UNIVERSITAS GADJAH MADA

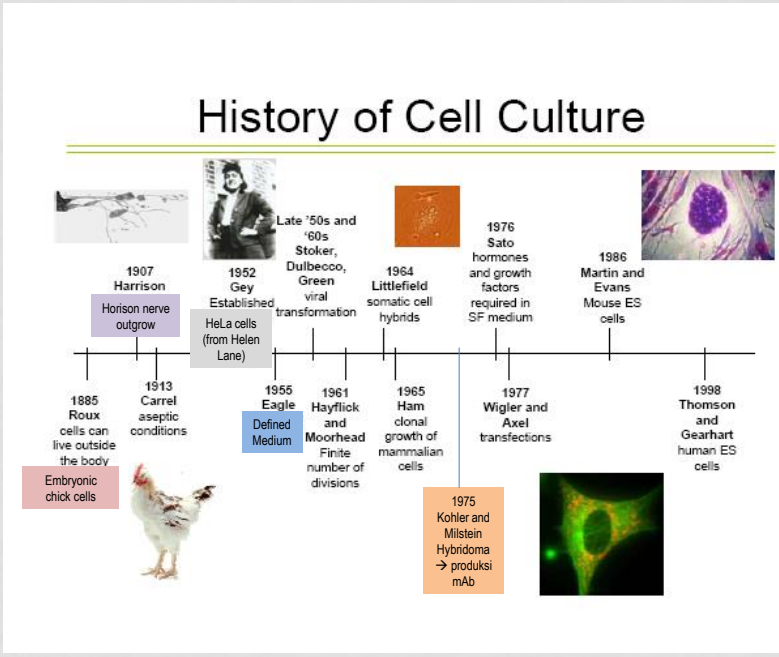
KULTUR SEL Dan SEL LINES

Sismindari, Farmasi-UGM

Kultur sel dan Kultur Jaringan

- Kultur sel merupakan proses dimana suatu organisme (prokariot, eukariot, tanaman) ditumbuhkan dalam kondisi yang terkontrol.
- Kultur jaringan menumbuhkan organ, jaringan dan sel secara in vitro dalam media yang mengandung nutrient dan growth factor pada temperature tertentu dmenggunakan incubator.
- Ada beberapa tipe sel yang dapat tumbuh dalam kultur, seperti misalnya fibroblasts, skeletal tissue, cardiac, epithelial tissue (liver, breast, skin, kidney) dan beberapa tipe sel tumor.

Sompol T.,MD.,PhD., Dept.of Physiology, Fac. Of Med. Siriraj Hospital



DIPERLUKAN UNTUK APA CELL CULTURE?

- **Model untuk**
  mempelajari dasar-dasar biologi sel, interaksi anatara penyakit dan pengaruhnyaterhadap sel
- **Toxicity testing**
  Mempelajarai efek suatu obat baru
- **Cancer research**
  Mempelajari efek senyawa kimia, virus, radiasi dalam merubah cell normal menjadi sel kanker, atau sebaliknya.

Virology

Kultivasi Virus untuk produksi vaksin: polio, rabies, chicken pox

- **Gene therapy**
  penggantian gena non fungsional dengan yang fungsional dalam sel

CELLS CULTURES
KLASSIFIKASI BERDASARKAN:

- *Asalnya (Origen)*
- *Sifat pertumbuhan (Manner of growth)*
- Shape of growth (Culture Morphology)

CATEGORIES OF CELL CULTURES BASED ON ORIGINS.

	Origin	Similarity to original tissue	Ease of maintenance	Doublings
Primary cells	Animal tissue, fetal or adult	Representative	Difficult	0 - 1
Finite cell lines	Animal tissue, usually fetal	Representative	Difficult	Fetal: 20-80; adult tissue: very limited
Continuous cell lines	Spontaneous tranformation of primary or finite cell lines	Not very representative; cell are less differentiated	Easy	Indefinite, with selection for higher growth rate
Transformed cell lines	Tumor tissue, spontaneous or viral transformation of continuous cell line	Not very representative; less differentiated than parent	Easy	Indefinite, with selection for higher growth rate
Hybridoma (monoclonal antibody production)	Fusion of antibody secreting B cells and myeloma cells	Not representative of either cell type	Difficult	Limited

- Semakin terdeferensiasi sel, semakin lambat tumbuh.

PRIMARY CELLS AND CONTINUOUS CULTURES

- **Kultur Sel Primer**
  - Sel yang tumbuh dan diambil dari sepotong jaringan
  - morfologi yang serupa dengan jaringan aslinya
  - waktu hidup yang terbatas
  - Sel macrophages dan neurons tidak membelah→ diambil dari sel primer
- ***Continuous cell lines***
  - Cells yang telah mengalami perubahan genetik dan kadang sifat in vitro nya tidak menggambarkan situasi in vivo.
  - Lebih mudah penanganannya (*easy to handle*)

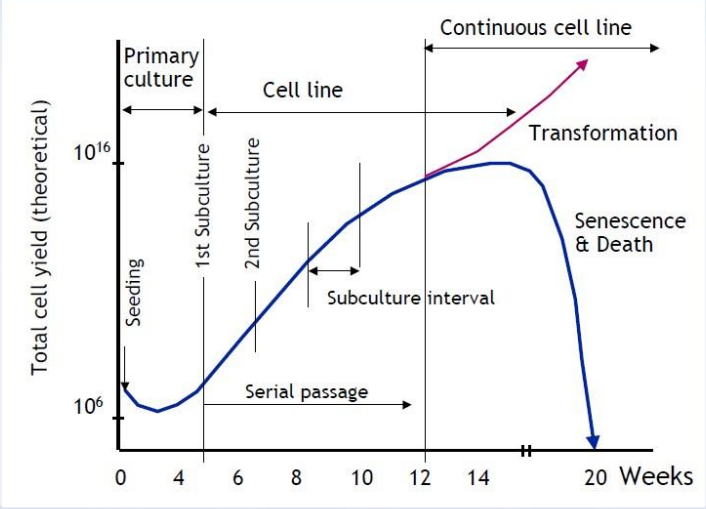
CONTINUOUS CELL LINE

- Cell line yang potensial untuk disubkulture secara tidak terbatas
  - Cell line yang tidak terbatas (infinite cell line)
  - Sel line yang abadi (Immortal cells line)
- Sel yang telah abadi sering disebut sebagai "transformed cells":
  - Cells yang pola tumbuhnya telah berubah

KEUNTUNGAN DAN KERUGIAN

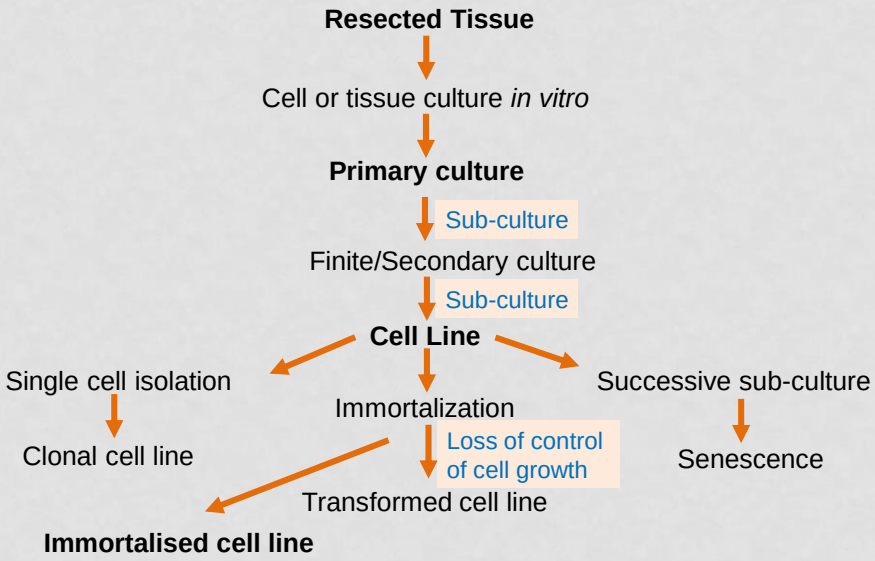
No	Sel	Keuntungan	Kerugian
1	Sel Primer	- Model penelitian sesuai kondisi *in-vivo* - Tidak mengalami *dedifferentiated*	- Sulit di dapat - Relatif mempunyai waktu hidup pendek - Mudah kontaminasi
	Sel primer tumor	- Lebih mudah ditumbuhkan - Kadang menghasilkan sendiri faktor pertumbuhan (autocrine)	- memerlukan jumlah sel yang lebih tinggi dalam awal menumbuhkan
2	*Continuous cell lines*	- Mudah dipelihara dan didapat dalam jumlah yang besar - Mudah dilakukan manipulasi ekspresi gena	Makin agressive cell line tersebut maka makin mudah berubah setiap waktu

PRIMARY CELLS AND CONTINUOUS CELL LINES



Growth of animal cell in cell culture

ISOLATION OF CELL LINES FOR *IN VITRO* CULTURE



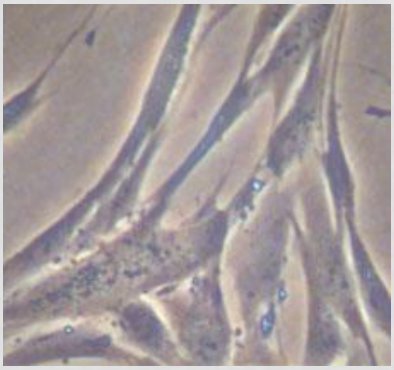
FINITE CELL LINES (SECUNDARY CELL CULTURE)

- Kultur sel yang tumbuh setelah subkultur yang pertama dari kultur primer
- Kulture tsb akan proliferasi utk waktu tertentu, setelah itu akan berhenti membelah dan menyerupai tahap senesce.
- kemungkinan mengalami kematian atau tumbuh secara stabil menjadi continous cell line dan mampu malakukan proliferasi tdk terbatas
- Perubahan tersebut diketahui sebagai

"in vitro transformation" or "immortalization"

FINITE CELL LINES

- MRC5 cells
  - Human embryonic lung fibroblasts
  - Bisa melakukan doubling time antara 60-70 kali sebelum senescence.



FINITE CELL LINES

- Keuntungan
  - Dapat diperoleh populasi dalam jumlah besar dari sel yang sejenis
  - Sebagian besar karakter sel dapat terjaga.
  - Can transform cells to grow indefinatly
- Kerugian
  - Cells mempunyai tendensi untuk terdiferensiasi dalam waktu yang lebih lama dalam culture.
  - Over time culture mempunyai tendensi menjadi sel dengan sifat berbeda (aberrant cell)

Klasifikasi Sel: *Manner of Growth*

SUSPENSION → Tersuspensi (*floating*) dalam medium (tidak menempel/attached)	ADHERENT GROWTH → membentuk monolayer (menempel/attached)	
Umumnya kultur sel darah, limfa, sum-sum tulang. Bentuk sel: bulat (bola). *Anchorage-independent*. Keuntungan: perolehan sel banyak, kemudahan panen sel. Contoh: HL-60 (human, peripheral blood, acute promyelocytic leukemia)	Umumnya kultur sel epitel. Bentuk sel bulat jika berada dalam suspensi, *flattened* dan beragam jika sudah *attached* (*round, triangular, squarish, elongated*). Keuntungan: mudah ditanam pada *coverslip*. Contoh: CHO-K1 (Chinese hamster, ovary) HT-29 (human, colon, colorectal adenocarcinoma)	
	ANCHORAGE-DEPENDENT	***ANCHORAGE INDEPENDENT***
	Harus *attached* untuk berproliferasi	Tidak harus *attached* untuk berproliferasi

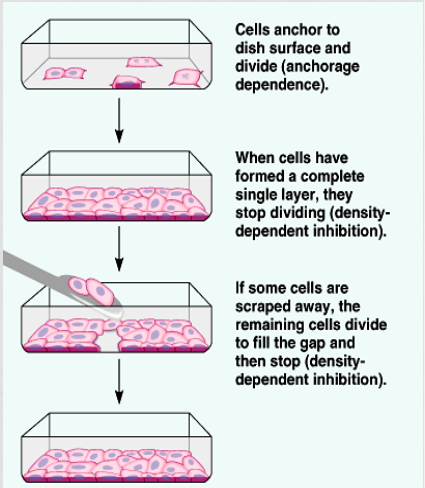
Prof.Dr.apt.Edy Meiyanto, Jakarta, 16 April 2013
Badan POM Republik Indonesia

CHARACTERIZATION BY CELL GROWTH

- **Suspension Cultures** sel hidup dan membelah saat disuspensikan dalam media cair dgn distirer.
  - Flasks
  - Spinner Cultures
  - Shaker Cultures
- Keuntungan: dapat diperoleh sel dalam jumlah besar dan mudah dipanen

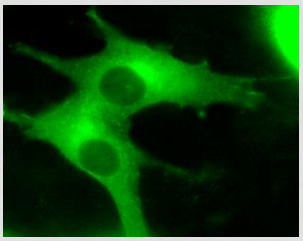
GROWING CELLS IN CULTURE

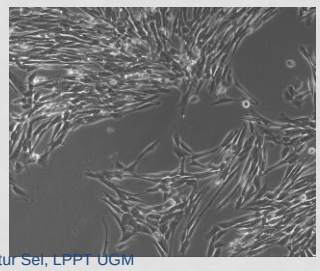
- Tempatkan sel pada plate kultur.
- Tambahkan nutrient, growth factors, dalam kondisi steril.
- Cells akan tumbuh memenuhi plate.
- Dapat di sub culture, dengan mengambil sedikit cells dan mulai ditumbuhkan sebagai kultur sel baru.
- Splitting cells dari satu plate ke plate yang lain adalah a passage.



NUMBER OF CELL DIVISIONS

- Ada keterbatasan waktu dalam kemampuan untuk membelah
- Normal cells mempunyai jumlah yang terbatas saat dikulturkan
- Jumlah tersebut berwariasi, umumnya antara 40 and 60 passages.



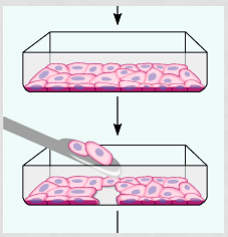


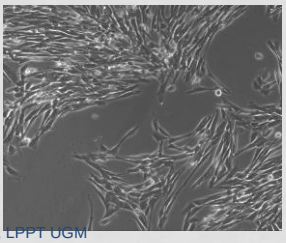
FENOMENA HAYFLICK'S DAN AGING

- Nampak ada korelasi antara "*the maximal number of passages and aging*".
- *The number of passages* menurun jika sel dipanen dari sel yang tua.

CONTACT INHIBITION

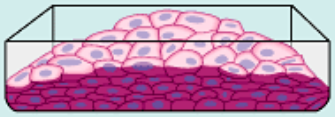
- Fenomena yang terlihat pada mamalia sel → berhenti membelah jika terjadi saling kontak antara sel
- Sel akan tumbuh dalam media yang sesuai sampai penuh dan berhenti berkembang
- Sel dapat dipacu untuk tumbuh kembali jika di sub kulturkan





CONTACT INHIBITION

- Sel kanker tidak terpengaruh proses contact inhibition.
- Dapat tumbuh berlapis-lapis (multiple layers)

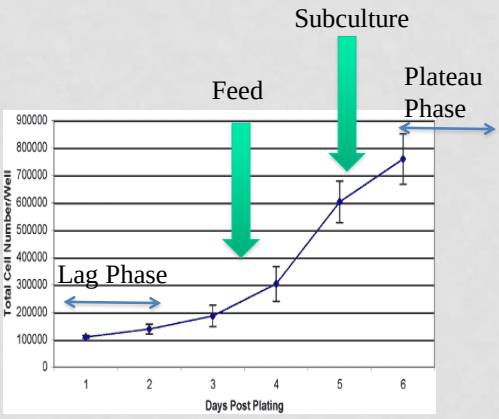


Cancer cells do not exhibit anchorage dependence or density-dependent inhibition.

GROWTH CYCLE IN ATTACHEMENT CULTURE

- Eukaryotic cells dalam kultur yang melekat mempunyai karakter spt bakteri
- Tumbuhnya terbagi dalam 3 phases.
  - Lag Phase
  - Log Phase
  - Plateau Phase

GROWTH CYCLE IN ATTACHEMENT CULTURE



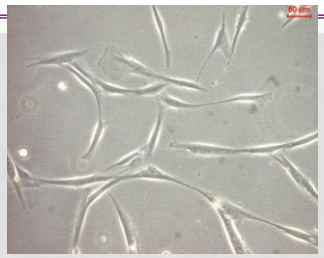
Days (in culture)	Cells/Well	Average # of Cells	Standard Deviation
Day 1	115000 115000 105000 105000	110000	5774
Day 2	117500 155000 152500 132500	139375	17722
Day 3	190000 237500 145000 175000	186875	38588
Day 4	380000 227500 287500 320000	303750	63656
Day 5	642500 532500 547500 690000	603125	75674
Day 6	675000 710000 885000 770000	760000	92105

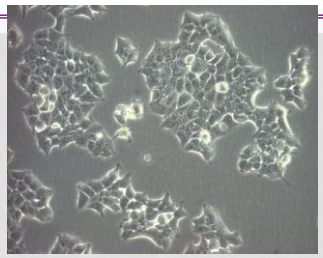
BERAPA LAMA SEL DAPAT DIGUNAKAN?

- Jika disubkultur berkali-kali → akan merubah karakter, morfologi dari sel
- Jika doubling time sel tersebut berubah → perlu diperhatikan kemungkinan sel telah berubah

KLASIFIKASI BERDASAR MORFOLOGI SEL

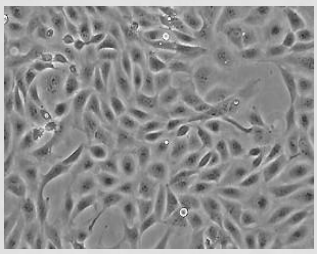
Fibroblastic

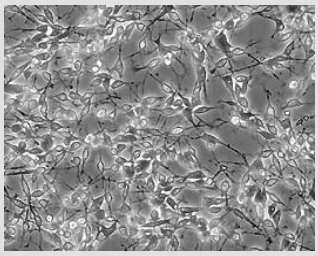




Epithelial

Endothelial

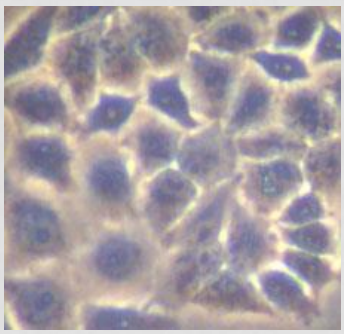




Neuronal

HELA CELLS

- Contoh klassik dari immortalized cell line.
  - Merupakan human sel epithelial dari fatal cervical carcinoma tertransformasi dengan human papillomavirus 18 (HPV18).



HAL YANG PERLU DIAMATI DALAM MENUMBUHKAN SEL

- Warna medium
  - Terlalu basa: *nongrowing culture*, kontaminasi fungi, CO_2 kurang
  - Terlalu asam: *overgrown culture*, kontaminasi bakteri, CO_2 terlalu tinggi
- Kekeruhan medium
  - Kontaminasi, *overgrown*
- Kondisi sel
  - Menggerombol, tunggal, *attached*, *non-attached*, bentuk, sedikit, konfluen, terlalu penuh

CELL CULTURE ENEMIES

Micro-organisms grow ~10-50 times faster than mammalian cells, which take ~8-16 hours to divide. They are more tolerant to variations in temperature, pH and nutrient supply than cells.

Cells are most vulnerable to contamination when our aseptic technique is bad and the culture becomes infected with bugs.

This can lead to the development of antibiotic resistant micro-organisms.

CELL CULTURE ENEMIES

Cells are more susceptible to infection at certain times

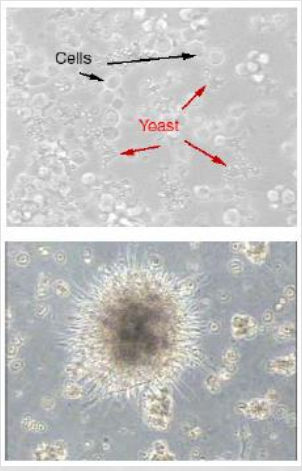
- When they have been stressed after recovery from liquid nitrogen
- Primary cells are often generated by enzymatic disruption and selection procedures
- Cultures prepared from live animals will often be accompanied by micro-organisms
- Splitting cells at too high a dilution can allow micro-organisms to dominate the culture
- Cells release Autocrine growth factors which condition the medium and favour cell growth

KONTAMINASI

- Kontaminasi Biologi
  - Mikroorganime,
    - bakteri, jamur (terlihat keruh, atau bisa diamati microscopy)
    - mycoplasma (*fluorescent staining* atau deteksi *mycoplasma-specific primer* (dengan PCR)
  - Kontaminasi silang, (microscopy)
- Kontaminasi kimia, *plasticizers*, *metal ions*, *traces of desinfectants*

Contamination

- Bacteria, mold, and yeast
- Mycoplasma
- Cross contamination

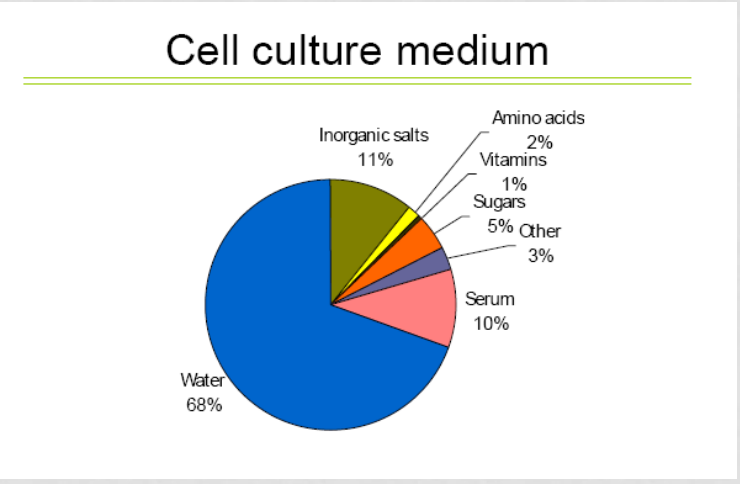


Alison Albee, ajalbee@wisc.edu, wiese lab.

MAJOR DEVELOPMENT'S IN CELL CULTURE TECHNOLOGY

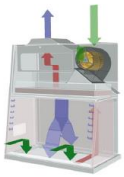
- First, development was the use of antibiotics which inhibits the growth of contaminants.
- Second, was the use of trypsin to remove adherent cells to subculture further from the culture vessel
- Third, was the use of chemically defined culture medium.

How to culture cells



Alison Albee, ajalbee@wisc.edu, wiese lab.

Biosafety Cabinet



(Cells are grown and maintained at an appropriate temperature and gas mixture typically, **37°C, 5% CO_2 for mammalian cells**) in a cell incubator.

Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes.

Incubator

- CO_2
- Water jacketed
- Humidity

Alison Albee, ajalbee@wisc.edu, wiese l

CULTURE MEDIA

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

WHY SUB CULTURING.?

- Once the available substrate surface is covered by cells (a confluent culture) growth slows & ceases.
- Cells to be kept in healthy & in growing state have to be sub-cultured or passaged
- It's the passage of cells when they reach to 80-90% confluency in flask/dishes/plates
- Enzyme such as trypsin, dipase, collagenase in combination with EDTA breaks the cellular glue that attached the cells to the surface

WORKING WITH CRYOPRESERVED CELLS

- Vial from liquid nitrogen is placed into 37 C water bath, agitate vial continuously until medium is thawed
- Centrifuge the vial for 10 mts at 1000 rpm at RT, wipe top of vial with 70% ethanol and discard the supernatant
- Resuspend the cell pellet in 1 ml of complete medium with 20% FBS and transfer to properly labeled culture plate containing the appropriate amount of medium
- Check the cultures after 24 hrs to ensure that they are attached to the plate
- Change medium as the colour changes, use 20% FBS until the cells are established

FREEZING CELLS FOR STORAGE

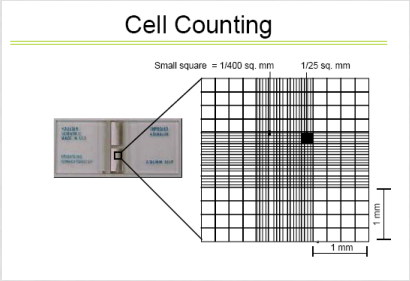
- Remove the growth medium, wash the cells by PBS and remove the PBS by aspiration
- Dislodge the cells by trypsin-versene
- Dilute the cells with growth medium
- Transfer the cell suspension to a 15 ml conical tube, centrifuge at 200g for 5 mts at RT and remove the growth medium by aspiration
- Resuspend the cells in 1-2ml of freezing medium
- Transfer the cells to cryovials, incubate the cryovials at -80 C overnight
- Next day transfer the cryovials to Liquid nitrogen

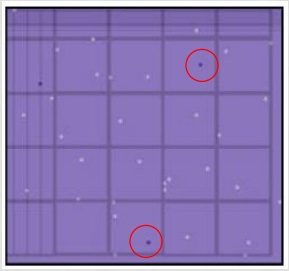
CELL VIABILITY

- Cell viability is determined by staining the cells with trypan blue
- As trypan blue dye is permeable to non-viable cells or death cells whereas it is impermeable to this dye
- Stain the cells with trypan dye and load to haemocytometer and calculate % of viable cells

$$\% \text{ of viable cells} = \frac{\text{Nu. of unstained cells} \times 100}{\text{total nu. of cells}}$$

Cell Counting



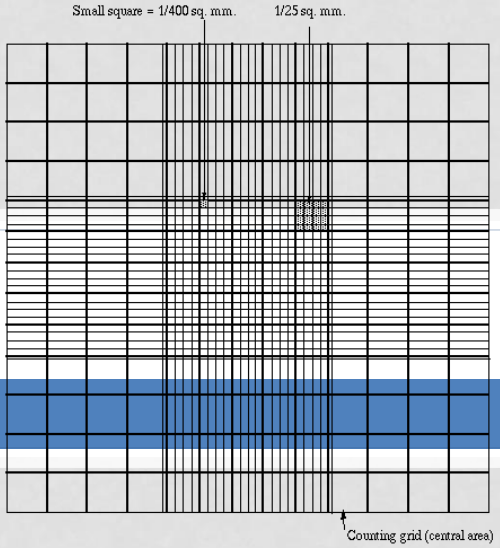


23 live cells
2 dead cells

Cells/mL = (# cells/square) x (dilution factor) x 10^4

Alison Albee, ajalbee@wisc.edu, wiese lab.

CELL COUNTS BY HEMOCYTOMETER

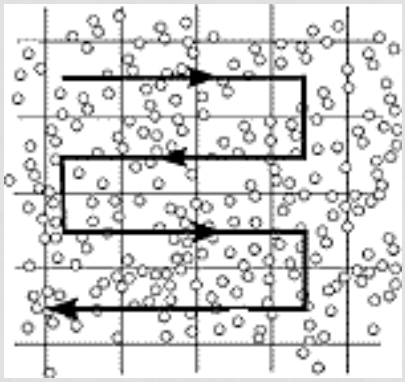


42

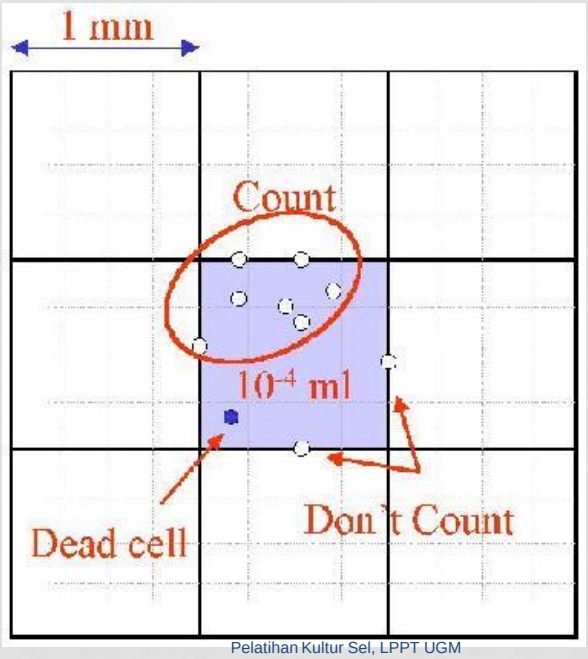
*daactarbhatti.weebly.com/uploads/3/5/1/6/.../neubauers_lecture.**ppt***

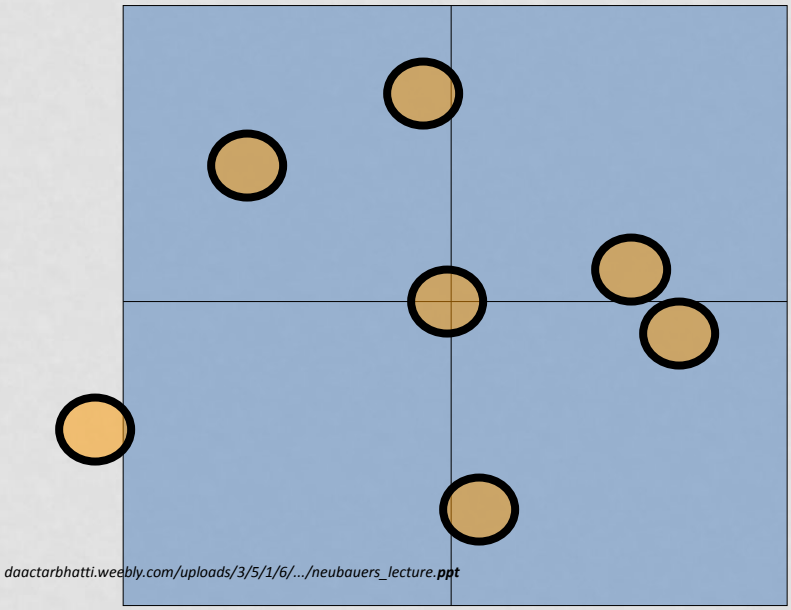
COUNTING RULE

- Do not count cells touching
  - Bottom line
  - Right line
  - ➢ This is to avoid double counting.



*daactarbhatti.weebly.com/uploads/3/5/1/6/.../neubauers_lecture.**ppt***





daactarbhatti.weebly.com/uploads/3/5/1/6/.../neubauers_lecture.**ppt**

Established cell lines

Cell line	meaning	Organism	origin tissue
HEK-293	*human embryonic kidney*	human	kidney (embryonic)
HeLa	*Henrietta Lacks*	human	Cervical cancer
CHO	*Chinese hamster ovary*	hamster	Ovary
Sf-9	*Spodoptera frugiperda*	Insect - *Spodoptera frugiperda* (Moth)	Ovary
NIH-3T3	NIH, 3-day transfer, inoculum 3 x 10^5 cells	mouse	embryo
bEnd.5	*brain endothelial*	mouse	brain
MCF-10A	*Michigan Cancer Foundation*	human	mammary gland
HMEC	*human mammary epithelial cell*	human	mammary gland
MDCK II	*Madin Darby canine kidney*	dog	kidney
COS-7	*Cercopithecus aethiops, origin-defective SV-40*	Ape - Cercopithecus aethiops	kidney
HL-60	*human leukemia*	human	Myeloblast
Jurkat		human	T-Cell-Leukemia

Alison Albee, ajalbee@wisc.edu, wiese lab.

EQUIPMENT

LAMINAR- FLOW HOOD

Horizontal Laminar Flow Cabinets

- These provide the most sterile environment for the cells, but offer no protection to the operator
- Filtered air enters at the back of the cabinet and is directed to the front, directly at the operator
- The most sterile part of the cabinet is at the back

LAMINAR- FLOW HOOD

- Routine maintenance checks of the primary filters are required (every 3-6 months).
- They might be removed and discarded or washed in soap and water.
- Every 6 months the main high efficiency particulate air (HEPA) filter above the work surface should be checked for airflow and hole
- Ultraviolet lights are used to sterilize the air and exposed work surfaces in laminar flow cabinets between use.
- Detergent
- 70% alcohol

INCUBATOR

- The incubators run at 37C and 5% Carbon Dioxide to keep the medium at the correct pH
- They all have meters on them to register temperature and gas level
- There are alarms to indicate when these deviate from set parameters
- Keep the door open for as short a time as possible
- It requires a controlled atmosphere with high humidity and super controlled of CO2 tension

CELL CULTURE INCUBATOR

AUTOCLAVE

- A simple bench-top autoclave may be sufficient, but a larger model with a timer and a choice of presterilization and poststerilization evacuation will give more capacity and greater flexibility in use

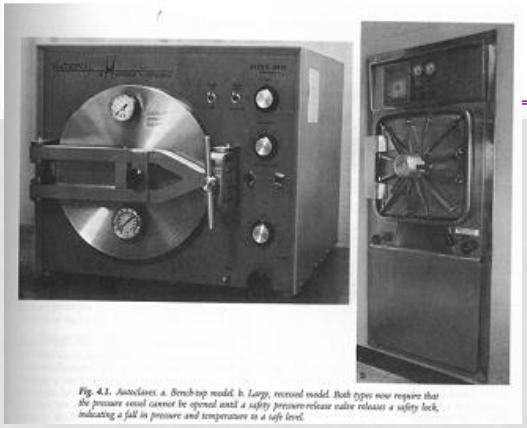


Fig. 4.1. Autoclaves. a. Bench-top model. b. Large, recessed model. Both types now require that the pressure vessel cannot be opened until a safety pressure-release valve releases a safety lock, indicating a fall in pressure and temperature to a safe level.

REFRIGERATORS AND FREEZERS

- 4C
- -20C
- -80C
- Liquid N2 tank

INVERTED & FLUORESCENCE MICROSCOPY

Inverted Microscopy

Large stage so plates and flasks can be used.
Magnification; 5X, 10X, 20X, 40X

Mikroskop fluorescence

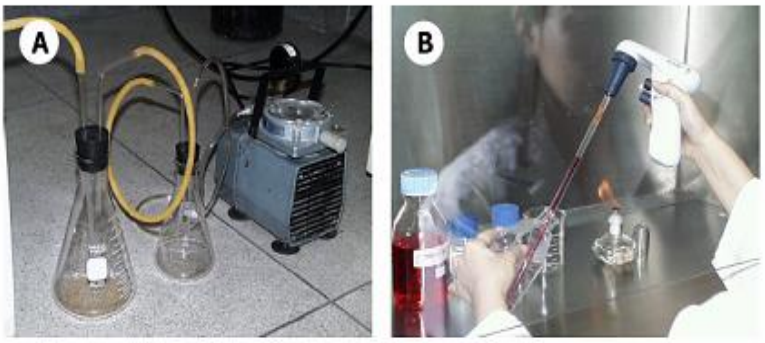
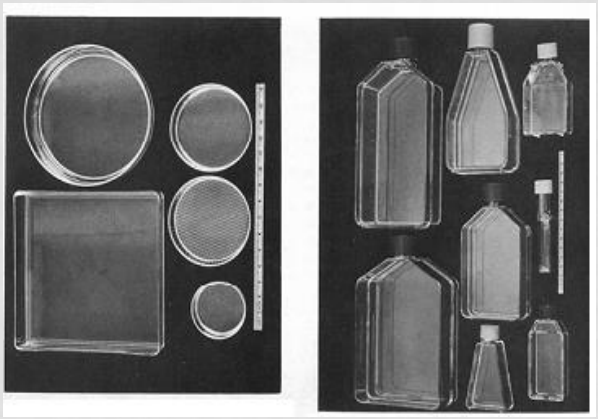


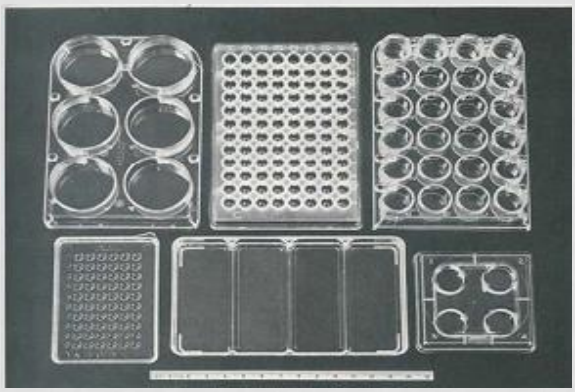
Figure 5: A: The vacuum pump with a 'trapping flask' protection. B: Glass or plastic pipettes with some form of suction aid are practical for transferring larger volumes.

Fig. 4.10. a. Pipetting aids. Tip to bottom: various plain bulbs and bulb with inlet and outlet valves; Gilson and Finn pipette micropipettes (tube special plastic tips); Bellco Hypump (standard pipettes); (see also Figs. 4.12, 5.3, and 5.6). b. Costar multipoint pipettor for loading microtitration plates.

TISSUE CULTURE DISH & FLASK



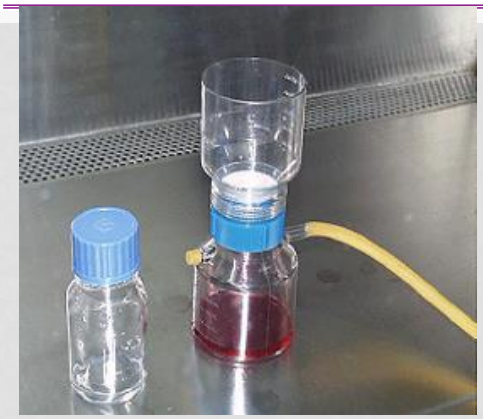
MULTIWELL PLATES



TANGKI NITROGEN

Hingga -200˚C

STERILISASI PENYARINGAN

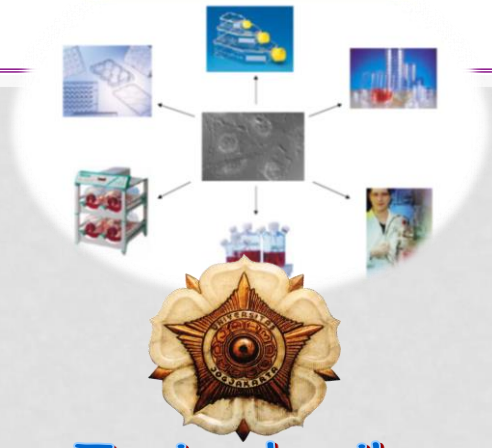


CENTRIFUGES

- There are centrifuges in each cell culture area which are refrigerated
- Human derived cells must be centrifuges in sealed rotors
- 100 x g is hard enough to sediment cells, higher g forces may damage cells
- If a tube breaks in the centrifuge, take the whole bucket into a cabinet and clean it there

- **Mammalian Cell Culture,** http://web.mnstate.edu/provost/biotech/Mammalian%20Cell%20Culture%20Lecture.ppt
- Introduction to Tissue culture, Sompol Tapechum M.D., Ph.D.,Department of Physiology, Faculty of Medicine Siriraj Hospital, http://www.ps.si.mahidol.ac.th/courseware/StoreResources/maram%20ali.ppt
- Basics of Cell Culture, **Paras Yadav[1], Annu Yadav[1], P. Kumar[1], J.S. Arora[1], T.K.Datta[1], S. De[1], S.L. Goswami[1], Mukesh Yadav[2], Shalini Jain[3], Ravinder Nagpal[4] and Hariom Yadav[3]**
  [1]Department of Animal Biotechnology, [3]Animal Biochemistry Division and [4]Dairy Microbiology Division, National Dairy Research Institute, Karnal 132001 (Haryana), India; [2]SOS in Chemistry, Jiwaji University, Gwalior-474011, M.P., India
  http://www.pitt.edu/~super7/32011-33001/32721.ppt

Cell culture vessels



Terimakasih
Semoga bermanfaat