Serial Dilution Protocols

Created: Friday, 30 September 2005

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Information

It is a common practice to determine microbial counts for both liquid and solid specimens---suspensions of *E. coli* in nutrient broth all the way to soil samples and hamburger meat. Most specimens have high enough numbers of microorganisms that the specimen has to be serially diluted to quantitate effectively.The following is a step-by-step procedure to working dilution problems, and includes some practice problems at the end.

The purpose can be determination of bacterial, fungal, or viral counts. This protocol is specific for bacterial counts (colony-forming units, CFUs), but can be modified for fungi (CFUs) and viruses (plaque-forming units, PFUs for viral counts).

History

Robert Koch is credited with identifying a method for bacterial enumeration, used first for the study of water quality. His article, *About Detection Methods for Microorganisms in Water*. was published in 1883.

The standard plate count is a reliable method for enumerating bacteria and fungi. A set of serial dilutions is made, a sample of each is placed into a liquefied agar medium, and the medium poured into a petri dish. The agar solidifies, with the bacterial cells locked inside of the agar. Colonies grow within the agar, as well as on top of the agar and below the agar (between the agar and the lower dish). The procedure described above produces a set of pour plates from many dilutions, but spread plates (sample spread on top of solidified agar) can be used also. The agar plate allows accurate counting of the microorganisms, resulting from the equal distribution across the agar plate. This cannot be done with a fluid solution since 1) one cannot identify purity of the specimen, and 2) there is no way to enumerate the cells in a liquid.

Principles

THE STANDARD FORMULA

$$\frac{\text{colony count (CFUs) on an agar plate}}{\text{total dilution of tube (used to make plate for colony count)}} \times \text{volume plated}$$

To work the problem, you need 3 values---a colony count from the pour or spread plates, a dilution factor for the dilution tube from which the countable agar plate comes, and the volume of the dilution that was plated on the agar plate.

PROTOCOL

STEP 1: Determine the appropriate plate for counting:

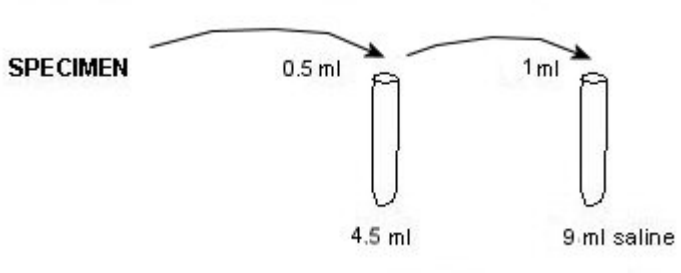
Look at all plates and find the one with 30-300 colonies (see COMMENTS & TIPS section at end for explanation).
Use the total dilution for the tube from where the plate count was obtained.
If duplicate plates (with same amount plated) have been made from one dilution, average the counts together.

STEP 2: Determine the total dilution for the dilution tubes:

Dilution factor = amount of specimen transferred divided by the total volume after transfer[amount of specimen transferred + amount of diluent already in tube].

Determine the dilution factor for each tube in the dilution series.
Multiply the individual dilution factor for the tube and all previous tubes.

To calculate this dilution series:



Determine the dilution factor of each tube in the set.

dilution factor for a tube = _____amount of sample_____
volume of specimen transferred + volume of diluent in tube

But after the first tube, each tube is a dilution of the previous dilution tube.

SO…..

total dilution factor = previous dilution factor of tube **X** dilution of next tube

FOR THE ABOVE DILUTION SERIES:

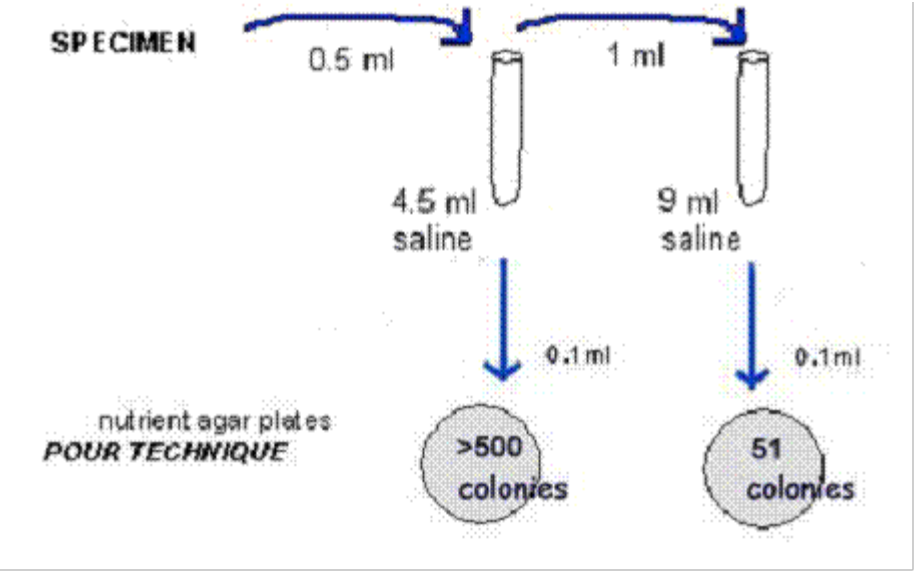
0.5 ml added to 4.5ml = 0.5/5.0 = 5/50 = 1/10 for 1st tube

1ml added to 9ml = 1/10 (2nd tube) **X** previous dilution of 1/10 (1st tube) = total dilution of 1/100 for 2nd tube.

STEP 3: Determine the amount plated (the amount of dilution used to make the particular pour plate or spread plate).

There is nothing to calculate here: the value will be stated in the procedure, or it will be given in the problem.

STEP 4: Solve the problem



1. The countable plate is the one with **51** colonies.

2. The total dilution of the 2nd tube from which that pour plate was made = $1/10^2$

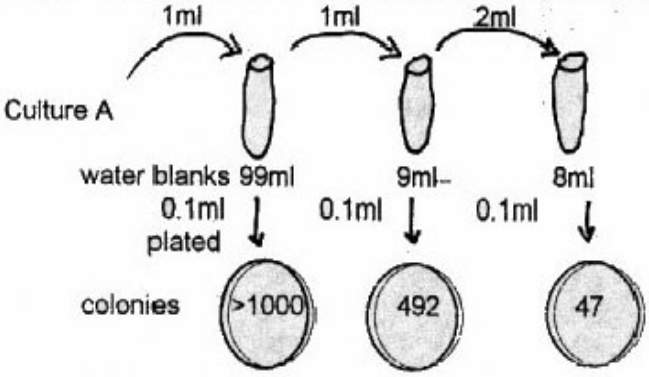
3. The amount used to make that pour plate = **0.1ml** (convert to 1/10 - it is easier to multiply fractions and decimals together).

51 colonies = 51×10^3 = **5.1×10^4** (scientific notation) OR **51,000 CFUs/ml**
$1/10^2$ X 1/10

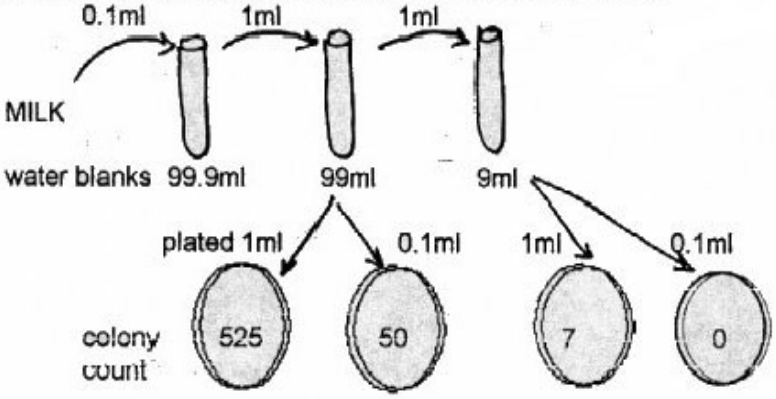
45 colonies = 45×10^4 = **4.5×10^5** (scientific notation) OR **450,000/ml**
$1/10^3$ X 1/10

SAMPLE PROBLEMS:

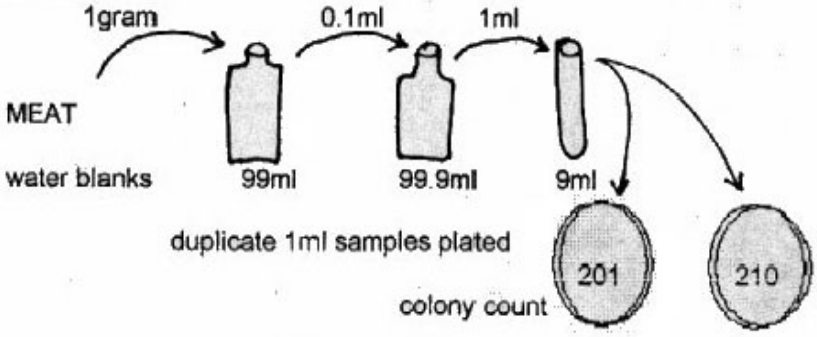
CALCULATE THE NUMBER OF BACTERIA PER MILLILITER OF CULTURE A.



CALCULATE THE NUMBER OF BACTERIA PER ML OF MILK.



CALCULATE THE NUMBER OF BACTERIA PER GRAM OF HAMBURGER MEAT.
(1 gram = 1ml)



SAFETY

Tubes and agar plates should be discarded properly in a biohazard container for proper sterilization. The pipettes will also be sterilized (washed first if using reusable glass pipettes.

Do not pipette by mouth.

Use sterile technique in the transfer of microorganisms from tube to tube, as well as in the production of the pour plates.

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

COMMENTS AND TIPS

Greater than 300 colonies on the agar plate and less than 30 leads to a high degree of error. Air contaminants can contribute significantly to a really low count. A high count can be confounded by error in counting too many small colonies, or difficulty in counting overlapping colonies.

Use sterile pipettes for the dilutions, and use different ones in between the different dilutions. To do otherwise will increase the chances of inaccuracy because of carry-over of cells.

Accuracy in quantitation is determined by accurate pipette use and adequate agitation of dilution tubes.

REFERENCES

There are no references at this time.

REVIEWERS

This resource was peer-reviewed at ASM Conference for Undergraduate Educators 2005.

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