Lab practice 1

25/26

Lab Safety

- **Emergency exit**
- **No food**
- **No drinks**
- ***Appropriate code of conduct***
- **No telephone, checking messages or smart phone calls or video, you can go outside**

Lab Safety

- **Wear Lab coat with name tag**
- **Wear goggles**
- **No open shoes and sandals**
- **No shorts, skirts**
- **No long pendents (earrings and neckless)**
- **No bracelets**
- **Tie your hairs**
- **Write your name on the foolscap (one per group)**
- **Take a break when you need it, inform your lab mates**

Vocabulary

Scale
Spatula
Petri dish
Tubes, 50 mL
Vials, 1 mL
L-spreaders
Micropipettes
Tips
Waste
Serological pipette
Pipettor
Saline solution
LB medium
Bunsen burner
Alu-foil
Ethanol
Parafilm

Proficiency in pipetting

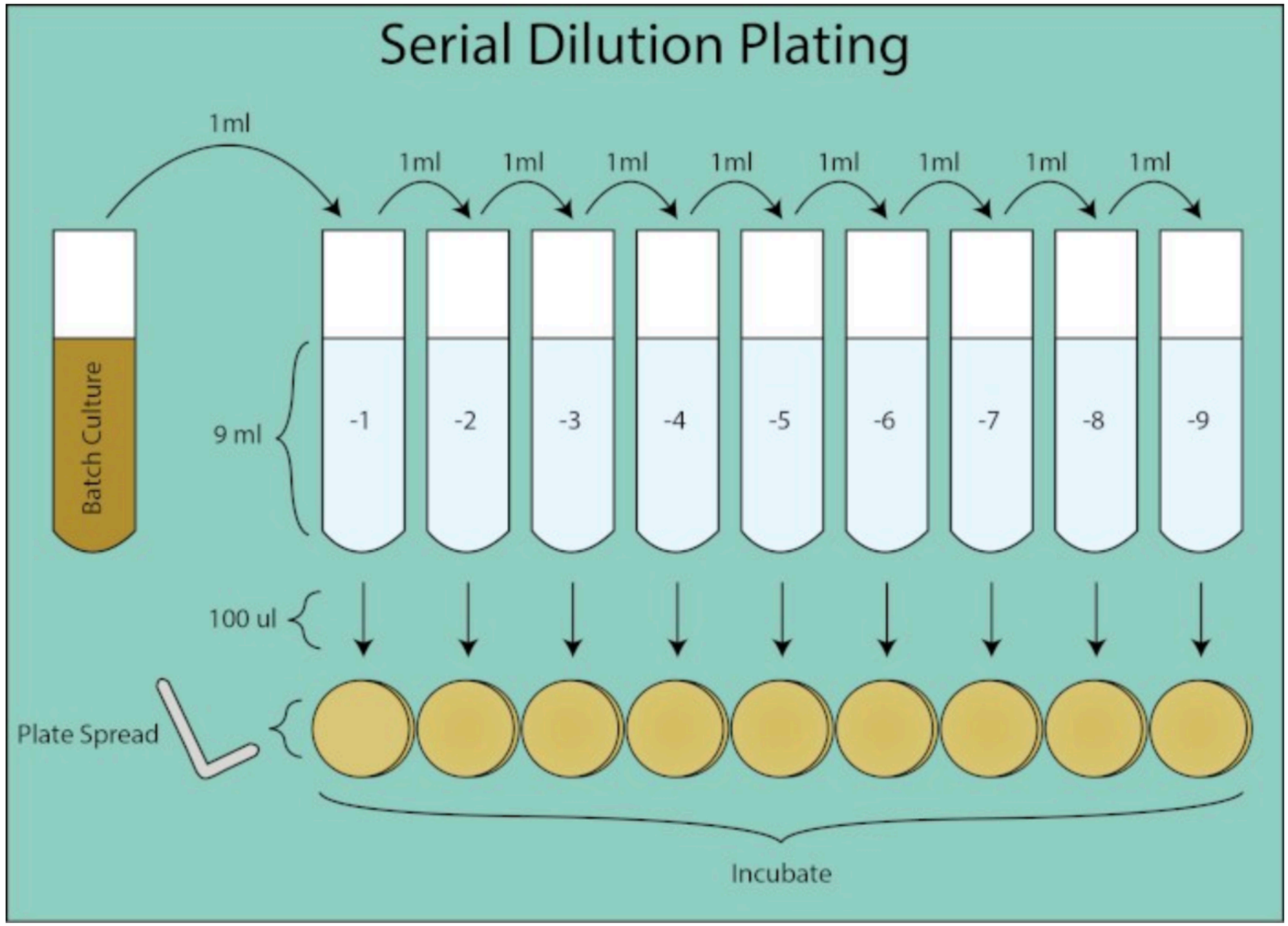
1 mL = 1000 µL

0.1 mL = 100 µL

0.01 mL = 10 µL

Color coding for tips and pipettes

Serial dilution



1. How many culturable soil/ freshwater-microbes there are on a nutrient-rich solid medium?

2. Are there more culturable microbes in the soil or in the freshwater sample?

Experiment flow 1

A. Samples collection

B. Measurement of soil and water

C. Dilution series

D. Plating on solid medium

E. Turn in the foolscap paper with A,B,C data

F. Incubating for 1 week and taking pictures

G. Counting colonies and plotting growth dynamic

H. Writing final RESULT report on e-book (pictures and number) and answer picture

Experiment 1 A-B

A. Samples collection B. Measurement of soil and water

- **Collect with the spatula some soil (no rocks or grass)**
- **Describe the sampling location on foolscap and annotate all the numbers and computations you make**
- **Weight sample from 0.5 to 1 g**
- **Add the sample into 10 mL saline solution**
- **Shake gently to make soil slurry**

Experiment 1C-D

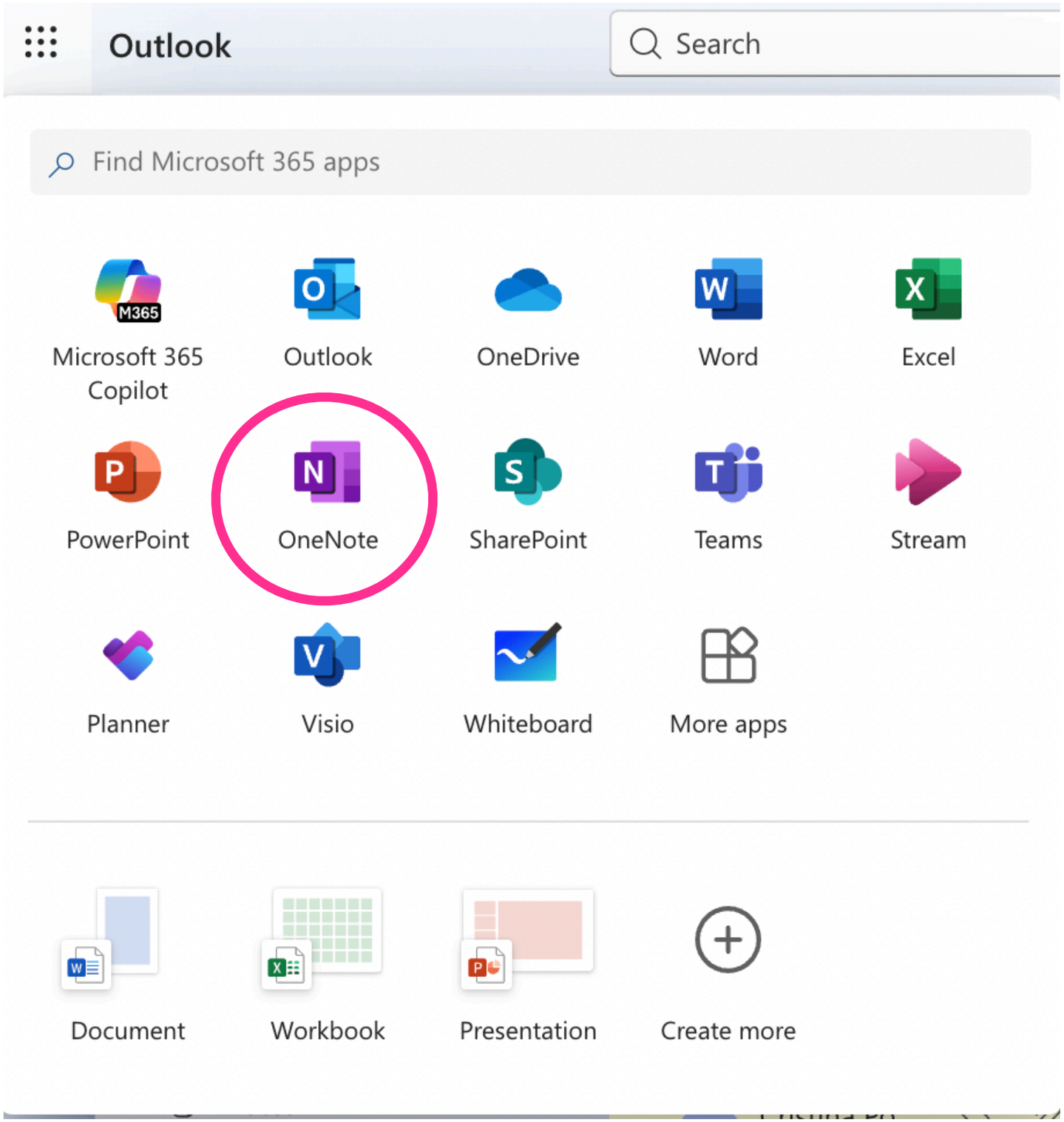
C. Dilution series D. Plating on solid medium

- **Prepare an appropriate number of tubes with 9 mL saline solution**
- **Pipette 1 mL of soil slurry into 9 mL of saline solution (use cut-out tip)**
- **Inverte gently the tube**
- **Repeat the step until it is necessary**
- **Plate 100 µL of the soil solution at the estimated correct dilution on to a LB plate twice to get 100 colonies on the plate**
- **Plate 100 µL of the higher dilution and lower dilution twice**

Experiment 1 E F G H

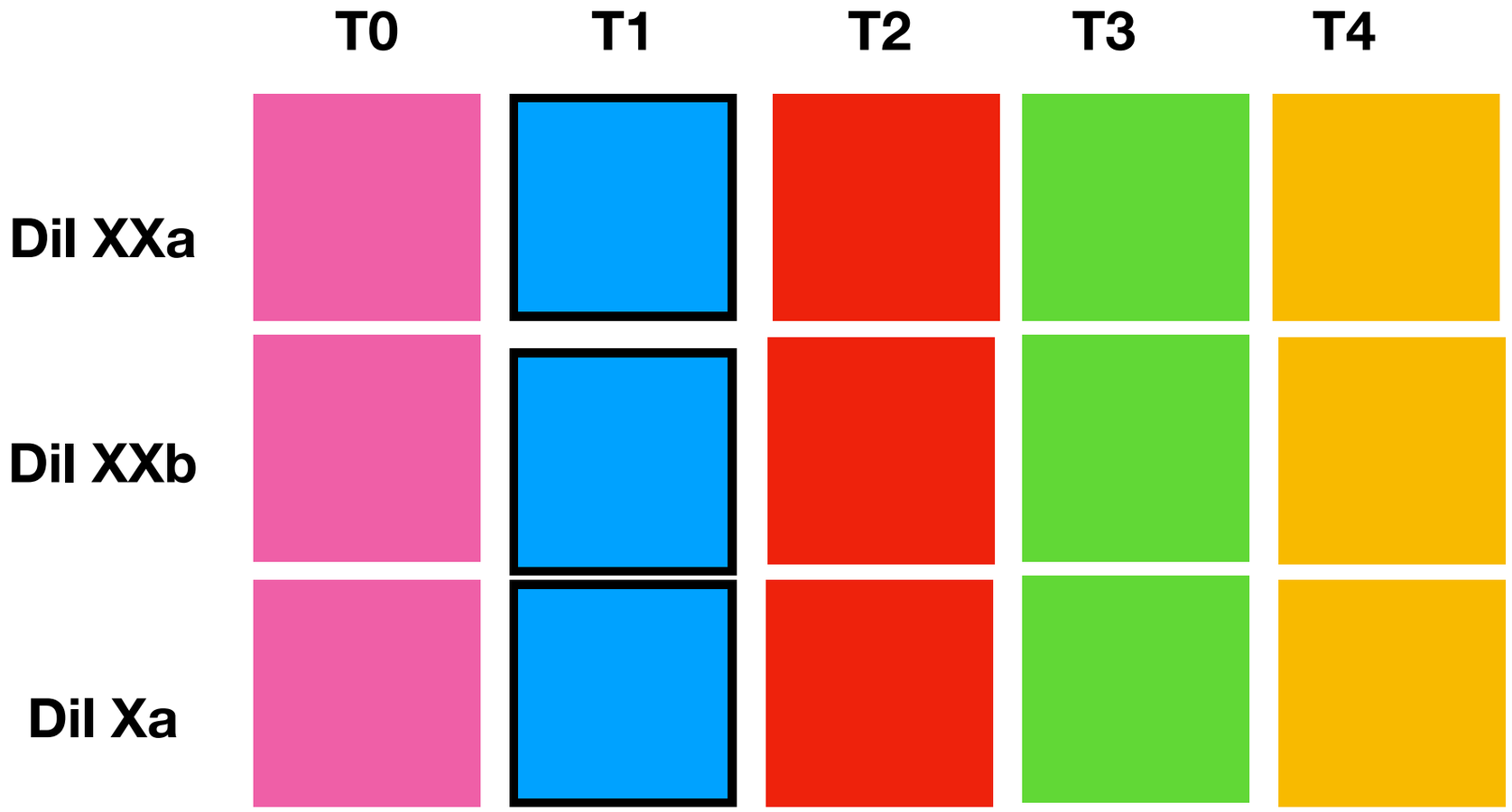
- **Package the plates (plastic bag with wet paper)**
- **Incubating plates in the box for 1 week**
- **Take pictures every day of the plates and change the wet paper**
- **Bring the plates back to class after one week**
- **On the e-book plot growth dynamic of colonies**
- **Writing final RESULT report on e-book (pictures and number) and answer picture**

e-BOOK



- **OneNote**
- **Create and share a notebook**

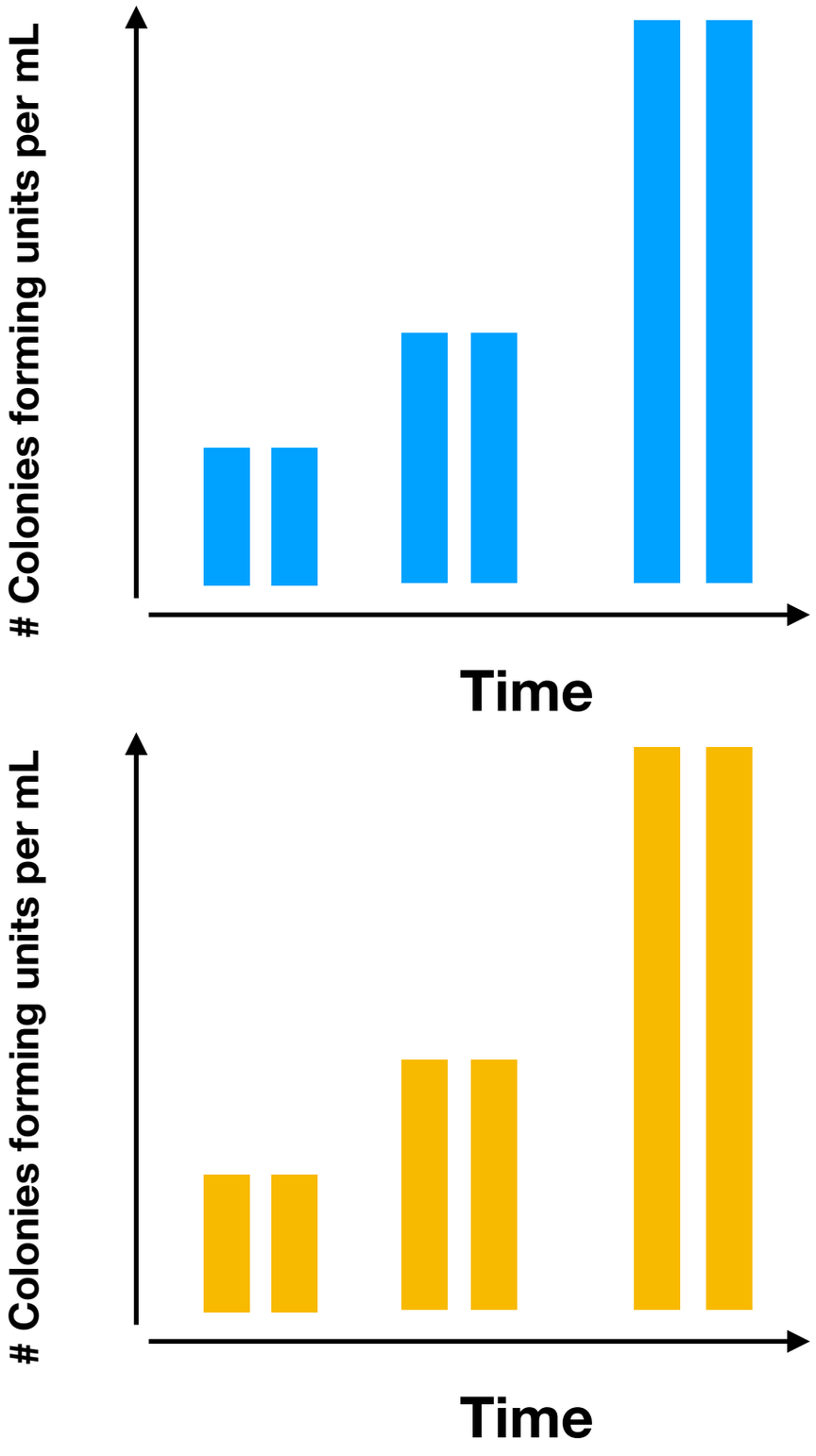
e-BOOK example: time series pictures

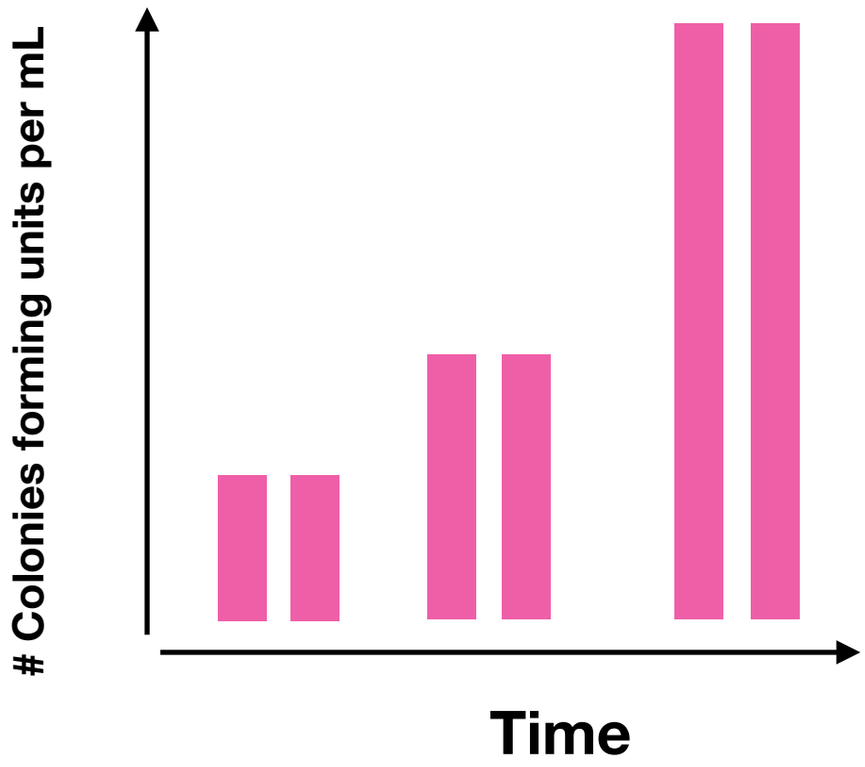


etc……

- **Soil plate pictures over time**
- **Water picture over time**

e-BOOK example: time series CFU/mL





- Plot bar graph for soil and water
- Compute average of CFUs over time
- Compute standard deviation of CFUs

e-BOOK example: answer questions based on your data

1. How many culturable soil/ freshwater-microbes there are?

2. Are there more culturable microbes in the soil or in the freshwater sample per unit of volume?

Goal

- **Assessing number of cultivable microbes via CFU (colony forming unit) plating method**
- **Assumption: 1 g of sediment has 1 billion microbes**
- **Colonies on the plates need to be countable —> how much to dilute?**
- **Importance of replicate**
- **Count the colonies over time and compute CFU/mL (number of colonies x dilution x plated microliters)**