Cellular Physiology
Cell Culture Laboratory

Laboratory 1
Primary Culture

PRIMARY CELL CULTURE

Separate cells directly from the tissue (kidney, liver, heart, skin etc)
Separated by enzymatic or mechanical method
Maintaining the growth of the cell in a culture medium using glass or plastic container

APPLICATIONS of Cell Culture

Excellent model system for studying

- The normal physiology, cell biology and biochemistry of cells
- The effects of drugs, radiation and toxic compounds on the cells
- Study mutagenesis and carcinogenesis

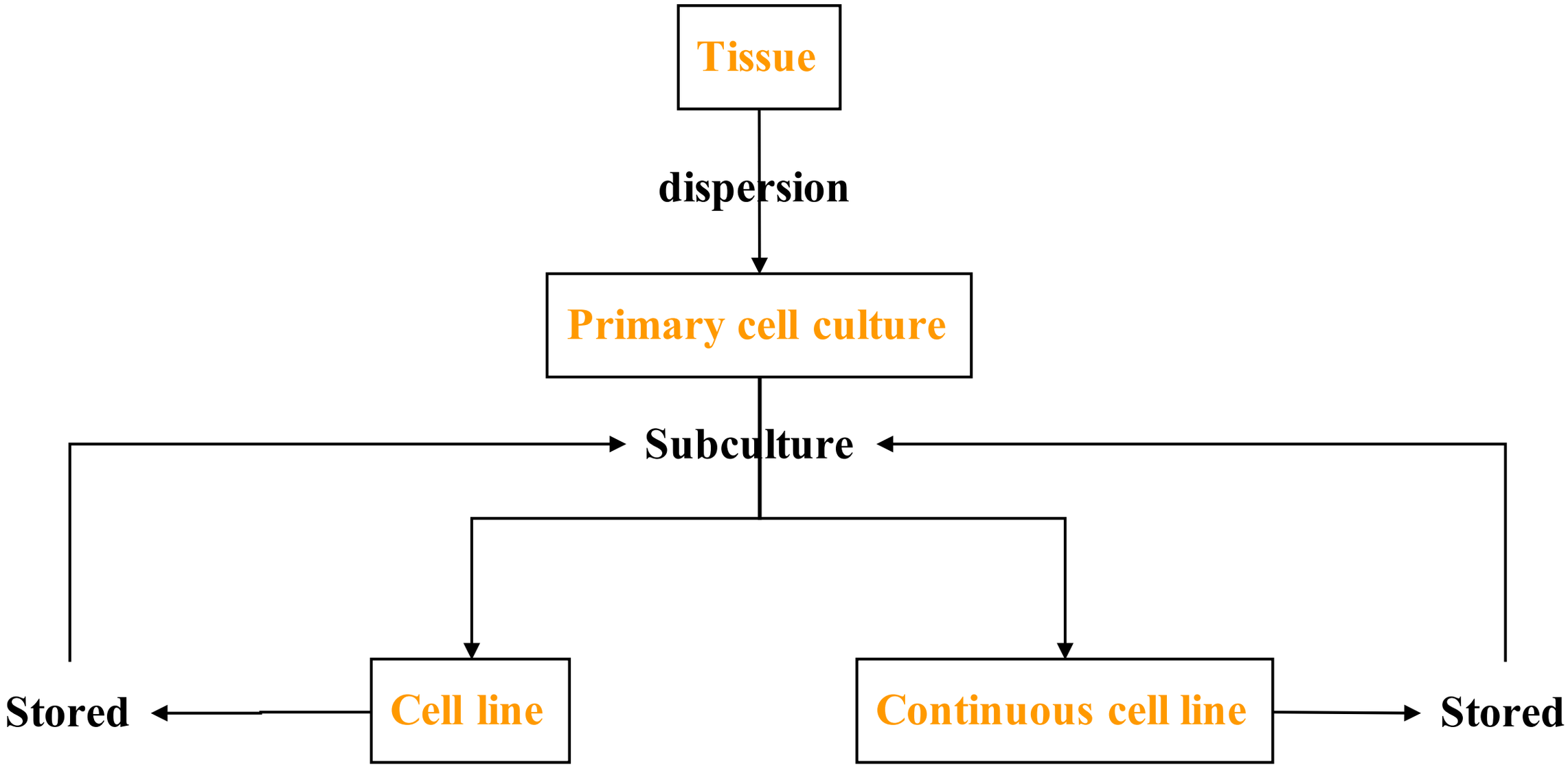
Used for gene transfer studies

Large scale manufacturing of biological compounds (vaccines, insulin, interferon, other therapeutic protein)

ADVANTAGES of Cell Culture

Absolute control of physical environment (pH, temperature, osmotic pressure, CO2 and O2 tension)
Homogeneity of sample
Less compound needed than in animal models

Initiation of Cell Culture



Type of cell culture

Primary Culture

A primary culture is that stage of the culture following isolation of the cells, but before the first subculture. They are usually more representative of the cell types in the tissue from which they were derived and in the expression of tissue-associated properties.

From the outgrowth of migrating cells from an explant
From tissue that is disaggregated mechanically or enzymatically

Subculture allows the expansion of the culture (now known as a cell line) but may cause a loss of specialized cells and differentiated properties (de-differentiation).

(+)Advantages:

Usually retain many of the differentiated characteristics of the cell *in vivo (*represent the tissue of their origin with regard to their properties*)*.

(-) Disadvantages:

Initially heterogeneous but later become dominated by fibroblasts.
The preparation of primary cultures is labor intensive and time consuming.

Can be maintained *in vitro* only for a limited period of time.

Cell culture morphology

Morphologically cell cultures take one of two forms:

Growing in suspension (as single cells or small free-floating clumps)
cell lines derived from blood (leukaemia, lymphoma)

Growing as a monolayer that is attached to the tissue culture flask.
cells derived from solid tissue (lungs, kidney), endothelial, epithelial, neuronal, fibroblasts

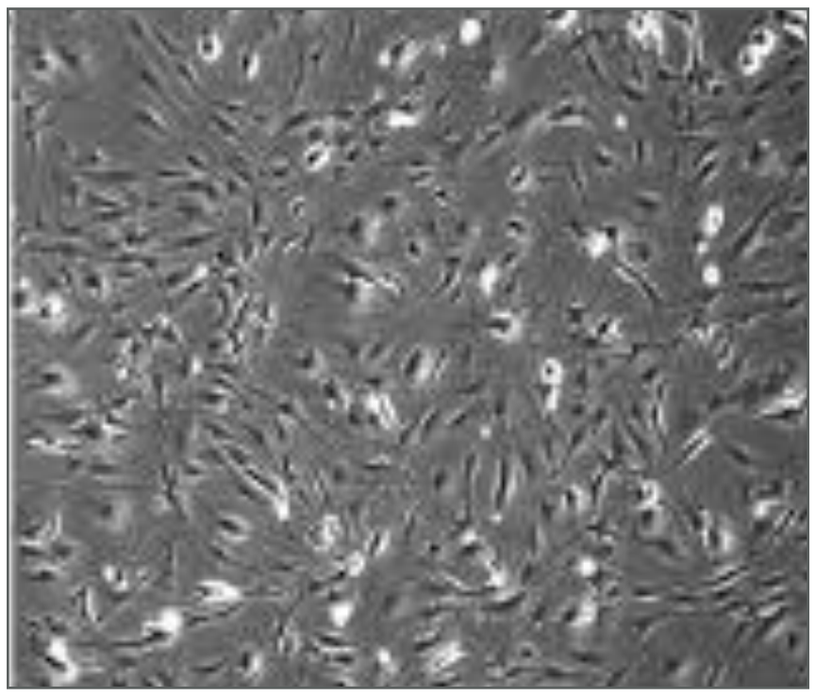
Adherent cells

Adherent cells are anchorage-dependent and usually propagate as a monolayer attached to the substrate.

The attachment is essential for proliferation

Adherent cells stop proliferating once they become confluent (i.e., when they completely cover the surface of the substrate) – a phenomena known as contact inhibition

Some cells will die if they are left in the confluent state for too long.



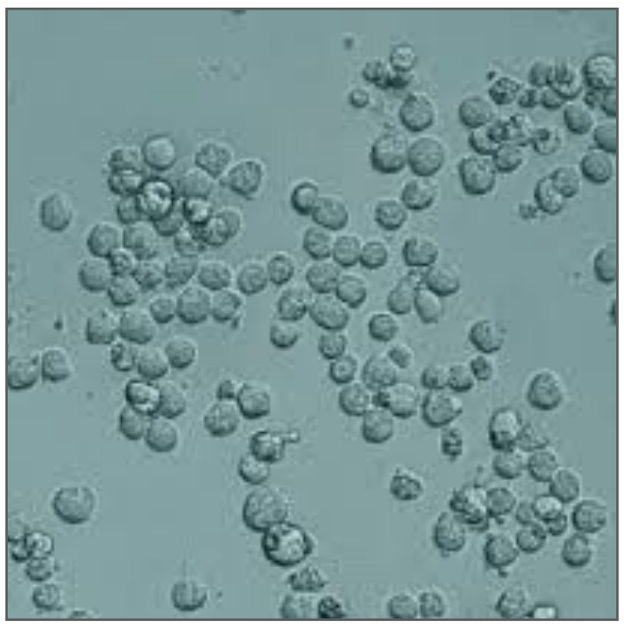
Suspension cells

Suspension cells can survive and proliferate without being attached to a surface – anchorage independence

Hematopoetic cells (derived from blood, spleen, or bone marrow) as well as some transformed cell lines and cells derived from malignant tumors can grow in suspension

Easier to passage as no need to detach them

As the suspension cells reach to confluency, aseptically remove 1/3rd of medium and add fresh one



Establishment of Primary Culture

Laboratory 1

Establishment of primary Culture

Materials:
Culture dish (non treated) - 2x
Culture dish (treated for cell culture) - 1x
1 pairs of scissors
1 needle
1 blade
15 ml tube - 3x (1 for MEM+10%FBS, 1 for PBS, 1 for tissue fragments)
1 eppendorf (for trypsin)
5-6 or more pasture pipette
Wast container

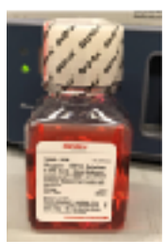
Reagents:
1 ml of Trypsin - (transfer into 1.5ml eppendorf and warm up in 37°C water batch)
10 ml of MEM medium + 10% FBS - prepared before - warm up in 37°C water batch
10 ml of PBS - Calcium, Magnesium free – phosphate buffered saline (PBS) - 10 ml (warm up in 37°C water batch)

Tissue from sheep foetus or adult tissue (small pice of tail or ear or skin).

Methods

1. Put on gloves and lab coat.
2. Sterilise/clean the laminar/bench by 70% ethanol.
3. Collect all materials/reagents required for experiment and prepare work place.
4. Isolate small piece (0.5 - 1cm) of tissue or adult tissue from sheep/mouse using scissors and/or blades.
5. Transfer tissue in culture dish (non treated) and wash with 1ml of PBS.
6. Remove hair, fat or death cells if you isolate piece of tissue from adult organs (ex. ear)
7. Transfer the tissue to the drop of trypsin (cover of non treated dish).
8. Cut tissue into small 1-2 mm pieces (keeping always in trypsin) (mechanical and enzymatic desegregation). Using needle, blade or scissors (as you prefer).
9. At the end of the above period add 5ml of medium containing serum and stir the contents for 1-2 more minutes to inactivate the action of trypsin.
10. Collect and transfer all the tissue/ trypsin/medium solution into a 15 ml tube (using Pasteur pipette).
11. Centrifuge at ~ 1000 rpm for 5mins.
12. Pour out the supernatant and resuspend the pellet in 5ml of warm PBS.
13. Centrifuge at ~ 1000 rpm for 5mins.
14. Pour out the supernatant and resuspend the pellet in 1ml of medium (MEM + 10% FBS).
15. Distribute all tissue piece equally to all the culture culture bottles and incubate at 37ºC + 5% CO2.
16. Observe culture every day under the microscope

Trypsin - is most commonly used **enzyme** in tissue culture to **detach** the adherent cells from culture vessel surface and/or to **disaggregate tissue** into single cell suspension.



Cell-cell and cell-matrix interaction depends on the **adhesion molecules**, which in turn, relies on the presence of calcium.
Trypsin weaken the function of adhesion molecules, EDTA is frequently included in the trypsin solution for its function as divalent cations chelator.
Tissue culture media contains calcium and magnesium ions, fetal bovine serum contains proteins that are trypsin inhibitors.
Both Mg2+ and Ca2+ inhibit trypsin.

PBS (phosphate buffered saline) is a **balanced salt solution** used for a variety of cell culture applications, such as **washing** cells before dissociation, **transporting** cells or tissue, **diluting** cells for counting, and preparing reagents.
PBS is formulated without calcium and magnesium for rinsing chelators from the culture before cell dissociation.

Culture **Minimal Essential Medium (MEM)** is a synthetic **cell culture medium** that can be used to maintain cells in **tissue culture.** It is based on 6 salts and glucose: (**calcium chloride, potassium chloride, magnesium sulfate, sodium chloride, sodium phosphate** and **sodium bicarbonate**), supplemented with 13 essential amino acids, and 8 vitamins: thiamine (vitamin B_1), riboflavin (vitamin B_2), nicotinamide (vitamin B_3), pantothenic acid (vitamin B_5), pyrodoxine (vitamin B_6), folic acid (vitamin B_9), choline, and myo-inositol (originally known as vitamin B_8).

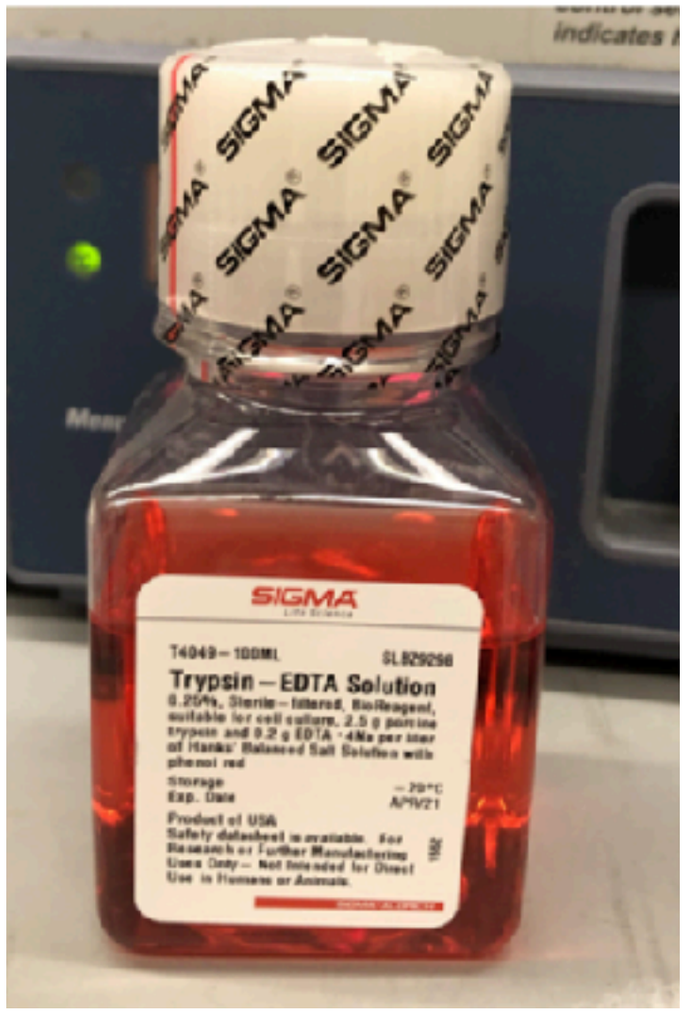
Reagents:

Trypsin - **warm up in 37°C water batch**
Phosphate buffered saline (PBS) - **warm up in 37°C water bath**
Minimal Essential Medium (MEM) containg 10% FBS - **warm up in 37°C water bath**

Phosphate buffered saline (PBS)

Store at 4°C

Trypsin

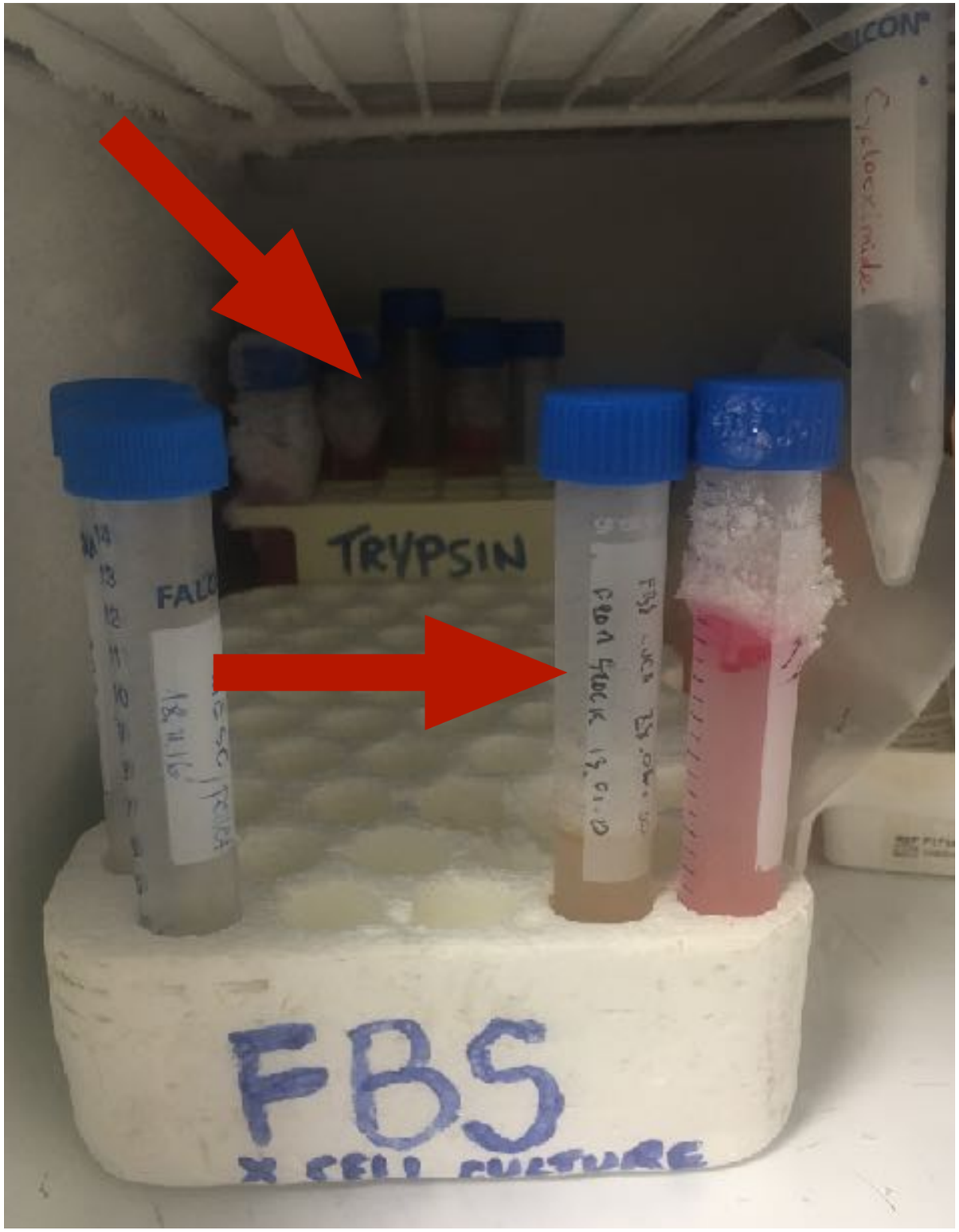


Store at 4°C, stock in - 20°C

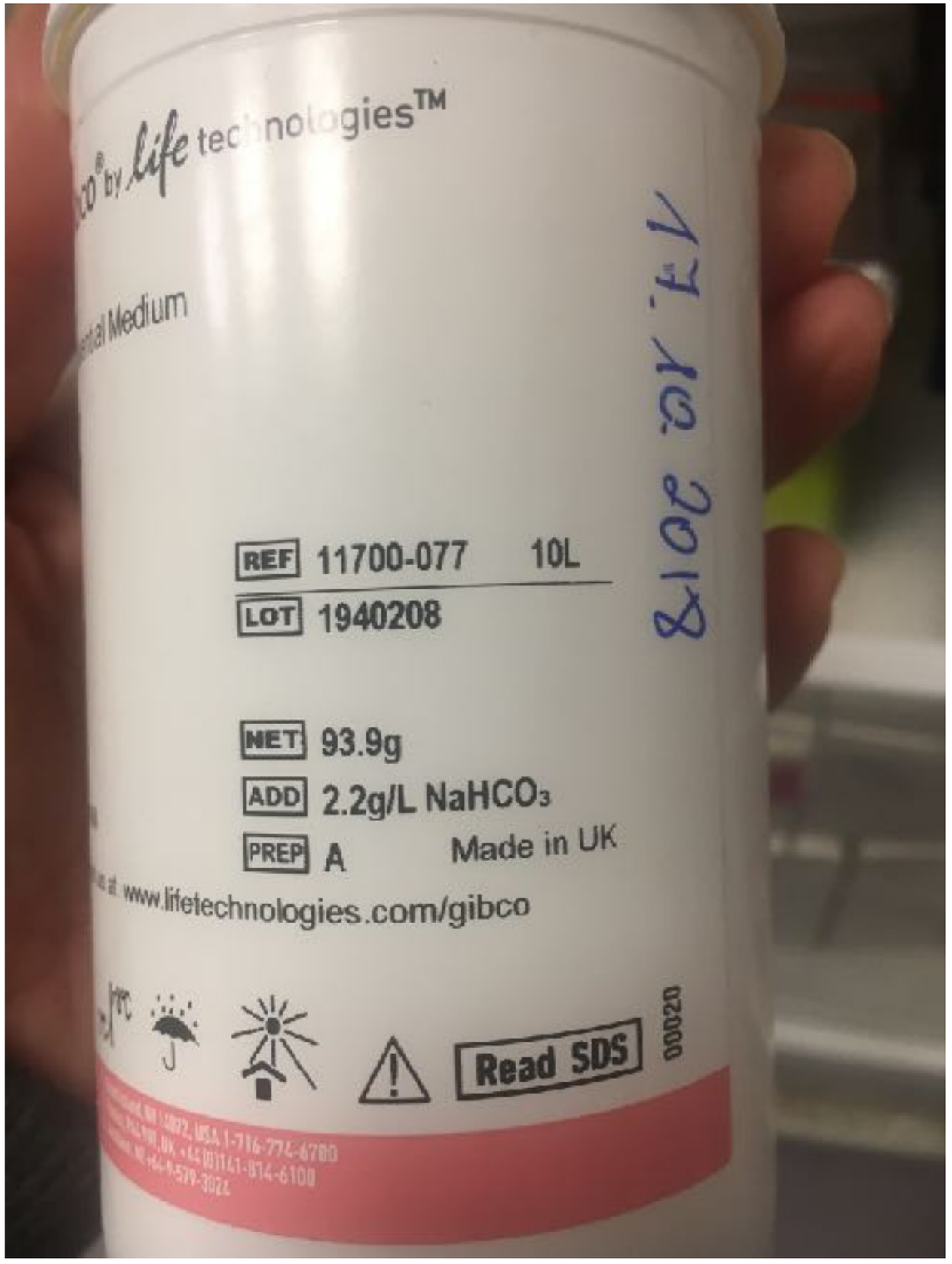
MEM - Minimum Essential Medium

Store at 4°C

Liquid media kept at 4°C; enyzmes (e.g. trypsin) and media components (glutamine and serum) at -20°C




CORNING
REF 15-010-CV 500 mL
MEM, 1X
(Minimum Essential Medium Eagle)
with Earle's salts
without L-glutamine
LOT 31819007
2°C 8°C
STERILE A
2021-05
For Research or Further
Manufacturing Use Only Not
for use in Diagnostic Procedures
Mediatech, Inc., 9345 Discovery Blvd.
Manassas, VA 20109 USA 1-800-492-1110
www.corning.com
Made in USA


life technologies™
Medium
REF 11700-077 10L
LOT 1940208
NET 93.9g
ADD 2.2g/L NaHCO3
PREP A Made in UK
www.lifetechnologies.com/gibco
Read SDS

Prepare 10 ml of MEM which contain 10% FBS

What we need:

Reagents:
Cell Culture medium - Minimum essential MediumM (MEM) ready to use (4°) which contain:
- Foetal bovine serum - FBS (-20°C)

Materials:
Membrane filter (0.2µm)
15 ml tube
Syringe
Pipet

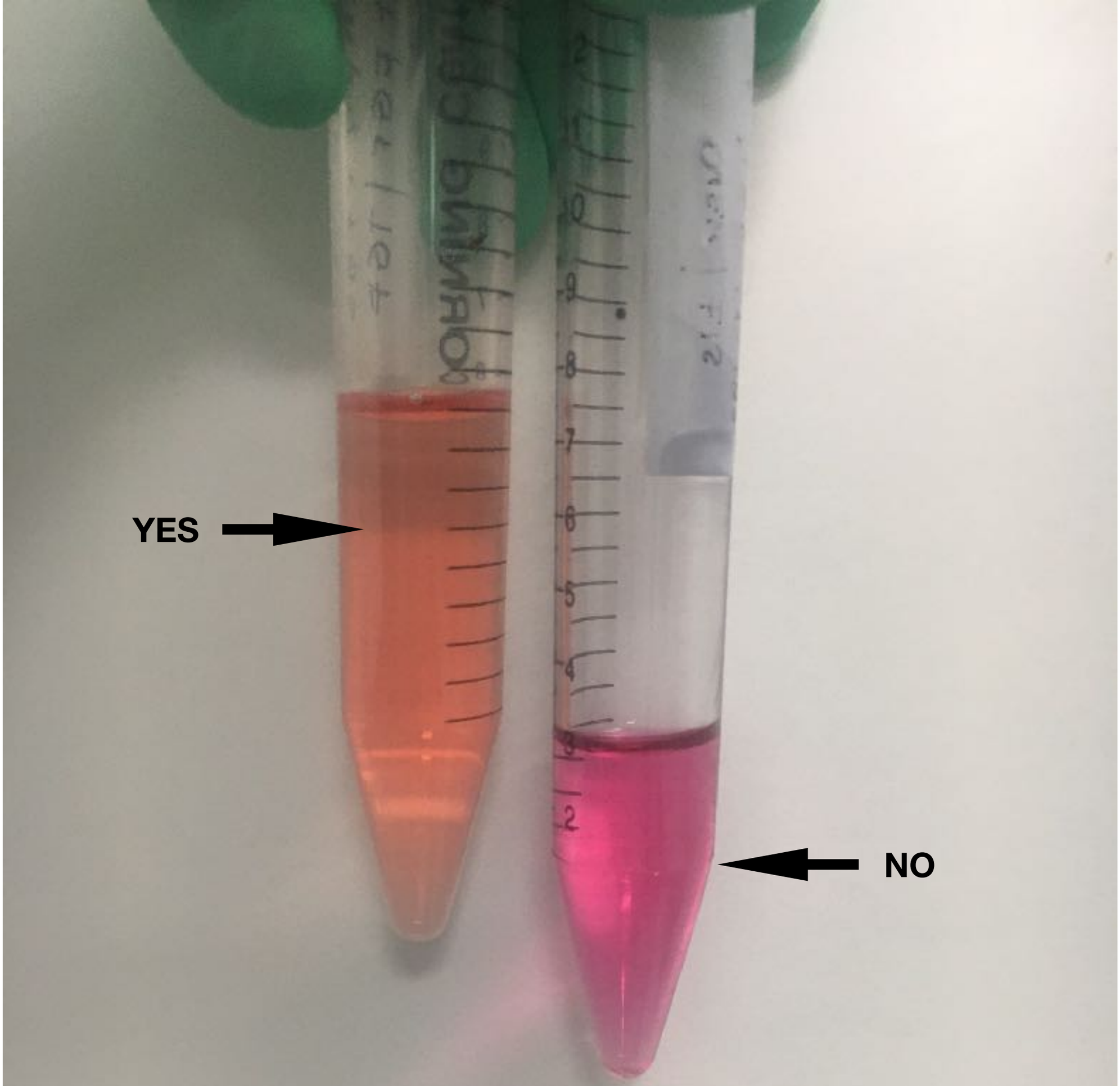
How to prepare culture medium

Sterilise the laminar/bench by 70% ethanol.

1. To prepare 10 ml of medium containing 10% foetal bovine serum (FBS) you need to take **9ml of MEM add 1ml of FBS**

2. Filter sterilise using 0,2 µm filter.

3. Warm up medium in water batch for at least 20min.


YES
NO

pH Control

pH can affect on:

Cell metabolism
Growth rate
Protein synthesis
Availability of nutrients

pH

- For Most Cells pH=*7.4 - 7.7* (while transformed cells may be better at pH *7.0 - 7.4*, epidermal cells could grown at pH 5.5
- Phenol Red is Used as pH Indicator
- Purple at ph>7.8, ph<6.5 yellow (see Fig. 1)

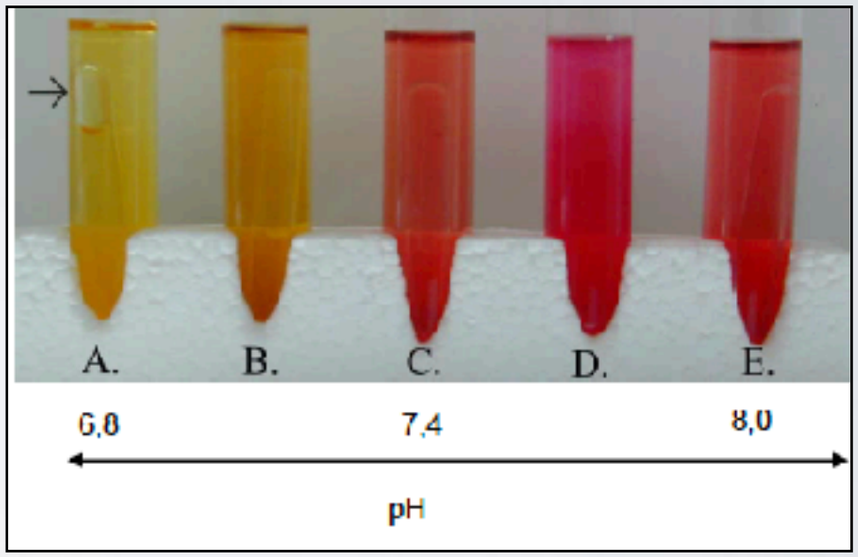


Figure 1. Phenol red is commonly used for visible detection off pH in the medium

- pH of the medium is dependent on the delicate balance of dissolved carbon dioxide (CO_2) and bicarbonate (HCO3), changes in the atmospheric CO_2 can alter pH of the medium
- necessary to use exogenous CO_2 when using medium buffered with CO_2-bicarbonate basal buffer

Warm up in 37°C water batch

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DONE

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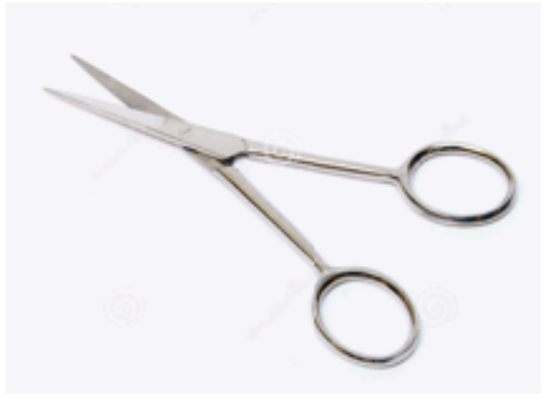
Culture dish (non treated)

pairs of scissors



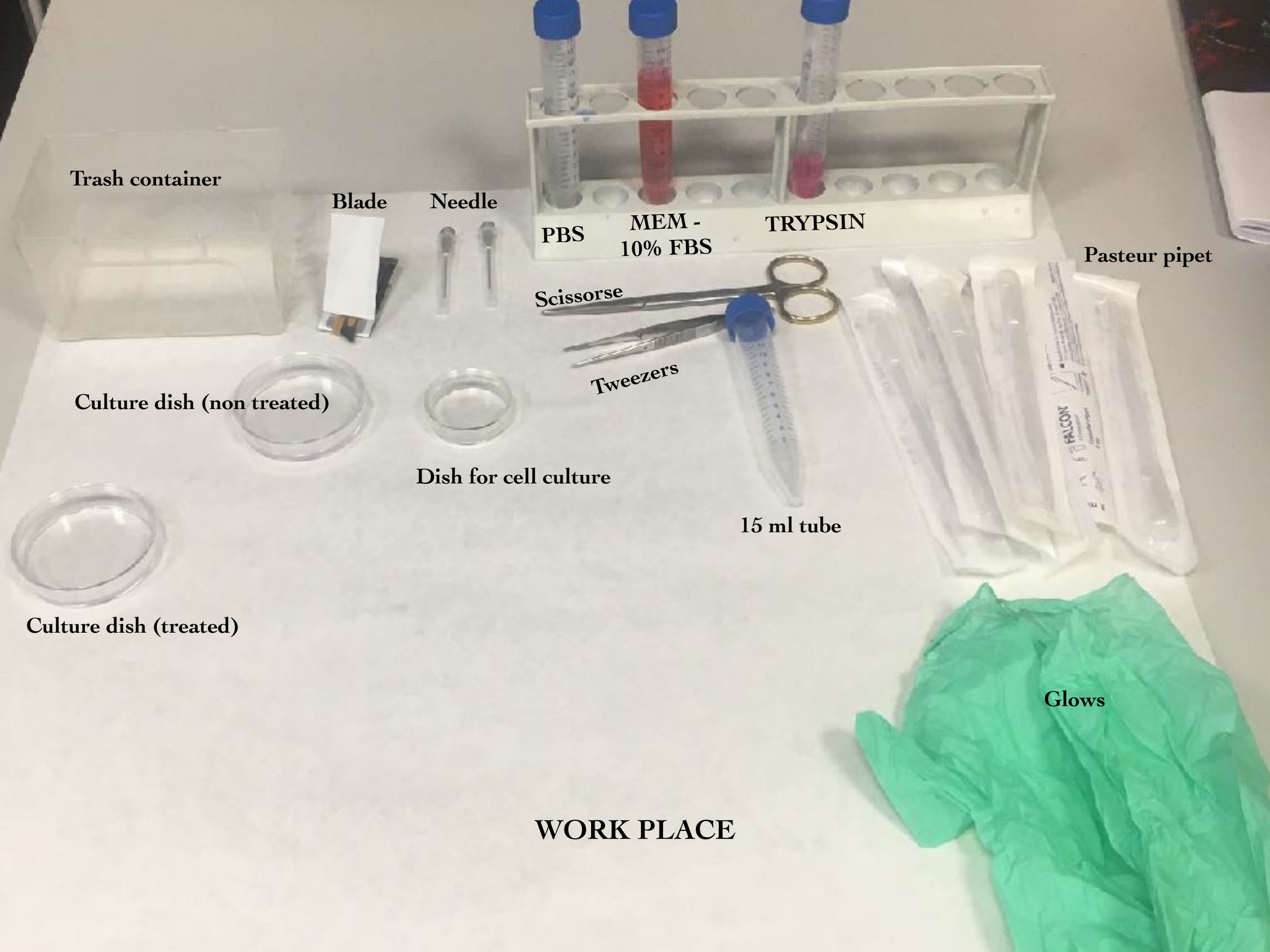
15 ml tube

5-6 or more pasture pipette

Culture dish (treated for cell culture)

blade

Wast container


Trash container
Blade
Needle
PBS
MEM -
10% FBS
TRYPSIN
Pasteur pipet
Scissorse
Tweezers
Culture dish (non treated)
Dish for cell culture
15 ml tube
Culture dish (treated)
Glows
WORK PLACE

Establishment of primary Culture

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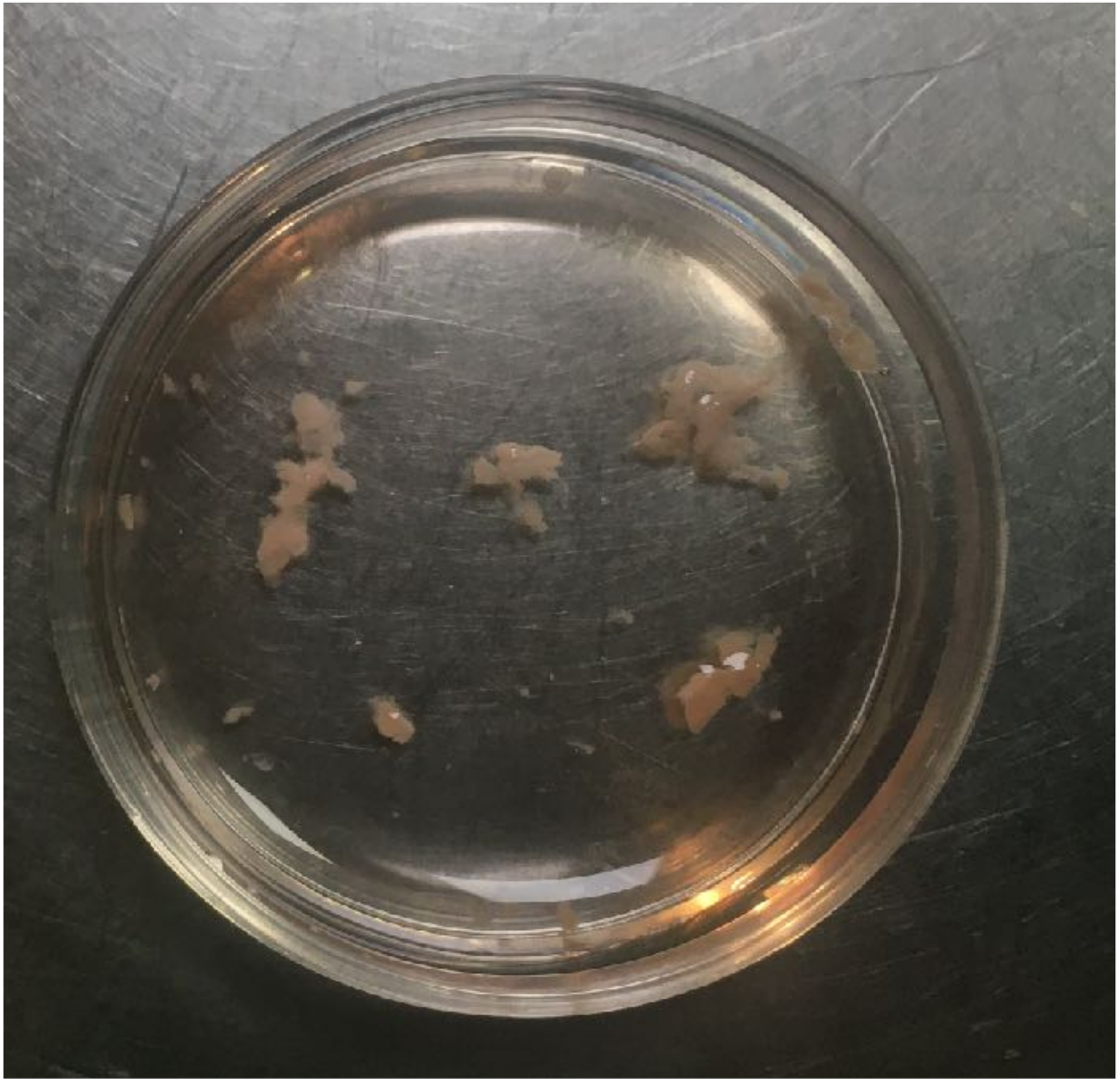
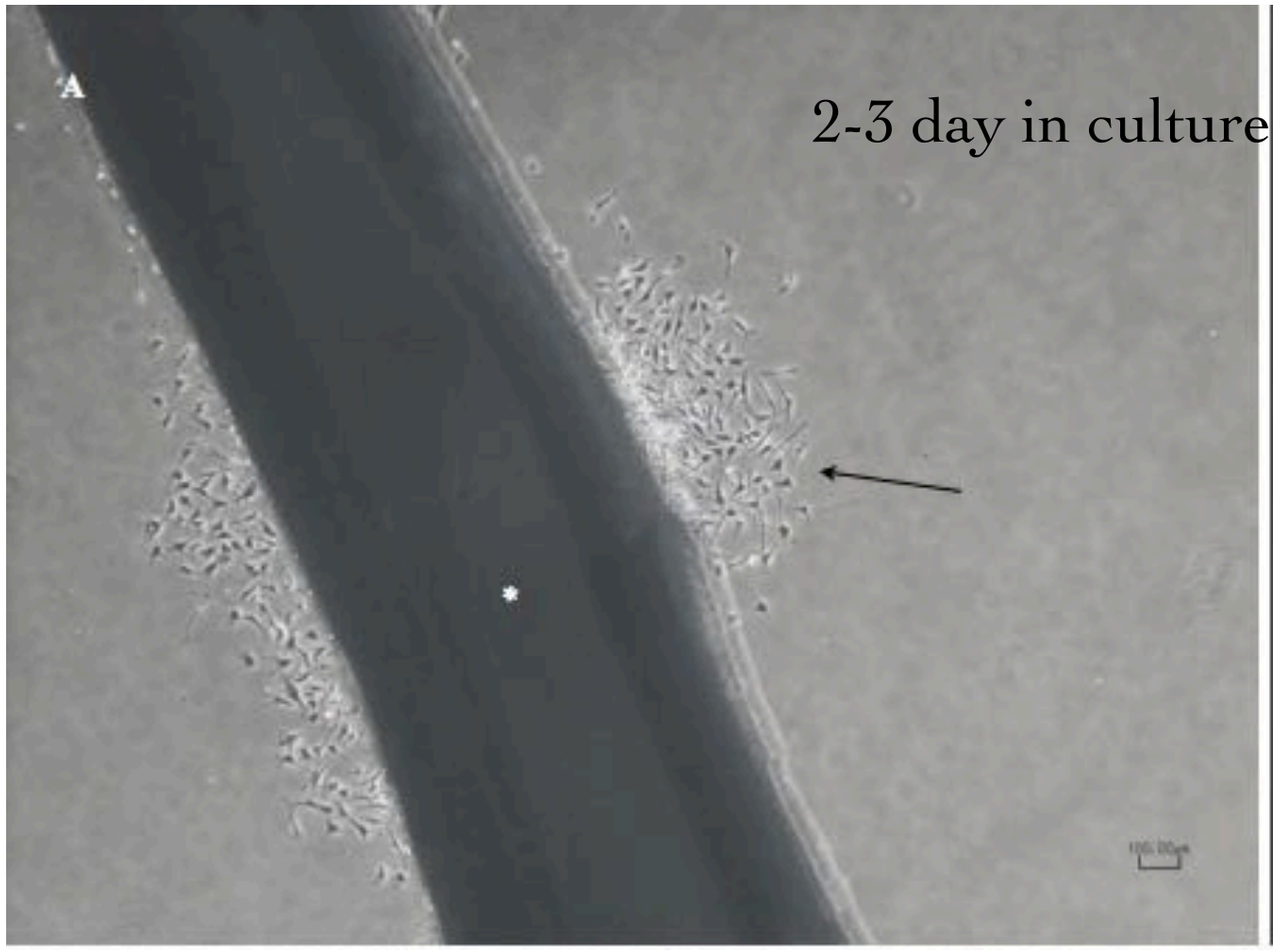
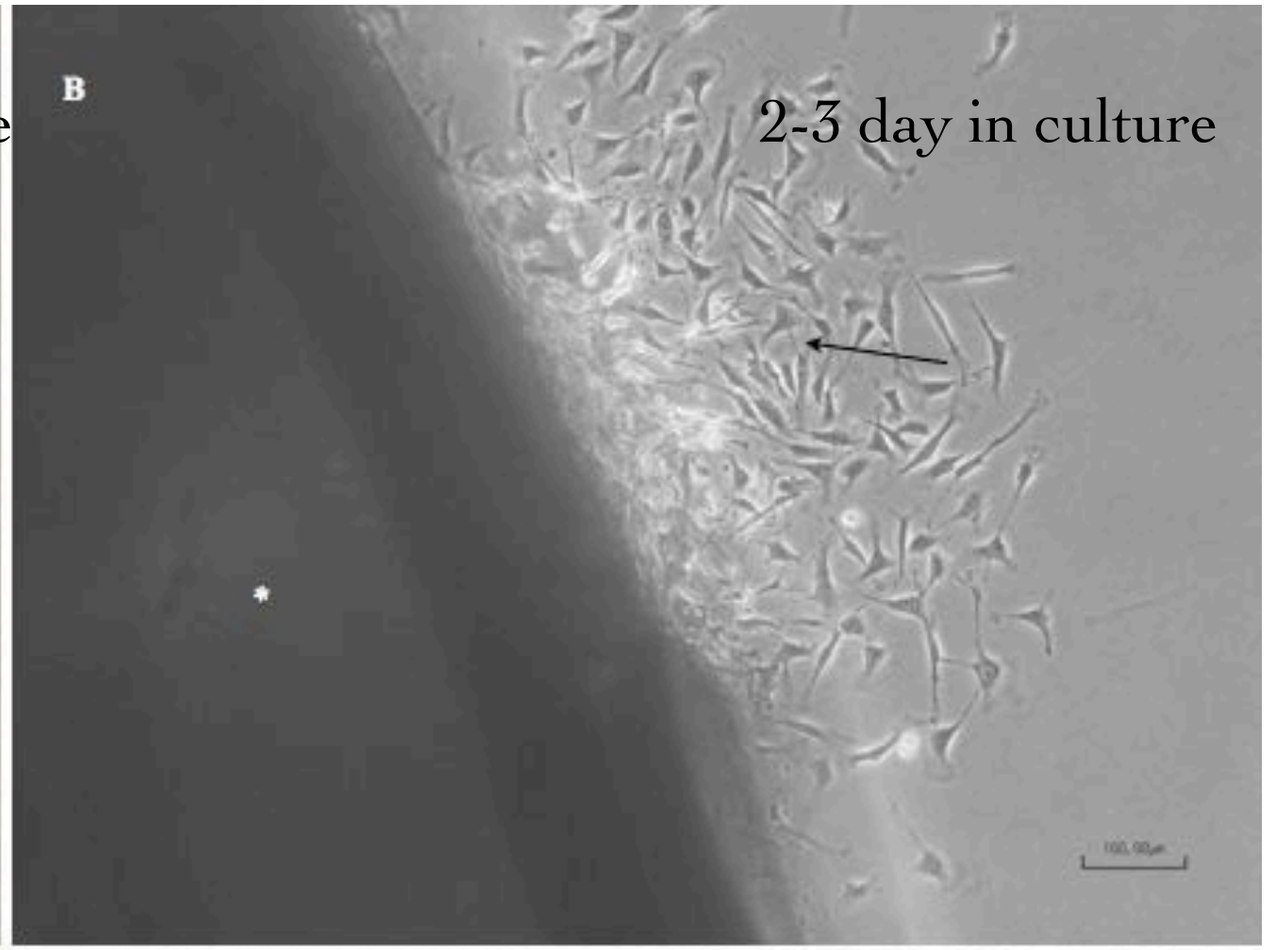
Small pieces

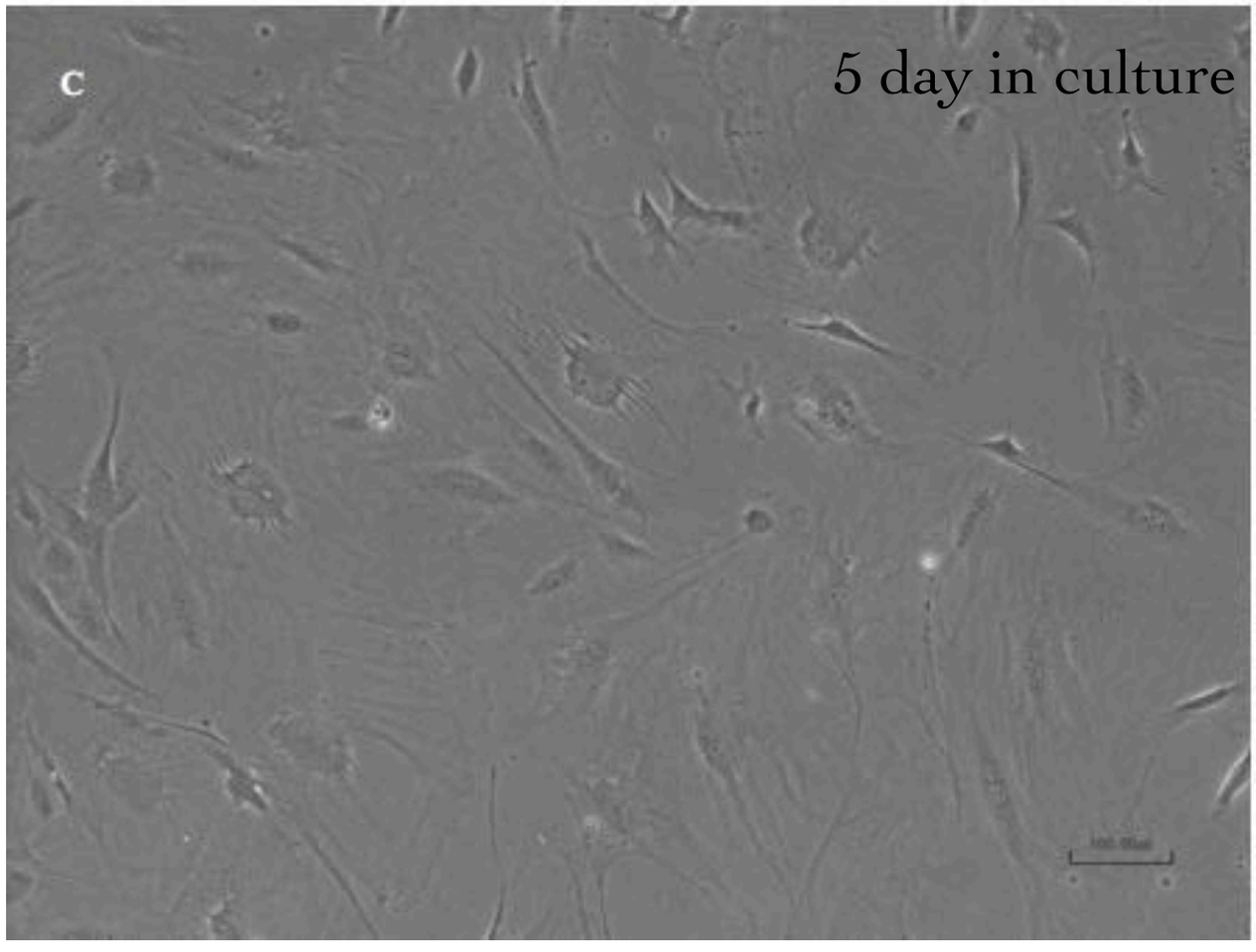
Separate from each other

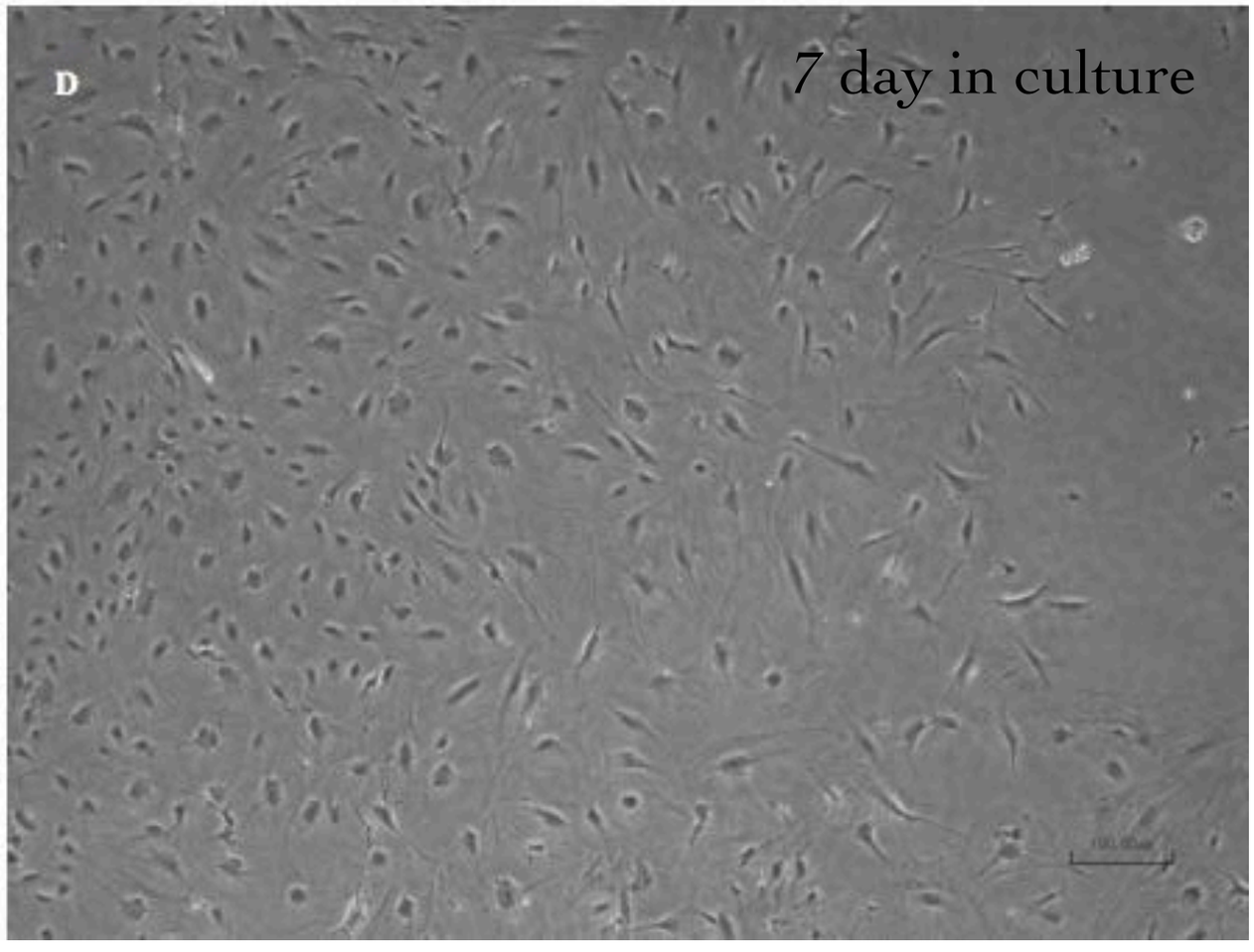
Small volume of medium

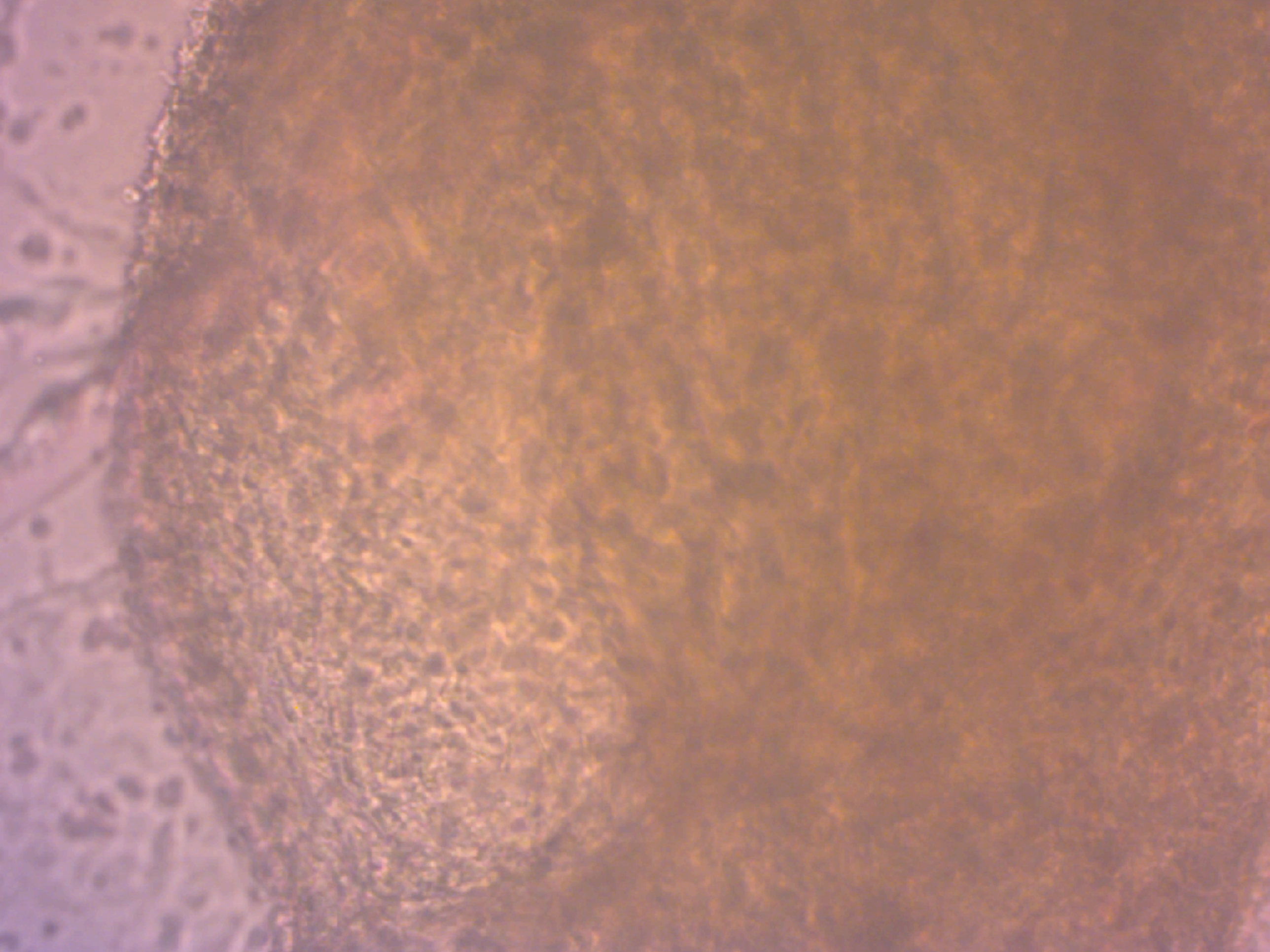


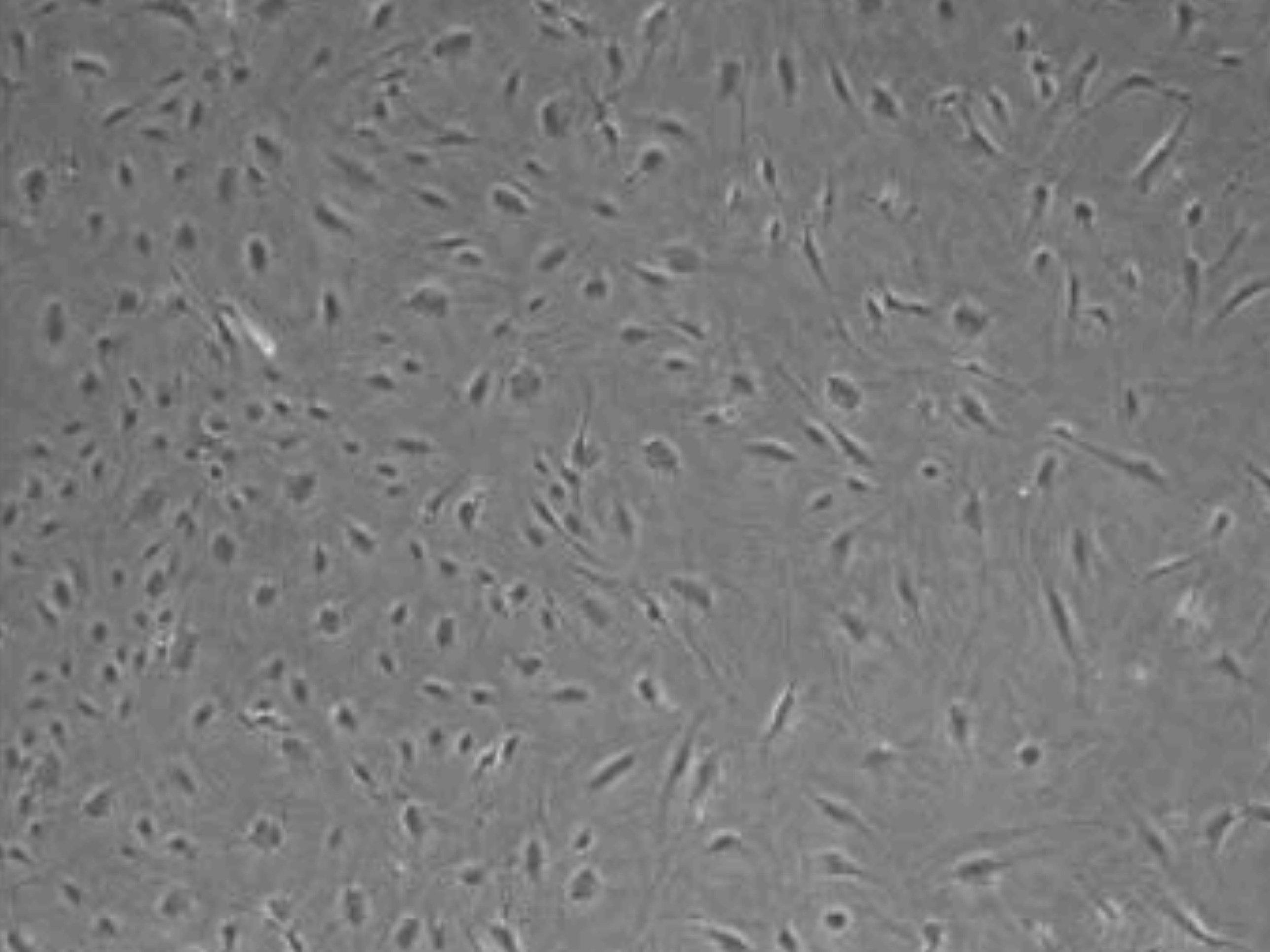

A
2-3 day in culture


B
2-3 day in culture


C
5 day in culture


D
7 day in culture





SECONDARY CELL CULTURE

When primary cell is sub cultured then it is **secondary cell**

Sub culture:
Transfer of cells from one culture dish to other

Method:
- remove growth medium
- detach adherent cells using enzyme (Trypsin, colagenase)

Why required:
- to provide fresh nutrients
- to provide more space

Establishment of primary Culture

Subculturing

https://www.youtube.com/watch?v=k2PMEET4Smo

Remove spent media from the culture vessel.

Add the pre-warmed dissociation reagent such as trypsin. Gently rock the container to get complete coverage of the cell layer.

Incubate the culture vessel at room temperature for approximately 2 minutes.

Add equivalent of 2 volumes of pre-warmed complete growth medium. Disperse the medium by pipetting over the cell layer surface several times.

Then split the cells into 2 or 3 flasks containing complete media.

Incubate the cells.

DETECTION OF CONTAMINATION

- In general indicators of contamination are **turbid culture media**, change in growth rate, abnormal pH, poor attachment, vacuolization, cell lysis…

- Yeast, bacteria and fungi usually shown visible effect on the culture (change in medium turbidity or pH)

- Mycoplasma detected by direct DNA staining with intercalating fluorescent substance eg. Hoechst 33258

TERMINOLOGIES

PRIMARY CELL CULTURE: When cells are sugically removed from and organism and placed into a suitable culture environment they will attach, divide and grow

CELL LINE: when the primary culture is subcultured and they show an ability to continously propagation

ANCHORAGE DEPENDENCY: Cells grow an monolayer adhering to the surface (glass/plastic)

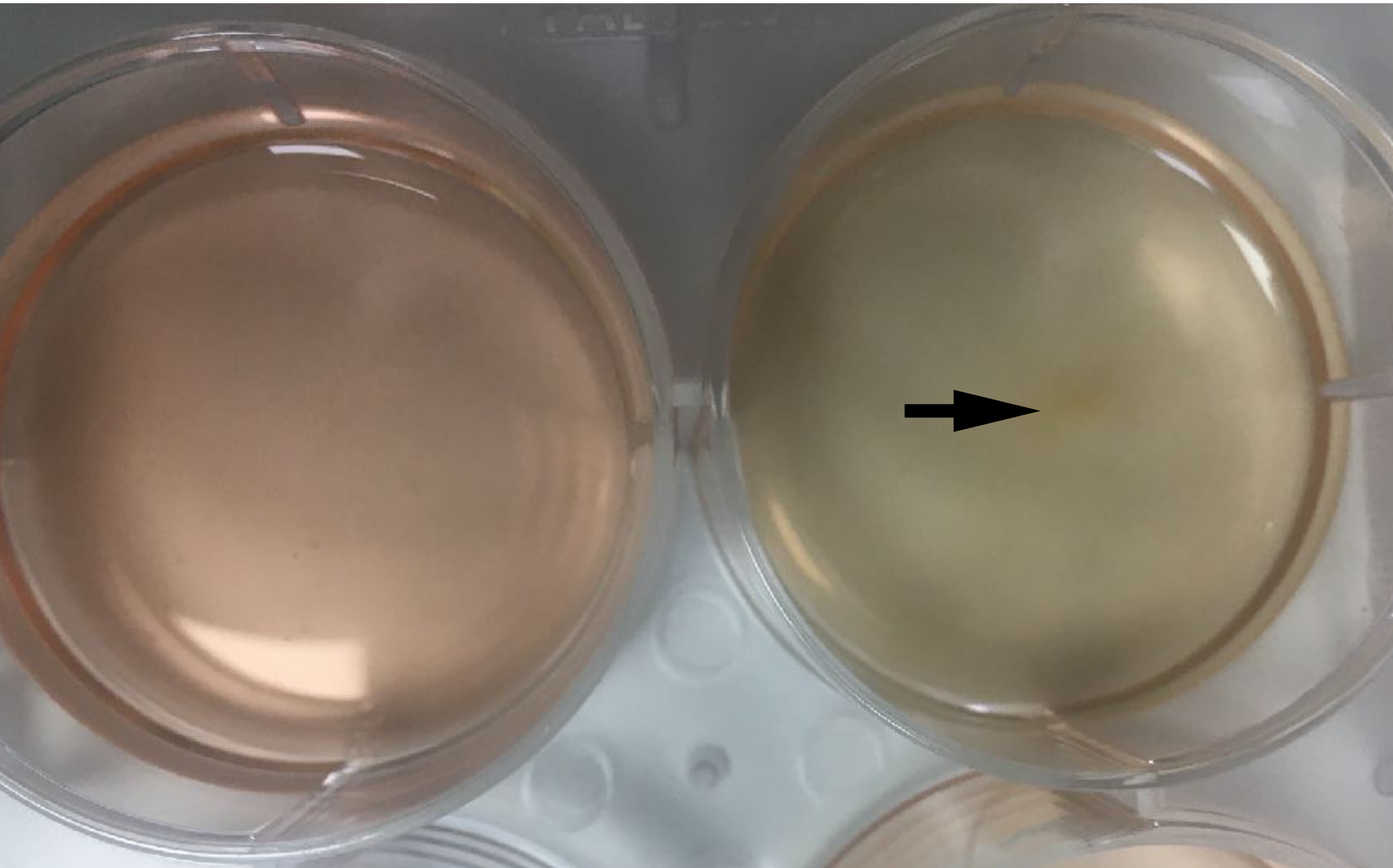
PASSAGING/SUBCULTURING: The process of spliting the cells

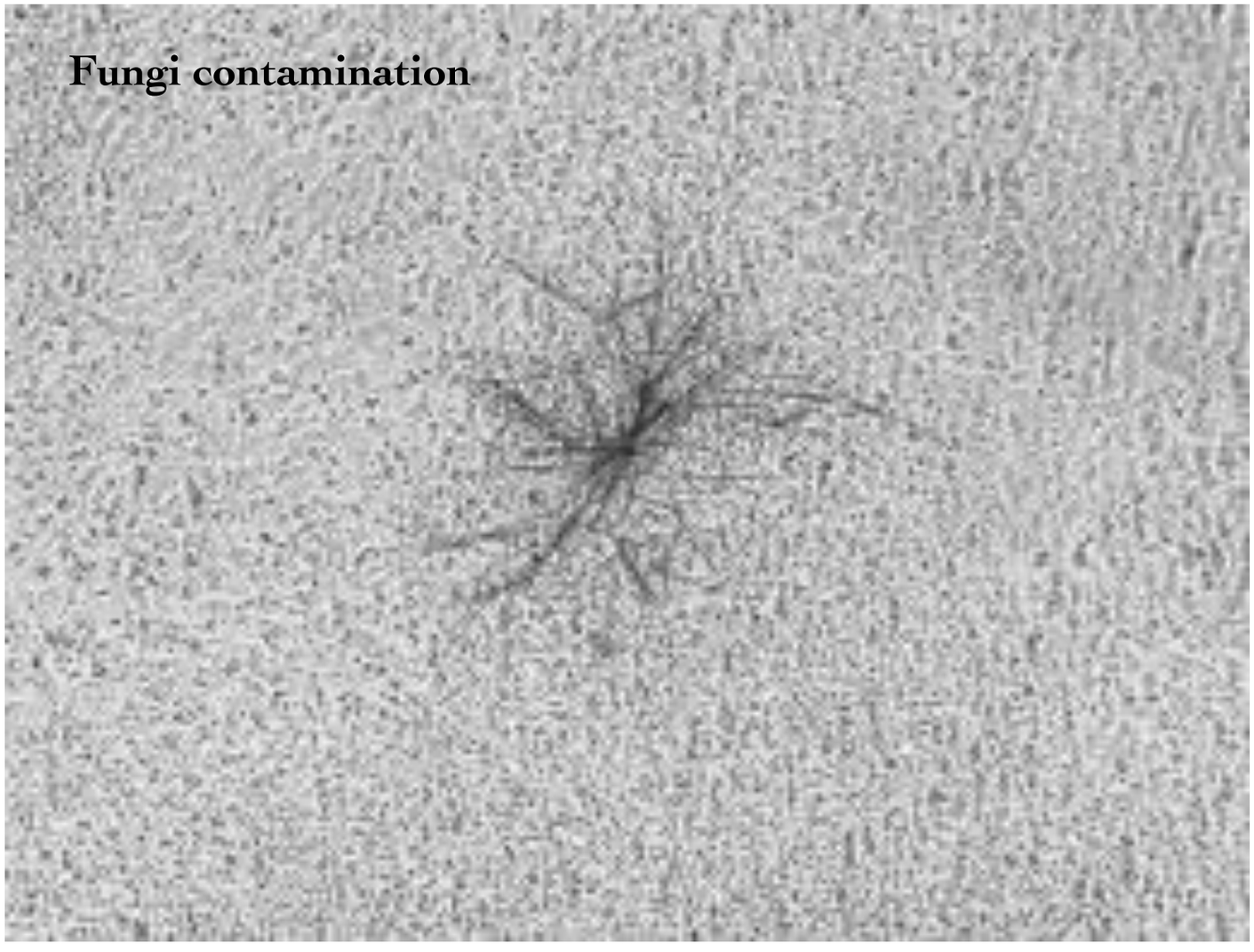
FINITE CELLS: When the cells has a finite life span

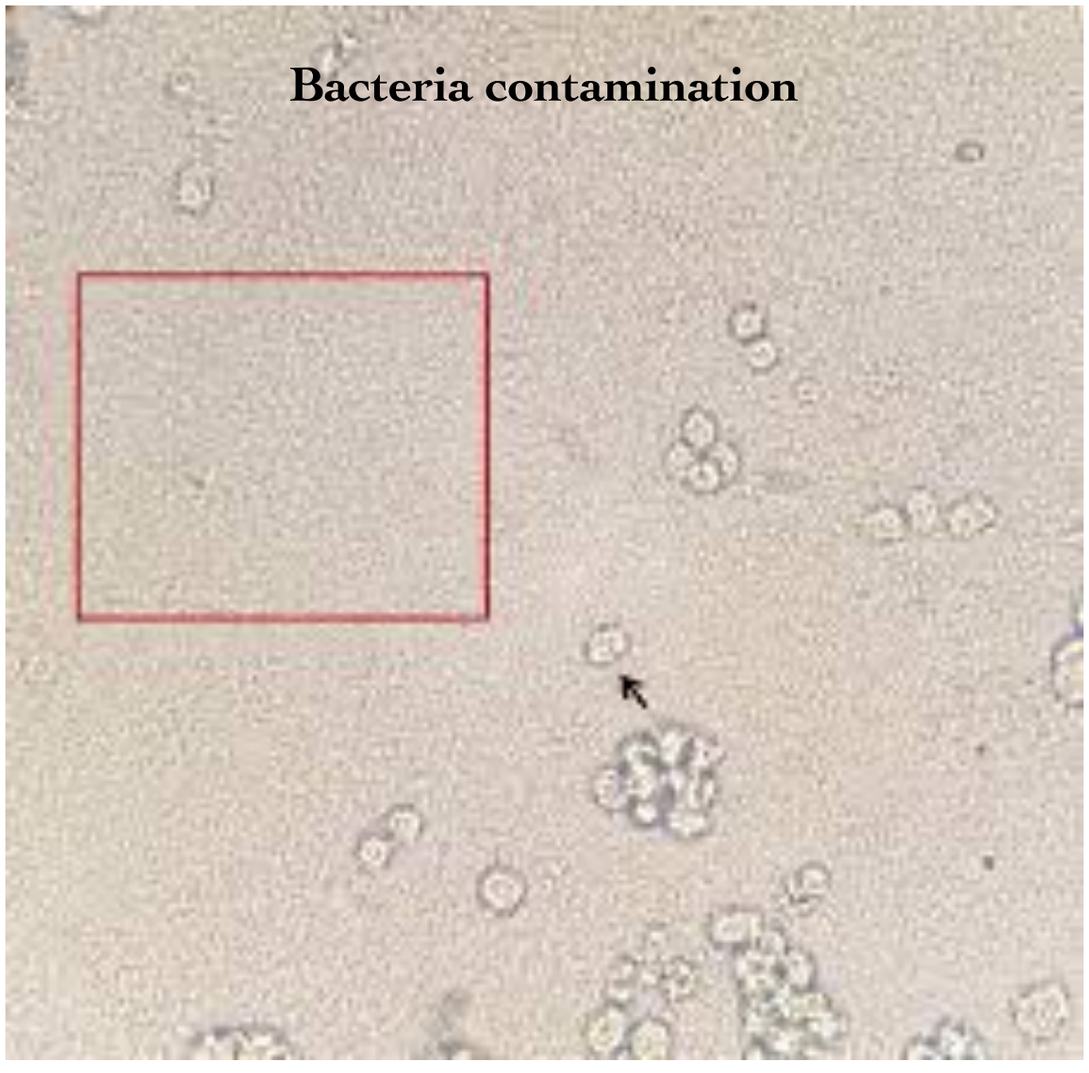
CONTINUOUS CELL LINE: When the cells can grow unpto infinite lifespan

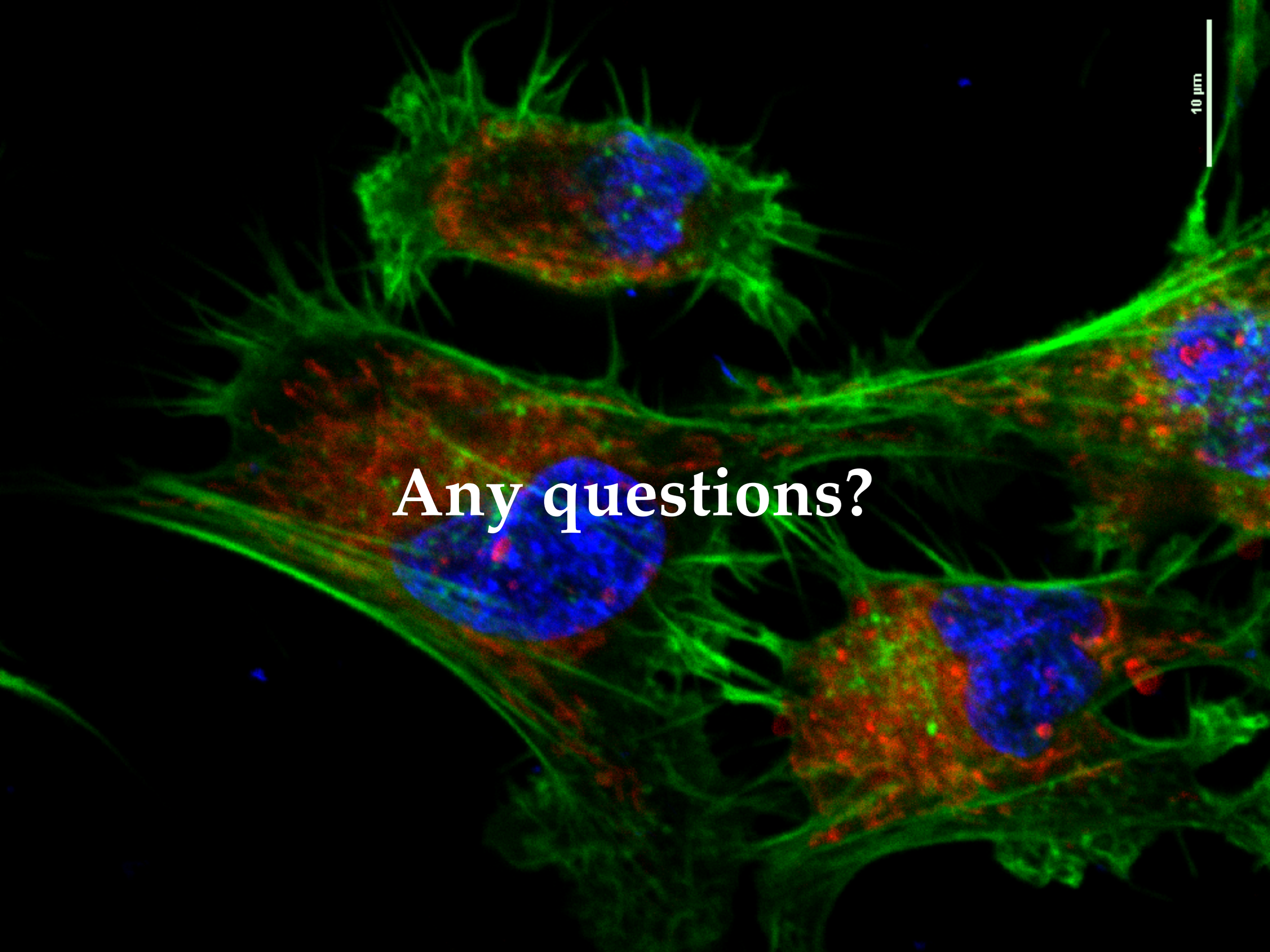
OK

CONTAMINATED




Fungi contamination


Bacteria contamination



Any questions?