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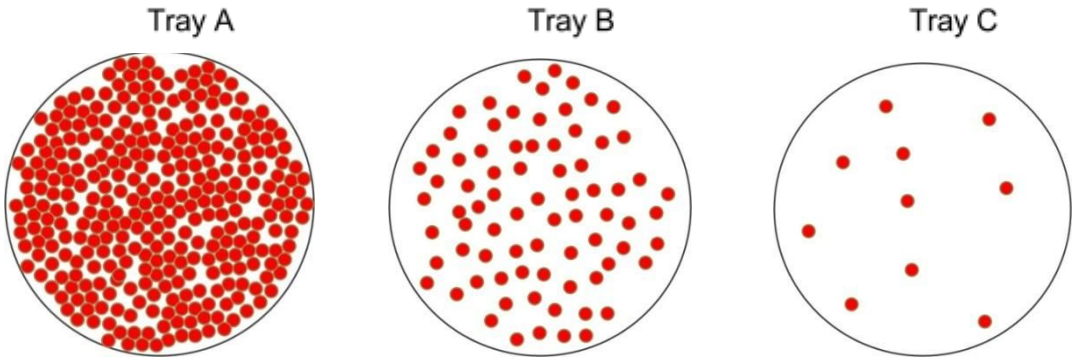
A221: Microbiology

Problem 5: Check it out! Part III

WORKSHEET

Question 1

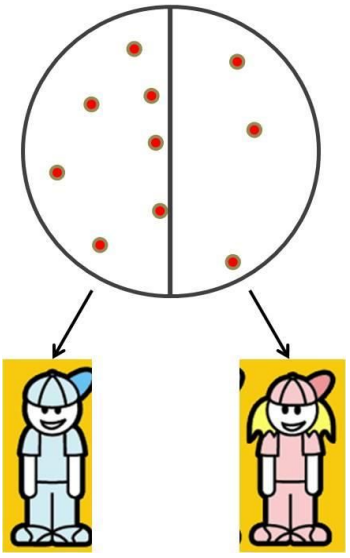
a) Different numbers of beads are placed in trays A, B and C respectively. If you were asked to count the number of beads in each of the trays, which is the tray that has the highest possibility to be miscounted? Why?
Tray A, as it has the too many beads in the tray.



b) The number of beads in tray B is 77, which makes up one fifth of that in tray A. If 10 trays of tray A are packed into one stack to be sold, what is the number of beads sold in one stack? Show your workings clearly.
77 x 5 = 385
385 x 10 = 3850 beads/stack

c) The number of beads in tray C is divided accordingly to the position of the beads in the tray as shown below, and given to the boy and girl.

Tray C



d) If the boy and the girl were told that they received beads from half of the tray and were asked to postulate the total number of beads in the whole tray (without consulting each other), what will be their numbers respectively?
Boy → 7 x 2 = 14
Girl → 3 x 2 = 6

e) Do they have the same number of beads on the whole tray? Explain why this is so.
No, the beads were not divided equally among them. Although they received half of the tray, there were more beads on the left half as compared to the right half

They have different total number of beads as the number of eads is too little; the beads are not even distributed on the tray

Question 2

An individual performed a coliform count on the proportion of contaminants in a water supply, 100µl of the water sample was plated and the following data was obtained:

Dilution Factor	Neat	10X	50X
CFU per plate	934	91	18

What is the concentration of coliforms in the original water sample? Show your working clearly and express your answer in CFU per ml.
Number of CFU in x 10 plate = 91
number of CFU in 100µl of the water sample= 91 x 10
= 910
Concentration of CFU = 910 CFU/100µl

= 910 CFU/0.1
= 9100 CFU/ml
= 9.1 x 10^3 CFU/ml

The more reliable result will be 9.1 x 10^3 CFU/ml, as the CFU for the neat and 50x diluted sample were either too numerous to be counted (TNTC) or too few to be counted (TFTC).

$=9.1 \times 10^3$ CFU/ml

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>300 (TNTC)
<30 (TFTC)

Question 3

The following 2 sets of data were obtained by 2 teams working on the same sample, where 100µl of the water sample was plated.

Team A

Dilution Factor	**Neat**	**5X**	**10X**
CFU per plate	**224**	**45**	**23**

Team B

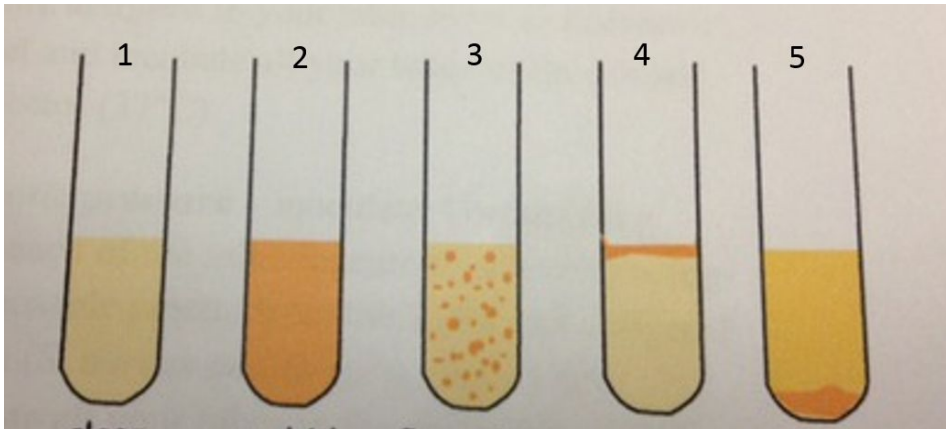
Dilution Factor	**Neat**	**5X**	**10X**
CFU per plate	**223**	**80**	**12**

Interpret the data and discuss the results. What conclusions can you make about the quality of the data?
The data is not accurate and hence not reliable as well. It is not accurate as both teams had highly varying results for 5X and 10X dilution. One of teams must have performed dilution inaccurately, causing either more or less colonies to form.

Assuming that the result of the neat sample is accurate, one can judge the accuracy of the plating by the correlation between the dilution factor and the CFU values. if a neat sample has 100 CFU, a 5X dilution will produce 20 CFU, then a 10X dilution should give rise to approximately 10 CFU. If the values observed show a high amount of variation from the expected number, it suggests that certain aspects of the experiment were not performed correctly. This would include inaccurate pipetting of volumes and insufficient mixing of samples.

Question 4

The picture below shows different growth pattern of bacteria in liquid broth.



With reference to the link below, complete the table to illustrate the type of growth and the respective description for each of the tubes shown above.
http://www.scienceprofonline.com/microbiology/use-of-liquid-nutrient-broth-media-for-growing-bacteria-2.html

Tube	Type of growth	Description
1	Clear (no growth)	Act as a control. No bacterial growth
2	Cloudy Uniform turbidity	Motile bacteria with flagella can swim. Their movement will cause the broth to become cloudy Growth of bacteria throughout the broth, creating a turbid cloudy broth
3	Flocculent growth	Some bacteria tend to stick together in clumps/flaky Growth of small masses of bacteria in the broth
4	Pellicle	A film-like growth on the surface of the media and interior of the test tube
5	Sediment	Non-motile bacteria sink to the bottom of the tube, forming sediment Growth of bacterial at the bottom of the tube

Going further

Even though microbes were discovered in the late 17th century by Leeuwenhoek and his microscope, and that it was believed that these microbes could cause disease, scientific techniques during that era were not able to conclusively prove this

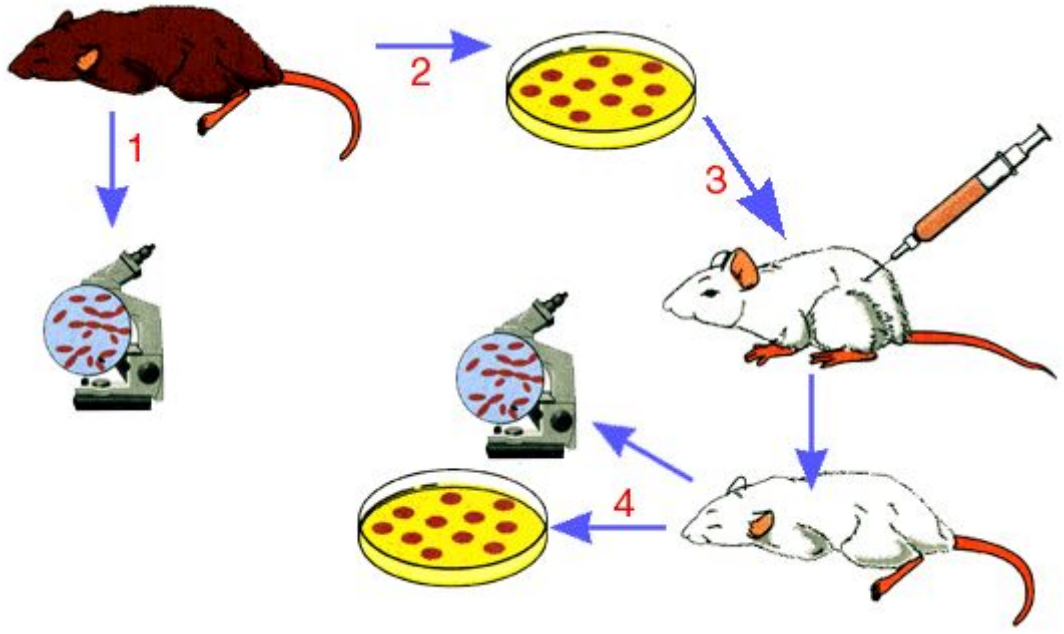
hypothesis. It was only when Robert Koch was able to perfect the technique of **pure culture** that convincing evidence of the germ theory of disease was accepted.

a) What is a pure culture and why was it important in the work of Robert Koch?
A pure culture is in which only one species of organisms is present. A pure culture is important in proving the work of Robert Koch because he wants to prove that only one type of bacteria caused the disease.

A pure culture is obtained when a single bacterium cell divides into a colony of its identical progeny. The culture would be homogenous and contain only the bacteria of interest.

Pure cultures are important in the identification of causative agents of disease.

Koch performed the following experiments represented in a diagrammatic fashion.



Koch observed the presence of *B. anthracis* bacteria in the blood and spleen of dead sheep that succumbed to Anthrax, the disease. He inoculated live mice with the infected sheep blood which resulted in the subsequent death of the mice (1). Upon observation under the microscope, he found the presence of *B. anthracis* in the blood and was able to recover the bacteria in pure culture (2). When a pure culture of the

bacteria recovered from (1) was inoculated into other live mice (3), it was once more able to result in the infection of the once healthy mice, and upon observation, similar cultures of *B. anthracis* could be obtained (4).

b) What could be inferred from Koch's experiments?

Koch wanted to prove only certain type of bacteria caused certain type of disease. So in his work, it is important to have pure culture. A pure culture is a culture that only has one type of bacteria in it. If there are many types of bacteria in the culture, Robert Koch cannot prove that the disease is caused by one type of bacteria.

Koch's postulates can be inferred

1) The microorganism must be present in every case of the disease.
2) The organism must be grown in pure culture from diseased hosts
3) The same disease must be produced when a pure culture of organisms is introduced into susceptible hosts.
4) The organism must be recovered from the experimentally infected hosts

In the above experiments, Koch showed that the microbe *B. anthracis* was found in every case of the disease, and it could be grown in pure culture from the dead mice/sheep. When introduced into a healthy host, the isolate resulted in the disease from which the microbe could once again be isolated.

Hence, in the above experiments, B *anthracis* is the cause of the disease that infected the hosts, and thus the causative agent of anthrax.

~End of worksheet~