Cell culture

Historical events in the development of cell culture

- 1878: Claude Bernard proposed that physiological systems of an organism can be maintained in a living system after the death of an organism.
- 1885: Roux maintained embryonic chick cells in a saline culture.
- 1897: Loeb demonstrated the survival of cells isolated from blood and connective tissue in serum and plasma.

1903: Jolly observed cell division of salamander leucocytes *in vitro*.

1907: Harrison cultivated frog nerve cells in a lymph clot held by the 'hanging drop' method and observed the growth of nerve fibers *in vitro* for several weeks. He was considered by some as the father of cell culture.

1910: Burrows succeeded in long term cultivation of chicken embryo cell in plasma clots. He made detailed observation of mitosis.

1911: Lewis and Lewis made the first liquid media; consisted of sea water, serum, embryo extract, salts and peptones. They observed limited monolayer growth.

1913: Carrel introduced strict aseptic techniques so that cells could be cultured for long periods.

1916: Rous and Jones introduced proteolytic enzyme trypsin for the subculture of adherent cells.

1923: Carrel and Baker developed 'Carrel' or T-flask as the first specifically designed cell culture vessel. They employed microscopic evaluation of cells in culture.

1927: Carrel and Rivera produced the first viral vaccine - Vaccinia.

1933: Gey developed the roller tube technique

1940s: The use of the antibiotics penicillin and streptomycin in culture medium decreased the problem of contamination in cell culture.

1948: Earle isolated mouse L fibroblasts which formed clones from single cells. Fischer developed a chemically defined medium, CMRL 1066.

1952: Gey established a continuous cell line from a human cervical carcinoma known as HeLa (Helen Lane) cells. Dulbecco developed plaque assay for animal viruses using confluent monolayers of cultured cells.

1954: Abercrombie observed contact inhibition: motility of diploid cells in monolayer culture ceases when contact is made with adjacent cells.

1955: Eagle studied the nutrient requirements of selected cells in culture and established the first widely used chemically defined medium.

1961: Hayflick and Moorhead isolated human fibroblasts (WI-38) and showed that they have a finite lifespan in culture.

1964: Littlefield introduced the HAT medium for cell selection.

1965: Ham introduced the first serum-free medium which was able to support the growth of some cells.

1965: Harris and Watkins were able to fuse human and mouse cells by the use of a virus.

1975: Kohler and Milstein produced the first hybridoma capable of secreting a monoclonal antibody.

1978: Sato established the basis for the development of serum-free media from cocktails of hormones and growth factors.

1982: Human insulin became the first recombinant protein to be licensed as a therapeutic agent.

1985: Human growth hormone produced from recombinant bacteria was accepted for therapeutic use.

1986: Lymphoblastoid γIFN licensed.

1987: Tissue-type plasminogen activator (tPA) from recombinant animal cells became commercially available.

1989: Recombinant erythropoietin in trial.

1990: Recombinant products in clinical trial (HBsAg, factor VIII, HIVgp120, CD4, GM-CSF, EGF, mAbs, IL-2).

Areas where cell culture technology is currently playing a major role.

- Model systems for

  Studying basic cell biology, interactions between disease causing agents and cells, effects of drugs on cells, process and triggering of aging & nutritional studies

- Toxicity testing

  Study the effects of new drugs

- Cancer research

  Study the function of various chemicals, virus & radiation to convert normal cultured cells to cancerous cells

Contd….

- Virology

  Cultivation of virus for vaccine production, studying their infectious cycle.

- Genetic Engineering

  Production of commercial proteins, large scale production of viruses for use in vaccine production e.g. polio, rabies, chicken pox, hepatitis B & measles

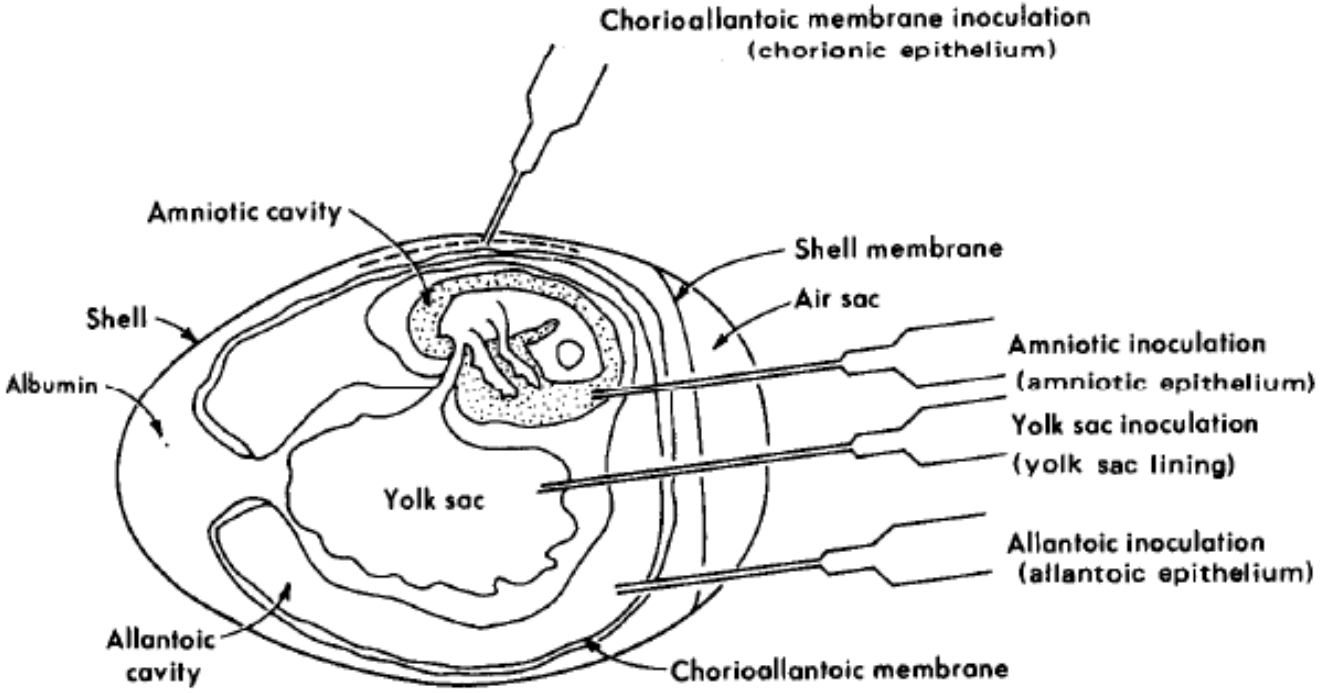
- Gene therapy

  Cells having a functional gene can be used to replace non-functional gene

Cultivation of viruses

- Animal inoculation
- Embryonated eggs
- Tissue culture

Growth of virus on embryonated eggs



Davis, Duylbecco, Eisen, Ginsberg "Microbiology" 4th ed, J.B. Lippincott 1990, Fig. 48-1

Tissue culture

1. Explant culture:

- Small bits of tissue attached to surface by plasma clot or warming
- Incubate with growth medium
- Cells migrate, multiply and form primary monolayer

- Eg. Adenoid tissue explant culture for adenovirus

- Advantage- for isolation of virus in the ‘latent’ state

Tissue culture

2. Organ culture:

- Small bits of organs maintained in vitro, preserving their original architecture & function

- Useful for isolation of some fastidious viruses

- Eg. Tracheal ring organ culture for isolation of new Rhinoviruses and Coronaviruses

Tissue culture

3. Cell culture:

- Cells dissociated from the tissue by proteolytic enzymes and mechanical shaking
- Washed, counted and suspended in growth medium in glass / polystyrene vessels
- Cells adhere to the surface
- On incubation, divide to form a monolayer

Essential equipment

- Biological safety cabinet
- CO_2 incubator
- Inverted microscope
- Pipette aid
- Aspiration pump
- Centrifuge, waterbath
- Refrigerator
- Cryopreservation equipment

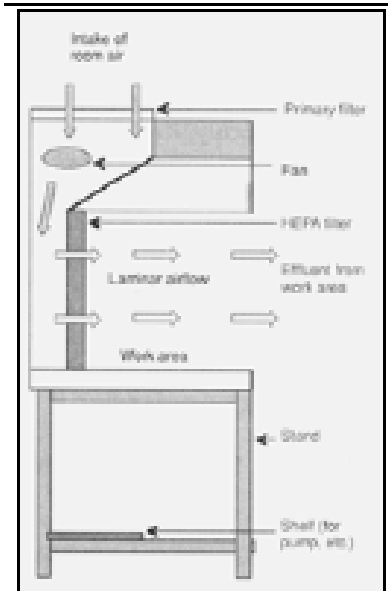
Additional equipment

- Sterilization equipment
- Balances, Vortex
- Water purification – distiller, deioniser
- Magnetic stirrer, pH meter
- Micropipettes
- Cell counter
- Video camera and monitor

Horizontal Laminar flow

Provides clean area, but air is directed towards the worker

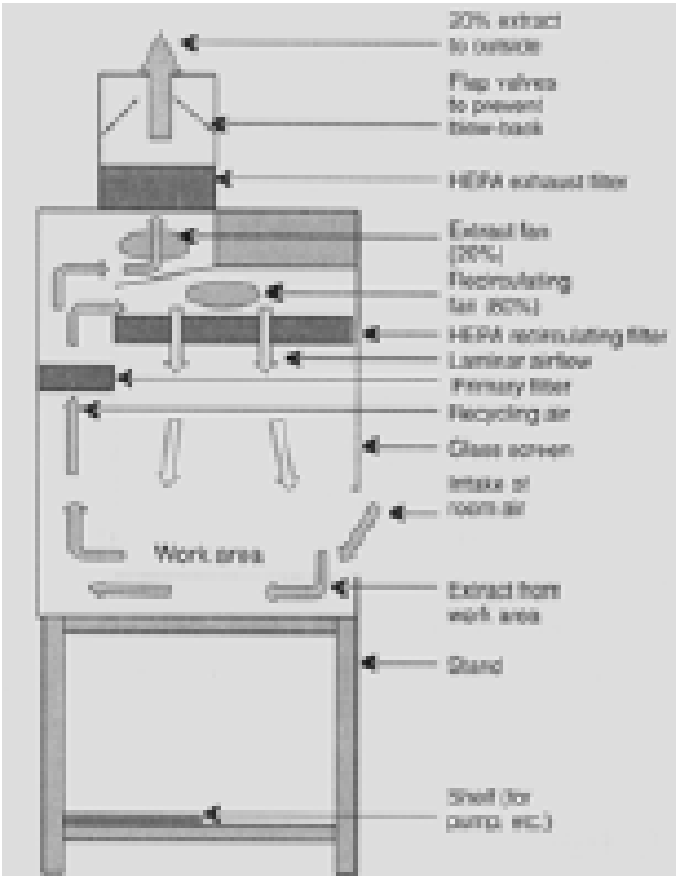
Work area is protected but not the worker



Vertical Laminar flow

Protects the work area and

the worker



Class I Microbiological Safety Cabinet

Class II Microbiological Safety Cabinet

Class III Microbiological Safety Cabinet

Incubator

Temp: 28 – 36^{0}C

Humidity: 95% - 100%

CO_2 level: 5 – 10%

Water jacket incubator or keep an open dish with distilled water on the bottom shelf

Incubator

Inverted microscope

- It is vital to look at the culture regularly
- A morphological change is often the first sign of deterioration in the culture
- Inverted because it is not good to open tissue culture dishes
- It should have phase contrast

  - Most cells are not dense enough to be visible in regular light without staining

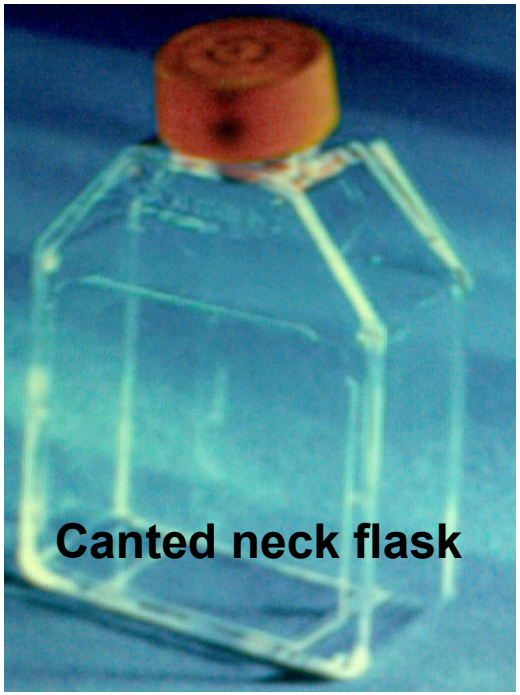
Fluorescence microscope

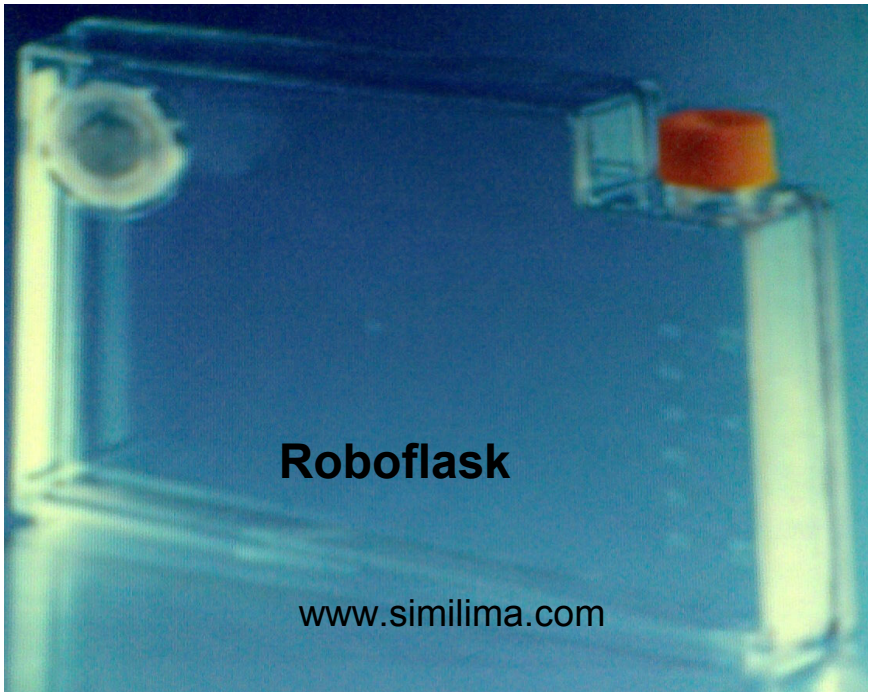
Equipment for cryopreservation

- Liquid nitrogen
  - Liquid phase (-196°C) or vapor phase (-156°C)
- Fancy freezing chambers to regulate the freezing rate
  - Styrofoam containers work too

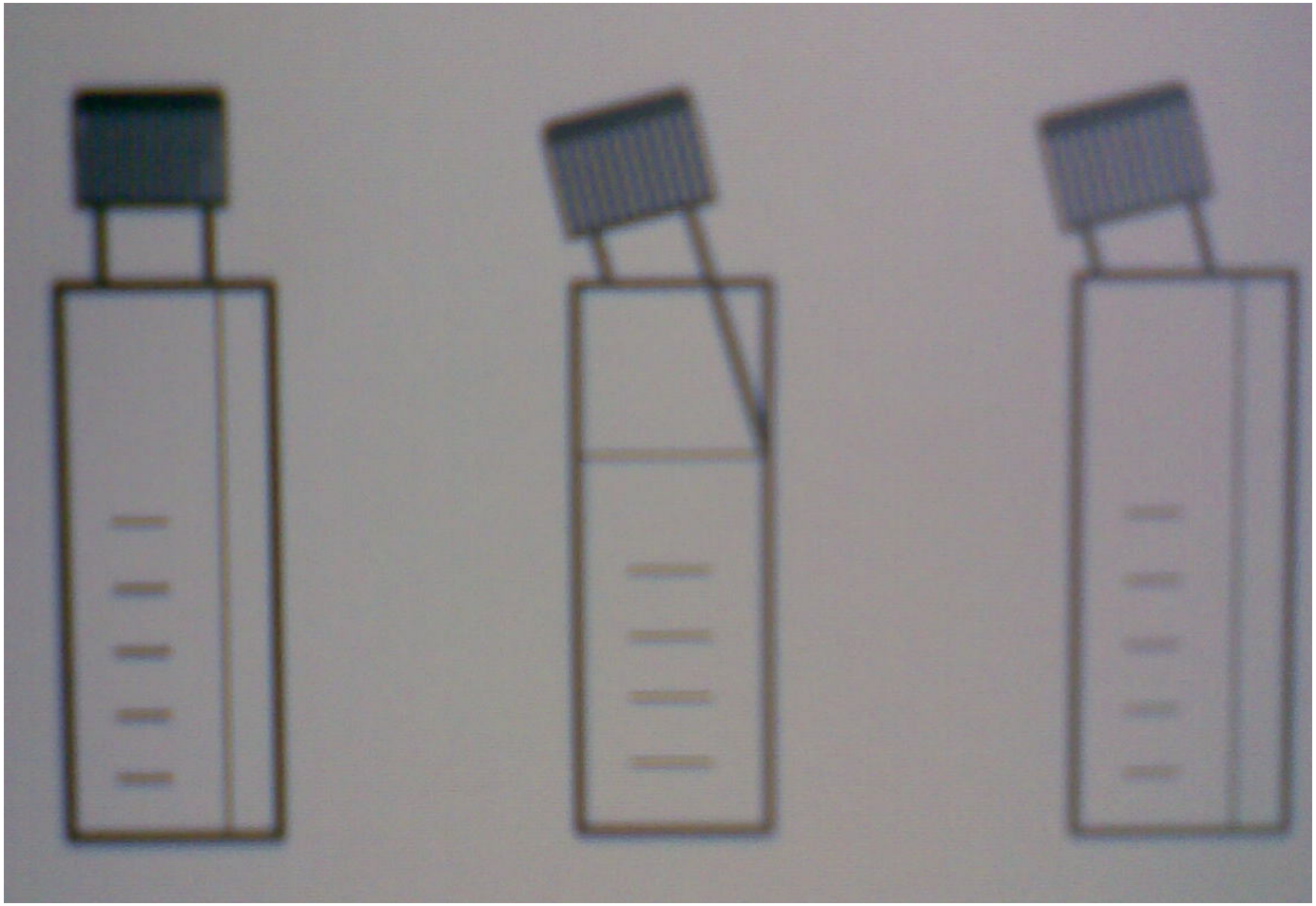
Choice of culture container

- Majority of vertebrate cells in vitro grow as monolayers on an artificial substrate
- Most cells need to spread out on a substrate in order to proliferate
- Overcrowding will inhibit proliferation
- But cells don't like to be lonely
- Cell yield is proportional to the available surface



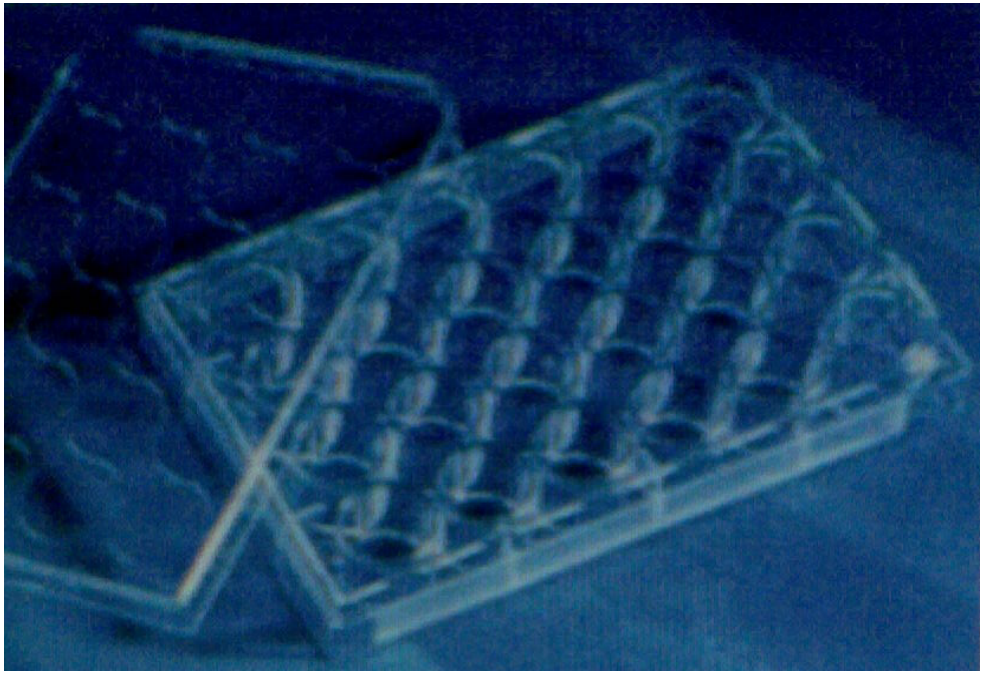


Flask neck styles

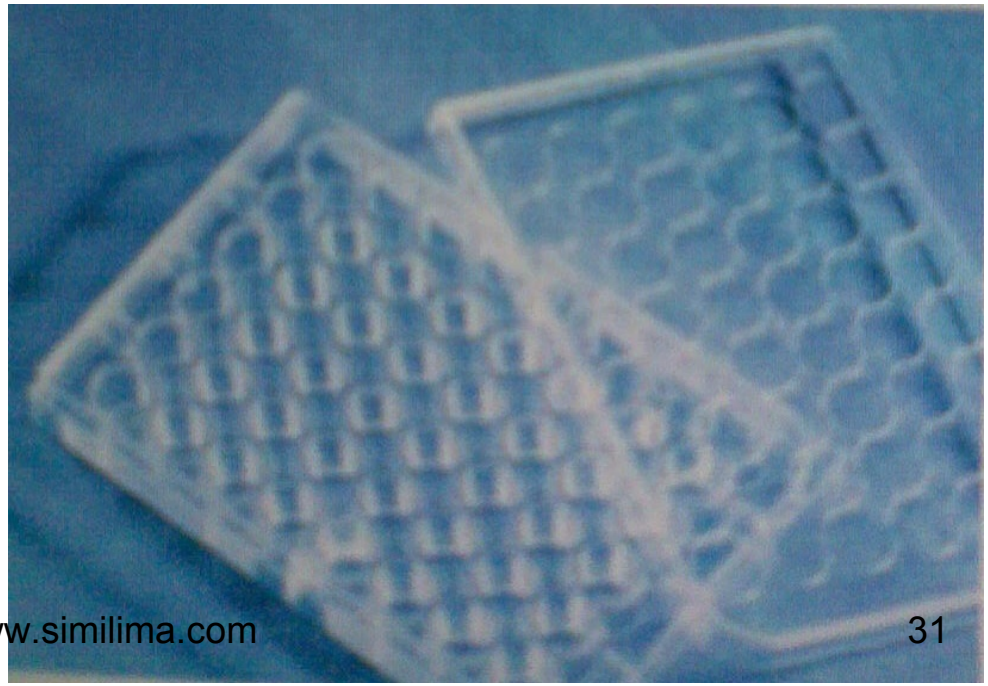


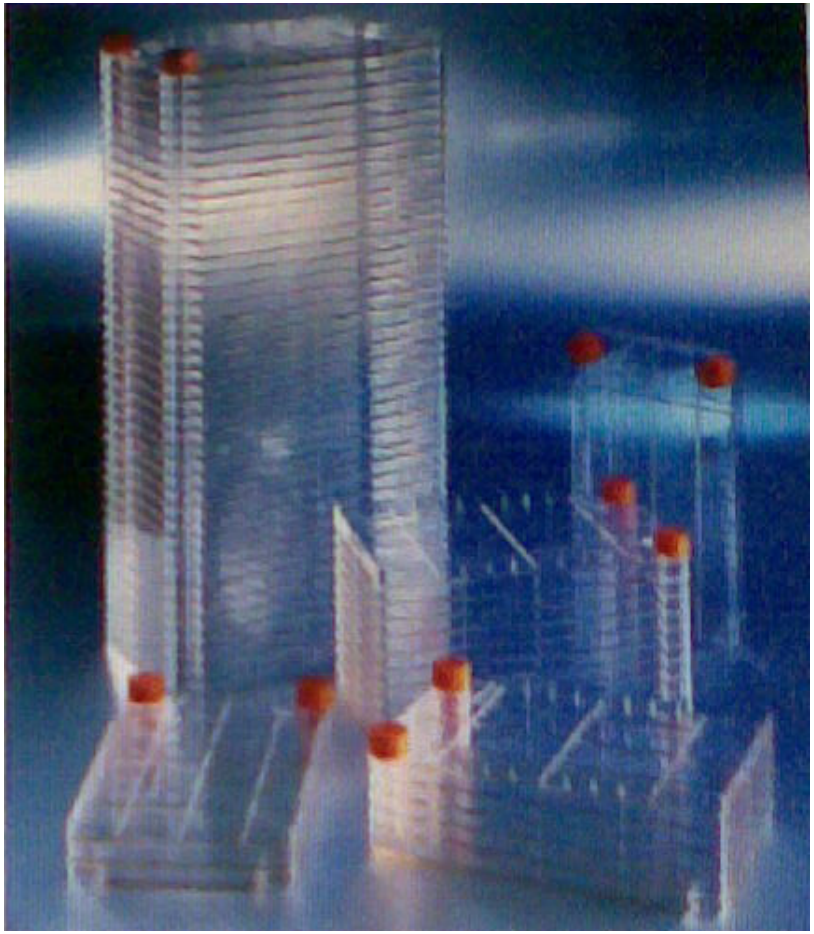
Straight neck **Canted neck** **Angled neck**

24-well plate



48-well plate

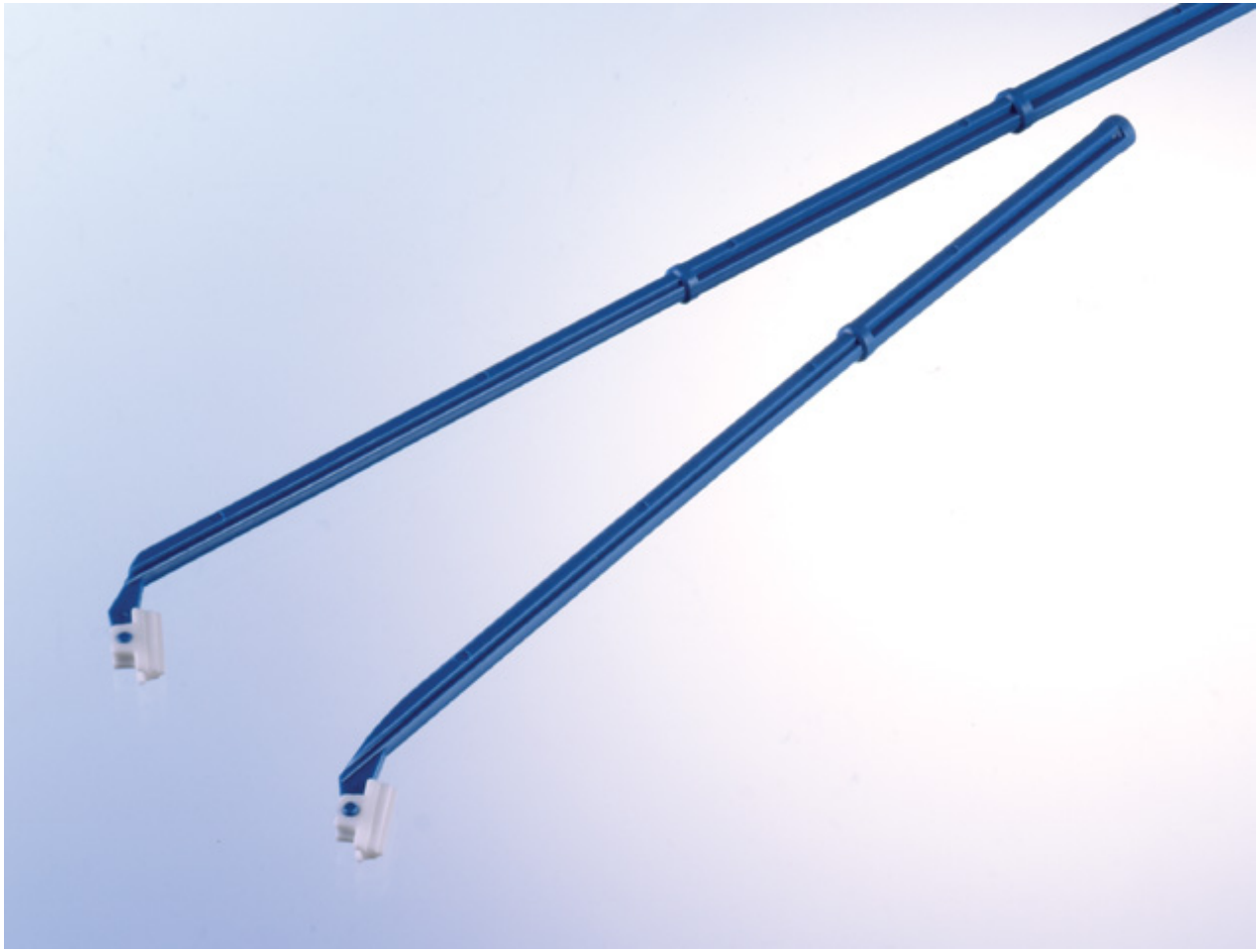




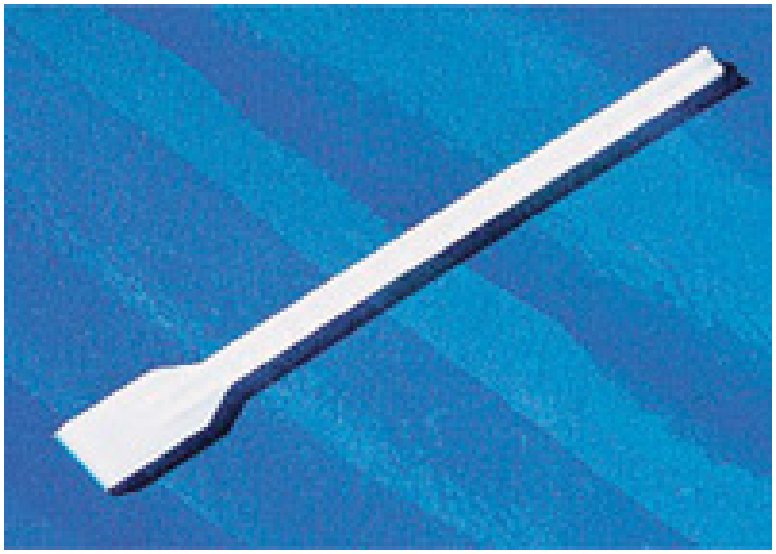
CellSTACK with 1 to 40 culture chambers

FIGURE 54-6 Inverted microscope used to examine cell monolayers growing attached to the inside surface beneath the liquid medium.

Greiner® cell scrapers
handle L 28 cm, blade W 1.8 cm



Corning® cell lifter designed for use with dishes and cell culture clusters.



blade L 1.9 cm, handle L 18 cm; sterility: sterile; γ -irradiated

Cell culture - types

- Primary cell cultures
- Diploid (Euploid) cell cultures (cell strains)

  (Semi-continuous cell cultures)
- Continuous cell cultures (cell lines)

  (Aneuploid / heteroploid cell lines)

- Primary
  - Heterogeneous – many cell types
  - Closest to animal
  - Technical hassle
- Diploid cell strain
  - Relatively homogeneous – fewer cell types
  - Further from animal
  - Technically less hassle
- Continuous cell line
  - Immortal
  - Most homogeneous
  - Genetically weird – furthest from animal
  - Hassle free
  - Suspension or monolayer

Primary culture

- Cells when surgically or enzymatically removed from an organism and placed in suitable culture environment will attach and grow
- Primary cells have a finite life span
- Primary culture contains a very heterogeneous population of cells
- Sub culturing of primary cells leads to the generation of cell lines
- Cell lines have limited life span, they passage several times before they become senescent
- Cells such as macrophages and neurons do not divide in vitro so can be used as primary cultures
- Lineage of cells originating from the primary culture is called a cell strain

Primary cell culture

- Cells dissociated from fresh human or animal tissue (human amnion, monkey kidney, rabbit kidney) by trypsin (0.22%), versene (0.02%) & shaking

- Washed & suspended in desired conc. in suitable vessels ($3x10^5$ viable cells/ml)

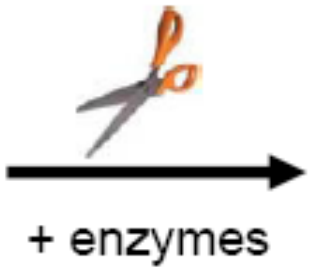
- Conc. of viable cells by counting a dilution in
  Trypan blue (Neubauer haemocytometer)
  (Vital stains – stain only dead cells)

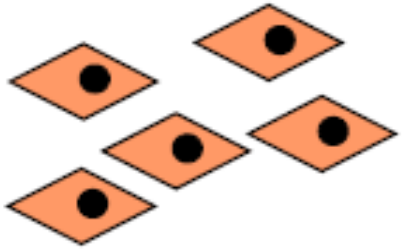
Erythrosin B, methylene blue, acridine orange, eosin, nigrosin, saffranin

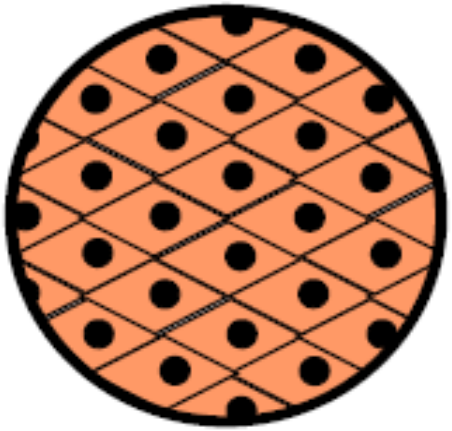
Primary cell culture …..

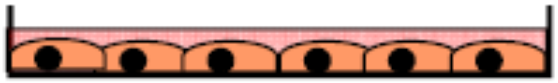
- Useful for isolation of ECHO & Orthomyxo viruses
- Slow metabolism, slow change in pH
- Have to be prepared from fresh tissue
- Maintained in vitro only for a short period
- Cell culture from different individuals of the same species may vary in their susceptibility to viral infection

Primary cell culture









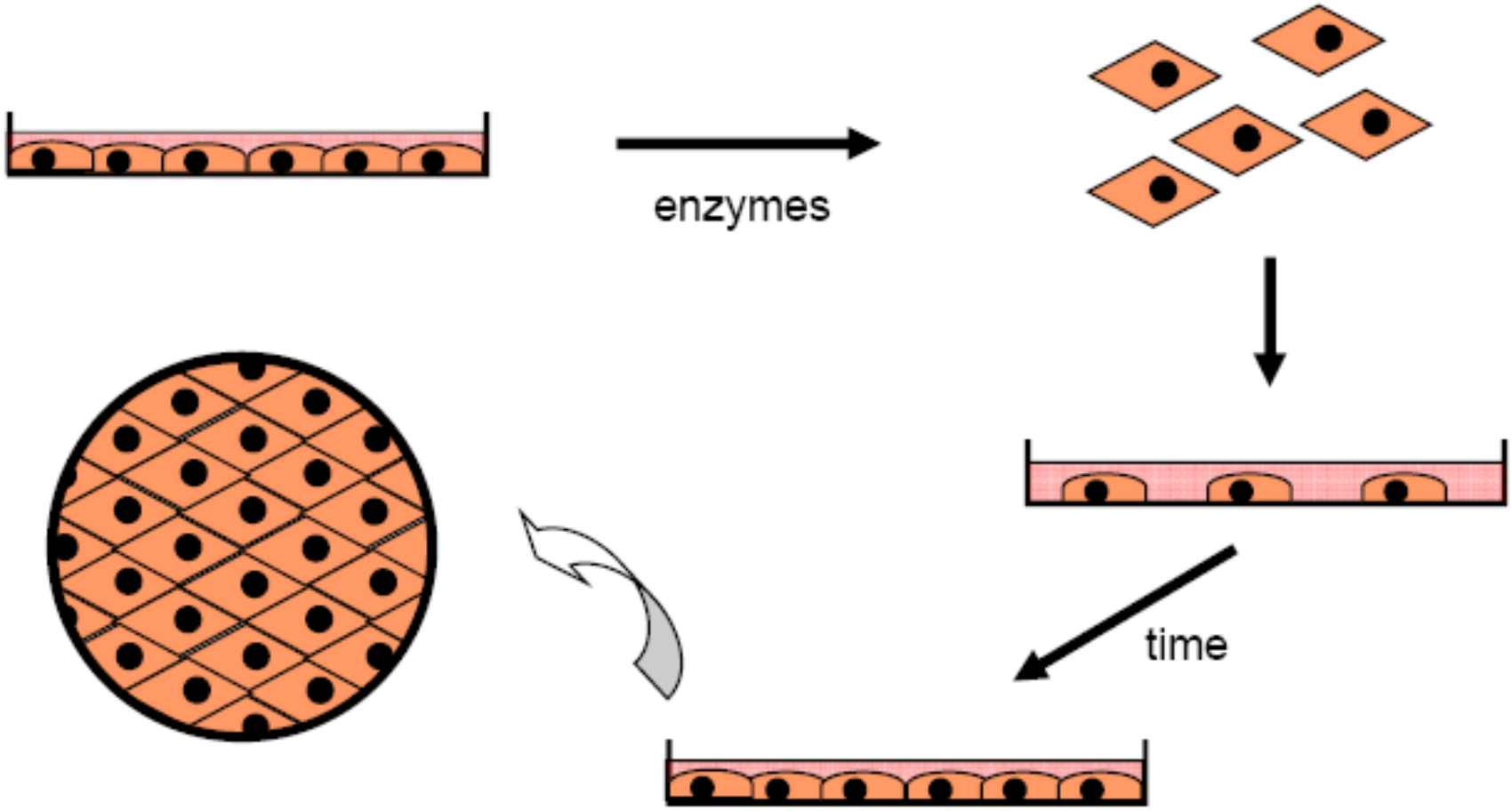
Passage (subculture)

- Remove medium, wash twice with PBS
- Add Trypsin-EDTA solution to cover cells
- Incubate at room temp for 1-3 mt.
- Cell sheet will appear opaque
- Remove the solution, give gentle shaking
- Add chilled GM, aspirate several times to suspend and separate cells
- Dilute a small sample of cell suspension, count
- Add to new tubes ($1x10^5$ cells/tube) with GM
- Replace GM with MM when monolayer is confluent

Subculture …

- Trypsin may be toxic to certain cells or may damage cell surface components
- In such situations, cells may be detached mechanically also, using a ‘rubber policeman’

Subculture



Diploid cell strain

- Established from human diploid fibroblasts (embryonic lung / neonatal foreskin)
- Remain virus sensitive for 20-50 passages
- May undergo 50 serial doublings before senescence
- MRC-5, WI-38, HEL, FS-9
- Subculture & feeding once a week.
- Maintained by freezing early passage cells in liquid nitrogen

Continuous cell lines

- By transformation (spontaneous or engineered) of cell strains or from tumours
- Loss of contact inhibition
- Indefinite subculture
- Chromosome No. not exact multiple of haploid No.
- High plating efficiency
- Faster growth rate; subculture twice weekly
- Frequent pH adjustment necessary

Continous cell lines

- Characteristics of continous cell lines

  -smaller, more rounded, less adherent with a higher nucleus /cytoplasm ratio

  -Fast growth and have aneuploid chromosome number

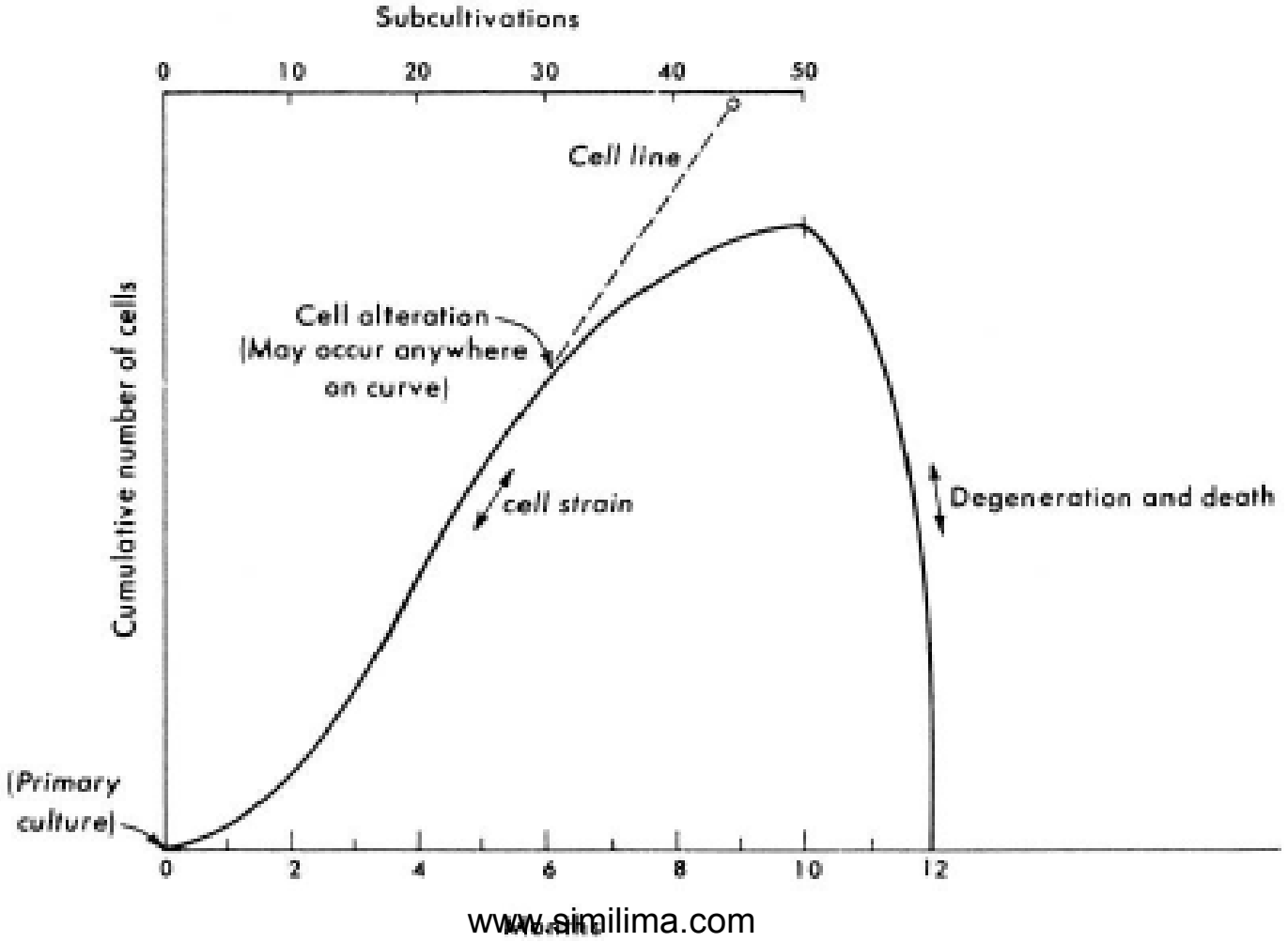
  -reduced serum and anchorage dependence and grow more in suspension conditions

  -ability to grow upto higher cell density

  -different in phenotypes from donar tissue

  -stop expressing tissue specific genes

Cell culture



Continuous cell lines

A549 – human alveolar cell ca (type II epithelial cells)

BGM – Baffalo green monkey kidney cells

BHK – baby hamster kidney cells

CV-1 – African green monkey kidney cells

HeLa – adenocarcinoma of cervix, HPV18 integrated

Propagated by George Otto Gey from biopsy of Cx of Henreitta Pleasant Lacks

George Otto Gey (1899-1970)

Head, Tissue Culture lab, Johns Hopkins University

Henrietta Lacks

Continuous cell lines ….

HEp-2 – sq. cell ca. of larynx, HPV16 transfected

LLC-MK2 – Rhesus monkey kidney cells

McCoy – mouse cell line of unknown origin

MDCK – Madin Darby Canine Kidney cells

ML – Mink lung cells

RD – human rhabdomyosarcoma cells

Vero - African green monkey kidney cells

Types of cells

Three types based on morphology or functional characteristics.

- Epithelial like - attached to a substrate and appears flattened and polygonal in shape
- Lymphoblast like - cells do not attach; remain in suspension with a spherical shape
- Fibroblast like - cells attached to a substrate; appears elongated and bipolar

Culture media

- Choice of media depends on the type of cell being cultured
- Commonly used media are GMEM, EMEM, DMEM etc.
- Media is supplemented with antibiotics viz. penicillin, streptomycin etc.
- Prepared media is filtered and incubated at 4^0 C

GMEM: Glasgow Minimum Essential Medium

EMEM: Eagle's MEM

DMEM: Dulbecco's Modified Eagle Medium

Suspension cultures

- Prepared from human / mouse leukaemias, ascites tumours
- Incubated on shakers or roller drums
- Special spinner apparatus to force normally adherent cells to grow in suspension
- Usually for large volume purposes
- Suspension cultures of human peripheral blood lymphocytes for EB virus

Microcultures

- 24, 48 or 96-well plates with flat bottoms and sealable lids

For

- Neutralization tests
- Tissue culture EIA
- Monoclonal Ab testing
- Screening assays

Shell vial cultures

- 15 x 45 mm vials with a 12mm cover slip at the bottom
- Add growth medium and cells
- Incubate to get a monolayer on the cover slip
- Readymade shell vial with monolayer available
- Inoculate sample (0.1 - 0.25ml) between 5-9 days
- Centrifuge at low speed (700g x 40 minutes at 35^0C)
- Add maintenance medium, incubate at 36 +/-1^0C for 16-24 hrs
- Cover slip examined by IF

New cell cultures

- Mixed cell lines (Hybrid)

R-Mix: A549 and ML with added trypsin
for respiratory pathogens
(Influenzas, parainfluenzas, RSV and Adeno)

E-Mix: BGM and A549 – for enteroviruses

Engineered cell lines

- Stable transformation of specific viral receptors for HIV-1 and EBV into non-permissive cell lines

- Engineering BGMK cells to express a receptor for enteroviruses (DAF)

- ELVIS (**E**nzyme **L**inked **V**irus **I**nducible **S**ystem): HSV-specific gene promoter sequence linked to a reporter gene (β-galactosidase) in transgenic BHK cell line. Infection by HSV causes accumulation of intracellular β-galactosidase. Application of a histochemical stain shows HSV infected cells as blue

Nutrition

- Maintenance medium- low serum conc.(0-2%)
  Keeps viability; do not promote multiplication
- Growth medium – high serum conc. (10-20%)
  Promotes multiplication
- Eagle's minimum essential medium in Earle's or Hanks' balanced salt solution
- Dulbecco's MEM, RPMI 1640
- Fetal calf serum

pH

- 7.0 to 7.4 (continuous cell lines)
- 7.4 to 7.7 (fibroblasts)

- Phenol red indicator:
  Purple in alkaline
  Red at 7.4
  Yellow at 6.5

- Buffer – $NaHCO_3/CO_2$ (require 5-10% CO_2),
  HEPES (no requirement of CO_2)

Chemical contamination

- Media – come from the reagents, ingredients, water or additives like sera - metal ions, endotoxins
- Free radicals genrated by photoactivation of tryptophan, riboflavin, HEPES (hydroxyethyl piperazine ethanesulfonic acid)
- Impurities in gas mixtures, residues of disinfectants

Biological contamination

- Bacteria, fungi, mycoplasma, viruses etc.
  - turbidity / colour change / microscopy
- Antibiotics:

  Benzyl penicillin 20-100 units/ml

  Gentamicin 16-50 mg/ml

  Tetracycline 10 mg/ml

  Amphotericin B 1.25 mg/ml or

  Nystatin 50 units/ml

Contamination …

- Mycoplasma may be detected by
  - culture
  - DNA staining with fluorescent dyes
  - mycoplasmal enzymatic assays
  - IFA
  - EIA
  - Hybridization probes
  - PCR assay

Contamination …

- Contamination by other cell lines: most commonly by HeLa
- Methods for checking cell identity:
  - Chromosome counting
  - Mixed haemagglutination test
  - Analysis of isoenzyme pattern
  - Immunofluorescence
  - Cytotoxicity test

Storage

- Cells packed by low speed centrifugation
- Resuspend in freeze medium (serum+DMSO)
- Dispense 1 ml volumes into cryovials
- Freeze slowly in controlled rate freezer (1^0 C / mt)
- Store in liquid nitrogen (-165^0 C)

Slow freezing without protectant:

> Water flows out of the cell

> Osmotic inbalance across the cell membrane

> cell death

Cryoprotectants:

- Protect the cell during freezing
- Minimize ice crystal formation
- Minimize the fragmentation of cell membranes

Recovery

- Rapidly thaw the cryovial in 37^0 C water bath
- Dilute cells with growth medium & centrifuge
- Resuspend in growth medium
- Transfer to culture flask & incubate

Specimen

- Aspirate or swabs (Herpes simplex lesions) in VTM
- VTM: DMEM with 1% fetal calf serum & antibiotics (Vanco 20 mg/ml, Genta 50 mg/ml, amphotericin 10 mg/ml)
- Vortex for 30 sec.; inoculate 0.1ml

Specimen: Blood, cerebrospinal fluid, dermal, ocular, genital, mucosal, oral, rectal, respiratory, stool, tissue, urine, biopsy

Viruses Typically Isolated From Clinical Specimens

Specimen	Virus1
Blood	CMV, enteroviruses 2, 3, HSV3, VZV3
CSF and CNS tissues	Enteroviruses, mumps virus, HSV, CMV
Dermal lesions	HSV, VZV, adenovirus, enteroviruses
Eye	HSV, VZV, adenovirus, enteroviruses, CMV
Genital	HSV, CMV
Mucosal	HSV, VZV
Oral	HSV, VZV
Rectal	HSV, VZV, enteroviruses

Viruses Typically Isolated From Clinical Specimens

Respiratory tract	
Upper	Adenovirus, rhinovirus, influenza, parainfluenza, enteroviruses, RSV, reovirus, HSV
Lower	Adenovirus, influenza, parainfluenza, RSV, CMV4
Stool	Enteroviruses, adenoviruses
Tissues	CMV, HSV, enteroviruses
Urine	CMV, adenoviruses, enteroviruses, mumps
1 Abbreviations:	
HSV - Herpes simplex virus	
CMV - Cytomegalovirus	
VZV - Varicella zoster virus	
RSV - Respiratory syncytial virus	
2 Enteroviruses: coxsackie virus, poliovirus, echovirus, and enterovirus 68-71.	
3 Rarely isolated.	
4 Usually in immunocompromised hosts.	

Specimen…

- Throat swab: Entero, Adeno & HSV
- Nasopharyngeal aspirate or swab: RSV, Influenza & parainfluenza
- Nasal swab: Rhinovirus
- Bronchial & brochoalveolar wash: Adeno & Influenza
- Rectal swab, stool: Rota, entero, enteric adenoviruses
- Urine: CMV, mumps, rubella, measles, polyoma, adenoviruses

Specimen….

- Skin & mucous membrane lesions: Entero, HSV, VZV, rarely CMV
- CSF, pleural fluids etc.: Entero, HSV, Influenza, CMV
- Blood: CMV, HSV, VZV, entero, adenoviruses

Avoid using calcium alginate swabs when collecting Herpes specimens as the fibers may inactivate the virus.
Avoid wooden shafted swabs, as they may inhibit viruses.

It is not possible to isolate Arboviruses from clinical specimens. In such cases serological studies are helpful.

Specimen should be collected during the acute phase of the disease as follows:

Throat swab: Carefully rub the posterior wall of the nasopharynx with a dry, sterile swab. Avoid touching the tongue or buccal mucosa. Place swab in the viral transport tube.

Refrigerate immediately.

Blood: Collect 5 mL whole blood into a heparinized tube. Consult with laboratory to which specimen will be sent.

Send @ room temperature.

Feces: Collect 4-8 g of feces (about the size of a thumbnail), and place in a clean, screw-capped container. Do **not** dilute the specimen or use preservatives. Viral swab is acceptable.

Refrigerate immediately.

Rectal Swab: Insert a sterile swab 2″ to 4″ into the rectum and rub the mucosa. Swab may be placed into cold virus transport medium.

Refrigerate immediately.

Cerebrospinal fluid: Collect 1 mL CSF aseptically in a sterile dry screw-capped vial. They do not require transport medium and should not be diluted.

Refrigerate immediately.

Skin lesions: Open the vesicle and absorb exudates into a dry swab, and/or vigorously scrape base of freshly exposed lesion with a swab to obtain cells that contain viruses. If enough vesicle fluid is available, aspirate the fluid with a fine gauge needle and tuberculin syringe, and place the fluid into cold viral transport medium. Use viral swabs for specimen collection.

Refrigerate immediately.

Urine: Collect clean-catch, midstream urine in a screw-capped, sterile, plastic container.

Refrigerate immediately.

Tissue: Use a fresh set of sterile instruments to collect each tissue. Place each specimen in separate dry, sterile non-toxic screw-capped container. To prevent the tissue from drying out, add a small amount of viral transport medium to the container. Identify each tissue with the patient's name, type of tissue, and date collected.

Refrigerate immediately.

Eye Swab or scraping: Use a viral swab to collect conjunctival material. Take conjunctival scrapings with a fine spatula and transfer the scraping to a viral transport medium.

Refrigerate immediately.

Genital swab: See skin.

Refrigerate immediately.

Nasopharyngeal aspirate

- Wash down the aspirate in 4-5 ml VTM
- Add antibiotic solution, vortex
- Inoculate 0.1 ml

Faeces

- Prepare 10-20% suspension in PBS or MM
- Centrifuge with glass beads at 1000g at 4^0C for 20 minutes
- Transfer 4 ml supernatant to sterile bottle, add 8 drops antibiotic solution
- Adjust pH to 7.0 with 8% $NaHCO_3$
- Inoculate 0.1 ml into suitable cell cultures

CSF

- Directly inoculate 0.1 ml to cell cultures

- Cervical, urethral, conjunctival, throat swabs are sent in VTM

Transport

Most viral specimens should be held @ 2-8°C (35-46°F) rather than frozen for short term, <72 hours, transit and storage. For delays exceeding 72 hours, freeze viral specimens @ -70°C (-94°F) or below.

Do not freeze @ -20°C (-4°F).

- When there is delay, transport in melting ice
- Better to keep sample in fridge than freezer
- For long delay, keep at -70^{0}C

Selection of cell cultures

- Basic requirements:

  - Human lung mucoepidermoid cells NCI-H292 (previously PMK for myxoviruses and enteroviruses

  - Human diploid fibroblast cell strain (HEL, MRC-5, WI-38 for CMV, Rhino, VZV, HSV)

  - Human continuous cell line (HEp-2, A549, KB or HeLa) for Adeno & RSV)

  - Human RD for broadest coverage of viruses

Inoculation

- Inoculate directly into the medium

- Aspirate GM, add inoculum, incubate at 35-36^0 C for 30-60 mt., add MM, continue incubation (adsorption enhances sensitivity)

Trypsinisation

- For optimum sensitivity to ortho- and paramyxoviruses
- MDCK and LLC-MK2 cell lines
- Maintain cells in medium containing 1mg/ml trypsin
- After inoculating sample, centrifuge at 36-37^0 C for I hr, change maintenance medium

Incubation

- Usually at 36-37^0 C
- Incubate at 33-35^0 C for for less invasive viruses (corona, rhino & enteroviruses that cause haemorrhagic conjunctivitis)
- Tube cultures of MK, AGMK, Vero, all diploid fibroblast cell cultures and NCI-H292 should be incubated in roller drums (one revolution in 3 minutes)
- Two weeks incubation usually sufficient

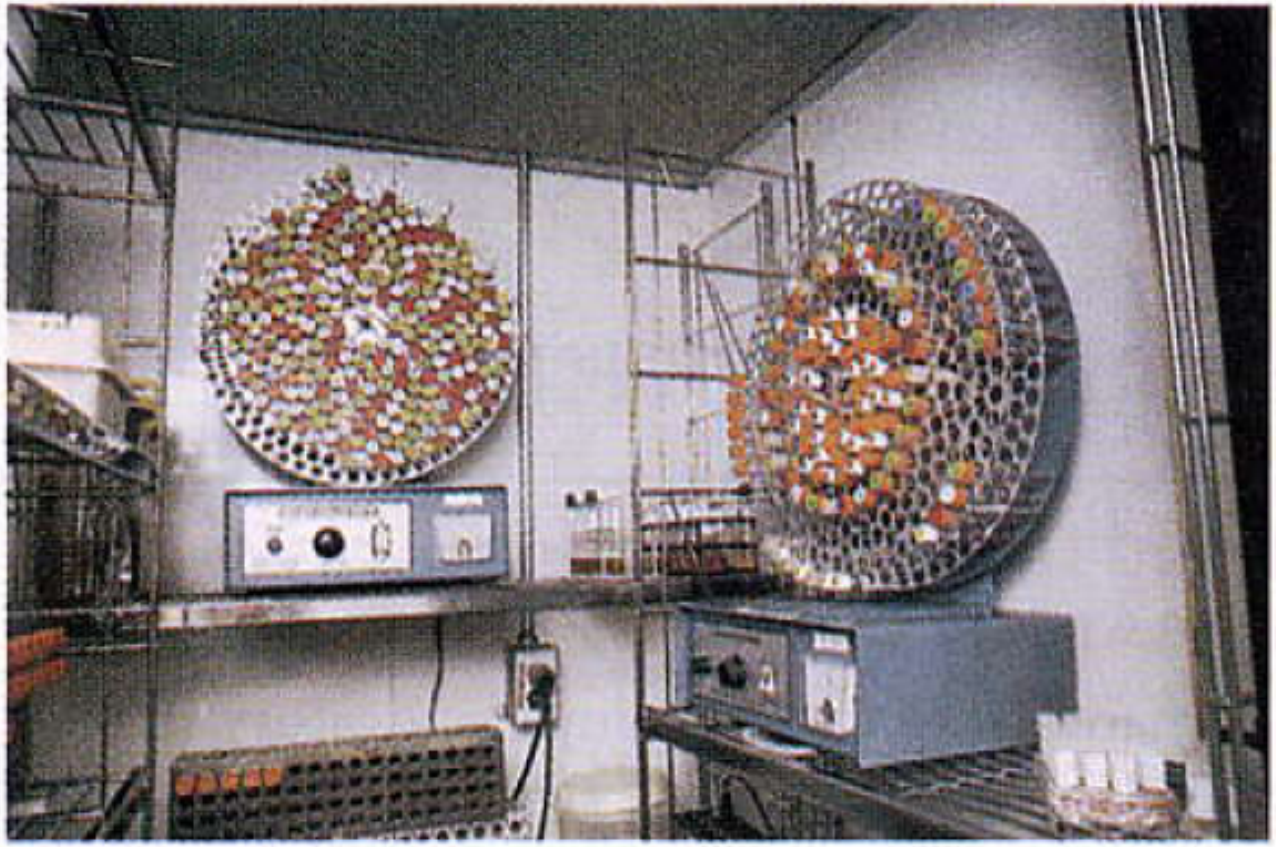


FIGURE 54-5 Roller drums used to hold cell culture tubes during incubation. Slow rotation continually bathes cells in medium. (Courtesy Children's Hospital Medical Center of Akron, Akron, Ohio.)

Examination of cultures

- Cell sheets are examined under low power 1 day after inoculation and then on alternate days for CPE
- Medium change every 3-4 days
- Continuous cell lines show overgrowth & metabolic degeneration by 14 days
- In the absence of CPE by 14 days, transfer 0.1ml of cells and medium to new vessels (Blind passage)
- Primary and diploid cell strains maintain morphology for 28 days

Use of 48-well culture plate

A	HEL		HEL.		HEL		HEL	
B	A549		A549		A549		A549	
C	BGM		BGM		BGM		BGM	
D		HEL		HEL		HEL		HEL
E		A549		A549		A549		A549
F		BGM		BGM		BGM		BGM
	1	2	3	4	5	6	7	8

Fig. 40.1 Suggested layout of cells in a 48-well cell-culture plate used for isolation of viruses from throat, skin, mucous membrane and conjunctival samples. Specimens (one per column) are inoculated in a staggered array to minimize cross-contamination. Up to eight specimens can be accommodated on each plate. Inoculation of duplicate plates allows the sample to be cultured at 37 and 33°C.

Detection of viral growth

- CPE
- Metabolic inhibition
- Haemadsorption
- Interference
- Transformation
- Immunofluorescence

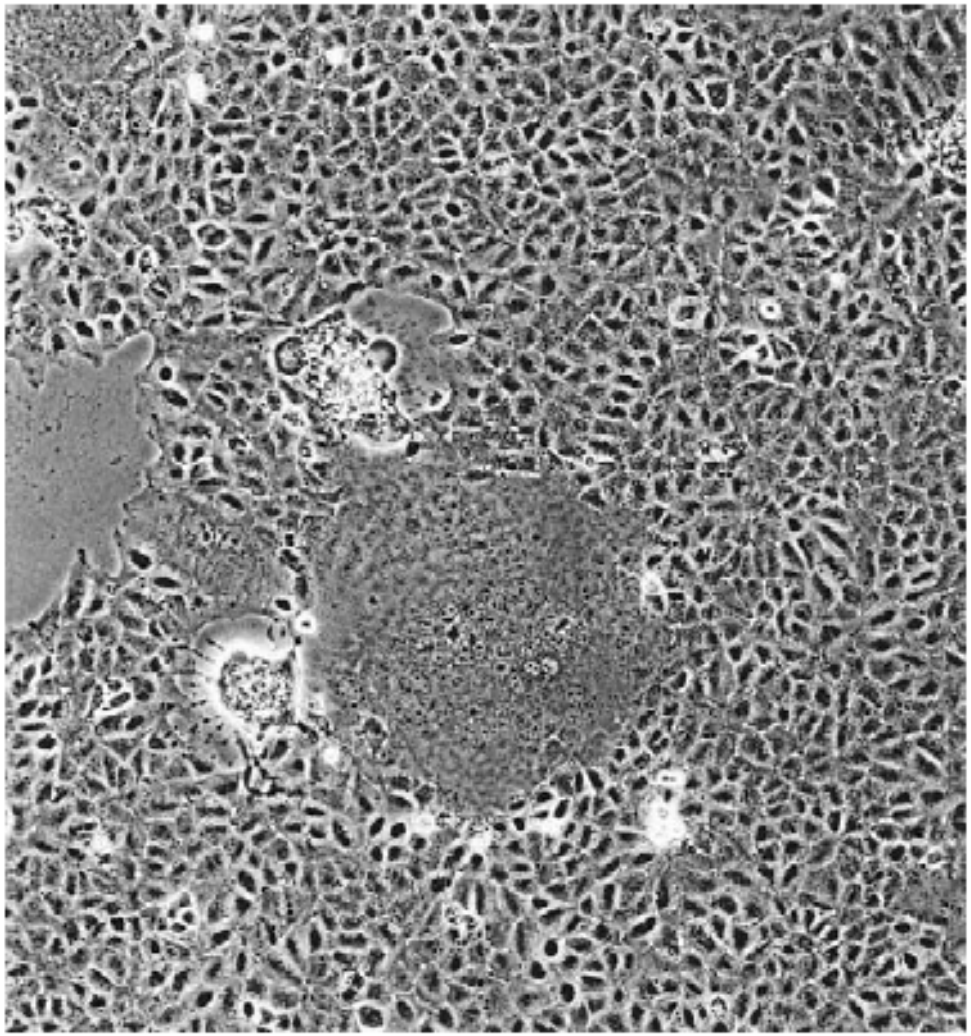
CPE produced by different viruses

VIRUS	PMK	HEp-2	HDF	CPE DESCRIPTION	RATE OF GROWTH (DAYS)
Respiratory syncytial virus (RSV)	+	+++	+	Syncytia in HEp-2	3-10
Rhinovirus	++	—	+++	Characteristic refractile rounding of cells. In PMK, CPE is identical to that produced by enteroviruses	4-10
Varicella-zoster	—	—	++	Discrete foci of rounded, swollen, refractile cells. Slowly involves entire monolayer	5-28

CPE, Cytopathic effects; *FA*, fluorescent antibody; *PMK*, primary monkey kidney; *RBCs*, red blood cells.

VIRUS	PMK	HEp-2	HDF	CPE DESCRIPTION	RATE OF GROWTH (DAYS)
Adenovirus	++*	+++	++	Rounding and aggregation of infected cells in grapelike clusters	2-10
Cytomegalovirus (CMV)	—	—	++++	Discrete, small foci of rounded cells	5-28
Enterovirus	++++	+	++	Characteristic refractile angular or tear-shaped CPE; progresses to involve entire monolayer	2-8
Herpes simplex (HSV)	+	++++	++++	Rounded, swollen refractile cells. Occasional syncytia, especially with type 2. Rapidly involves entire monolayer	1-3 (may take up to 7)
Influenza	++++	—	±	Destructive degeneration with swollen, vacuolated cells	2-10
Mumps	+++	±	±	CPE, usually absent. Occasionally syncytia are seen	5-10
Parainfluenza	+++	—	±	CPE, usually minimal or absent	4-10

CPE: Measles on human lung carcinoma (A549)



Fields Vriology (2007) 5th edition, Knipe, DM & Howley, PM (Editor in Chief) Lippincott Williams & Wilkins, Philadelphia Fig. 2.5

Metabolic inhibition

- Due to normal cellular metabolism, there will be acid production, and colour of phenol red indicator will change to yellow

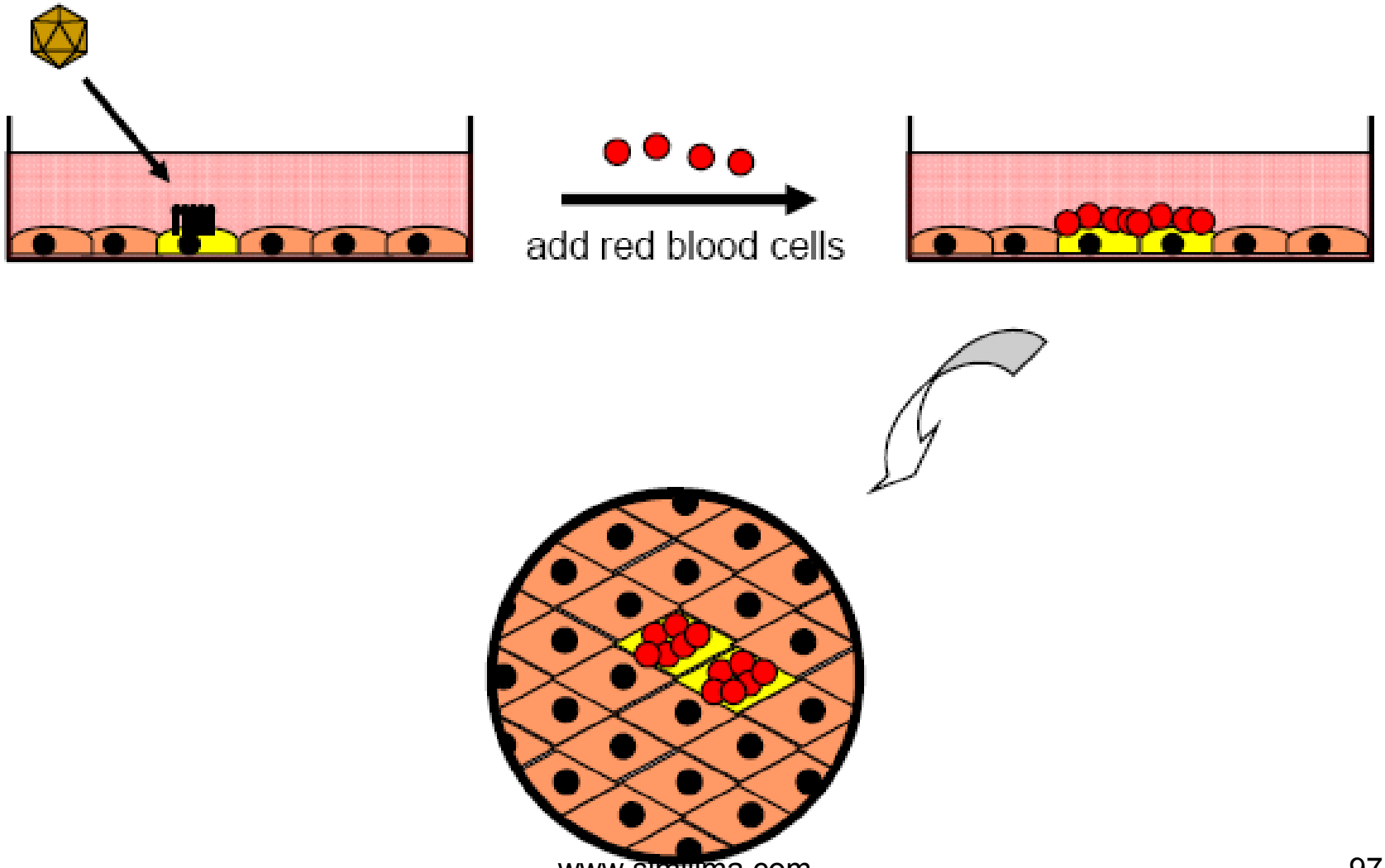
- When viruses grow in cell culture, cellular metabolism is inhibited, there is no acid production, phenol red will not become yellow

Haemadsorption

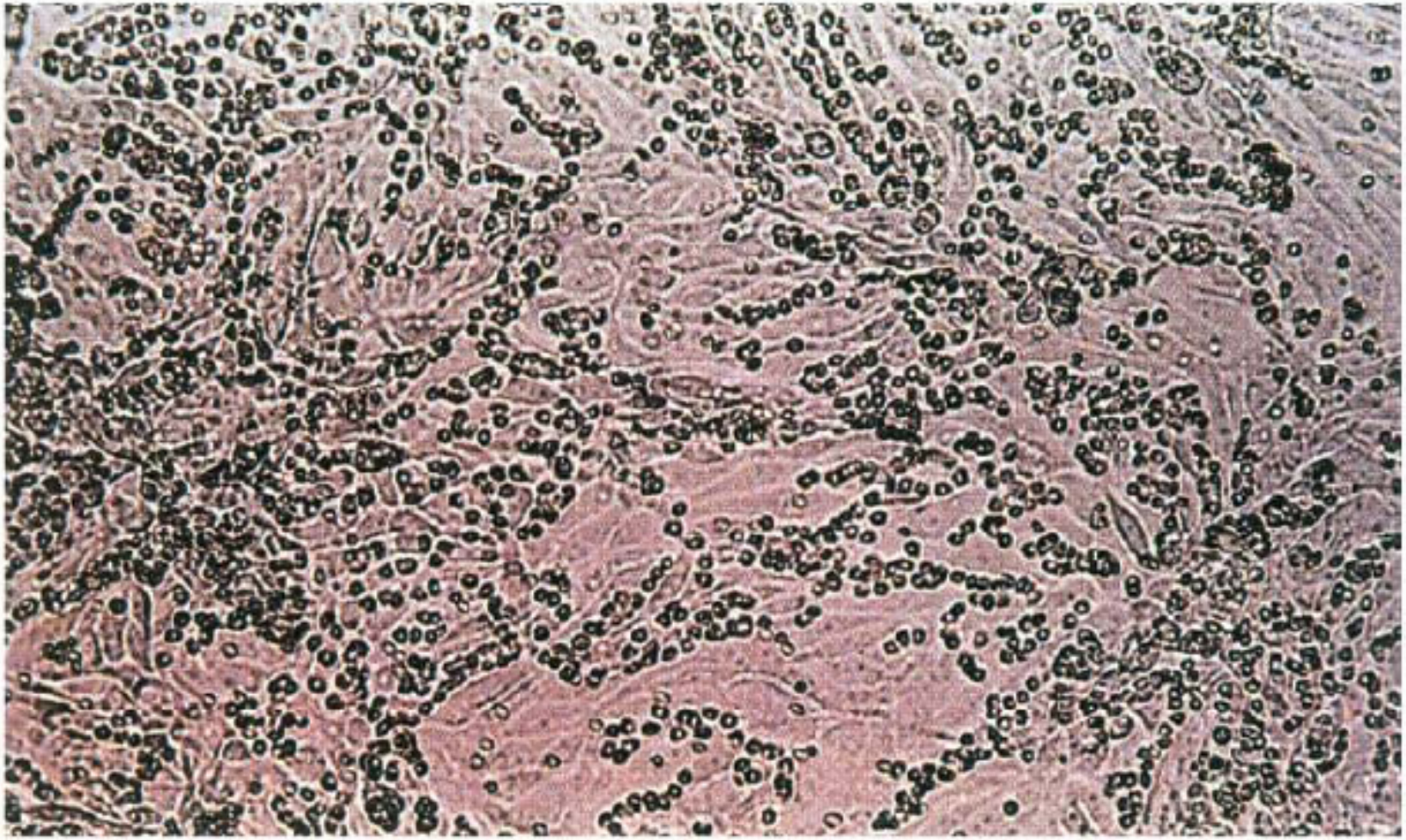
- When Influenza & parainfluenza viruses grow in cell cultures, haemagglutinins are expressed on cell surface.

- If guinea pig RBCs are added, they are adsorbed on to cell surface

Hemadsorbtion



Hemadsorption



Hemadsorption of erythrocytes to cells infected with influenza viruses, mumps virus, parainfluenza viruses, or togaviruses. These viruses express a hemagglutinin on their surfaces, which bind erythrocytes of selected animal species. (From Medical Microbiology, 5th ed., Murray, Rosenthal & Pfaller, Mosby Inc., 2005. Fig. 51-5.)

Interference

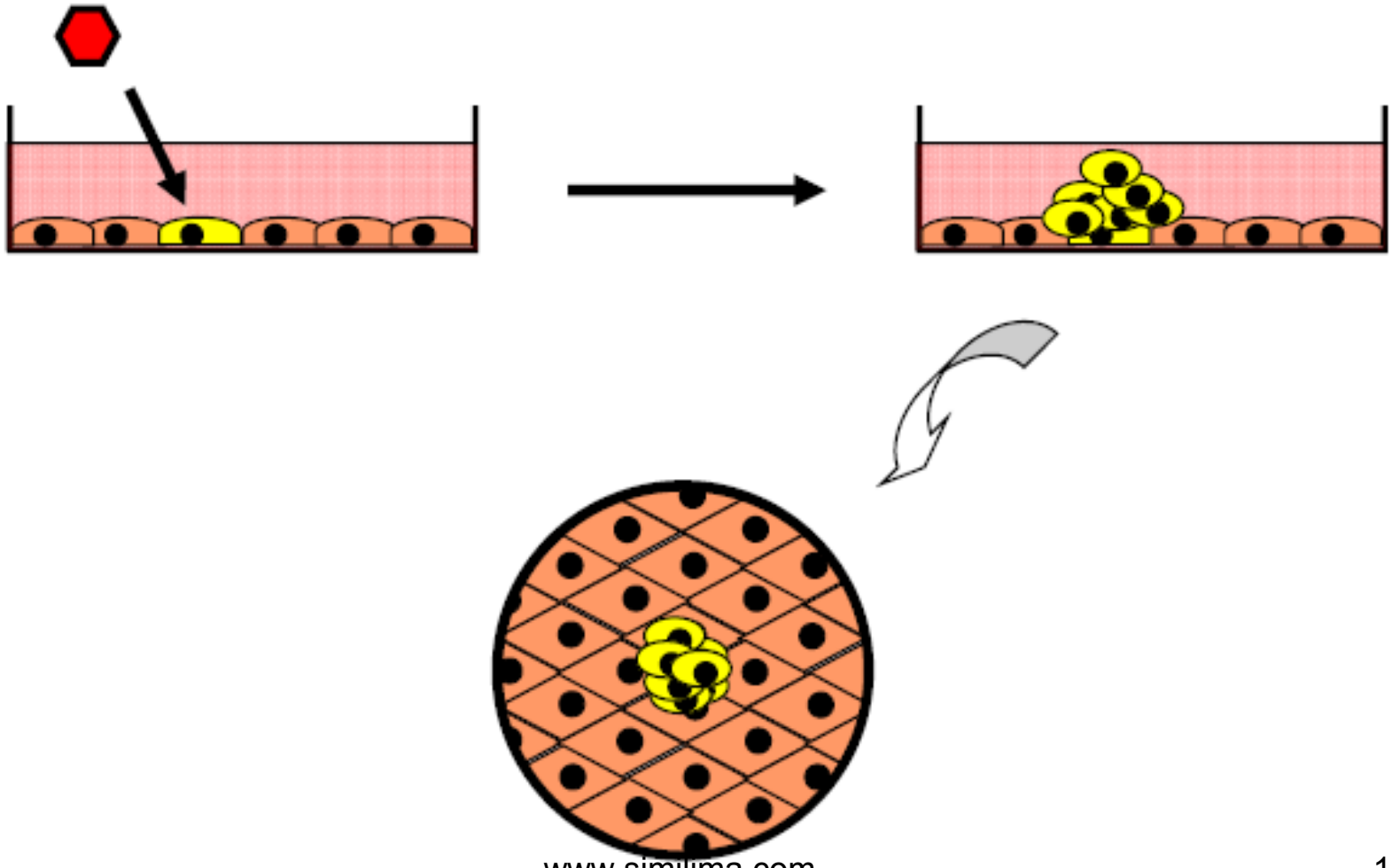
- When a noncytopathogenic virus is growing in a cell culture, inoculation of a cytopathogenic virus will not produce any CPE as infection by the second virus is prevented by interference

Transformation

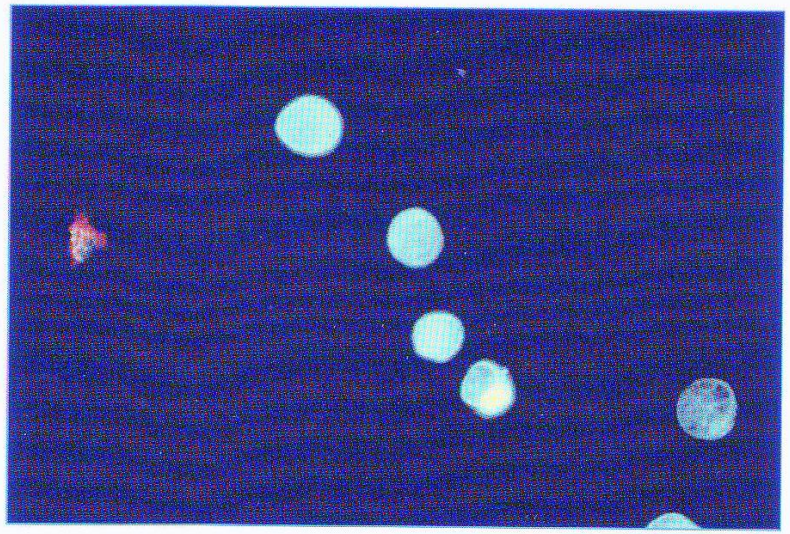
- Oncogenic viruses cause cell transformation
- Loss of contact inhibition
- Cells grow in heaped-up fashion producing microtumours

Transformation

loss of contact inhibition

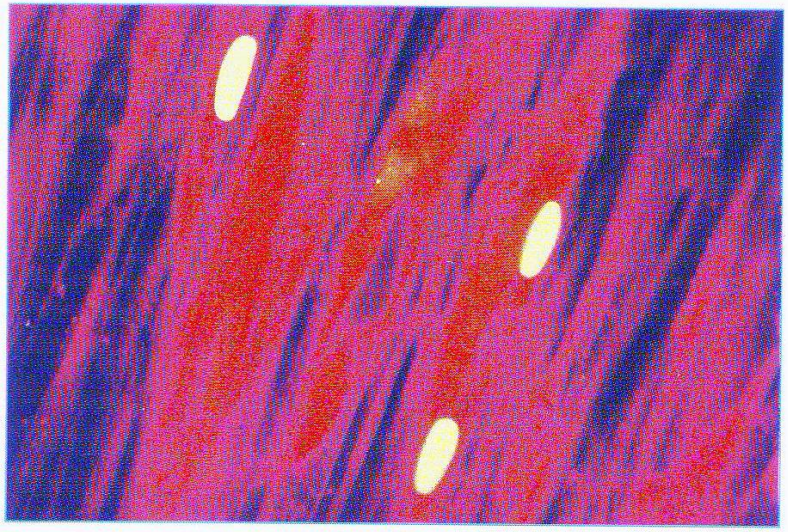


Immunofluorescence



D

HEp-2 cells infected with HSV type 1



F

CMV infected human diploid fibroblasts

Thank you