Fermentation reactions
a. are what make vinegar if certain bacteria are present
b. involves something other than oxygen at the end of the electron transport chain
c. only produces alcohol
d. is only performed by prokaryotes
e. occurs during log phase

12) Which of the following statements about antibiotics you tested is TRUE?
1. tetracycline was the first antibiotic discovered, by Fleming in the 1929
b. ampicillin is a semi-synthetic, more broad spectrum derivative of penicillin
c. polymyxin B is an antibiotic made by Streptomycin
d. tetracycline affects the cell wall
e. polymyxin B is a broad spectrum antibiotic that affects the ribosome

13) As described in lecture, beer involves all EXCEPT
a. malting, the activation of amylases from barley
b. adding variable levels of hops, done exclusively to impart flavor
c. mashing, the conversion of starch to glucose
d. pitching S. cerevisiae to produce ales
e. pitching S. carbergensis to produce lagers

14) Pseudomonas
a. are Gram Positives
b. cause diarrhea
c. are the only major prokaryote that ferments alcohol
d. only metabolize simple monomeric sugars
e. are important industrial pollution indicators and bioremediators

15) Campylobacter jejuni
a. is a lactose negative Enteric that could have grown on MacConkey plates
b. is a spirilla that is only carried and transmitted by human feces
c. uniquely causes a high fever about 1 week after ingestion
d. produces a secreted toxin that shuts down eukaryotic ribosomes
e. is mostly associated with diarrhea in developing/third world nations

16) Salmonella
a. is a lactose positive Enteric that could have grown on MacConkey plates
b. is represented by less than 10 major strains
c. relies heavily on changing its pili and flagella to confuse your defenses
d. is only carried and transmitted by human feces
e. produces a secreted toxin that converts ATP to cAMP, releasing salt and water

17. Shigella
a. is a spirilla that is only carried and transmitted by human feces
b. was used by Rajneesh cult members in what remains the largest bioterrorist event on US soil
c. affects nearly half of victim households because of its profuse diarrhea and low infectious dose
d. is genetically almost identical to Salmonella
e. produces a secreted toxin that converts ATP to cAMP, releasing salt and water

18. Enterohemorrhagic E. coli
a. is only associated with ground beef transmission
b. typically produces up to 20 bloody bowel movements a day in its victims
c. LPS causes massive inflammation and autoimmune complications
d. produces a secreted toxin that has the same action as the Shigella or Shiga toxin
e. causes the most cases of bacterial diarrhea in the US

19) 6 pts. Name and explain 2 ways to enumerate bacteria using the following table.

<table>
<thead>
<tr>
<th>Name/Describe Method</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

20) 2 pts. each. Mixed Vocabulary

STATIONARY PHASE

DAPI

ANTIBIOTIC

BT TOXIN

HUS

21) 6 pts. ALL OR NOTHING. You mix 1 gram chicken 100 ml water and, from this mixture, perform SIX 10-fold dilutions (#1-6, in descending dilution). You plate 0.1 ml from each, observing the following data:

<table>
<thead>
<tr>
<th>Tube/Plate 1: too many to count</th>
<th>Tube/Plate 4: too many to count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube/Plate 2: too many to count</td>
<td>Tube/Plate 5: 142</td>
</tr>
<tr>
<td>Tube/Plate 3: too many to count</td>
<td>Tube/Plate 6: none</td>
</tr>
</tbody>
</table>

Question: How many bacteria were in the original sample. Show all math. You are advised to draw pictures.

22) 6 pts. ALL OR NOTHING. Using a hemocytometer, you obtain the following counts by counting 3 grids. The grid size is a 0.005 cm square that is 0.002 cm thick:

Grid 1 = 245  Grid 2 = 218  Grid 3 = 256

Question: How many cells were in the original sample, expressed as cells/ml. Show all math.

23) 9 pts. True/False - Nitrogen Cycle

_____ Your final Rhizobium colonies were actively fixing nitrogen.

_____ Both the Rhizobium and Azotobacter media contained high levels of sugar to promote capsule formation.
The source for your Rhizobium inoculation was soil.

Two different microbes are required to convert ammonia into nitrate.

Nitrate reducers were the only example of a lithotrophic nitrogen transformation that we studied.

Beijerink's historic late-1800's nitrogen enrichment was the first isolation of Nitrobacter.

Disinfectants, not considered tissue-safe, generally sterilized the nodules better than antiseptics.

Nitrogenase is found in only the prokaryotic domains of life.

Nitrogenase is a high-fidelity enzyme that only breaks down the triple bond in N₂ gas.

24) 7 pts. During the phototroph enrichment lab, the class - as a whole - obtained only 1 group of phototrophs. Name this group and provide FOUR specific lines of evidence that you used to support this argument and partially identify your enrichment.

PHOTOTROPH GROUP - be as descriptive as possible

Evidence 1
Evidence 2
Evidence 3
Evidence 4

25) 8 pts. Describe 4 ways you assessed your fermentation, making sure to briefly explain how you carried each out and why.

Method 1
Method 2
Method 3
Method 4

26) 6 pts. ALL OR NOTHING. Using the following chart and the provided data, solve your unknown:

Data Observations: (in no particular order) WHO AM I?

Golden layer after addition of Kovacs
Black after stabbing golden tube
Bubble in Durham tube of red tube
Blue after streaking green plate
Light/white after streaking purple plate

<table>
<thead>
<tr>
<th></th>
<th>Sulfur</th>
<th>Indole</th>
<th>Lactose</th>
<th>Glucose-Gas</th>
<th>Citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Citrobacter intermedius</td>
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<tr>
<td>Salmonella typhimurium</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Klebsiella pneumoniae</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Proteus mirabilis</td>
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<td>+</td>
<td>var</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>var</td>
</tr>
</tbody>
</table>

27) 6 pts. Draw a simple flow chart that explains how you plated and then sorted for river Coliform Enterics, Non-Coliform Enterics, and Pseudomonads. Explain/identify/name specific features that distinguish each.