
In The name of God

Cell Culture Media , Buffers , Antibiotics, Serums, Supplements and Surfaces

Starting with **plasma**, **lymph** , **serum**, and **embryonic extracts**

Chemically defined media based on analyses of **body fluids** and **nutritional biochemistry.**

Eagle's Basal Medium [Eagle, 1955] and, subsequently, **Eagle's Minimal Essential Medium (MEM)** [Eagle, 1959] became widely adopted. supplemented with **calf, human, or horse serum, protein hydrolysates, and embryo extracts**.

Cell Culture Media

Essential nutrients and energy source

- Amino acids, fatty acids, trace elements, salts, vitamins and cofactors and carbon source

Physicochemical properties

- pH and osmolarity mostly through ions and bicarbonate

Growth Function Phenotype

pH

Most cells grow well at **pH 7.4**

For some **normal fibroblast lines** the best is **pH 7.4 – 7.7** for **transformed cells** may **7.0–7.4 is better**

Temperature influence pH:

0.2 units lower at room temperature than at 37°C

Buffering systems

- **CO_2 and Bicarbonate**

Closed or open systems, low toxicity & cost, nutritional benefit

- **HEPES** is a **much stronger buffer** in the pH 7.2–7.6 (**10–20 mM**)

Concentration :more than double that of bicarbonate. **Toxic and expensive**

- **Phosphate**
- **Sera**
- **Other components such as pyruvate**

CO_2 and Bicarbonate

$$H_2O + CO_2 \longrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$$

$$H_2O + CO_2 + NaHCO_3 \longleftrightarrow H^+ + Na^+ + 2HCO_3^-$$

For each kind of medium proper amount of :
buffering system concentrations and CO_2 tension to achieve
correct pH and osmolality

Compound	Eagle's MEM Hanks's salts	Low HCO_3^- + buffer	Eagle's MEM Earle's salts	DMEM
$NaHCO_3$	4 mM	10 mM	26 mM	44 mM
CO_2	Atmospheric and evolved from culture	2%	5%	10%
HEPES* (if required)	10 mM	20 mM	50 mM	—

Oxygen

Level of O_2 ➡ Risk of free radicals

Free radical scavengers (glutathione, 2-mercaptoethanol or thiothreitol

Depth of the culture medium = Rate of oxygen diffusion

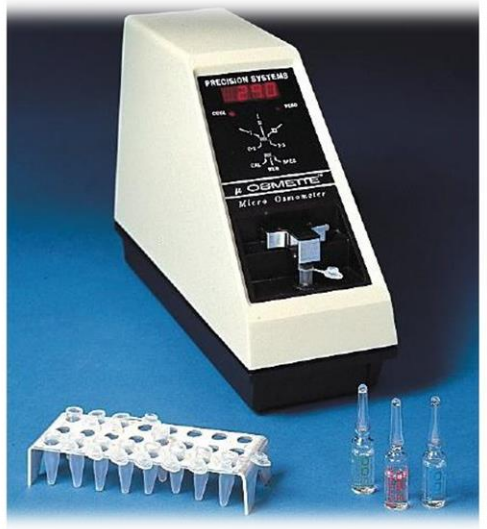
Depth :within the range 2–5 mm (0.2–0.5 mL/cm_2)

Osmolality

For most cells 260 mosmol/kg and 320 mosmol/kg

HEPES , drugs dissolved in strong acids and bases and their subsequent neutralization affect osmolality

Serum effect



Balanced Salt Solutions (BSS)

Use for:

- **Washing cells and tissues and disecting.**
- **Incubation for short time(glucose, up to 4 hours)**
- **Base of cell culture media**
- **Diluent fluid** for **amino acids** and **vitamins** to maintaining **intra and extracellular osmotic balance.**

Balanced Salt Solutions (BSS)

Provides:

Cells water and **inorganic ions** for normal cell metabolism and **energy source**(glucose),

Buffering system **:physiological PH range (7.2 - 7.6)** and

Osmotic pressure to treat the tissues and cells with various agents.(HEPES-NaCl)

Balanced Salt Solutions (BSS)

Mixture of inorganic salts and other components

Most commonly include : sodium, potassium, calcium, magnesium, and chloride ,

may include sodium bicarbonate,

Sometimes glucose as an energy source

and phenol red as a pH indicator

TABLE 9.2. Balanced Salt Solutions

Component		Earle's BSS		Dulbecco's PBS: Without Ca^{2+} and Mg^{2+} (D-PBSA)		Dulbecco's PBS: With Ca^{2+} and Mg^{2+}		Hanks's BSS		Spinner salts (as in S-MEM)	
	M.W.	g/L	mM	g/L	mM	g/L	mM	g/L	mM	g/L	mM
Inorganic salts											
$CaCl_2$ (anhydrous)	111	0.02	0.18			0.2	1.80	0.14	1.3		
KCl	74.55	0.4	5.37	0.2	2.68	0.2	2.68	0.4	5.4	0.40	5.37
KH_2PO_4	136.1			0.2	1.47	0.2	1.47	0.06	0.4		
$MgCl_2 \cdot 6H_2O$	203.3							0.1	0.5		
$MgSO_4 \cdot 7H_2O$	246.5	0.2	0.81			0.98	3.98	0.1	0.4	0.20	0.81
NaCl	58.44	6.68	114.3	8	136.9	8	136.9	8	136.9	6.80	116.4
$NaHCO_3$	84.01	2.2	26.19					0.35	4.2	2.20	26.19
$Na_2HPO_4 \cdot 7H_2O$	268.1			2.2	8.06	2.16	8.06	0.09	0.3		
$NaH_2PO_4 \cdot H_2O$	138	0.14	1.01							1.40	10.14
Total salt			147.9		149.1		154.00		149.4		158.9
Other components											
D-glucose	180.2	1	5.55					1	5.5	1.00	5.55
Phenol red	354.4	0.01	0.03					0.01	0.0	0.01	0.03
Gas phase		5% CO_2				Air		Air		5% CO_2	

Amino Acids

- The essential amino acids **cysteine**, **arginine**, **glutamine**, and **tyrosine**
- Requirements for amino acids **vary from one cell type to another**
- **Nonessential amino acids** :for a particular cell type's **incapacity to make** or lost by **leakage into the medium**.

Amino Acids

- The concentration of amino acids usually limits the maximum cell concentration

- glutamine is also used by cultured cells as a source of energy and carbon

(stability:1 month in 4°C &1week in 37°C)

- Glutamax (Invitrogen) is an alanyl-glutamine dipeptide which is more stable than glutamine(more than 4month in 4°C)

Vitamins:

Requirements vary from one cell type to another

Different media, different formulations for vitamins

Organic Supplements

Hormones and Growth Factors

Salts

- **Osmolality** (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-,$SO4^{2-}$, $PO4^{3-}$ and HCO^{3-})
- **Membrane potential regulation(** Na^+, K^+, and Cl^-)
- **Matrix** and **nutritional precursors for macromolecules(**$SO4^{2-}$, $PO4^{3-}$, and HCO^{3-})
- **Ca^{2+}**

1. **Cell adhesion, signal transduction , proliferation** and **differentiation**
2. **Reduced in suspension cultures** :**minimize cell aggregation** and **attachment**

Water

Quality(purity)

Inorganic compounds (heavy metals, iron, calcium, and chloride),

Organic compounds (detergents),

microbial-related contaminants (endotoxins and pyrogens),

particles and colloids from several different origins

water for injection (WFI) and for **purified water (PW)**

Sterility (mycoplasma)

Conductivity <0.1 μs/cm^2

pH= 5-7

Antibiotics

Different contaminants,

Different antibiotics,

Different mechanisms

- **Disadvantages:**

- **Antibiotic resistant organisms.**
- **Hidden Low-level, cryptic contaminants**
- **Cell toxicity**
- **Hidden Mycoplasma**

Antibiotics is restricted to:

- **primary cultures.**
- **large-scale labor-intensive experiments** with a high cost of consumables.
- **Contaminants eradication**

GCCP: antibiotics **unnecessary**

Antibiotics for cell culture

Antibiotic	Concentration, µg/mL (unless otherwise stated)		Activity against
	Working	Cytotoxic	
Amphotericin B (Fungizone)	2.5	30	Fungi, yeasts
Ampicillin	2.5		Bacteria, gram positive and gram negative
Ciprofloxacin	100		Mycoplasma
Erythromycin	50	300	Mycoplasma
Gentamycin Gentamicin	50	>300	Bacteria, gram positive and gram negative; mycoplasma
Kanamycin	100	10 mg/mL	Bacteria, gram positive and gram negative; mycoplasma
MRA (ICN)	0.5		Mycoplasma
Neomycin	50	3,000	Bacteria, gram positive and gram negative
Nystatin	50		Fungi, yeasts
Penicillin-G	100 U/mL	10,000 U/mL	Bacteria, gram positive
Polymixin B	50	1 mg/mL	Bacteria, gram negative
Streptomycin SO_4	100	20 mg/mL	Bacteria, gram positive and gram negative
Tetracyclin	10	35	Bacteria, gram positive and gram negative
Tylosin	10	300	Mycoplasma

Serum

- Attachment factors (Fibronectin, Laminin, etc.)
- Survival/Growth factors (Insulin, IGF-1, etc.)
- Hormones
-
- Vitamins
- Cofactors

Serum

Advantage:

Increases buffering capacity

Chelator, Carrier proteins

Binding and neutralizing toxins

Protease inhibitoring

Promotes attachment of cell to substratum

Source of Intermediate metabolites,

Hormone and growth factor

Disadvantage:

Variable from lot to lot

Contaminants or degradative enzymes

Infectious agents (viruses, prions)

Expensive

Choice of Sera

- Country of origin(South America and Newsland)
- Certificates (contaminants)
- Heat inactivation
- Special applications (Stem cells, Gene manipulated cells, …)
- Known , reliable companies

Choice of media

- **Cell type:**

Species , organ, attachment, CO_2 requirement

- **Your accessibilities and application**

Commonly used Media:

Minimum Essential Medium (MEM)

Dulbecco's Modified Eagle Medium (DMEM)

RPMI-1640 (Roswell Park Memorial Institute ,for suspension cell culture)

Leibovitz' L-15 Medium

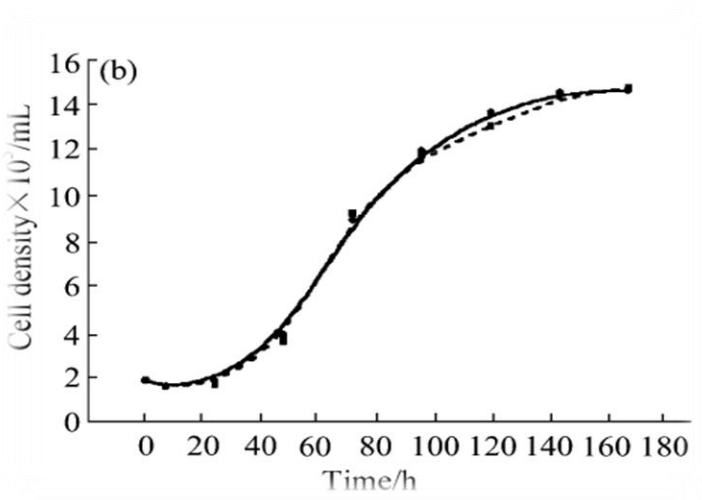
Quality control of cell culture media and sera

-Sterility test (incubate the first & final sample of each lot of medium in Co_2Incubator for 14 days Result → No Growth (pass test)

-Growth promotion test

Determine the growth curve of cell line and comparison by approved media

Result → similar with approved media



Medium Stability and Storage

- **4-8 ºC, No light**
- **Sterilize with filtration**
- **Separate aliquot for each cell line**

-Serum, **proteins**, **L- glutamine**, **ascorbic acid** , **NEAA** and **antibiotics** can be prepared and aliquot separately and add to the medium before use.

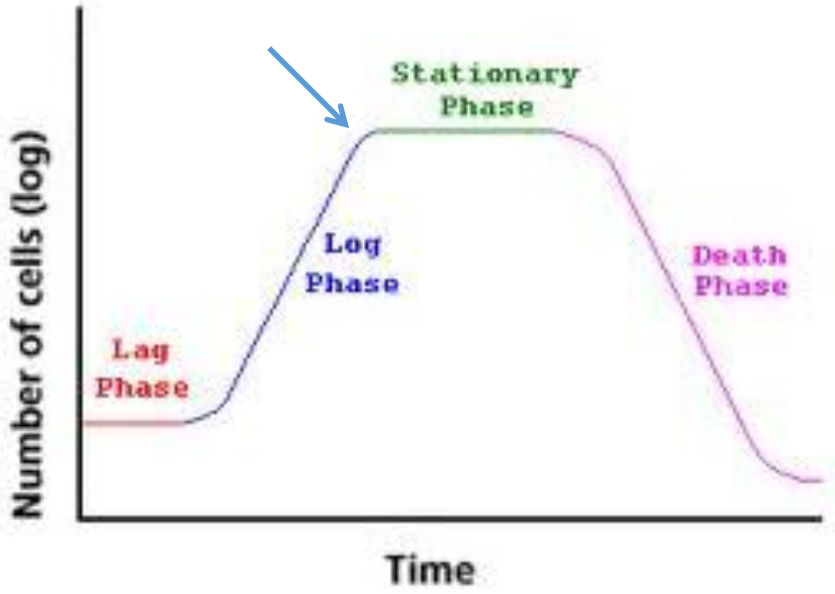
- **What is the meaning of passage number?**
- **The number of times the cells have been removed (or “split”) from the plate and re-plated**

1. **What is the meaning of subculture?**

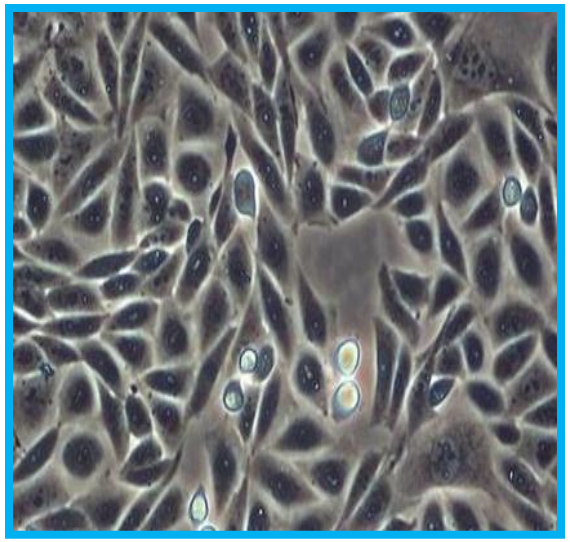
Diluting cell number in order to keep cells actively growing.

2. **What is the best time for sub culture?**

Near to enter the stationary phase.

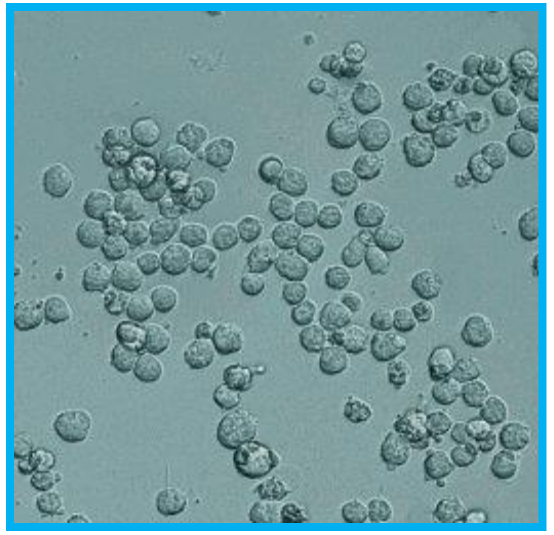


Different types of cells



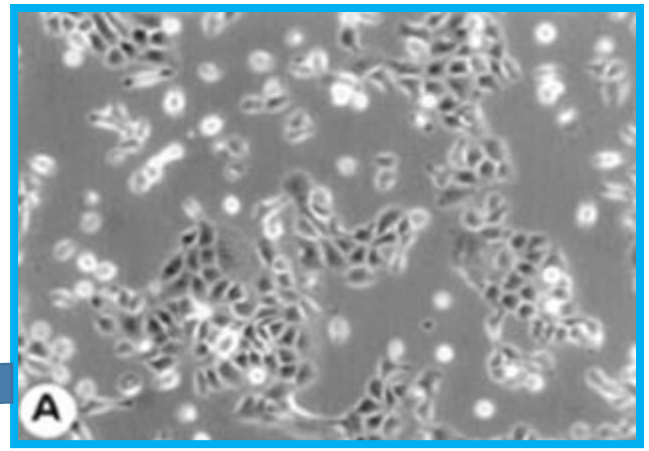
Adherent cells

Semi adherent cells

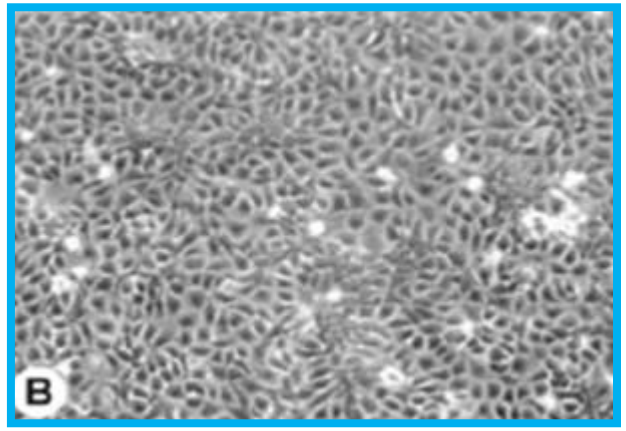


Suspension cells

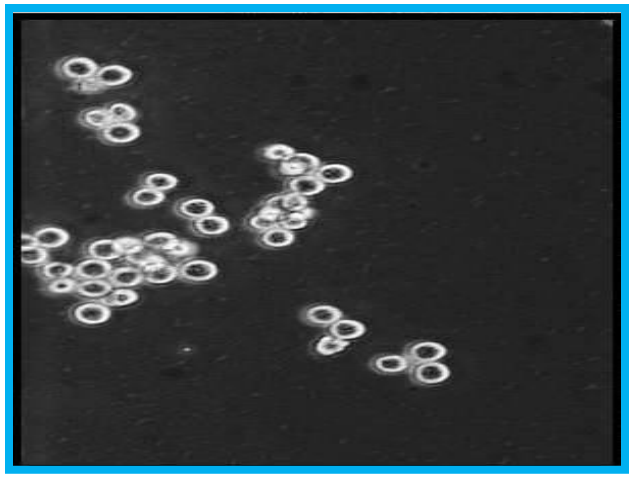
Confluency VS Concentration



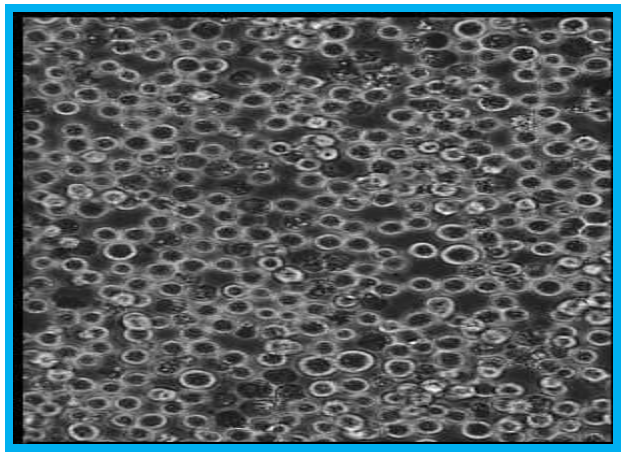
Semi confluent



confluent



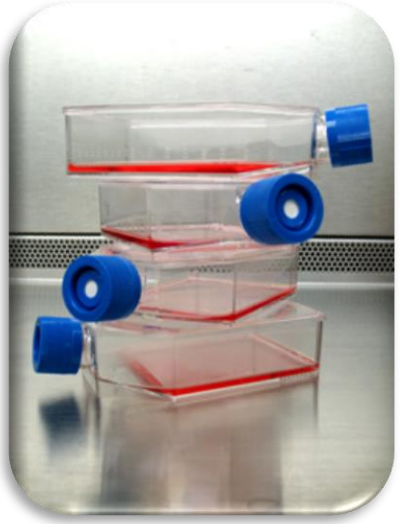
Low concentration

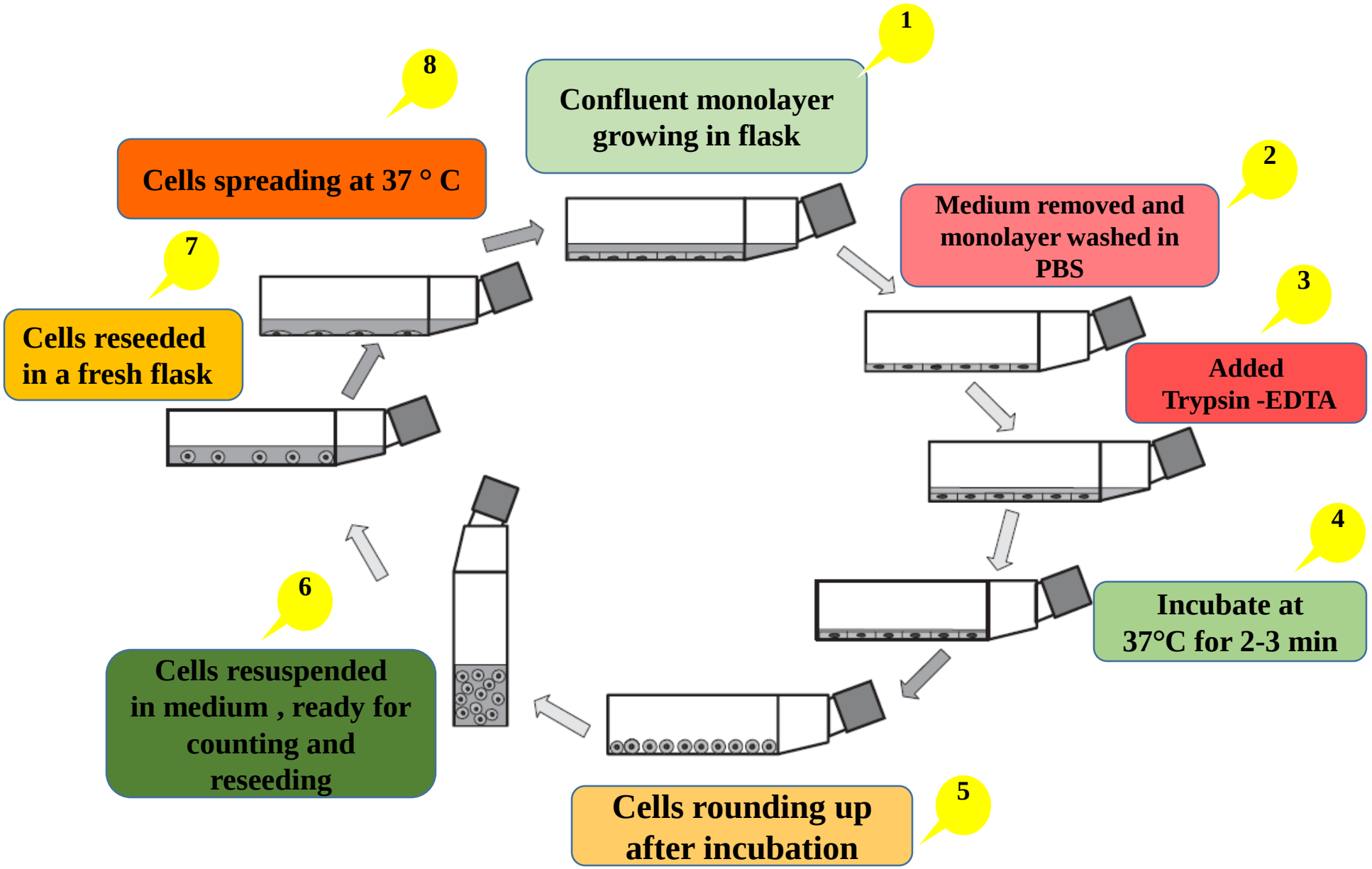


High concentration

Steps of subculture

- Examination
- Cell washing
- Cell harvesting
- Cell counting
- Plating
- Incubation




1
Confluent monolayer growing in flask
2
Medium removed and monolayer washed in PBS
3
Added Trypsin -EDTA
4
Incubate at 37°C for 2-3 min
5
Cells rounding up after incubation
6
Cells resuspended in medium , ready for counting and reseeding
7
Cells reseeded in a fresh flask
8
Cells spreading at 37 ° C

Freeze

Continues Cell Culture Problems:

- Contamination
- Age-related or environmentally- induced changes

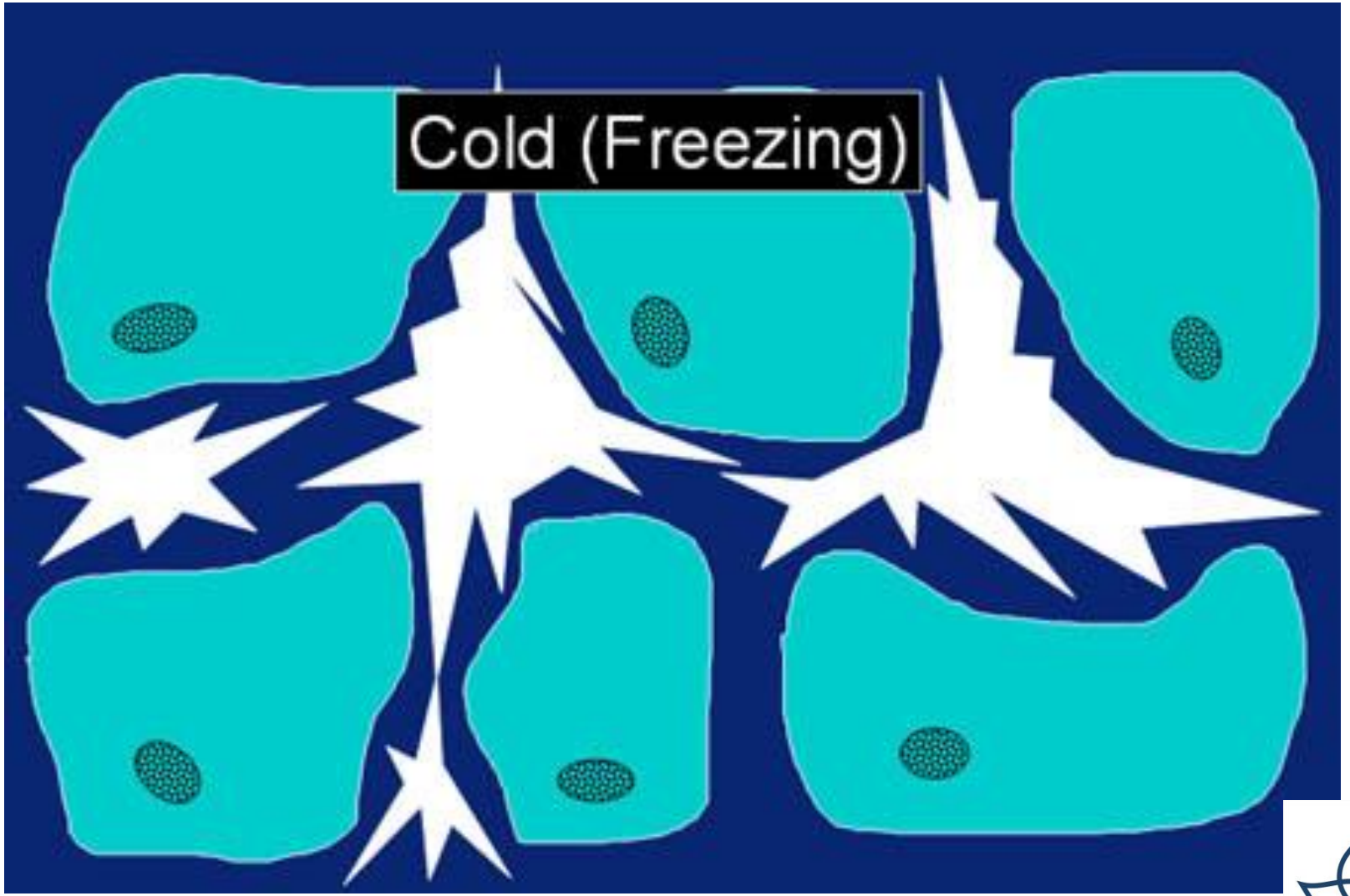
Solution:

Cryopreservation

Advantages of Freezing Cell Cultures

- ***less work - saves time -and money***
- serves as a ***backup-supply*** for emergencies
- provides a more homogeneous population by ***minimizing culture aging and evolution***

Growing ice causes cells to dehydrate and shrink



To combat freeze-induced cellular damage:

Addition of extracellular somatically active molecules (cryoprotectant)

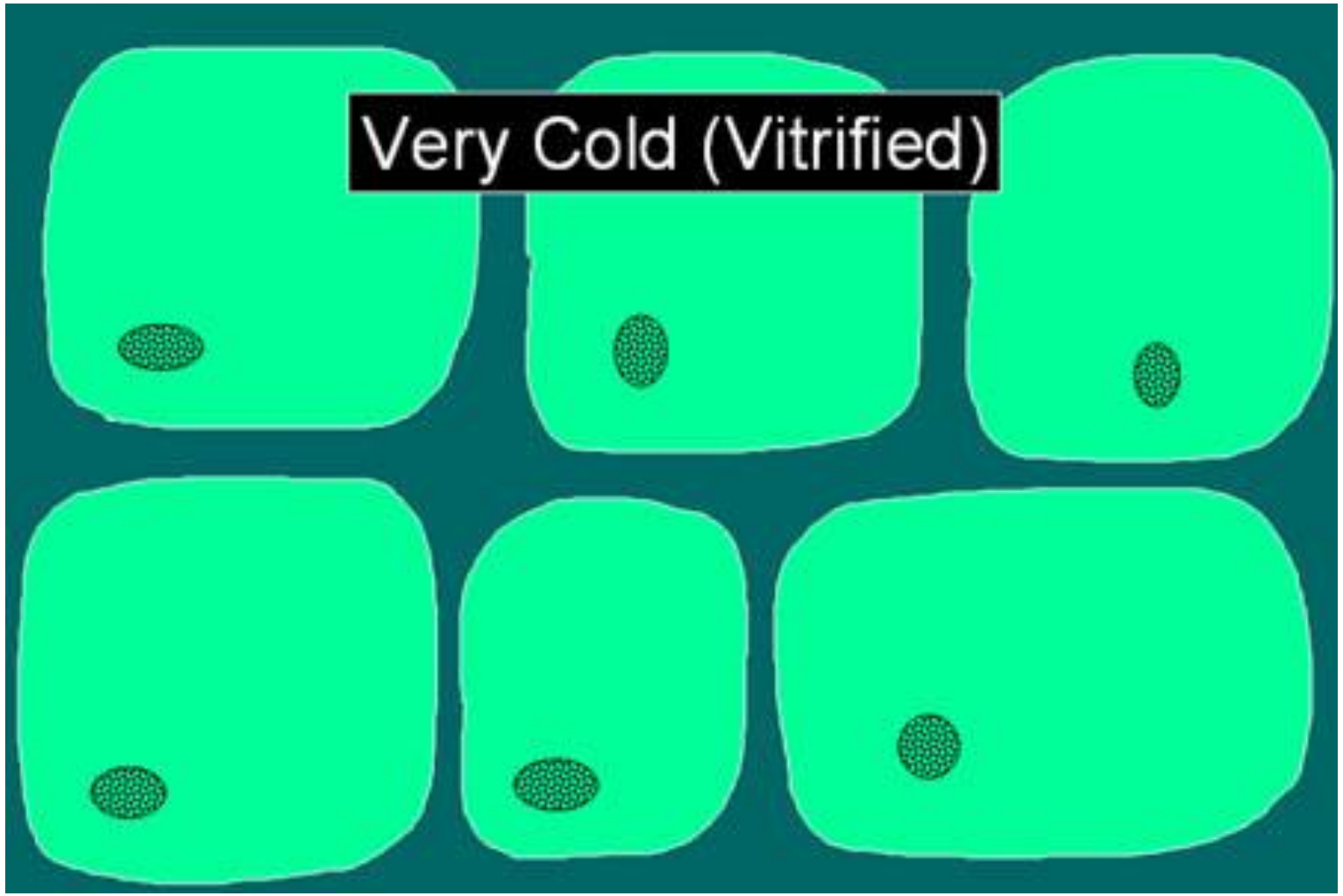
↓

Replace intracellular water

↓

Prevent intracellular ice formation and cellular shrinkage

Finally cells are vitrified and biological time is stopped



What are cryoprotectants?

- **Cryoprotectants use in cryopreservation include those materials which are compatible with biological systems.**

- **They are classified into two groups:**
  - **Intracellular cryoprotectants**
  - **Extracellular cryoprotectants**

Intracellular cryoprotectants

- **Their MW is low.**
- **They penetrate and permeate into the cell.**
- **DMSO, Ethylene glycol, Glycerol are some examples in this category.**
- **They are more effective in slowly freezing cells.**

Extracellular cryoprotectants

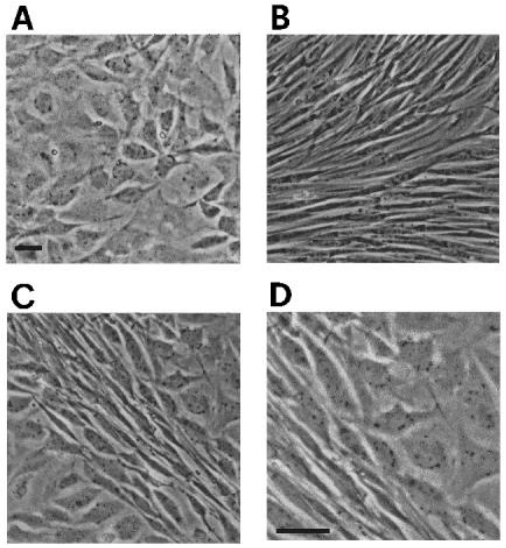
- **They are large macromolecules.**
- **They don’t penetrate and permeate into the cell.**
- **Hydroxy-ethyl starch (HES), polyvinylpyrolidone (PVP), polyethylene oxide (PEO), sucrose, trehalose and dextran are some examples in this category.**
- **They are more effective in rapid cooling conditions for cells.**

Freezing Medium

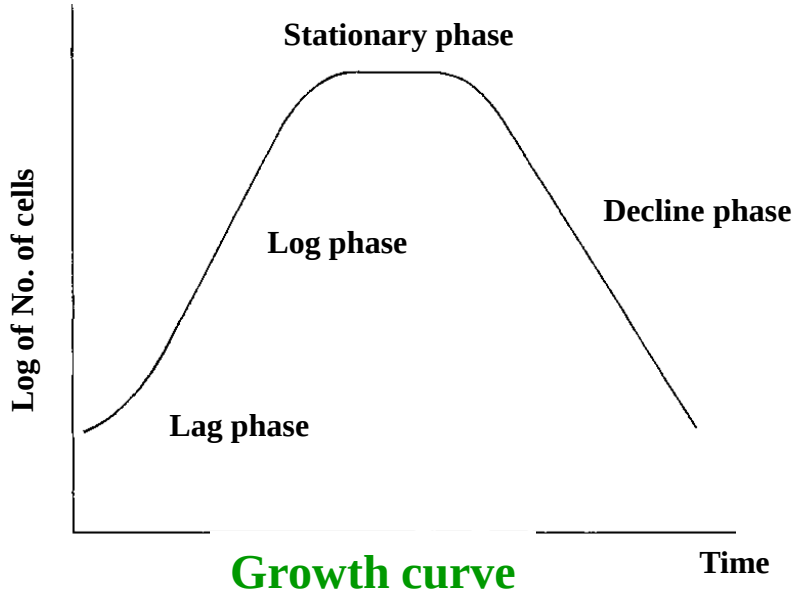
- Serum concentration in freezing medium is 40%, 50%, or even 100%.

- Usually 90% serum with 10% DMSO

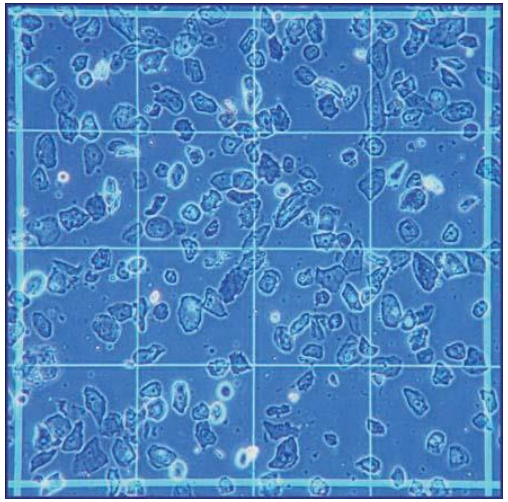
Primarily Study of Cells



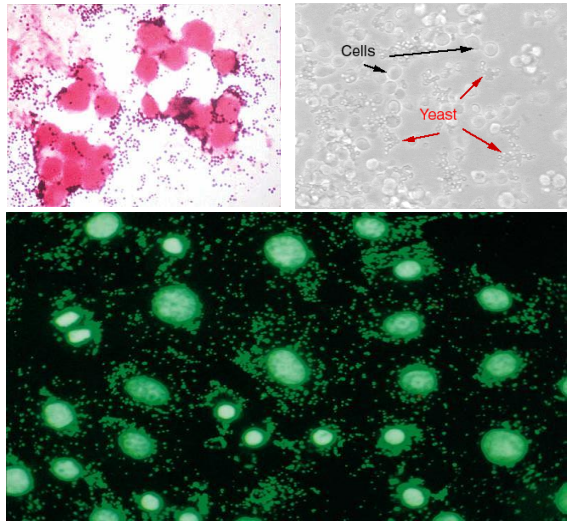
Morphology



Growth curve



Viability

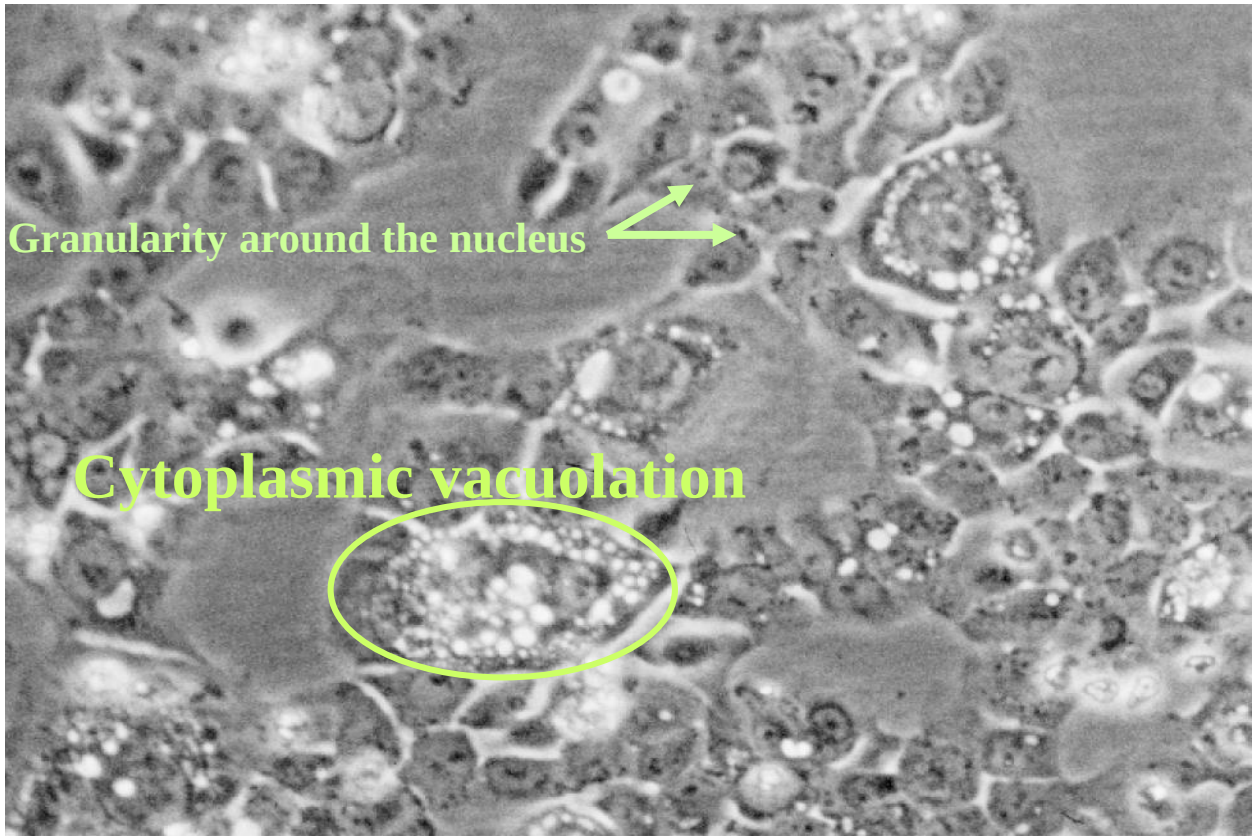


Microbial contamination

Significance of Cell Morphology

Signs of Deterioration of Cells

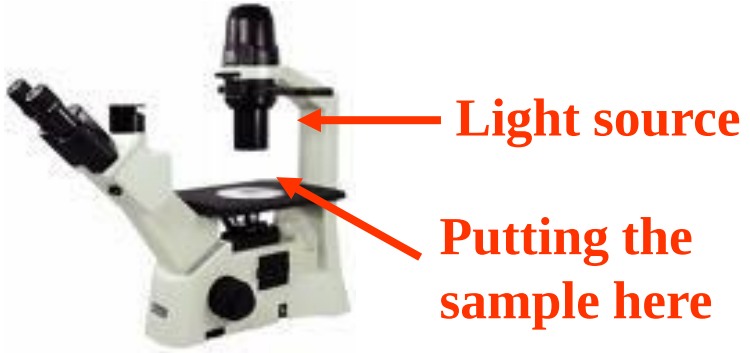
1. Granularity around the nucleus
2. Cytoplasmic vacuolation
3. Rounding up of the cells with detachment from the substrate



Methods of Study the Morphology of Cells

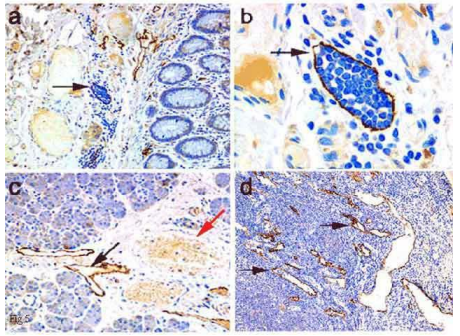
Microscopy

Inverted microscope is one of the most important tools in tissue culture laboratory.



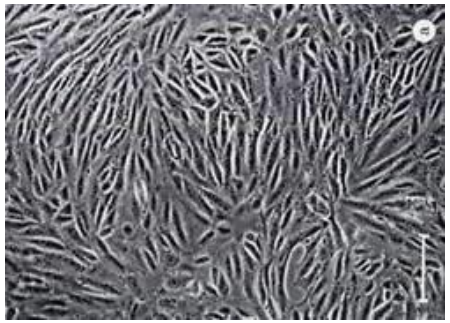
I. Staining

Giemsa stain provides a convenient method of preparing a stained culture.



II. Culture vessels for cytology:

Monolayer cultures



Significance of cell viability

Cell viability is a determination of living or dead cells ,based on a total cell sample.

Application of cell viability:

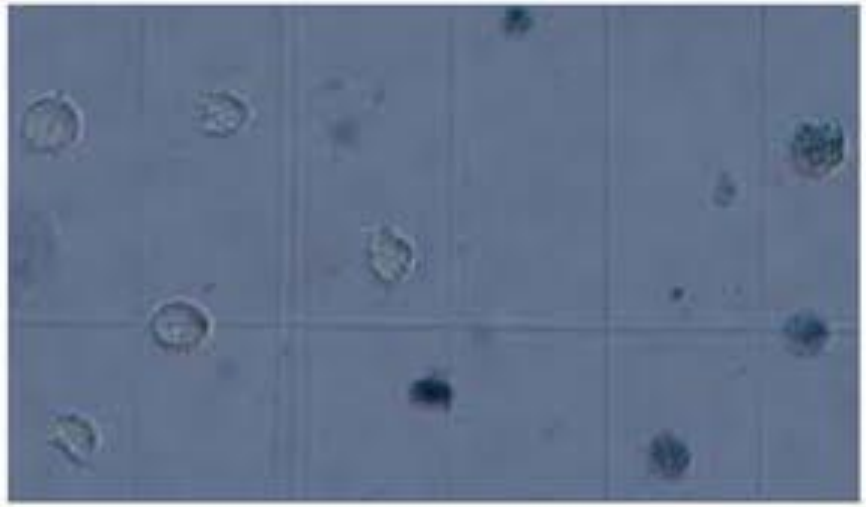
1. ***To evaluate the death or life of cancerous cells*.**

2. To evaluate the rejection of implanted organs

4. To evaluate environmental damage due to toxins

To bank any kind of cells, the number of viable cells are necessary to make a plan for next step of a research or a treatment.

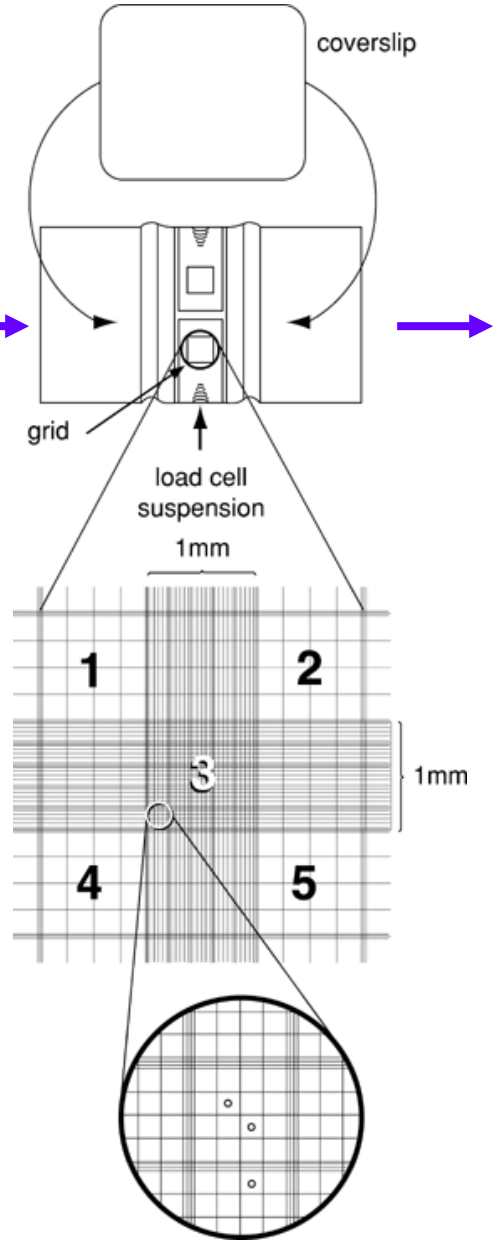
Using trypan blue staining to assess cell viability by microscope.

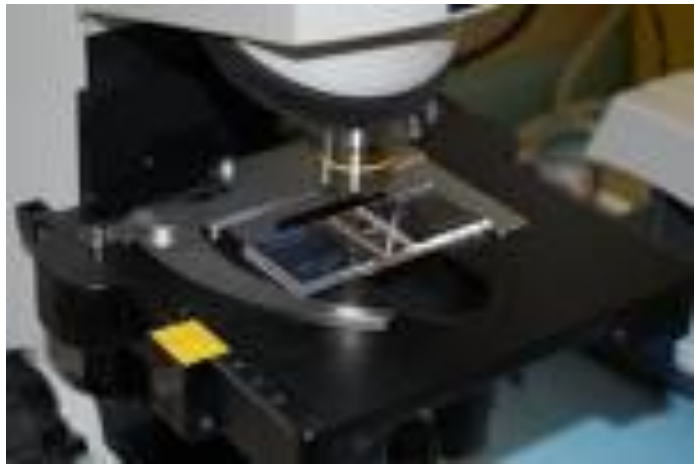


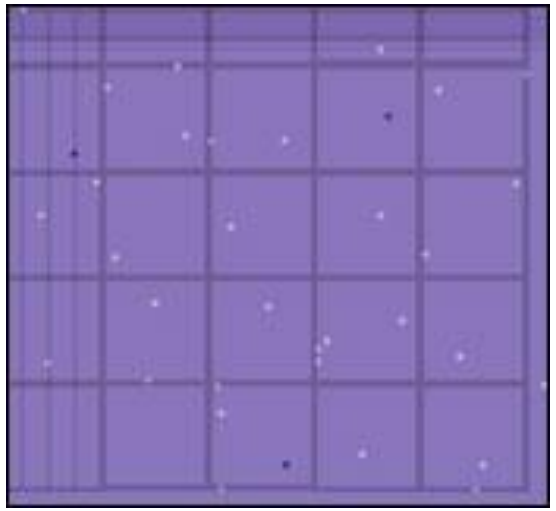
Viable cells have intact membranes and exclude the dye

Nonviable cells are labeled with dye

Cell Viability Assessment by Using Neubauer Slide







Calculate the cell viability

Calculate the cell viability using the equation:

$$\% \text{ Cell Viability} = \frac{\text{number of unstained (living) cells} \times 100\%}{\text{Total cells counted (stained + unstained)}}$$

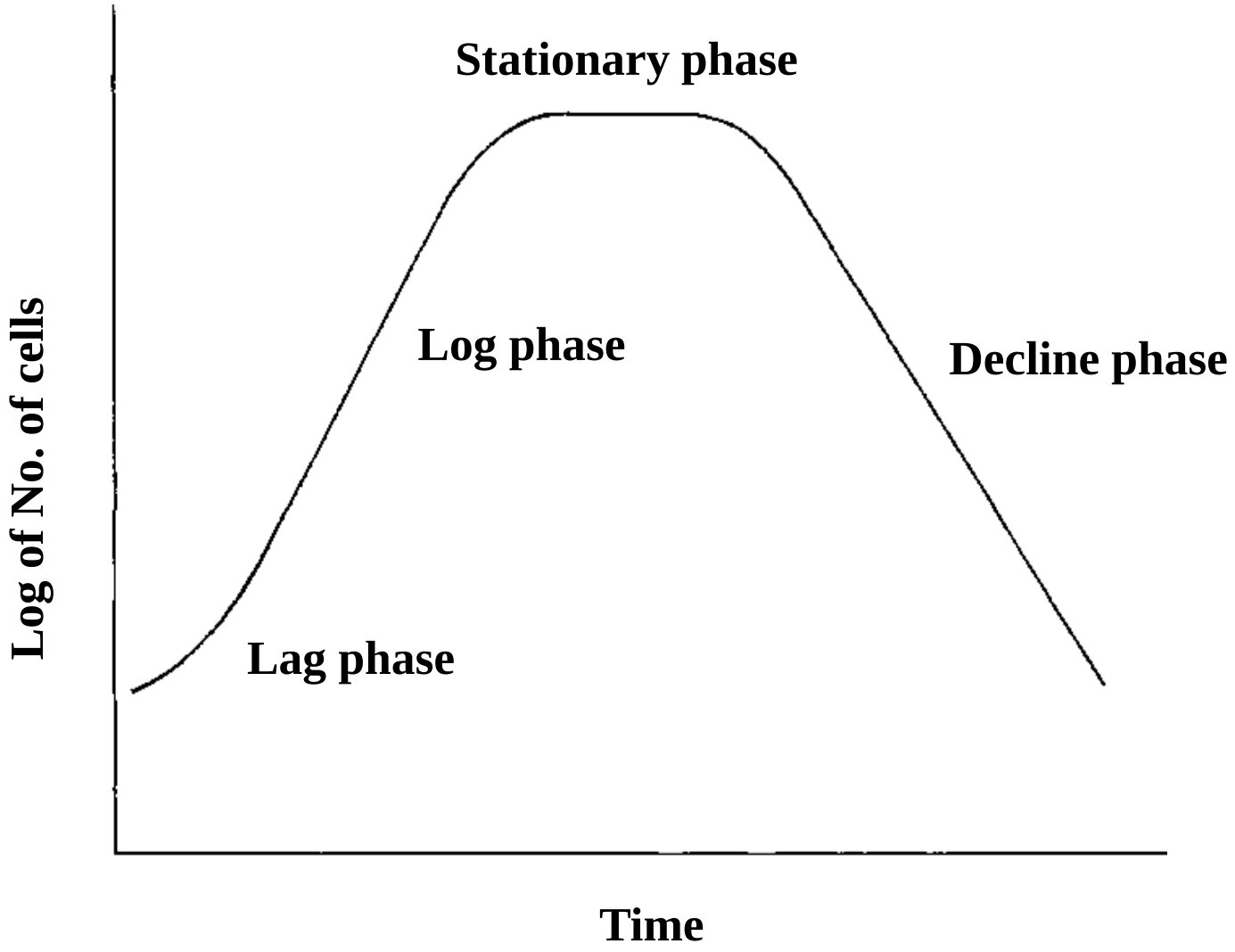
Example: If a total of 300 cells (stained + unstained) are counted and 200 are identified as living cells (unstained), then the viability is calculated as:

$$\% \text{ Cell viability} = \frac{200 \text{ live cells}}{300 \text{ total cells}} \times 100\% = 67\%$$

Cell Viability Assessment by Cell counter

Growth Curve

Growth curve



Significance of Growth curve

A reduced cell count after 5 days could be caused by a reduced growth rate of some or all of the cells.

Growth curve is useful for:

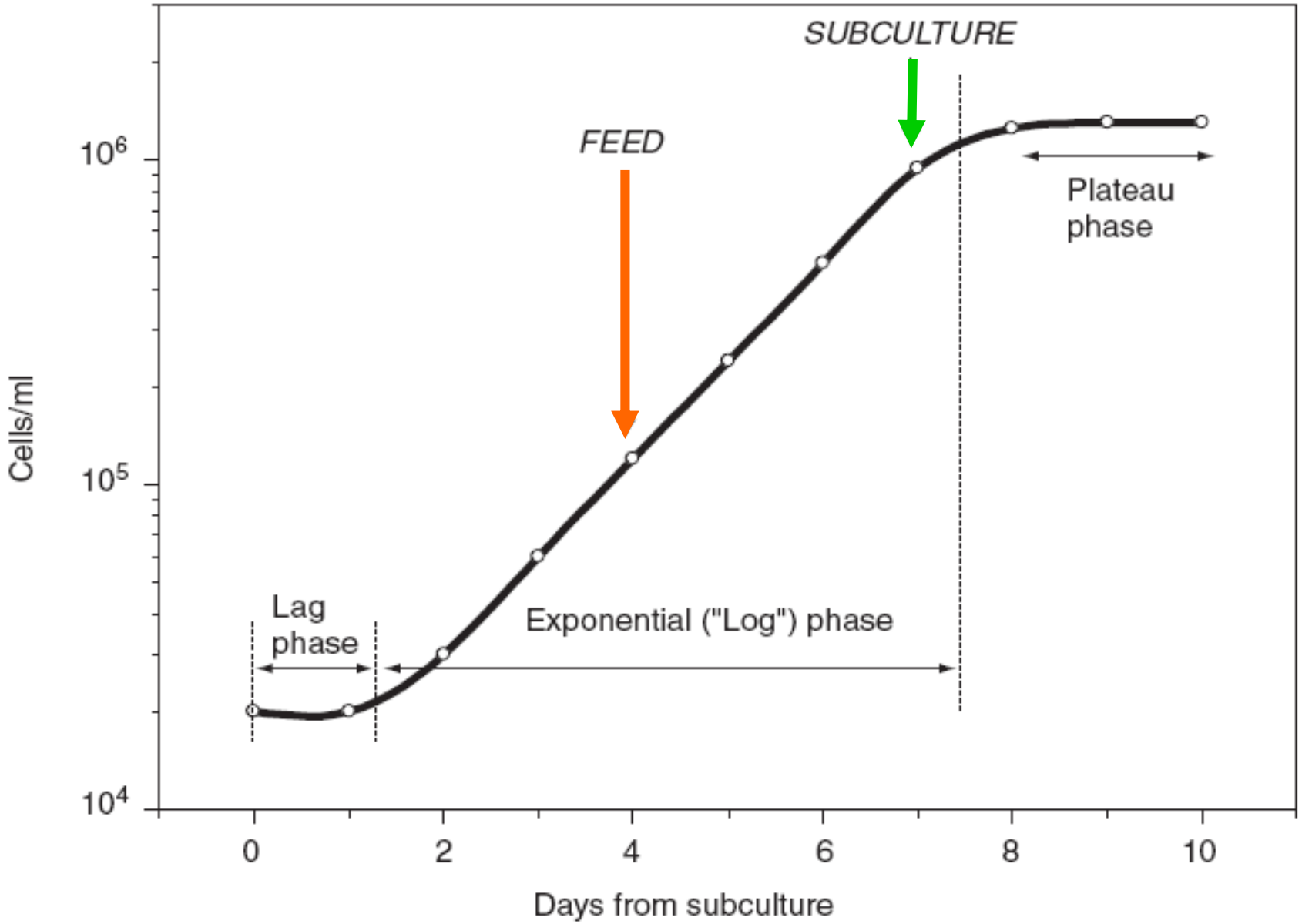
1. **To determine feeding and subculture time.**

2. **To test media, sera, growth factors and some drugs.**

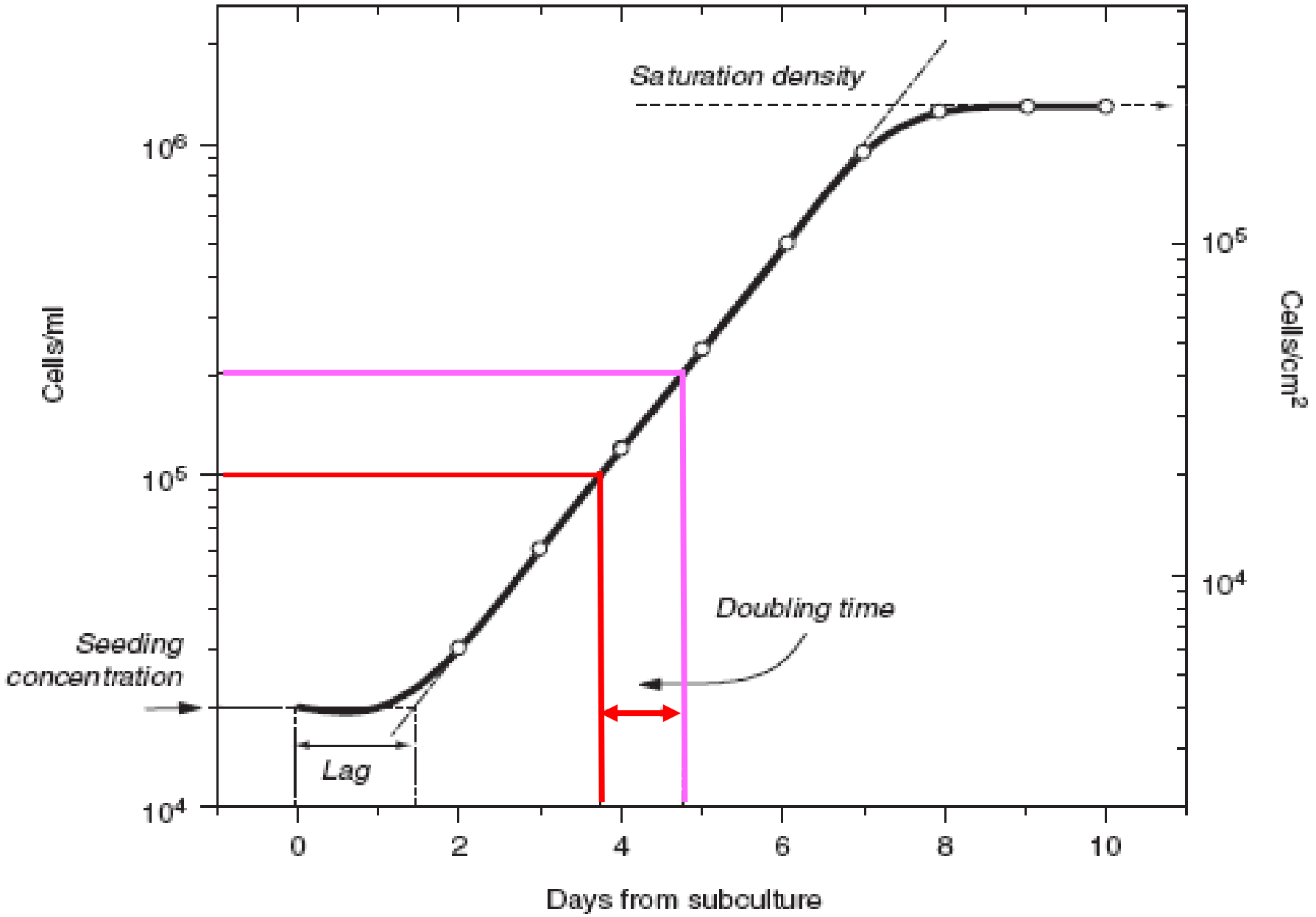
3. **To find out proliferation doubling time (PDT) which is known to be in mid-log phase.**

4. **To determine the saturation density.**

Growth Curve and Maintenance



Population Doubling Time



Interpretation of Growth Curve

*The long lag implies that the culture has to adapt to the serum.

** Short doubling times are preferable if you want a lot of cells quickly.

*** A high saturation density will provide more cells for a given amount of serum and will be more economical.


Thanks For your Attention