UE: In-vitro Techniken und Zellkultur, Kurs 3 / 4

2SStd., Krammer / Kiesslich; 437239 / 437240

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lecture part Kiesslich

further reading**: Fundamental techniques in cell culture**

see additional links at**: www.uni-salzburg.at/pdt** (lectures)

Content:

1. Cell culture basics
    - design and equipment for cell culture laboratories
    - microbiological safety cabinets
    - centrifuges
    - incubators
    - culture receptacles
    - basic cell culture procedures
    - general aspects of medium composition
    - sterile working: the do`s and dont`s of cell culture
    - safety aspects

2. Examples of commonly used cell lines and the A431 cell line
    - morphology
    - maintenance

3. Basic cell culture techniques (practical examples within this course)

1. Cell culture basics

Design and equipment for cell culture laboratories

laboratory design: ideally divided into two areas:
an working area reserved for handling of new material (quarantine area)
an working area known to be free of contaminants (main tissue culture facility)

ideally, tissue culture is performed in a facility specially designed for and exclusively devoted to this kind of work.

- all new material should be handled as 'quarantine material' until it has been shown to be free of contaminants.

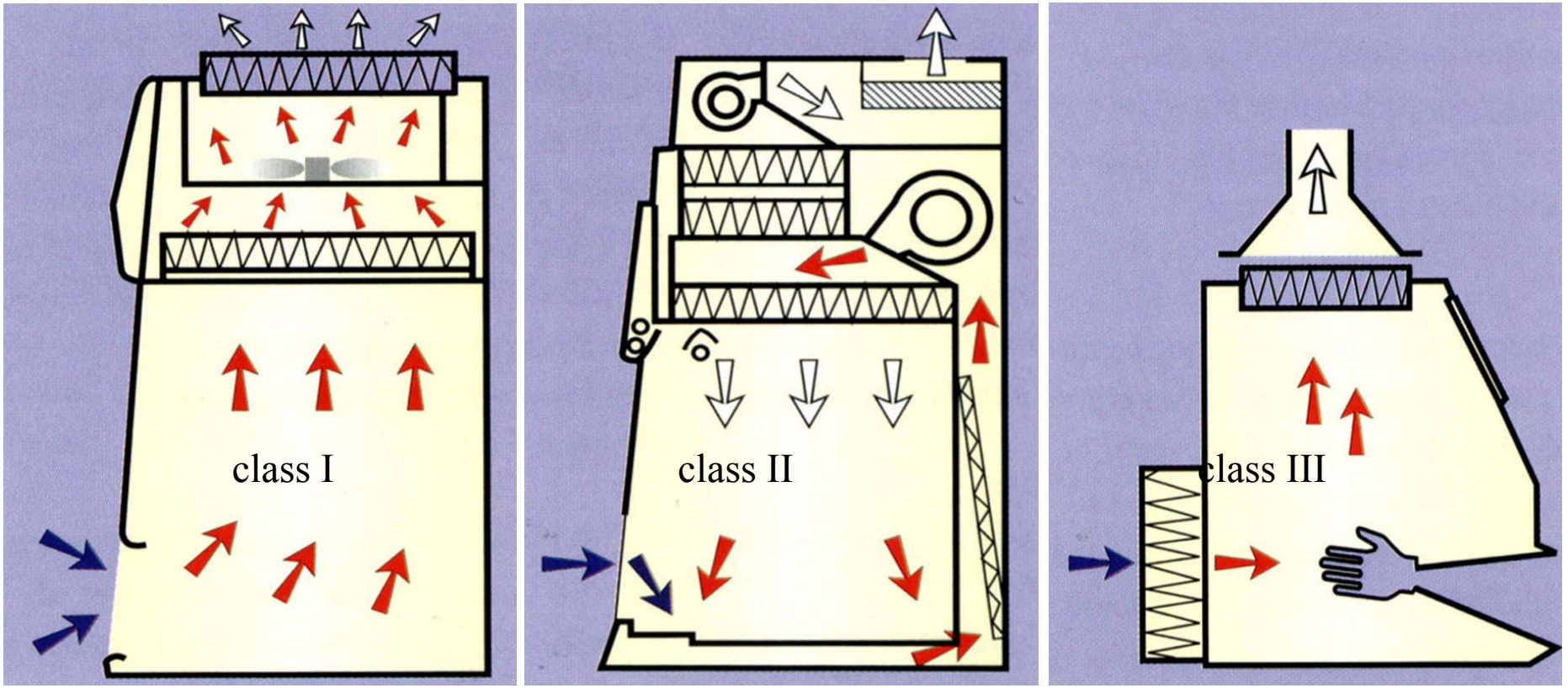
- all work surfaces should be cleaned between activities

- the nature of the cell material used may require additional considerations such as
  - the type of work surfaces and flooring
  - the type of microbiological safety cabinet
  - provision of hand washing facilities
  - provision of air pressure negative to corridors

see guidelines for handling of different kinds of cell material (Biosafety levels et al.) below…

Microbiological safety cabinets (Sterilbänke, Reinraumbänke)

- provision of a clean working environment for the product
- protection of the operator from aerosols
- level of protection / containment varies according to the class of cabinet used (I, II, III)
- product / operator protection is provided through the use of HEPA filters
  (high efficiency particulate air)



Centrifuges

by their very nature, centrifuges produce aerosols; when working with dangerous material, this risk can be minimised by model that use

- **sealed buckets**
- **a clear lid** (allows checking the condition of the load before opening)

Careful balance the load and do not ovefill tubes.

Incubators

...provide correct and stable growth conditions for the cell material such as:

- **temperature**: 28° C (insect cell lines) to 37° C (mammalian cells)
- **CO_2**: contributes to pH regulation in bicarbonate / CO_2 buffering systems (5-10%)
- **humidity**: carbonate-buffered media requires gas exchange with the atmosphere of the culture receptacle through loosely closed / permeable culture stoppers; therefore, evaporation of the media must be prevented to ensure correct osmolarity by provision of high humidity in the incubator (~100%). This can be achieved by
  * a water bath within the incubator, or
  * incubators with integrated humidity control.
- **copper-coated incubators:** reduction of the risk of microbiological infection by the inhibitory action of copper on microbes.

Culture receptacles

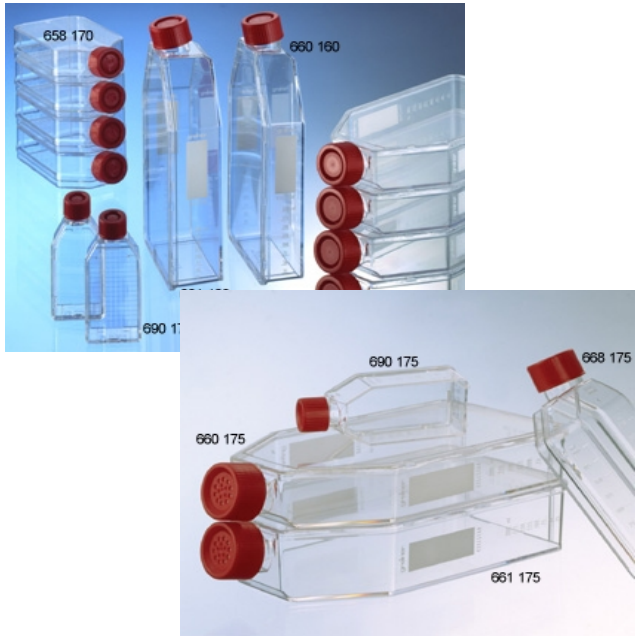
For anchorage-dependent cells (adherent cells), only culture dishes / flasks with a specially designed surface are needed. ‘tissue culture treated’ material contains a hydrophilic surface to facilitate anchorage of cells.

Any type of culture vessel and additional consumables such as tubes and pipettes is available as single-use, steril-packed plasticware.

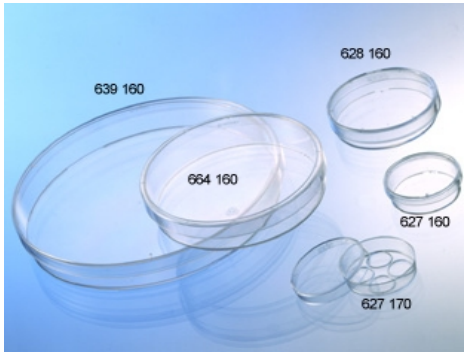
Culture flasks are available with closed caps (including a filter in the cap; this types requires to open the cap (1/2 turn) for incubation in a CO_2-incubator) or with permeable (filter-) caps.

	purpose	steril working
Culture flasks	Maintenance of cell cultures / cell lines	+++
Petri dishes	Experimentation	+
Multi-well plates	Experimentation	++

- tissue culture flasks
(12.5, 25, 75, 175 cm²)

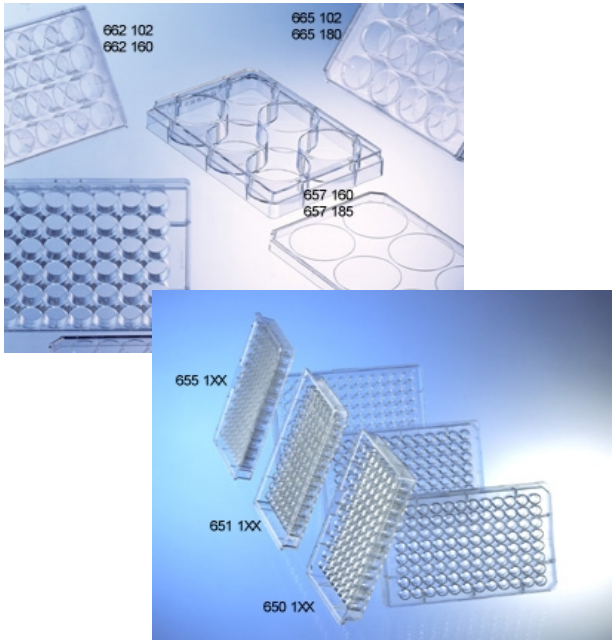


- petri dishes
(30, 60, 100 mm)



[Images from Greiner-BioOne
(any other supplier is suitable as well)]

- multi-well plates
(6, 12, 24, 48, 96, 384)



Basic cell culture procedures

…a little glossary of commonly used terms:

split, subcultivate, passage: (maintenance of a cell line) transfer of a certain amount of cell from one culture vessel to another.

passage number: number of subcultivations (e.g. starting from a primary culture); caution (!): some characteristics, e.g. the sensitivity toward cellular stress may change with increasing passage number.

adherent / suspension cultures: cells that are anchorage-dependent or not, respectively.

primary culture: this material is derived directly from animal tissue either as an explant culture or after dissociation into a cell suspension by enzyme digestion. Such cultures are usually heterogeneous and have a limited life span. Many characteristics of differentiation are maintained during the (short) in vitro cultivation.

cell line / continuous cultures: a well characterised, and (commercially) available type of cell. Cells with limited lifespan show characteristics of differentiation and can be propagated for only approx. 30 cell divisions; thereafter, they senesce. Other cell lines can be propagated infinitely by subcultivation.

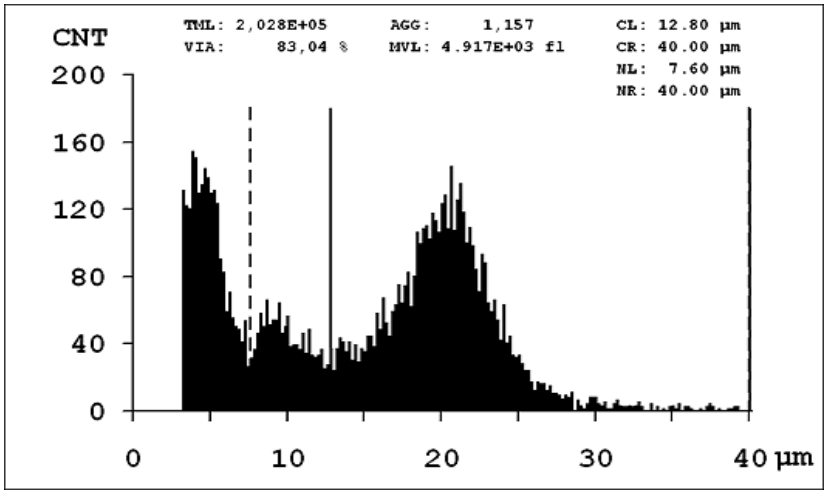
transformed cells: cell cultures with unlimited lifespan usually represent transformed cell lines (they are either derived from actual clinical tumours or transformation is induced by viral oncogenes or chemical treatments); transformed cell lines have the advantage of unlimited availability, but the disadvantage of having retained very little of the original in vivo characteristics.

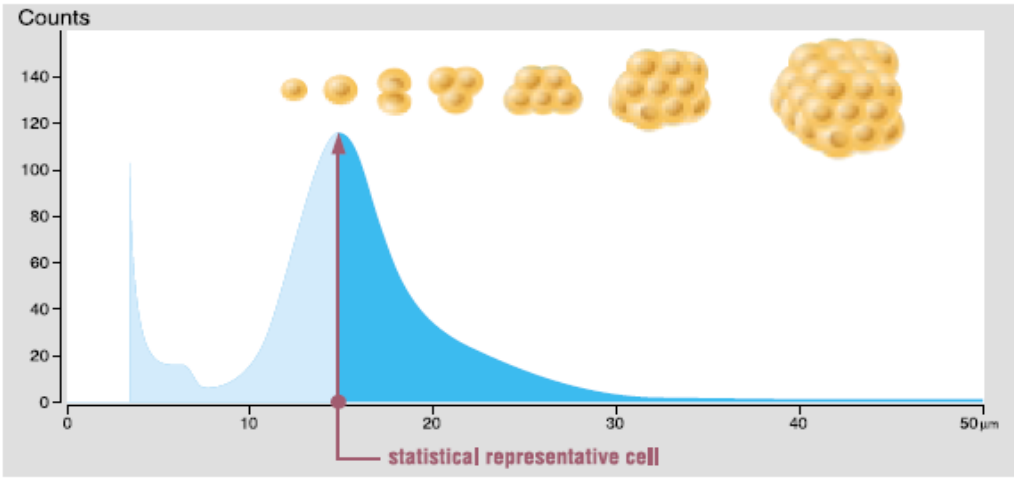
►Cell counting

Electronic cell counting (Schaerfe-systems' CASY-1 TT) is based on the measurement of the conductance / resistance of a cell solution passing through a capillary. Every cell generates an increase in resistance depending on the diameter, that is counted as an event; finally, a histogram is generated – count number versus diameter.

advantage: high sample number processed

disadvantage: no discrimination between dead and alive cells; aggregates of cells need careful evaluation.





►Subcultivation

Typical working steps of subcultivation:

- after removal of the old medium, the cell layer is washed with PBS (phosphate buffered saline)
- most cultures are detached from the substrate by the use of proteases such as trypsin. EDTA (a chelator) is added to facilitate cell detachment, since several cell-matrix proteins require the presence of divalent cations (such as Mg^{++} or Ca^{++})
- after centrifugation, cells are resuspended in an appropriate volume, counted and an aliquot is transferred into a new flask / dish.

Key Points:

- [aseptic technique / sterile working]
- trypsin must be inactivated to prevent damage to cells; trypsin is inhibited in the presence of serum (FCS) - ~1/10 volume of FCS is sufficient to stop trypsin.
- apply trypsin only as long cells attach to the substrate (can be accelerated by shaking the flasks/dishes)
- adherent cells need some time to attach again; activities such as change of the media require fully attached cells (~8-12 hrs post subcultivation).

►Cryopreservation of cell lines

Cell lines are obtained (commercially) as frozen cultures (at -80° C (dry ice) or -196° C (liquid nitrogen)). Cryopreservation of cell stocks are performed similarly.

Typical working steps of cryopreservation:

- harvest cells as usual, spin down, count and resuspend in an appropriate volume of freeze medium (usual FCS concentration including 10% v/v DMSO (dimethyl sulfoxide))
- transfer 1ml-aliquots in cryo-vials labeled with name of the cell line, passage number, date and cell density.
- freeze solutions in a -80° C freezer; thereafter the vials to the gaseous phase of a liquid nitrogen storage vessel and record the location.

Key Points:

- [aseptic technique / sterile working]
- DMSO or glycerol is added to the freeze-medium as a cryoprotentant which helps to avoid development of ice crystals within the cells during freeze / thaw procedure.
- DMSO (dimethyl sulfoxide) is toxic to cells above 4° C; freezing to -80° C helps to shorten the time required.
- a certain number of cells is killed by the freeze / thaw process; therefore, the cell number should be large enough to ensure sufficient cell density after thawing.

►Resuscitation of frozen cell lines

Typical working steps of resuscitation:

- thaw ampoule of frozen cells in a 37° C water bath
- transfer solution containing cells into a large volume of pre-warmed medium
- after centrifugation, cells are resuspended in an appropriate volume and transferred into a new flask / dish.
- check cells after 24hrs post resuscitation

Key Points:

- [aseptic technique / sterile working]
- DMSO or glycerol is added to the freeze-medium as a cryoprotentant which helps to avoid development of ice crystals within the cells during freeze / thaw procedure.
- DMSO (dimethyl sulfoxide) is toxic to cells above 4° C; after thawing the cultures, the solution needs to be diluted as soon as possible with a large volume of pre-warmed medium.
- all thawing / freezing steps should be performed as quickly as possible to minimize the exposure of cells to DMSO.

General aspects of medium composition

Basic Constituents of media: Inorganic salts, Carbohydrates, Amino Acids, Vitamins, Fatty acids and lipids, Proteins and peptides, Serum

Inorganic Salts:

The inclusion of inorganic salts in media performs several functions. Primarily they help to retain the osmotic balance of the cells and help regulate membrane potential by provision of sodium, potassium and calcium ions. All of these are required in the cell matrix for cell attachment and as enzyme cofactors.

Carbohydrates:

The main source of energy is derived from carbohydrates generally in the form of sugars. The major sugars used are glucose and galactose however some media contain maltose or fructose. The concentration of sugar varies from basal media containing 1g/l to 4.5g/l in some more complex media. Media containing the higher concentration of sugars are able to support the growth of a wider range of cell types.

Buffering Systems:

Most cells require pH conditions in the range 7.2 - 7.4 and close control of pH is essential for optimum culture conditions. There are major variations to this optimum. Fibroblasts prefer a higher pH (7.4 - 7.7) whereas, continuous transformed cell lines require more acid conditions pH (7.0 - 7.4). Regulation of pH is particularly important immediately following cell seeding when a new culture is establishing and is usually achieved by one of two buffering systems; (i) a "natural" buffering system where gaseous CO_2 balances with the CO_3 / HCO_3 content of the culture medium and (ii) chemical buffering using a zwitterion called HEPES.

Cultures using natural bicarbonate/CO_2 buffering systems need to be maintained in an atmosphere of 5-10% CO_2 in air usually supplied in a CO_2 incubator. bicarbonate/CO_2 is low cost, non-toxic and also provides other chemical benefits to the cells.

HEPES has superior buffering capacity in the pH range 7.2 - 7.4 but is relatively expensive and can be toxic to some cell types at higher concentrations. HEPES buffered cultures do not require a controlled gaseous atmosphere.

Most commercial culture media include phenol red as a pH indicator so that the pH status of the medium is constantly indicated by the color. Usually the culture medium should be changed / replenished if the color turns yellow (acid) or purple (alkali).

Vitamins

Serum is an important source of vitamins in cell culture. However, many media are also enriched with vitamins making them consistently more suitable for a wider range of cell lines. Vitamins are precursors for numerous co-factors. Many vitamins especially B group vitamins are necessary for cell growth and proliferation and for some lines the presence of B12 is essential. Some media also have increased levels of vitamins A and E. Vitamins commonly used include riboflavin, thiamine and biotin.

Proteins and Peptides

These are particularly important in serum free media. The most common proteins and peptides include albumin, transferrin, fibronectin and fetuin and are used to replace those normally present through the addition of serum to the medium.

Fatty Acids and Lipids

Like proteins and peptides these are important in serum free media since they are normally present in serum. e.g. cholesterol and steroids essential for specialized cells.

Trace Elements

These include trace elements such as zinc, copper, selenium and tricarboxylic acid intermediates. Selenium is a detoxifier and helps remove oxygen free radicals.

Serum

Serum is a complex mix of albumins, growth factors and growth inhibitors. The most commonly used serum is fetal bovine serum. (Other types: newborn calf serum / horse serum. The quality, type and concentration of serum can all affect the growth of cells and it is therefore important to screen batches of serum for their ability to support the growth of cells.

+ Serum is also able to increase the buffering capacity of cultures (important for slow growing cells or where the seeding density is low (e.g. cell cloning experiments)).
+ A further advantage of serum is the wide range cell types with which it can be used despite the varying requirements of different cultures in terms of growth factors. In addition serum is able to bind and neutralize toxins.
- However, serum is subject to batch-batch variation that makes standardization of production protocols difficult.
- There is also a risk of contamination associated with the use of serum.

These risks can be minimized by obtaining serum from a reputable source since suppliers of large quantities of serum perform a battery of quality control tests and supply a certificate of analysis with the serum. Heat inactivation of serum (incubation at 56ºC for 30 minutes) can help to reduce the risk of contamination since some viruses are inactivated by this process.

Media type	**Examples**	**Uses**
Balanced salt solutions	PBS, Hanks BSS, Earles salts, DPBS, HBSS	Form the basis of many complex media
Basal media	MEM	Primary and diploid cultures.
	DMEM	Modification of MEM containing increased level of amino acids and vitamins. Supports a wide range of cell types including hybridomas.
	GMEM	Glasgows modified MEM (defined for BHK-21 cells)
Complex media	RPMI 1640	Originally derived for human leukaemic cells. It supports a wide range of mammalian cells including hybridomas
	Iscoves DMEM	Further enriched modification of DMEM which supports high density growth
	Leibovitz L-15	Designed for CO_2 free environments
	TC 100, Grace's Insect Medium, Schneider's Insect Medium	Designed for culturing insect cells
Serum Free Media	CHO	For use in serum free applications.
	Ham F10 and derivatives Ham F12, DMEM/F12	These media must be supplemented with other factors such as insulin, transferrin and epidermal growth factor. These media are usually HEPES buffered
Insect cells	Sf-900 II SFM, SF Insect-Medium-2	Specifically designed for use with Sf9 insect cells

Inorganic Salts	mg/l
Calcium Chloride anhydrous	200
Ferric(III)-Nitrate · 9H2O	0.1
Potassium Chloride	400
Magnesium Sulphate anhydrous	97.7
Sodium Chloride	6400
Sodium Dihydrogen Phosphate · H2O	125
Sodium Bicarbonate	3700
Amino Acids	
L-Arginine · HCl	84
L-Cysteine	48
Glycine	30
L-Histidine · HCl · H2O	42
L-Isoleucine	105
L-Leucine	105
L-Lysine · HCl	146
L-Methionine	30
L-Phenylalanine	66
L-Serine	42
L-Threonine	95
L-Tryptophan	16
L-Tyrosine	72
L-Valine	94
Vitamins	
D-Pantothenic Acid (hemicalcium)	4
Choline Chloride	4
Folic Acid	4
myo-Inositol	7.2
Nicotinamide	4
Pyridoxal · HCl	4
Riboflavin	0.4
Thiamine · HCl	4
Other Components	
D-Glucose anhydrous	4500
Phenol Red	15

E15-011, DMEM from
PAA laboratories
http://www.paa.at/

Sterile working: The do`s and dont`s of cell culture

the DOs

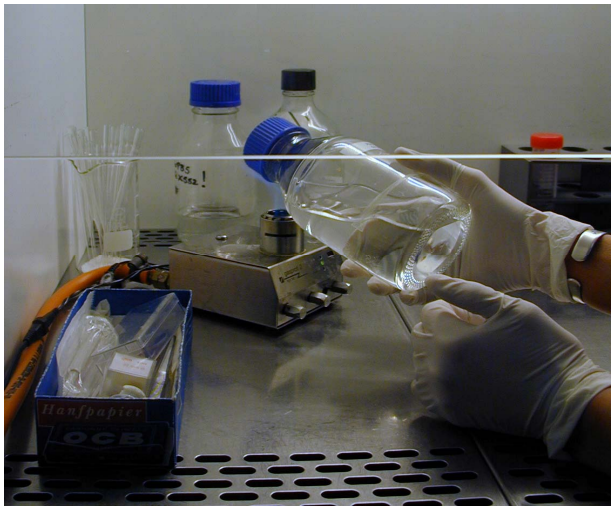
- inform personnel about potential risks when handling with cell material and reagents.
- use personal protective equipment at all times (lab coat, gloves)
- keep all work surfaces free of clutter
- correctly label all solutions / material including the date of preparation
- only work with one cell line at a time (wait for 15 min between working steps using different cell lines)
- wash your hands after leaving the lab (every time)
- newly established cell cultures should be handled in quarantine until quality control checks are complete
- whenever possible, maintain separate bottles of reagents / media for different cells
- examine cells daily for signs of contamination

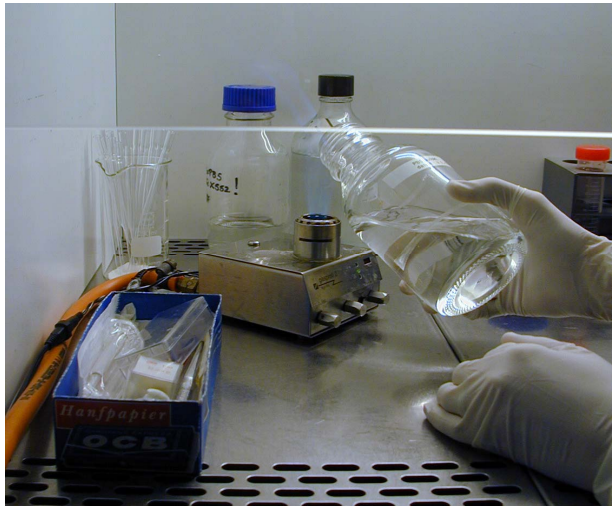
the DONTs

- don’t work with material / reagents of unknown potential danger
- don’t allow waste to accumulate especially in the safety cabinet
- don’t have too many people in the lab at one time
- don’t keep cells continuously in culture without returning to a frozen stock
- avoid cultures becoming fully confluent (subcultivate at 70-80% confluency)
- don’t mix cultures at different passage numbers

Sterile working:

- clean working area (safety cabinet) before handling with cell cultures (70% ethanol)
- perform sterile work in the middle of the safety cabinet (within the laminar air circulation)
- don't use plastic ware of unknown quality (replace in case of *any* doubt)
- pipettes, media etc. must not get in contact with any material, e.g. the outside of the media / buffer bottles
- don't speech, sneeze or cough into the air stream of the safety cabinet
- move slowly in the safety cabinet to ensure proper air circulation
- sterilize bottle / plastic cups and threads *every time* the bottle / plastic tube is opened or closed (also with cell culture flasks and cups) by swinging through a propane gas flame (see images)
- don't vortex and / or invert bottles, tubes etc. by shaking





Safety aspects

For animal cell culture the level of risk is dependent upon the cell line to be used and is based on whether the cell line is likely to cause harm to humans. Classification of risks are given below (fundamental techniques in cell culture, Sigma-Aldrich):

low risk:
non human / non primate continous cell lines and some well characterized diploid line of finite lifespan.

medium risk:
poorly characterized mammalian cell lines

high risk:
- cell lines derived from human / primate tissue or blood
- cell lines with endogenous / exogenous pathogens (classification is dependent upon the pathogen)

Safety guidelines using 'Biosafety level' (1-4) classification are available from the CDC (center of disease control) at

http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm
biosafety level 1 guidelines quoted from the above link:

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

The following standard and special practices, safety equipment and facilities apply to agents assigned to Biosafety Level 1:

A. **Standard Microbiological Practices**

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.
9. A biohazard sign can be posted at the entrance to the laboratory whenever infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the investigator.
10. An insect and rodent control program is in effect (see Appendix G).

B. **Special Practices None**

C. **Safety Equipment (Primary Barriers)**

1. Special containment devices or equipment such as a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.
2. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.

3. Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.

4. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.

2. Each laboratory contains a sink for handwashing.

3. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.

4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.

5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.

6. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

2. Examples of commonly used cell lines:

Source: ATCC (http://www.lgcpromochem.com/atcc/)

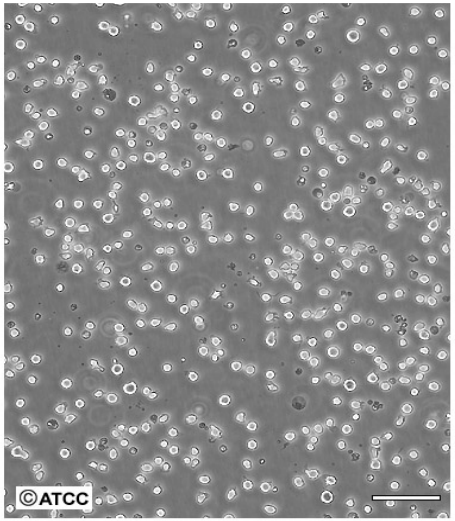
• **U-937:** *Homo sapiens* (human), Growth Properties: suspension, Morphology: monocyte; The U-937 cell line was derived by Sundstrom and Nilsson in 1974 from malignant cells obtained from the pleural effusion of a patient with histiocytic lymphoma. ATCC Number: CRL-1593.2. Biosafety Level 1.

• **3T3-Swiss albino:** *Mus musculus* (mouse) (murine), Growth Properties: adherent, Morphology: fibroblast; The 3T3 cell line was established by G. Todaro and H. Green in 1962 from disaggregated Swiss mouse embryos. ATCC Number: CCL-92. Biosafety Level 1.

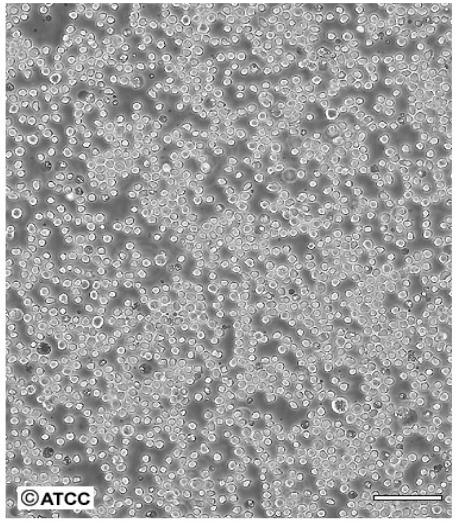
• **HeLa:** *Homo sapiens* (human), Growth Properties: adherent, Morphology: epithelial; Organ: cervix, Cell type: epithelial, Disease: adenocarcinoma . ATCC Number: CCL-2. Biosafety Level 2.

• **CHO:** *Cricetulus griseus* (hamster, Chinese), Growth Properties: adherent, Morphology: epithelial; The CHO-K1 cell line was derived as a subclone from the parental CHO cell line initiated from a biopsy of an ovary of an adult Chinese hamster by T. T. Puck in 1957. ATCC Number: CCL-61. Biosafety Level 1.

ATCC Number: **CRL-1593.2**
Designation: **U-937**



Low Density Scale Bar = 100µm



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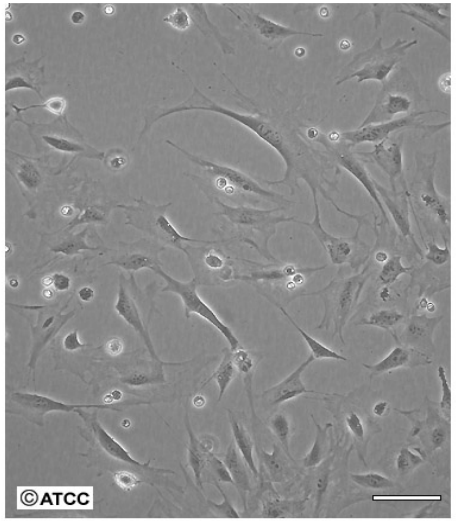
ATCC Number: **CCL-92**
Designation: **3T3 Swiss Albino**



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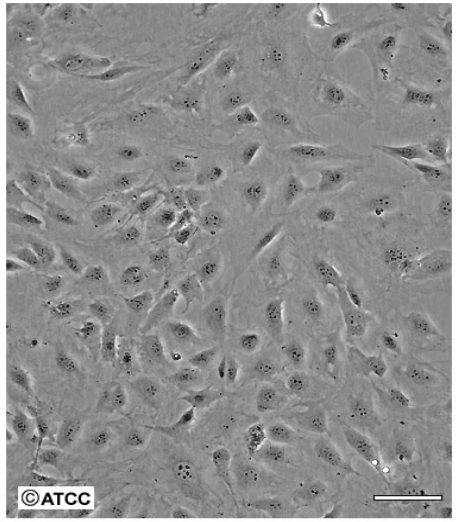

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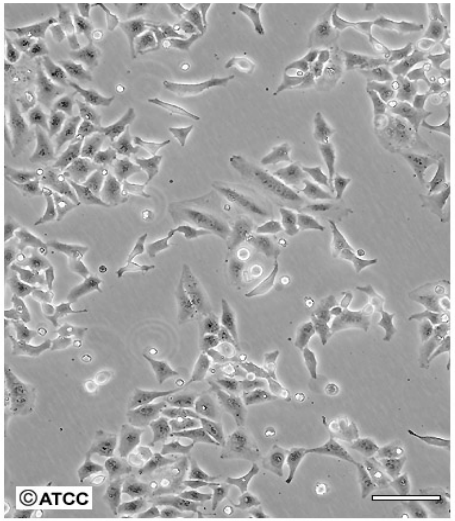
ATCC Number: **CCL-2**
Designation: **HeLa**



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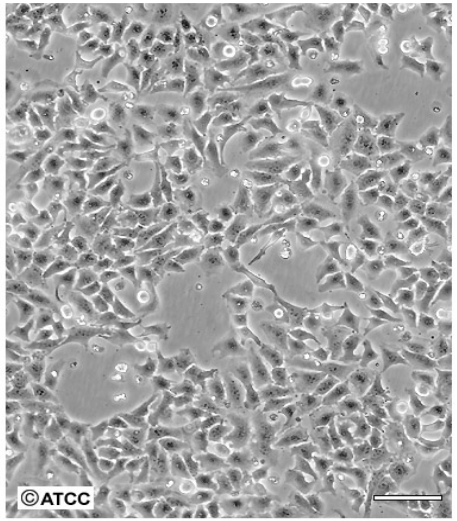
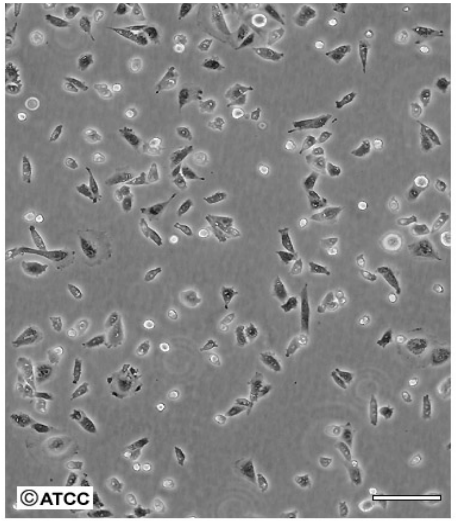


High Density Scale Bar = 100µm

ATCC Number: **CCL-61**
Designation: **CHO-K1**



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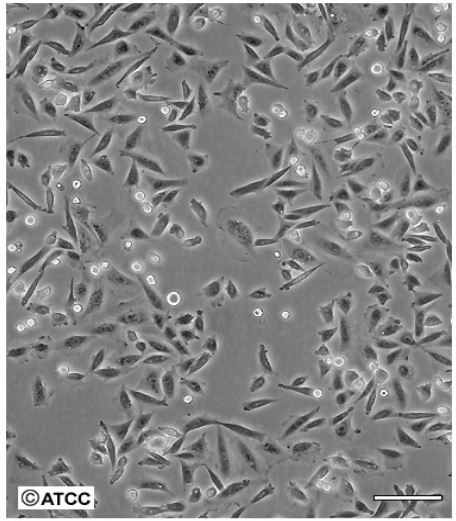
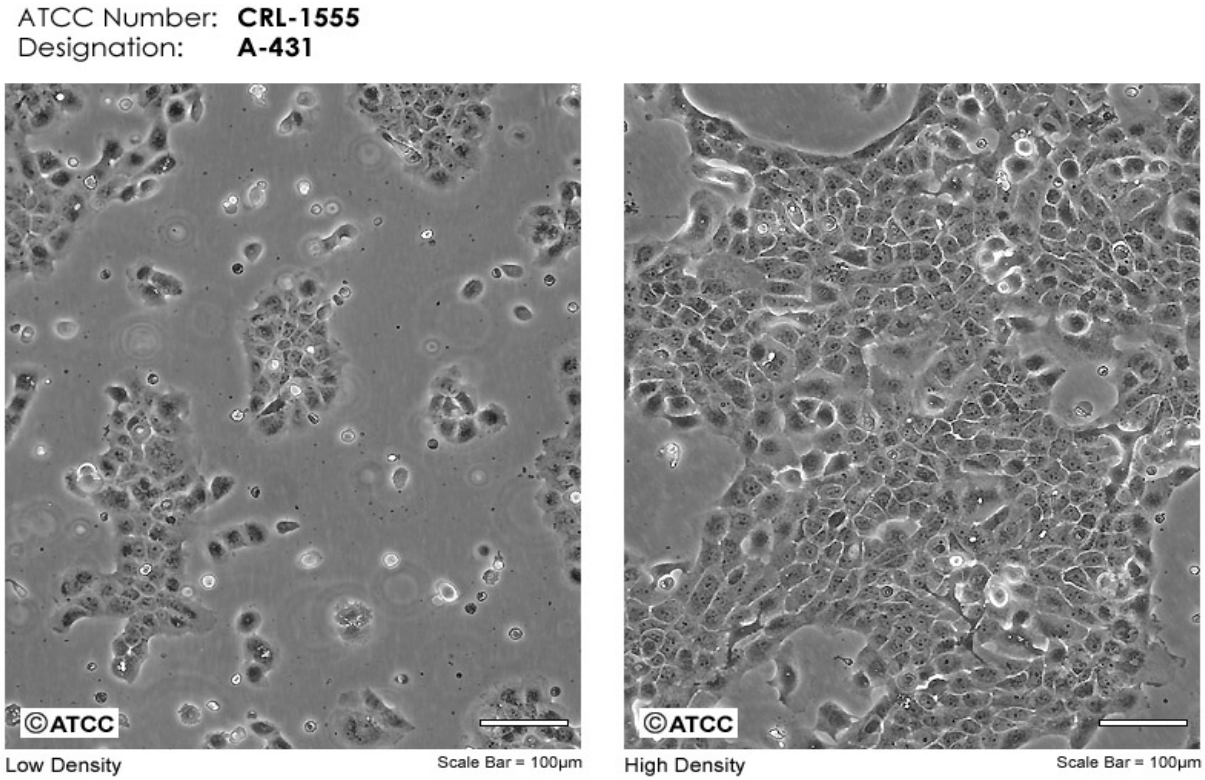


High Density Scale Bar = 100µm

The A431 cell line: human epidermoid carcinoma cell line

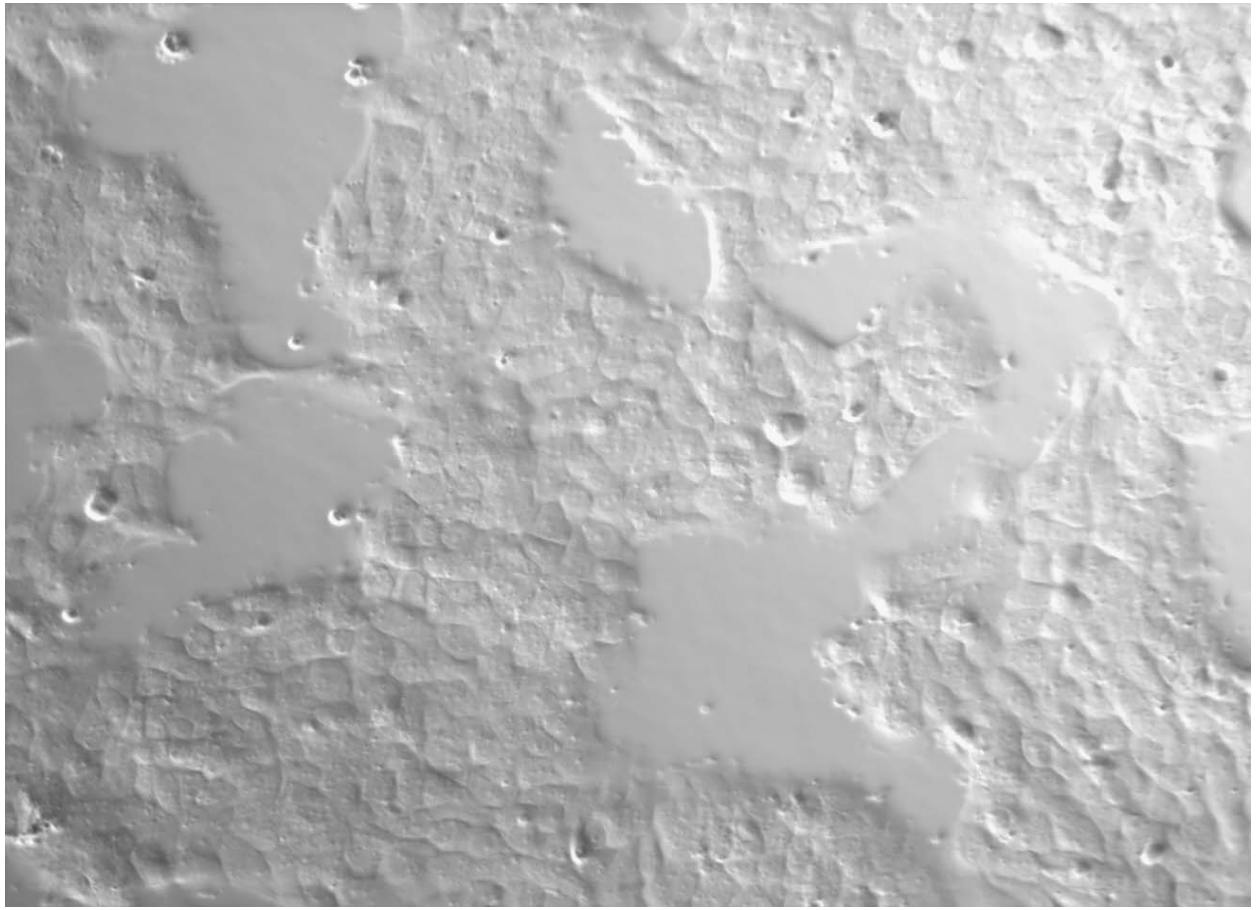
Homo sapiens (human), Growth Properties: adherent, Morphology: epithelial; The epidermoid carcinoma cell line A-431, derived from an 85-year-old female, is one of a series of cell lines established from solid tumors by D.J. Giard, et al.. ATCC Number: CRL-1555. Biosafety Level 1.

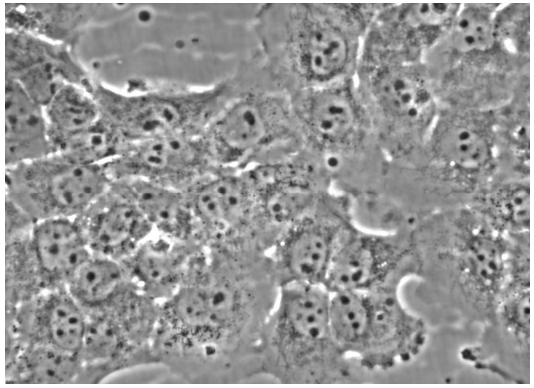
Organ: skin
Tissue: epidermis
Disease: epidermoid carcinoma

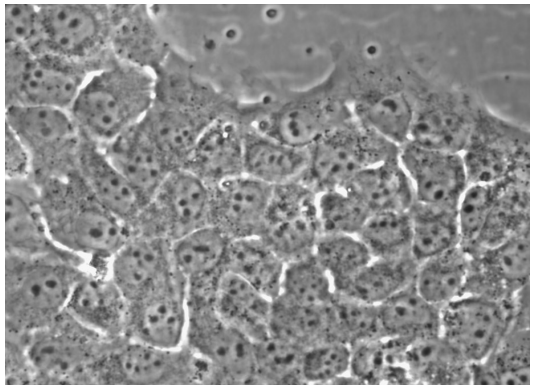


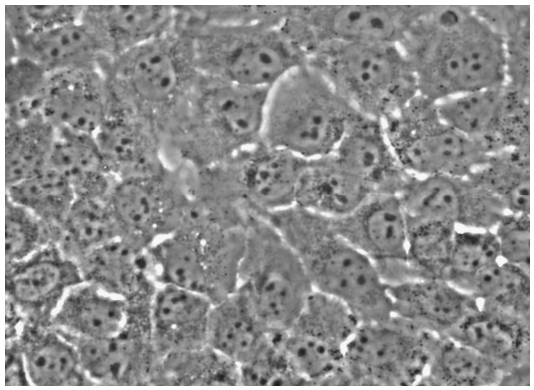
J Natl Cancer Inst. 1973 Nov;51(5):1417-23. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP.

Morphology:







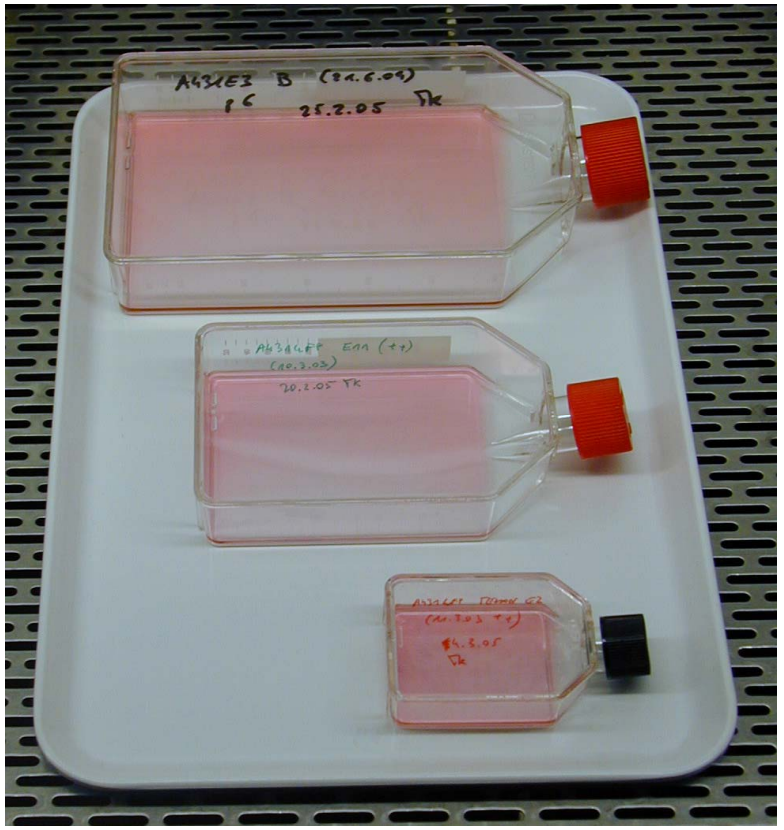


Phase contrast images were recorded by a SPOT-2 digital camera (Diagnostic instruments, Michigan, USA) mounted to a inverted fluorescence microscope (IX-70 Olympus).

Maintenance:

- **Medium**: Dulbecco's modified Eagle's Medium (DMEM; Sigma-Aldrich, Vienna, Austria) containing 4.5 $g.l^{-1}$ glucose supplemented with
  - –10 mM HEPES
  - –4 mM L-glutamine
  - –1 mM Na-pyruvate
  - –100 $U.ml^{-1}$ penicillin
  - –0.1 $mg.ml^{-1}$ streptomycin and
  - –5% (v/v) fetal calf serum (FCS)

(all from PAA-laboratories, Linz, Austria), in a humidified atmosphere at 37° C and 5% CO_2.
Volume: sufficient to completely cover the growth surface, see image.



- **Culture dishes**: petri dishes, culture flasks, multi-well plates designed for culture of adherent cells (‚tissue culture treated')

- **Split ratio / subcultivation**: maximum split ratio ~1:10 to 1:15

3. Basic cell culture techniques (course examples I)

Cell culture (Tutor: Mag. A. Moder / Mag. T. Kiesslich)

aim:

• cell culture practice, sterile working

procedure:

• passaging and counting cells

evaluation & discussion:

• description of the protocol; comparison of the counting methods

life – dead assays (Tutor: Mag. A. Moder / Mag. T. Kiesslich)

aim:

• comparison of two methods for quantization of the cell number (viability, cell death), namely the MTT assay and ALAMAR blue assay

procedure:

• a dilution series of A431 cells (seeded in on prev. day) is measured using both methods

evaluation & discussion:

• two diagrams showing the relationship between signal and cell number

• evaluation and comparison the dynamic range and upper / lower detection limit of the two assays

3. Basic cell culture techniques (course examples II)

FACS Analysis (Tutor: Mag. A. Moder[IV])

aim:

- principle of flow cytometric analysis (FACS)

procedure:

- Specific staining of surface antigens (GR-1 & CD8) for identification of neutrophil granulocytes and t-cells in spleen cells.
- necrosis detection (PI-stain of EtOH-treated samples, A431).

evaluation & discussion:

- description of the protocol
- evaluation (plot) of the different populations

3. Basic cell culture techniques (course examples III)

Protein determination (Tutor: Mag. T. Kiesslich)

aim:

•protein quantitation (BCA assay), determination of protein conc. in an unknown sample

procedure:

• dilution series of a protein (BSA, bovine serum albumine) of known concentration and some dilutions of FCS are measured with the BCA assay

evaluation & discussion:

• a diagrams showing the relationship between signal and protein concentration
• determination of the protein concentration in FCS

Intracellular GSH determination (Tutor: Mag. T. Kiesslich)

aim:

• determination of the intracellular concentration of GSH (glutathione) in A431 cells

procedure:

• a dilution series of A431 cells and a dilution series of GSH is measured (normal cells and glc-free samples)

evaluation & discussion:

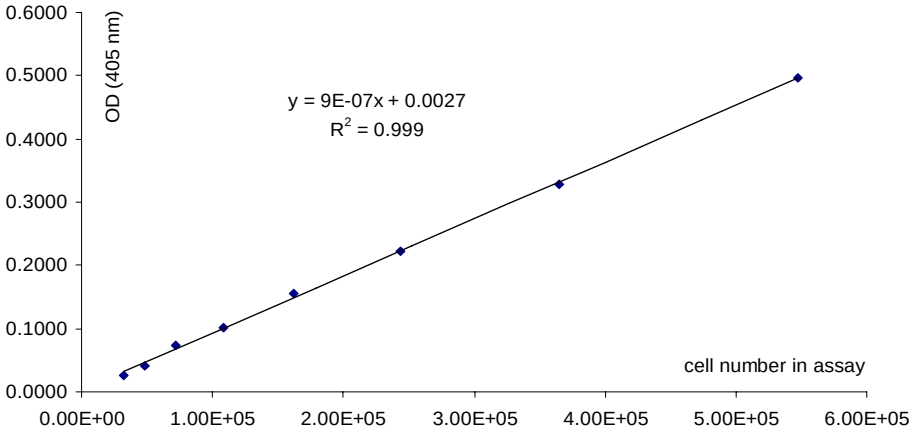
• two diagrams showing the relationship between signal and cell number / GSH
• calculation of the intracellular concentration [mol/l, M] (for an example see below…)

cell volume:

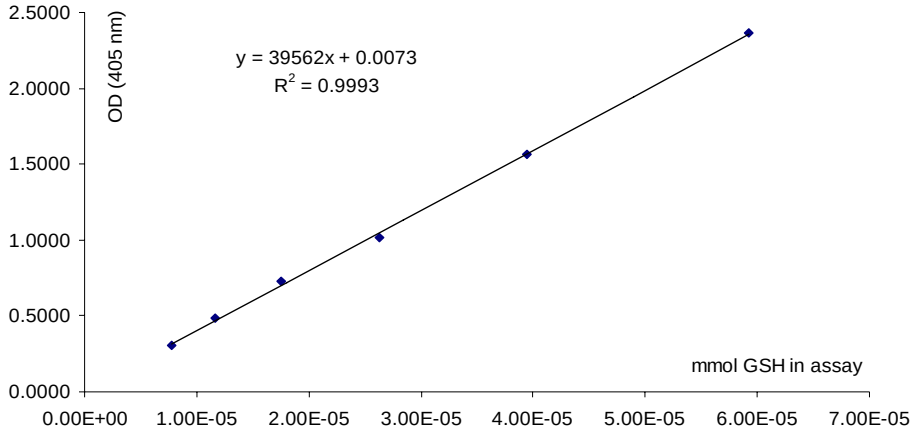
the cell volume needed for calculation of the intracellular concentration in moles per liters is obtained from cell counting (CASY cell counter); usually in the range of 18 µm. The calculation is based on the assumption that a cell represents a sphere whose volume can be calculated by the following formula:

$$V_{sphere} = \frac{4}{3} r^3 \pi$$

cell dilution series



GSH dilution series



relation cell number – OD:

500,000 cell give a certain OD (calc. from formula):

y(OD)=9E-7*x(cell number) + 0.0027

x=5E5, therefore y=9E-7*5E5+0.0027=0.4527 OD for 5E5 cells

calculation of the amount GSH in 5E5 cells:

500,000 cell give 0.4527 OD, this is converted into mmol GSH using the formula of the second diagram:

y(OD)=39562*x(mmol GSH) + 0.0073

x=(y-0.0073)/39562=(0.4527-0.0073)/39562=1.13E-5 mmol = 1.13E-8 mol GSH

calculation of the intracellular concentration:

500,000 cell contain 1.13E-8 mol GSH. The volume of one cell is:

$V=4/3*r^3*\pi=4/3*(9E-6)^3*\pi=3.05E-15\ m^3=3.05E-12$ l

The volume of 5E5 cells is: 3.05E-12 l * 5E5 = 1.53E-6 l

The molar concentration is c=n/v; therefore, the intracellular concentration of GSH in A431 cells is:

c=n/v=1.13E-8 mol GSH / 1.53E-6 l = 0.00738 mol/l = **7.38 mM**

UE: In-vitro Techniken und Zellkultur, Kurs 3 / 4

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April 2006

lecture part Kiesslich

further reading**: Fundamental techniques in cell culture**

see additional links at**: www.uni-salzburg.at/pdt** (lectures)