Organizacja i wyposażenie pracowni biotechnologii rozrodu

Dr Ricardo Faúndez
Katedra Chorób Dużych Zwierząt z Kliniką
Zakład Rozrodu Zwierząt, Andrologii i Biotechnologii Rozrodu

The assisted conception treatment cycle

- Consultation: history, examination, investigations, counseling, consent(s)
- Drug scheduling regimen: GnRH agonist pituitary downregulation or oral contraceptive pill to schedule withdrawal bleed
- Baseline assessment at start of treatment cycle
- Gonadotropin stimulation
- Follicular phase monitoring, ultrasound/endocrinology
- Induction of ovulation
- Oocyte retrieval (OCR)
- In-vitro fertilization/ICSI
- Embryo transfer
- Supernumerary embryo cryopreservation
- Luteal phase support
- Day 15–18 pregnancy test
- Ultrasound assessment to confirm gestational sac/fetal heartbeat

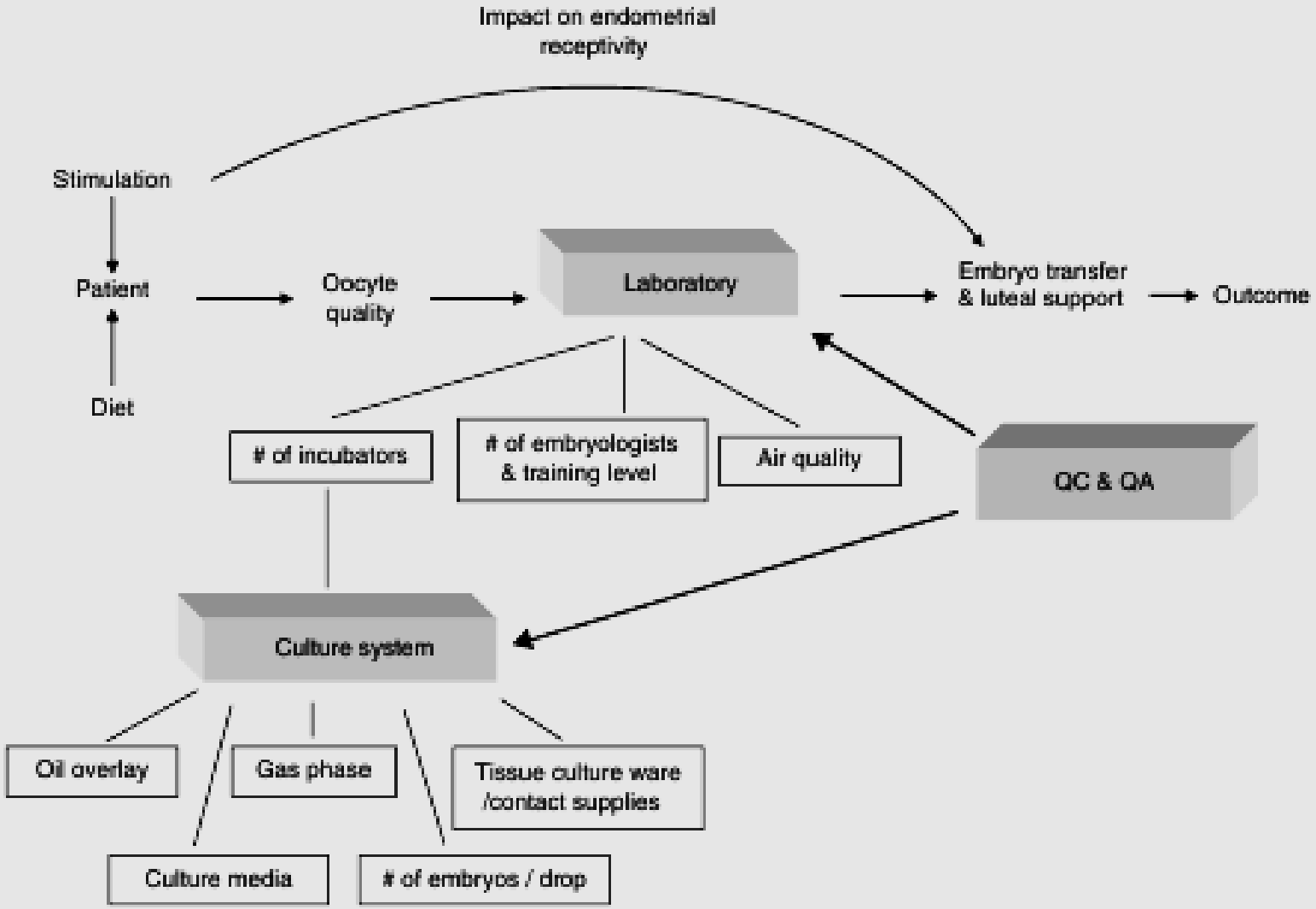


Figure 9.1 Critical elements of an ART treatment cycle (with thanks to David Gardner, Melbourne, Australia).

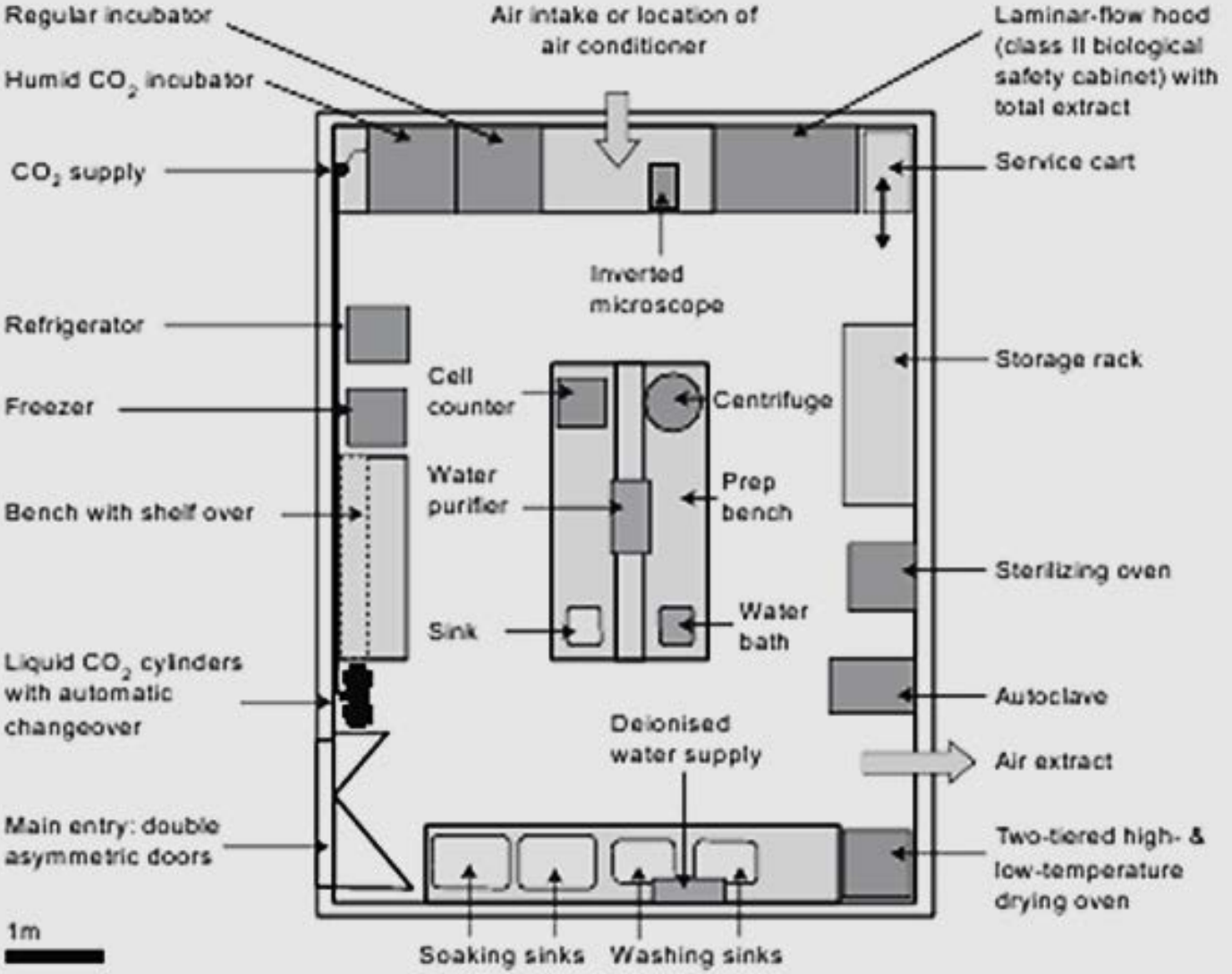


Fig. 4.1. Small Tissue Culture Laboratory. Suggested layout for simple, self-contained tissue culture laboratory for use by two or three persons. Dark-shaded areas represent movable equipment, lighter-shaded areas fixed or movable furniture. Scale 1:100.

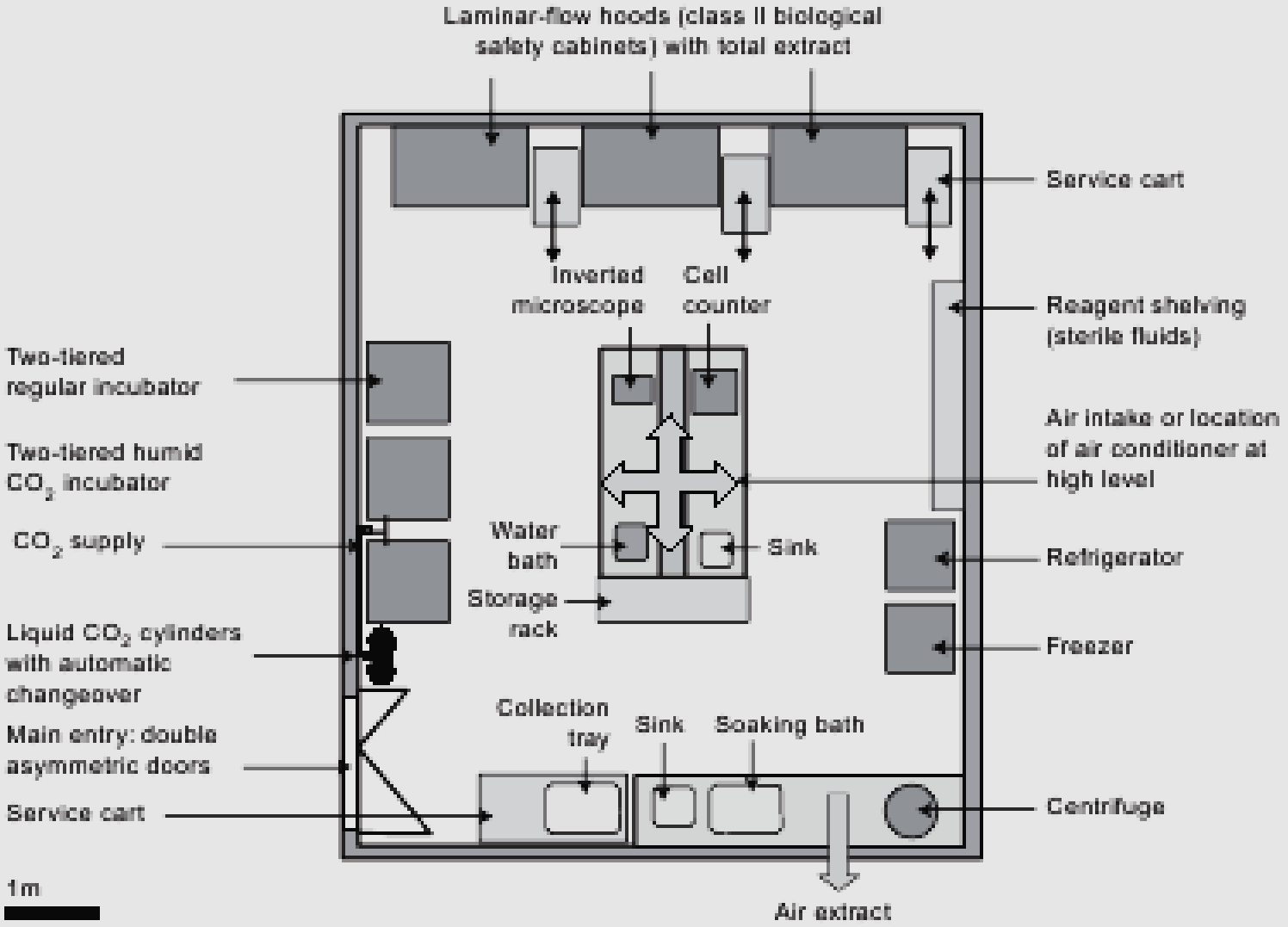


Fig. 4.2. Medium-Sized Tissue Culture Laboratory. Suitable for five or six persons, with washing up and preparation facility located elsewhere. Dark-shaded areas represent movable equipment, light-shaded areas movable or fixed furniture. Scale 1:100.

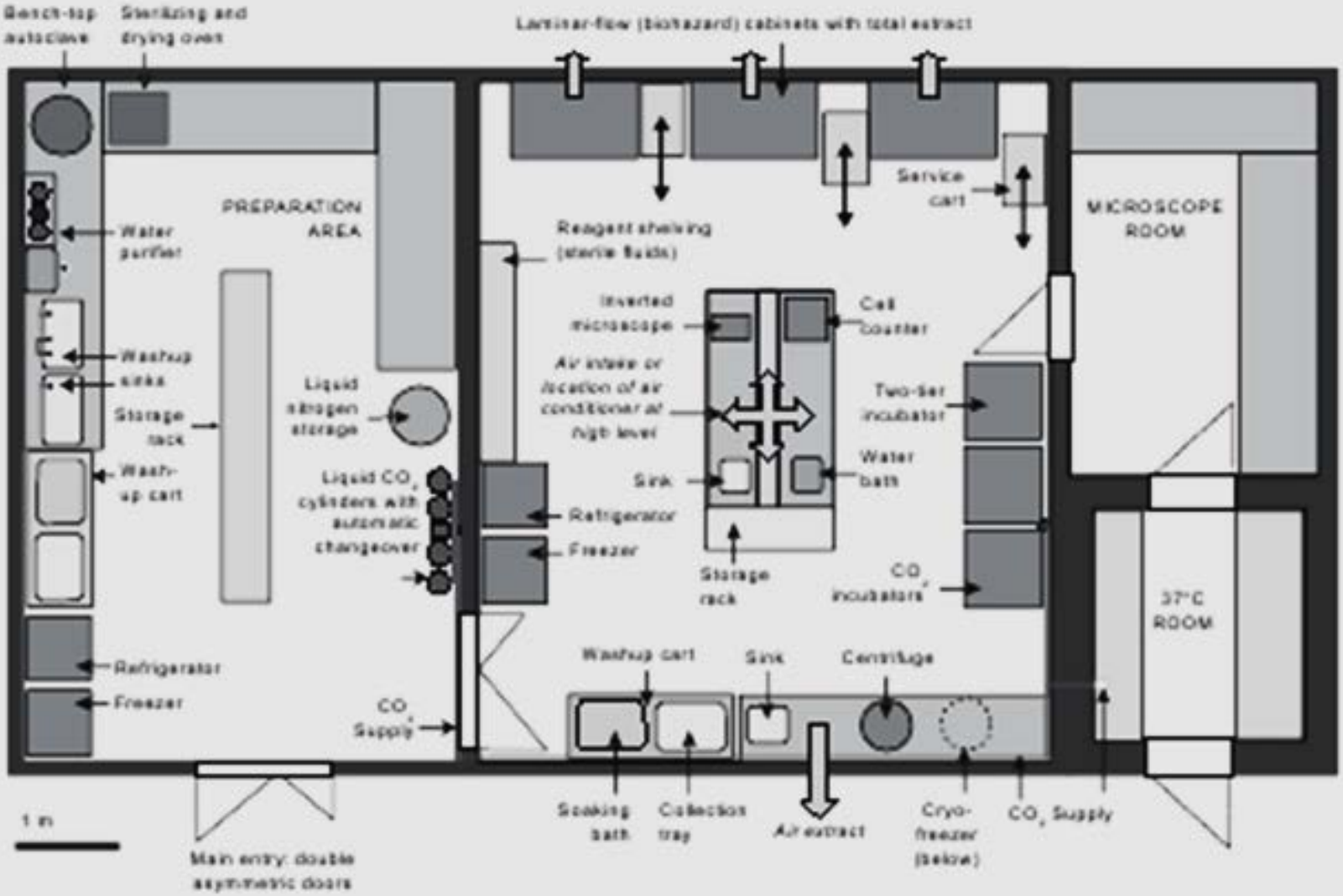


Fig. 4.3. Tissue Culture Lab with Adjacent Prep Room. Medium-sized tissue culture lab (*see* Fig. 4.2), but with attached preparation area, microscope room, and 37°C room. Scale 1:100.

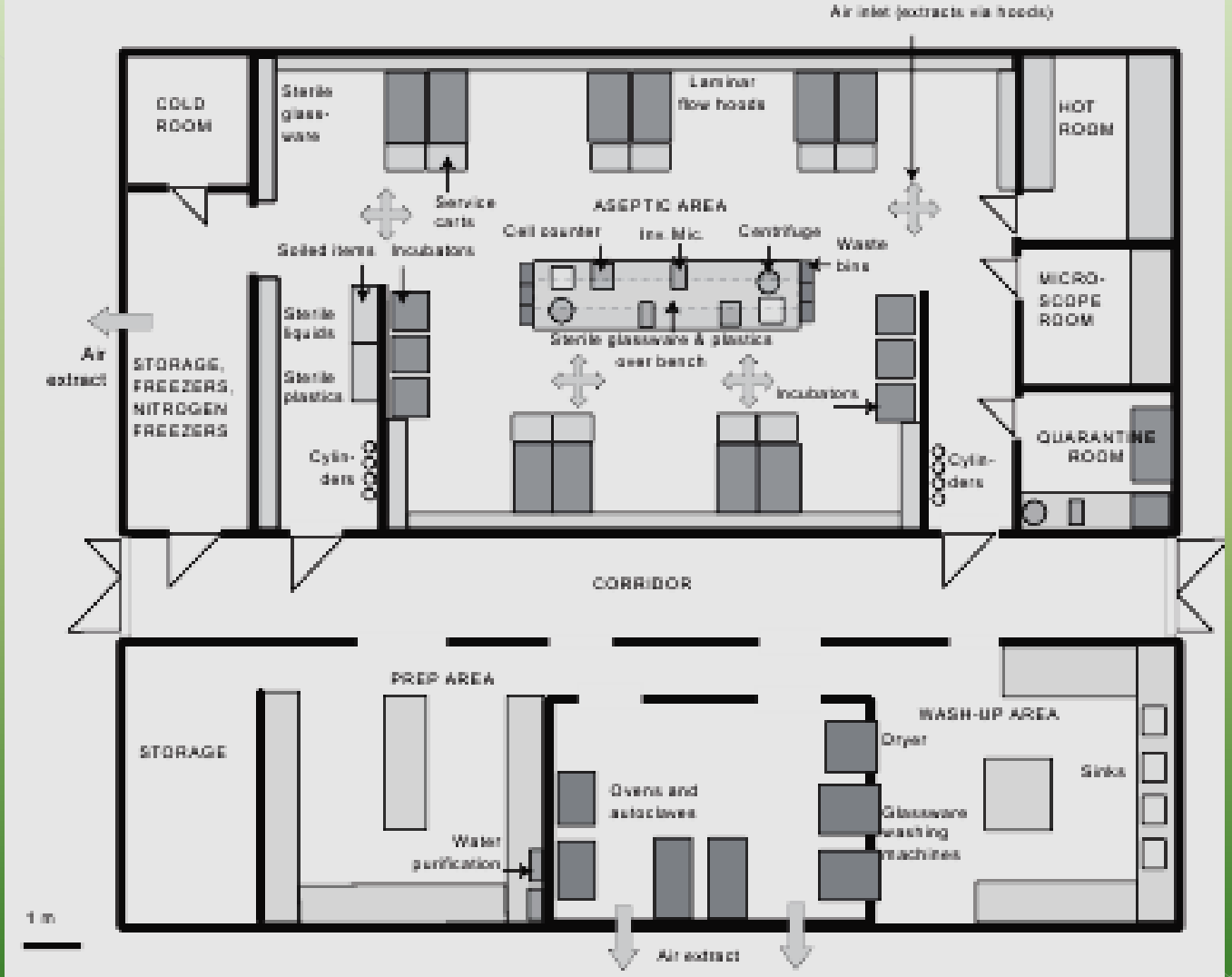


Fig. 4.4. Large Tissue Culture Laboratory. Suitable for 20 to 30 persons. Adjacent washing up, sterilization, and preparation area. Dark-shaded areas represent equipment, light-shaded areas fixed and movable furniture. Scale 1:200.

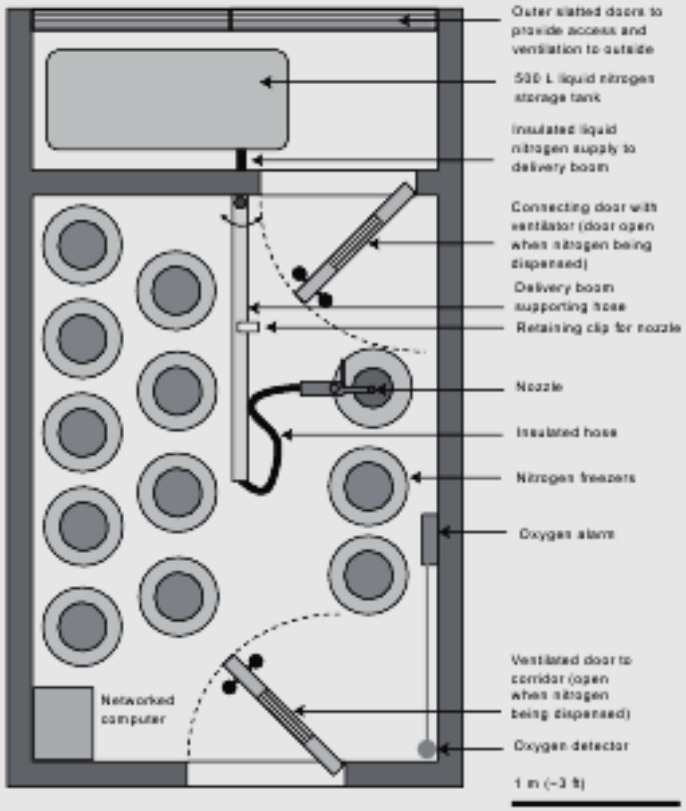


Fig. 4.7. Liquid Nitrogen Store and Freezer Store. The liquid nitrogen store is best located on an outer wall with ventilation to the outside and easy access for deliveries. If the freezer store is adjacent, freezers may be filled directly from an overhead supply line and flexible hose. Doors are left open for ventilation during filling, and a wall-mounted oxygen alarm with a low-mounted detector sounds if the oxygen level falls below a safe level.

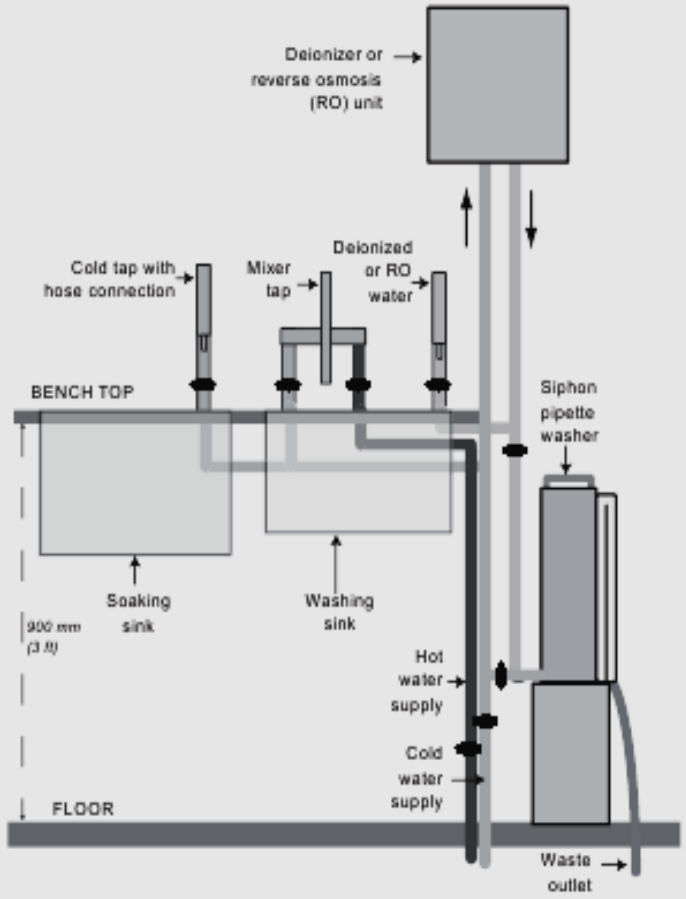


Fig. 4.6. Washing Up Sink and Pipette Washer. Suggested layout for soaking and wash up sinks, with hot, cold, and deionized water supplies. Scale 1:16.

Table 8.1(b) Classification of clean areas in terms of airborne particles

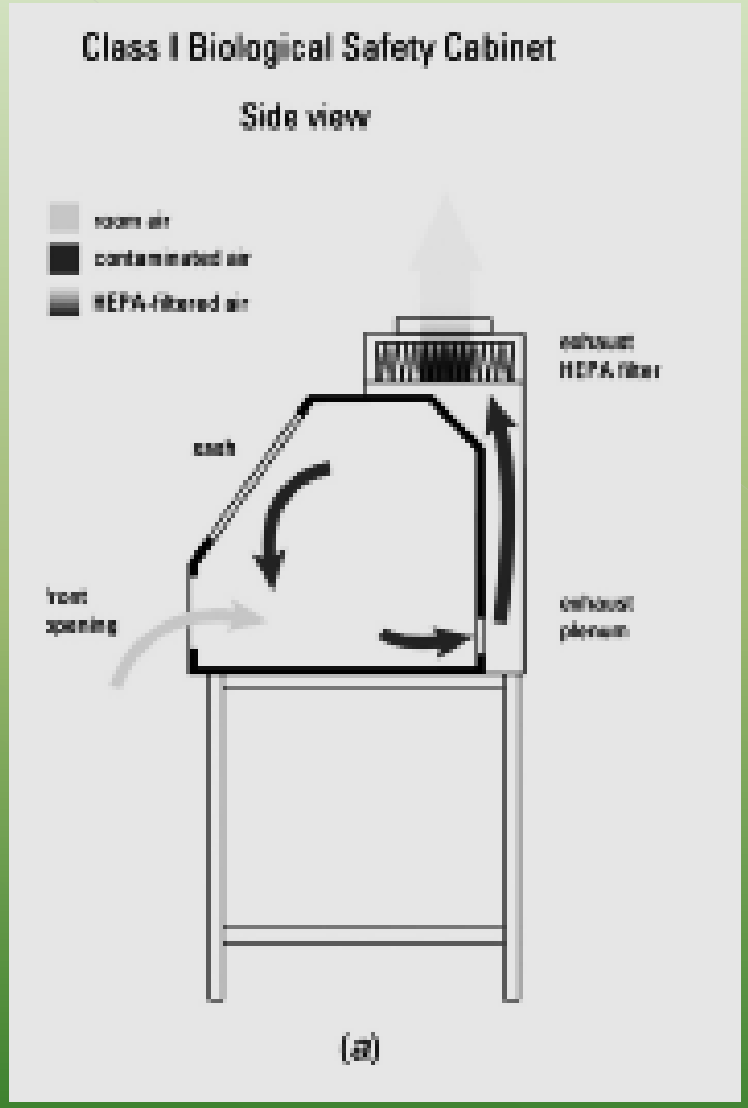
	At rest		In operation	
	Maximum permitted number of particles/m³			
Grade	0.5–5.0 µm	>5 µm	0.5–5.0 µm	>5 µm
A	3 500	0	3 500	0
B	3 500	0	350 000	2 000
C	350 000	2 000	3 500 000	20 000
D	3 500 000	20 000	Not defined	Not defined

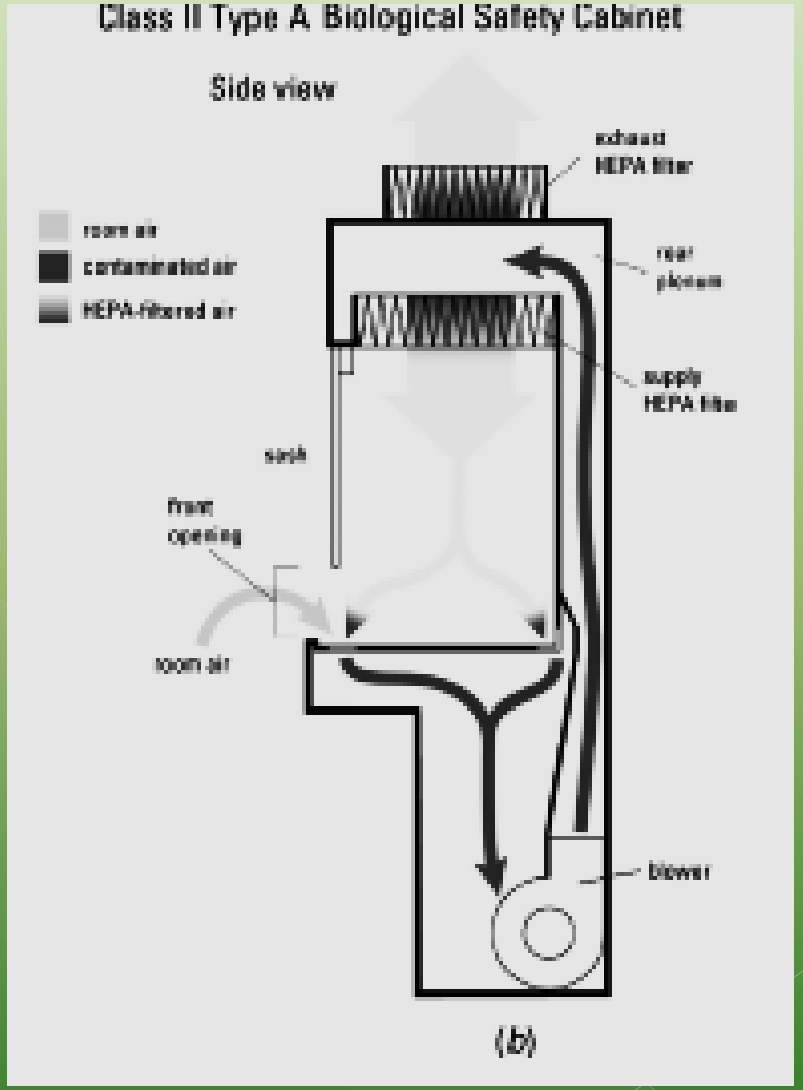
"At rest" = equipment installed and operating; "in operation" = installed equipment functioning and specified number of personnel present.

Table 9.1 Average values for limits of microbial detection in areas of defined air quality

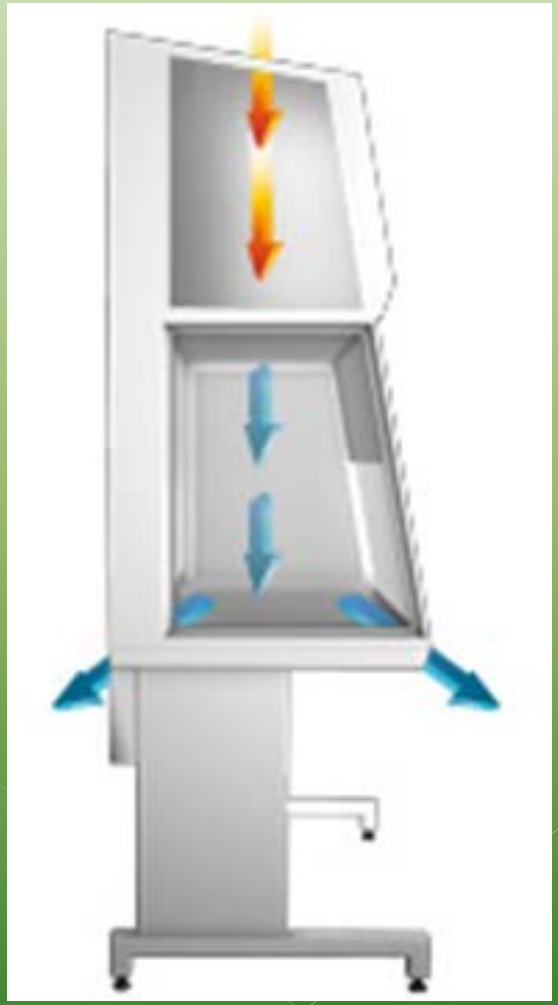
Grade	Air sample (CFU/m³)	Settle plates (90 mm diameter) (CFU/4 hours)	Contact plates (55 mm diameter) (CFU/plate)	Glove print (5 fingers) (CFU/glove)
A	<3	<3	<3	<3
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

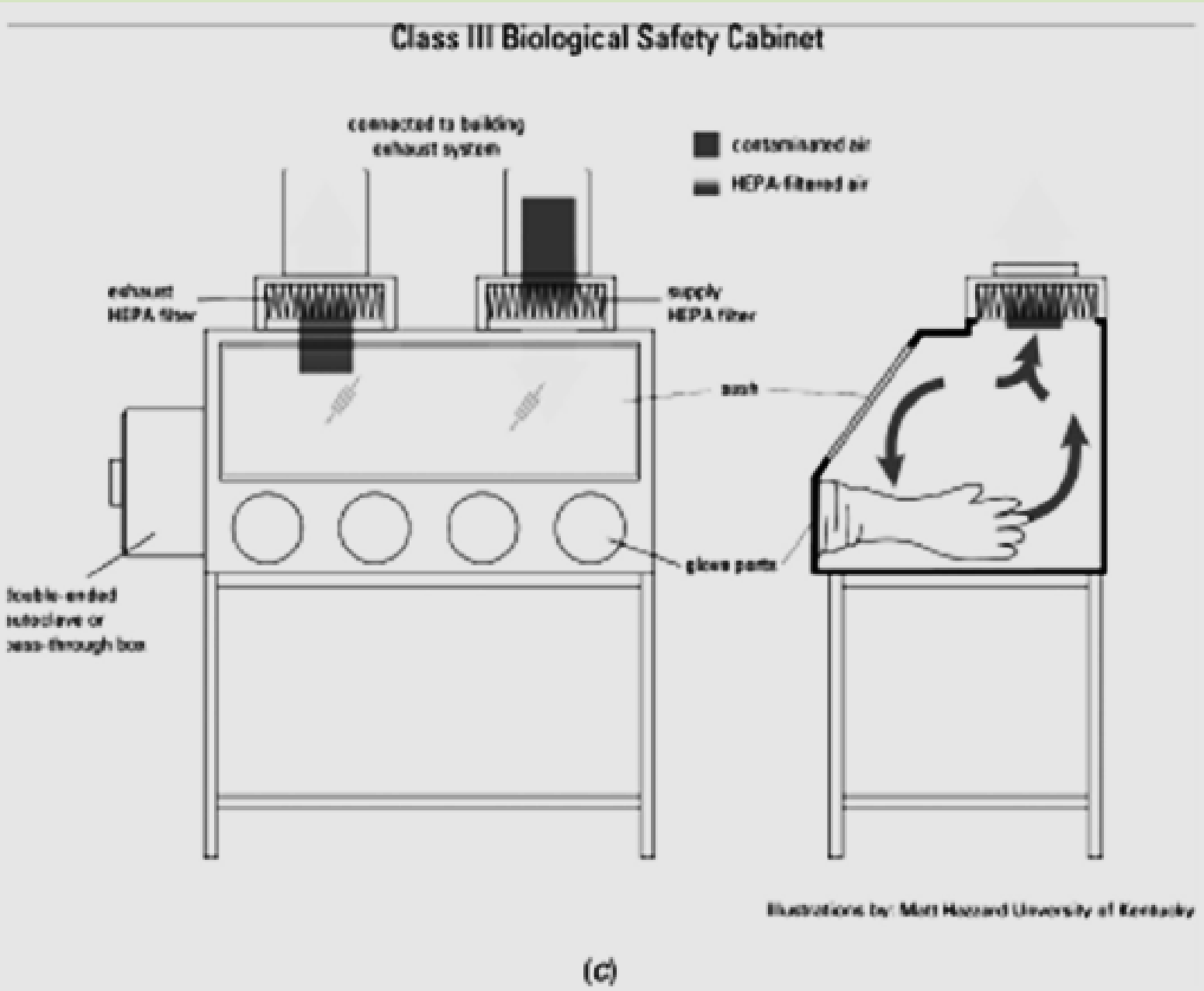
CFU, colony forming units.


Class I Biological Safety Cabinet
Side view
room air
contaminated air
HEPA-filtered air
exhaust HEPA filter
sash
front opening
exhaust plenum
(a)


Class II Type A Biological Safety Cabinet
Side view
exhaust HEPA filter
room air
contaminated air
HEPA-filtered air
rear plenum
supply HEPA filter
sash
front opening
room air
blower
(b)


IVFtech




Class III Biological Safety Cabinet
connected to building exhaust system
contaminated air
HEPA-filtered air
exhaust HEPA filter
supply HEPA filter
sash
glove ports
double-ended autoclave or pass-through box
Illustrations by: Matt Hazzard University of Kentucky
(c)

Equipment and supplies for embryology

- CO_2 incubator
- Dissecting microscope
- Inverted microscope
- Heated surfaces for microscope and manipulation areas
- Heating block for test-tubes

Laminar flow cabinet
Oven for heat-sterilizing
Small autoclave
Water bath
Pipette 10–1000 mL Eppendorf
Refrigerator
Supply of medical grade CO_2
Supply of 5% CO_2 in air (or special gas mixtures)
Wash bottle + Millex filter for gas
Rubber tubing
Pipette canisters
Clinical grade mineral or paraffin oil
Culture media
Glassware for media preparation
Osmometer (for media preparation)
Weighing balance
Tissue culture plastics: (Nunc, Corning, Sterilin)
Flasks for media and oil: 50 mL, 175 mL
Culture dishes: 60, 35 mm
OCR (oocyte retrieval) needles
Test-tubes for OCR: 17 mL disposable
Transfer catheters and stylets: embryo, GIFT, IUI
Syringes
Needles
Disposable pipettes: 1, 5, 10, 25 mL
"Pipetus" pipetting device
Eppendorf tips, small and large
Millipore filters: 0.22, 0.8 mm
Glass Pasteur pipettes (Volac)
Pipette bulbs
Test-tube racks
Rubbish bags
Tissues
Tape for labeling
7X detergent (Flow)
70% ethanol
Sterile gloves, latex and non-latex
Oil: Boots, Squibb, Sigma, Medicult
Supply of purified water: Milli-Q system or Analar
Glassware for making culture media: beakers, flasks, measuring cylinder

TABLE 4.1. Tissue Culture Facilities

Minimum requirements	Desirable features	Useful additions
Sterile area, clean, quiet, and with no through traffic	Filtered air (air-conditioning)	Piped CO_2 and compressed air
Separate from animal house and microbiological labs	Service bench adjacent to culture area	Storeroom for bulk plastics
Preparation area	Separate prep room	Quarantine room
Wash up area (not necessarily within tissue culture laboratory, but at least adjacent to it)	Hot room with temperature recorder	Containment room (could double as quarantine room)
Space for incubator(s)	Separate sterilizing room	Liquid N_2 storage tank (~500 L) and separate storeroom for nitrogen freezers
Storage areas:	Separate cylinder store	Microscope room
Liquids: ambient, 4°C, −20°C		Darkroom
Glassware (shelving)		Vacuum line
Plastics (shelving)		
Small items (drawers)		
Specialized equipment (slow turnover), cupboard(s)		
Chemicals: ambient, 4°C, −20°C; share with liquids, but keep chemicals in sealed container over desiccant		
CO_2 cylinders		
Space for liquid N_2 freezer(s)		
Sink		

TABLE 5.1. Tissue Culture Equipment

Basic requirements	Nonessential, but beneficial	Useful additions
Laminar-flow hood (biohazard if for human cells)	Cell counter	Glassware washing machine
Incubator (humid CO_2 incubator if using open plates or dishes)	Peristaltic pump	Low-temperature ($\leq -70^{\circ}C$) freezer
5% CO_2 cylinder (for gassing cultures)	Pipettor(s)	Conductivity meter
Liquid CO_2 cylinders, without siphon (for CO_2 incubator)	PH meter	Osmometer
Balance	Sterilizing oven	Polyethylene bag sealer (for packaging sterile items for long-term storage)
Sterilizer (autoclave, pressure cooker)	Hot room	Computer for freezer records and cell line database
Refrigerator	Temperature recorders on sterilizing oven and autoclave and in hot room	Colony counter
Freezer (for $-20^{\circ}C$ storage)	Phase-contrast, fluorescence microscope	High-capacity centrifuge (6×1 L)
Inverted microscope	Pipette plugger	Digital camera and monitor for inverted microscope(s)
Soaking bath or sink	Pipette drier	Time-lapse video equipment
Deep washing sink	Automatic dispenser	Cell sizer (e.g., Schärfe, Coulter)
Pipette cylinder(s)	Trolleys or carts	Portable temperature recorder for checking hot room or incubators
Pipette washer	Drying oven(s), high and low temperature	Plastics shredder/sterilizer
Still or water purifier	Roller racks for roller bottle culture	Controlled-rate cooler (for cell freezing)
Bench centrifuge	Piped CO_2 supply from cylinder store	Fluorescence-activated cell sorter
Liquid N_2 freezer (~35 L, 1,500–3,000 ampoules)	Automatic changeover device on CO_2 cylinders	Confocal microscope
Liquid N_2 storage Dewar (~25 L)		Microtitration plate scintillation counter
Slow-cooling device for cell freezing (see Section 20.3.4)		Centrifugal elutriator centrifuge and rotor
Magnetic stirrer racks for suspension cultures		
Hemocytometer		

TABLE 7.1. Elements of Risk Assessment

Category	Items affecting risk
Operator	
Experience	Level
	Relevance
	Background
Training	Previous
	New requirements
Protective clothing	Adequate
	Properly worn (buttoned lab coat)
	Laundered regularly
	Repaired or discarded when damaged
Equipment	
Age	Condition
	Adherence to new legislation
Suitability for task	Access, sample capacity, containment
Mechanical stability	Loading
	Anchorage
	Balance
Electrical safety	Connections
	Leakage to ground (earth)
	Proximity of water
Containment	Aerosols:
	Generation
	Leakage from hood ducting
	Overspill from work area
	Toxic fumes
	Exhaust ductwork:
	Integrity
	Site of effluent and downwind risk
Heat	Generation
	Dissipation
Maintenance	Frequency
	Decontamination required?
Disposal	Route
	Decontamination required?
Physical Risks	
Intense cold	Frostbite
	Numbing
Electric shock	Loss of consciousness
	Cardiac arrest
Fire	General precautions
	Equipment wiring, installation, and maintenance
	Incursion of water near electrical wiring
	Fire drills, procedures, escape routes
	Solvent usage and storage (e.g., do not store ether in refrigerators)
	Flammable mixtures
	Identification of stored biohazards and radiochemicals

TABLE 7.1. Elements of Risk Assessment (*Continued*)

Category	Items affecting risk
Chemicals (including gases and volatile liquids)	
Scale	Amount used
Toxicity	Poisonous
	Carcinogenic
	Teratogenic
	Mutagenic
	Corrosive
	Irritant
	Allergenic
	Asphyxiative
Reaction with water	Heat generation
	Effervescence
Reaction with solvents	Heat generation
	Effervescence
	Generation of explosive mixture
Volatility	Intoxication
	Asphyxiation
Generation of powders and aerosols	Dissemination
	Inhalation
Import, export, and transportation	Breakage, leakage
Location and storage conditions	Access by untrained staff
	Illegal entry
	Weather, incursion of water
	Stability, compression, breakage, leakage
Biohazards	
Pathogenicity	Grade
	Infectivity
	Host specificity
	Stability
Scale	Number of cells
	Amount of DNA
Genetic manipulation	Host specificity
	Vector infectivity
	Disablement
Containment	Room
	Cabinet
	Procedures
Radioisotopes	
Emission	Type
	Energy
	Penetration, shielding
	Interaction, ionization
	Half-life
Volatility	Inhalation
	Dissemination
Localization on ingestion	DNA precursors, such as [^{3}H]thymidine
Disposal	Solid, liquid, gaseous
	Route
	Legal limits

TABLE 7.1. Elements of Risk Assessment (*Continued*)

Category	Items affecting risk
Special Circumstances	
Pregnancy	Immunodeficiency
	Risk to fetus, teratogenicity
Illness	Immunodeficiency
Immunosuppressant drugs	Immunodeficiency
Cuts and abrasions	Increased risk of absorption
Allergy	Powders, e.g., detergents
	Aerosols
	Contact, e.g., rubber gloves
Elements of Procedures	
Scale	Amount of materials used
	Size of equipment & facilities and effect on containment
	Number of staff involved
Complexity	Number of steps or stages
	Number of options
	Interacting systems and procedures
Duration	Process time
	Incubation time
	Storage time
Number of persons involved	Increased risk?
	Diminished risk?
Location	Containment
	Security and access


Heraeus
BBD 6220

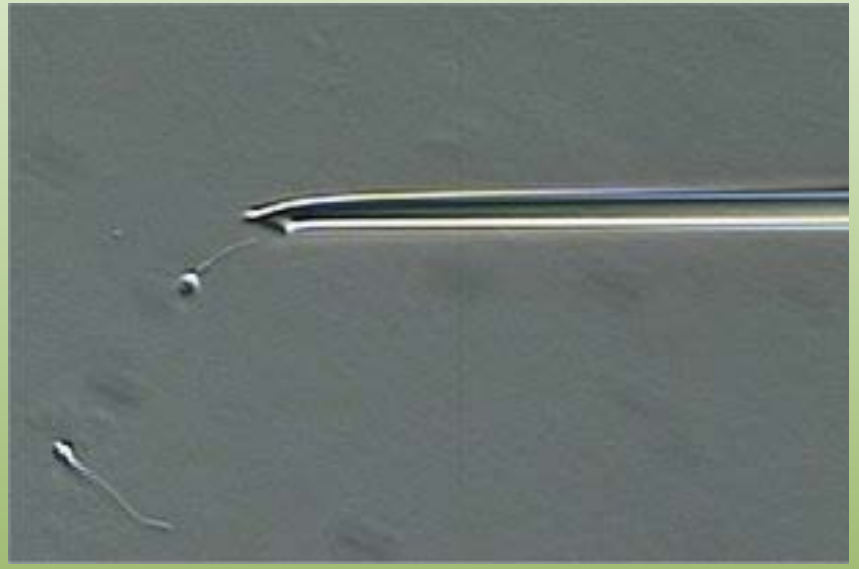

HERA
cell
4

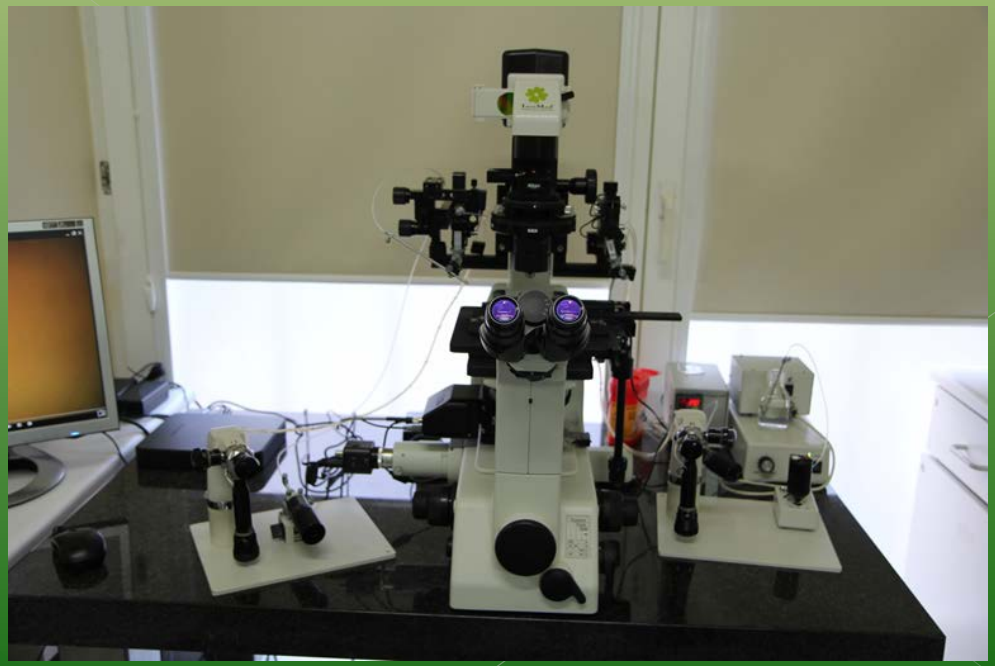

36.7
5.1
Heraeus
InviMed
HERA
cell
4

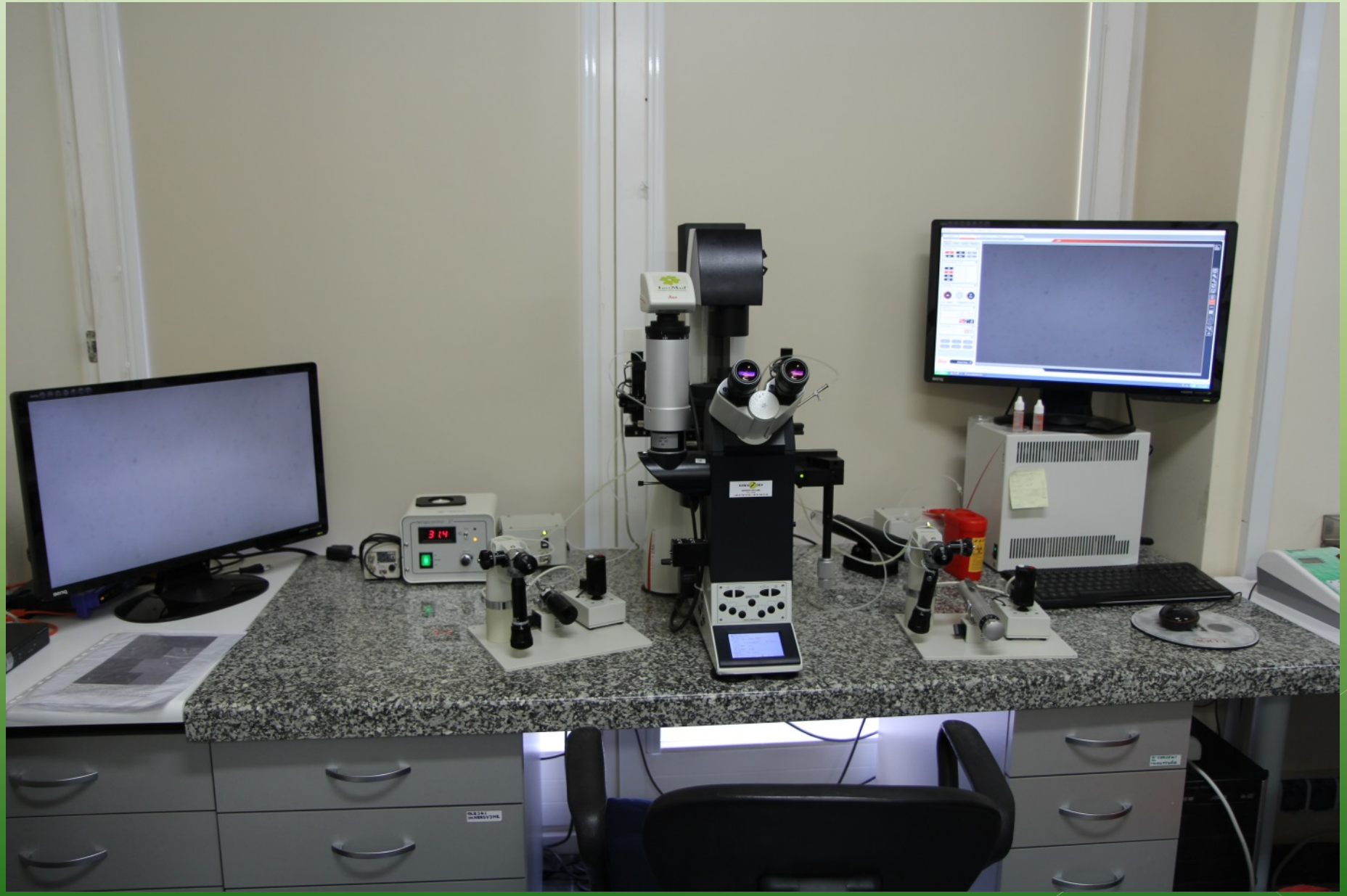

Heraeus
37.2
5.5
78.0
MIDURA
JOANNA
5) 03.10

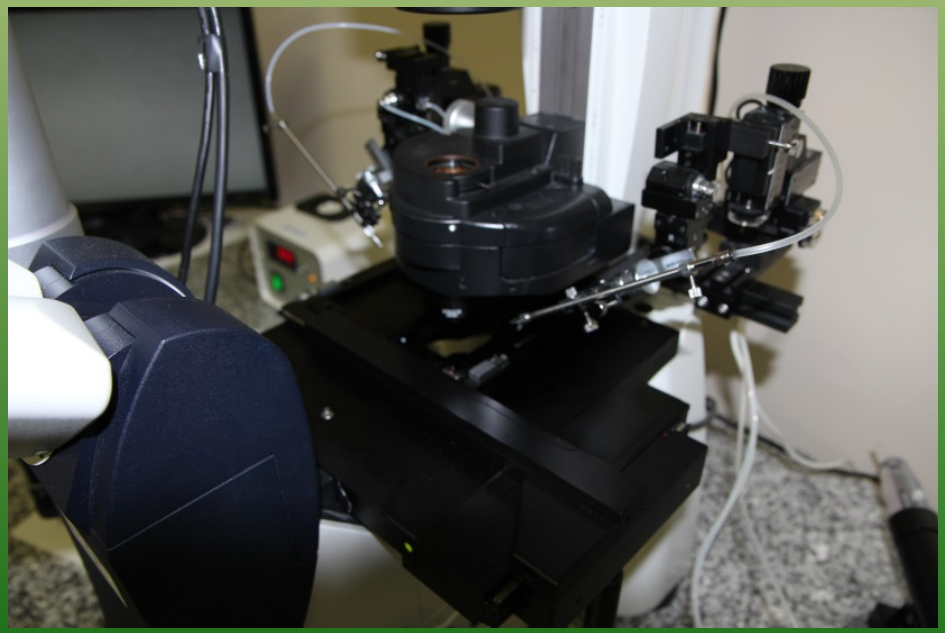

Fidelbank
Fidelbank
Fidelbank

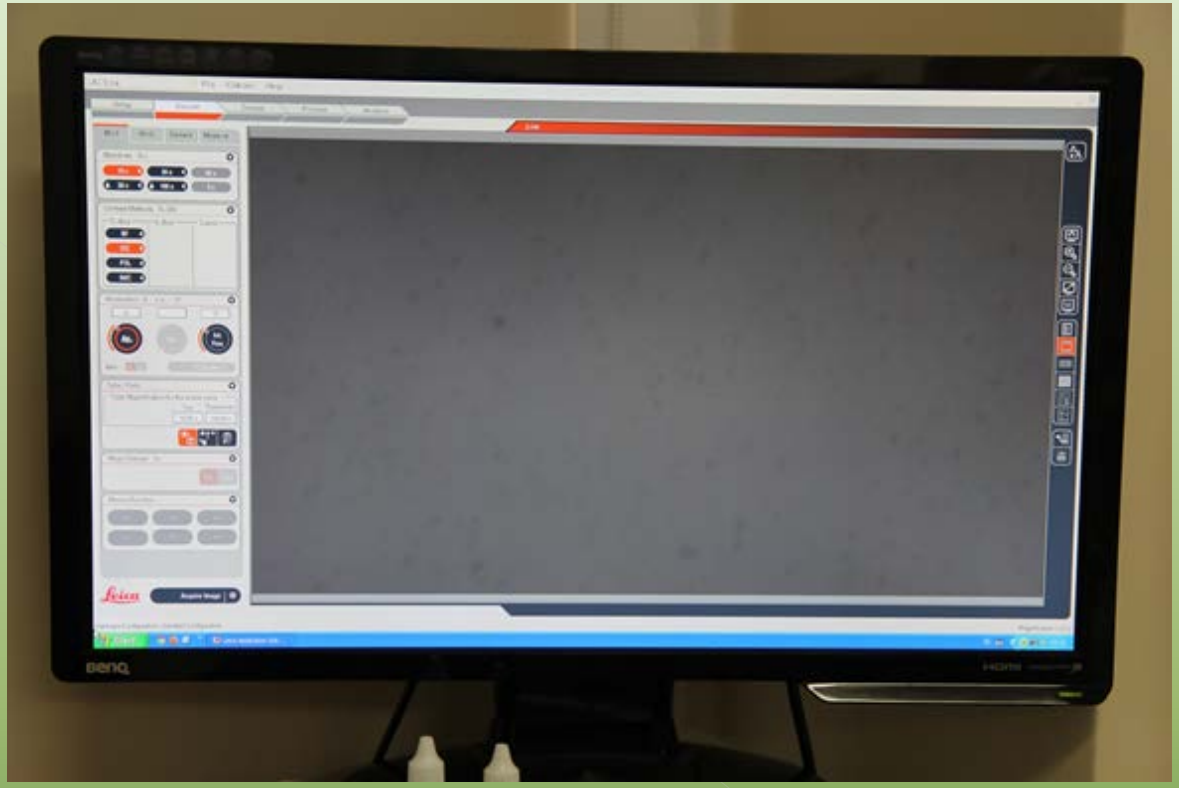

IVFtech
IVFtech
Warming blocks
for small and larger test tubes: 14/17 cc or 6 cc.
IVFtech
Warming blocks for petri dishes and mediaflask
Warming blocks may be used to secure the temperature in the plastic dish while used at the temperated tabletop.
IVFtech
IVFtech
Glass bubble flask
IVFtech
Glass hood
for CO_2 flow to the culture dishes which are to be used to equilibrate the culture medium.





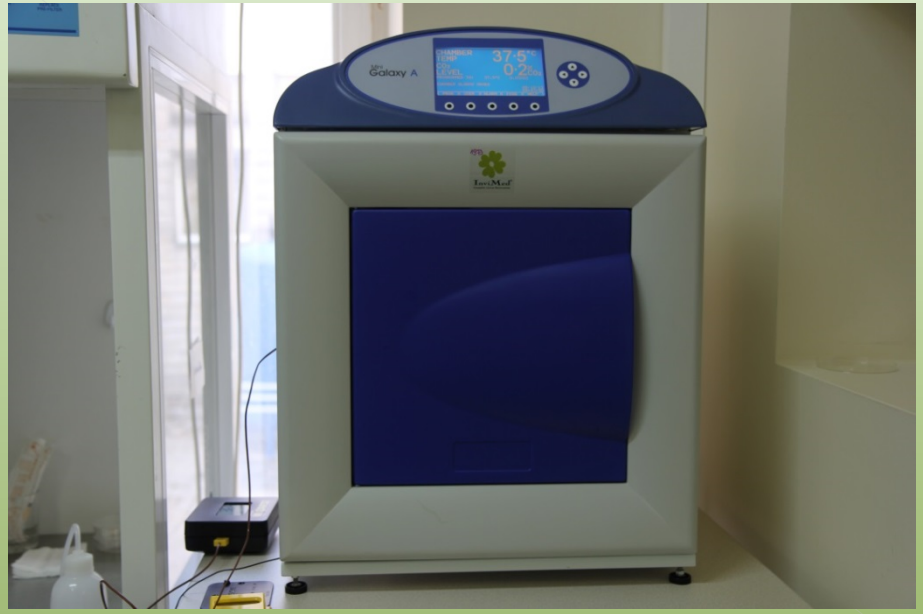





benq


PECON
tempcontrol - 37
32.7
°C


Coda Tower.
<genX>
international


Galaxy A
37.5
0.2


Galaxy A
CHAMBER TEMP 37.3°C
CO2 LEVEL 0.2 xCO2
DOOR OPEN FOR 00:06


6100

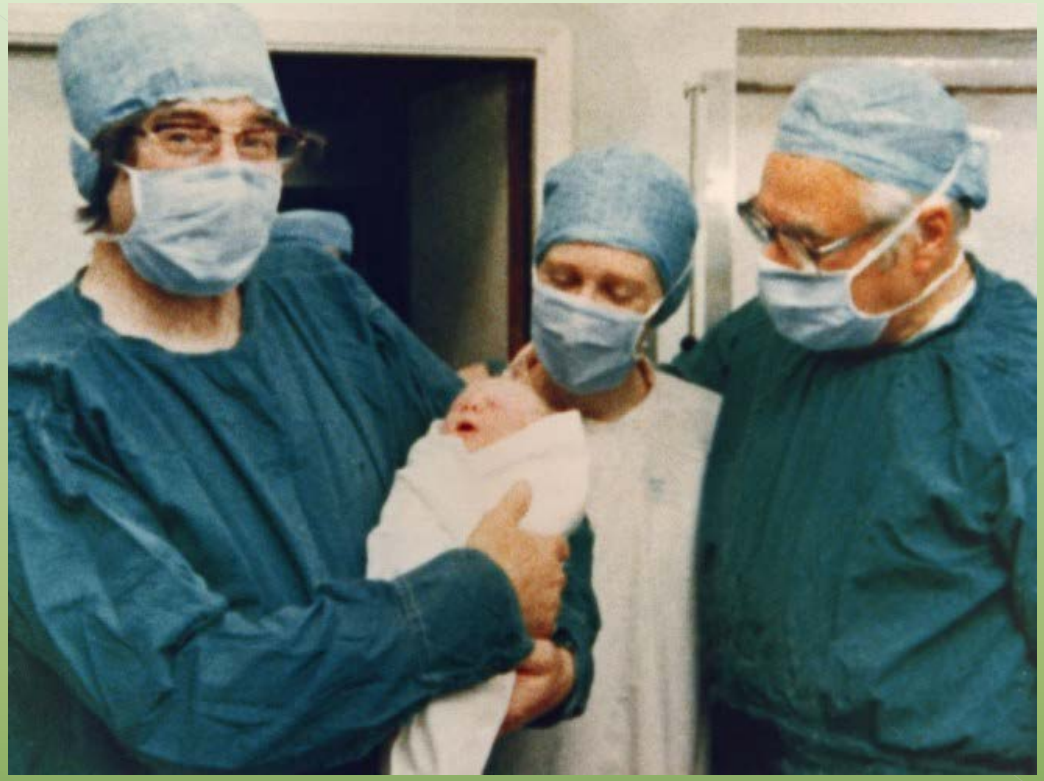

FREEZE CONTROL
CryoBath


WIZARD2


WIZARD2


KITAZATO


BRADY
TLS2200



Dziękuję za uwagę