Infrastructure and Personnel Preparation for Serology and Molecular Laboratory Testing for SARS CoV-2

July Kumalawati

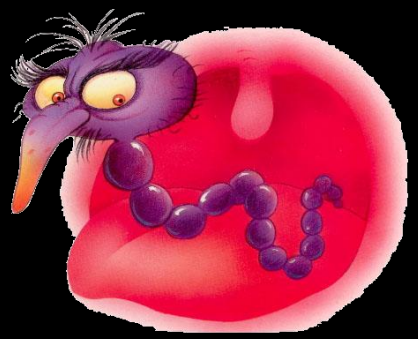
Overview

- **Hazards in laboratory**
- **Levels of laboratory biosafety**
- **Requirements for level 1-3**
- **Infrastructure**
- **Molecular laboratory requirements**

Hazards in medical laboratories

- **Biological hazards**
- **Chemical hazards**
- **Physical hazards:**
  - **fire hazards**
  - **electrical hazards**
  - **extreme temperatures**
  - **radiation**
  - **other environmental hazards such as slippery floor, poor lighting, poor ventilation, and poor laboratory layout**

Biological hazards



Routes of transmission:

- Percutaneous
- Mucous membrane
- Ingestion
- Inhalation

Biological hazards

Laboratory biosafety levels:

- Biosafety level 1 (BSL 1)
- Biosafety level 2 (BSL 2)
- Biosafety level 3 (BSL 3)
- Biosafety level 4 (BSL 4)

} **Medical / Clinical Laboratories**

Biosafety level 1

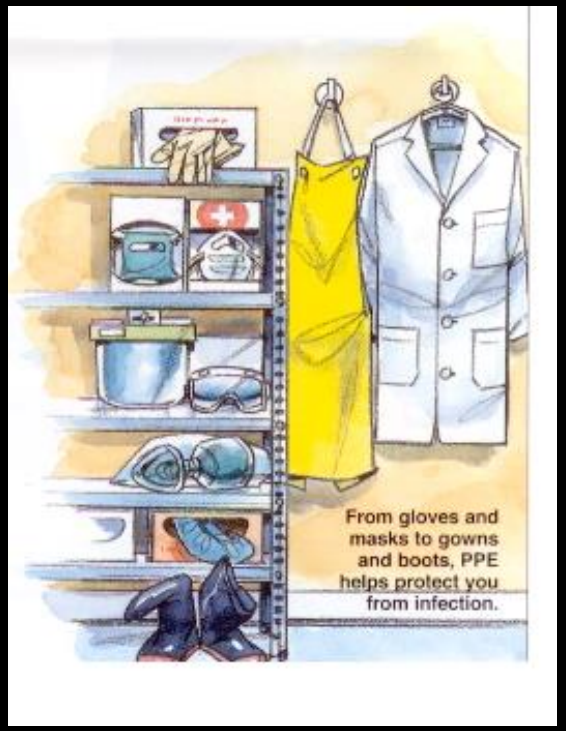
- **Needs:**
  - **Standard microbiological practices**
  - **No special practices**
  - **Safety equipment (primary barriers)**
  - **Laboratory facilities (secondary barriers)**

Standard microbiological practices

- **Limited/restricted access to the laboratory when work in progress**
- **Washing hands**
- **No eating, drinking, smoking, handling contact lenses, applying cosmetics and storing food in work area**
- **No mouth pipetting**
- **Policies for safe handling of sharps are instituted**
- **Procedures are performed carefully to minimise splashes & aerosols**
- **Work surfaces and laboratory equipment decontaminated**
- **Potentially infectious wastes decontaminated prior to disposal**
- **Insect & rodent control**

Safety equipment

- **Laboratory coats / gowns**
- **Gloves**
- **Protective eyewear**



Laboratory facilities

- Doors for access control
- Sink for hand-washing
- Can be cleaned easily. No carpets or rugs
- Bench-tops are impervious to water and resistant to moderate heat, organic solvents, acids, alkalis and chemical used for decontamination
- Furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets and equipments are accessible for cleaning
- Windows fitted with fly screen

Biosafety level 2

- **Suitable for work involving agents of moderate potential hazard to personnel and the environment**

Biosafety level 2

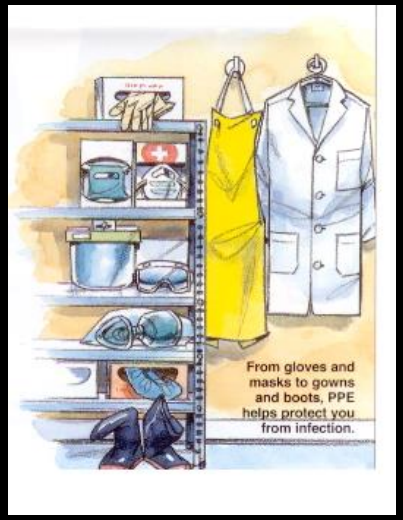
- **Needs:**
  - **Same as BSL 1**
  - **Specific trainings for laboratory personnel**
  - **Extreme precautions with contaminated sharp items**
  - **The use of biological safety cabinets when certain procedures in which infectious aerosols or splashes may be created are conducted**

Biosafety level 2

- **Special practices:**
  - **Immunisation for laboratory personnel**
  - **Tests on laboratory personnel: based line serum samples, periodically collected samples. Depending on the agents handled**
  - **High degree of precaution must always be taken with any contaminated sharp items (needles, syringes, slides, pipettes, capillary tubes, scalpels)**
  - **Broken glassware must not be handled directly by hand**
  - **Use of leak-proof containers during specimen collection, handling, transport or shipping**
  - **Biohazard warning sign**

Biosafety level 2

- **Safety equipments:**
  - **Same as BSL 1**
  - **Biological safety cabinets, preferable class II**
  - **Protective lab coats, or gowns. Not to be worn outside the laboratory**
  - **Face protection (goggles, mask, face shield or other splash guard)**



Biosafety level 2

- **Laboratory facilities:**
  - **Same as BSL 1**
  - **Lockable doors**
  - **Consider location away from public areas**
  - **Locate biological safety cabinets away from doors, windows, heavily traveled lab areas & other potentially disruptive equipment**
  - **Eyewash station**
  - **Adequate illumination**
  - **Chairs covered with non-fabric material that can be easily decontaminated**

Biosafety level 3

- **Applicable to clinical, diagnostic, teaching, research or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by inhalation route**

Biosafety level 3

- **Needs:**
  - **Same as BSL 2**
  - **Special practices:**
    - **Laboratory doors are kept closed when work in progress**
    - **No minors allowed in the laboratory**
    - **All open manipulations involving infectious materials are conducted in biological safety cabinets (class II or III) or other physical containment devices**
    - **No animals or plants not related to the work allowed in the laboratory**
    - **Frequent changing of gloves accompanied by hand-washing**
    - **Centrifugation in closed containers or rotors with lids**

Biosafety level 3

- **Safety equipments:**
  - **Same as BSL 2**
  - **Protective clothing: solid-front or wrap-around gowns, scrub suits or coveralls. Not to be worn outside the laboratory**
  - **Biological safety cabinets class II or III**
  - **Respiratory and face protection devices**

Biosafety level 3

- **Laboratory facilities:**
  - **Same as BSL 2**
  - **Laboratory is separated from areas that open to unrestricted traffic flow**
  - **Walls, floors & ceilings are constructed for easy cleaning and decontamination. Smooth, impermeable to liquids & resistant to chemicals and disinfectants**
  - **All windows are closed and sealed**
  - **Decontamination method in the facility**
  - **Ducted exhaust air ventilation system (negative pressure). No recirculation of air. Exhausted air HEPA-filtered**

Work Area

- 15 – 20 m^2 per personnel
- Plus 8 – 10 % for storage
- Footprints of instruments

Floor

- Able to withstand the weight of lab equipment
- Vibration damping
- Chemical resistant
- Non-slip
- Easy to clean, minimum gap
- Rounded to the wall

Wall

- Semi-permanent partition
- Easy to clean
- Never use tiles

Door

- Lockable
- Wide and high enough to bring in lab equipment
- Preferably using double door
- Width : 90 cm and 45 cm
- With glass panel

Window

- For light source and/or ventilation
- Also functions as emergency exit
- Open easily
- Easy to clean without disturbing lab activity
- Use insect screen if necessary

Work Benches

- Strong for the lab equipment weight
- Modular
- Surface is easily cleaned, chemical and heat resistant
- Bench height:
  - Sitting: 60-75 cm
  - Standing: 90 cm

Work Stool and Chairs

- Water and chemical resistant
- Easily cleaned
- Ergonomic
- Adjustable height

Cabinet and Drawer

- **Modular, under the bench**
- **Wall-hanged cabinets should not be used for heavy things**

Gaps

Gaps between benches, cabinets, and drawers should be able cleaned easily

Ventilation

- **Working temperature: 20 - 25°C**
- **Humidity: 40-60%**
- **Air changes: 6-12 times/hour**
- **HEPA Filtered exhausted air**
- **Positive or Negative Pressured**

Lighting

- Natural (window) or Artificial (light bulb) sources
- Preferably white daylight, coloured light may hinder colour recognition of test result
- Light intensity:
  - Work area: 600 lux (lumen/m^2)
  - Administration: 400 lux
  - Waiting room: 200 lux

Electricity

- ✘ Easily accessible
- ✘ Enough capacity for lab equipment
- ✘ Avoid using extension cables
- ✘ Back-up electricity supply
- ✘ Water and chemical vapour resistant
- ✘ Accessible switches
- ✘ Install safety circuit breakers

Water Supply

Potable water:

- Handwashing
- Lab equipment wasing

- Elbow or foot-operated
- Deep, scratched-proof and chemical resistant sink, prevent splashing

Reverse-osmosis:

- For analyzers: capacity in volume and pressure

Sewage

- **Liquid infectious and chemical waste should be decontaminated before released into the environment**
- **Blockage should be easily repaired**

Solid Waste

- Ordinary waste = household
  - Black-coloured plastic bag
- Infectious waste
  - Yellow or red-coloured plastic bag
  - Preferably autoclavable plastic bag
- Cytotoxic or hazardous chemical container waste
  - Purple-coloured plastic bag
- Sharps
  - Puncture-proof plastic container

Gas Supply

- **Stop-valves should be easily accessible**
- **Good room-ventilation to prevent gas built-up**
- **Gas tanks should be secured**

Electricity, water, and gas lines

- **Behind the benches**
- **Easily accessible for repairs**

Work Safety Environment

- Fume hoods and biological safety cabinet class II (preferable class IIB)
- Fire prevention equipment and emergency exits
- Lead wall for radioactive material
- Eye wash and emergency shower
- Segregation of “Dirty” area from “Clean” area

Chemical Storage

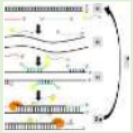
- Corrosive resistant
- Fire resistant

IDEAL DESIGN MOLECULAR LABORATORY

- 4 ROOM **(Mandatory)**

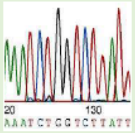
Reagen Preparation

Nucleic Acid Extraction



Amplification

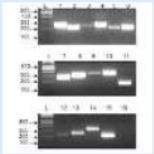
Post Amplification/Sequensing



- 3 ROOM

Reagen Preparation

Nucleic Acid Extraction



Amplification & post amplification

- 2 ROOM

Reagen Preparation & Nucleic Acid Extraction

Amplification & post amplification

Closed system

Courtesy of Miswar Fattah (IACC)

Minimum Instrument in Molecular Laboratory

- Reagen Preparation

-20°C Freezer (small, about 100 L capacity)
Centrifuge with rotor for 1.5 ml eppendorf tubes
Ice flaking machine (?) or cool box /ice box
Refrigerator
Autoclave / Pressure cooker (small)
Hot air oven (small)
Micropipettes (10µl, 100 µl and 1000µl capacity)

Spin down Centrifuge
PCR Cabinet for mastermix preparation

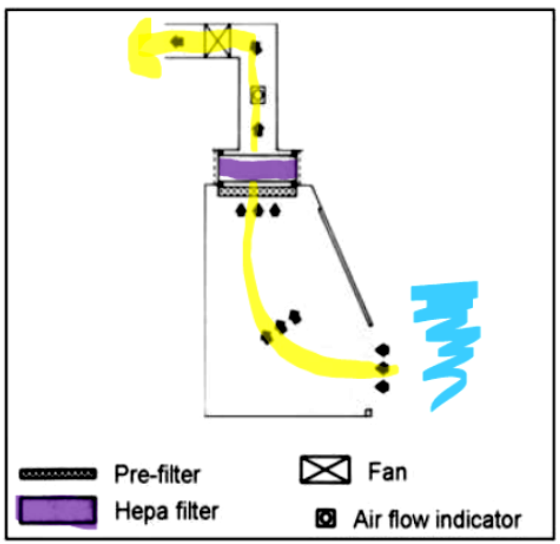
Courtesy of Miswar Fattah (IACC)

Minimum Instrument in Molecular Laboratory: Nucleic Acid extraction Room

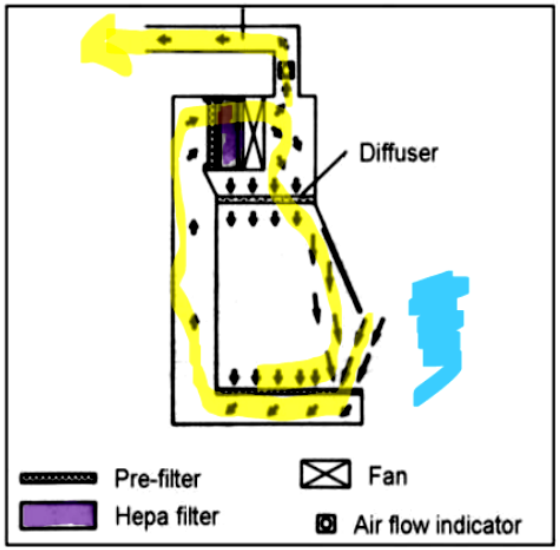
- Biosafety Cabinet
- Biohazard bin: layered with biohazard bag,
- Refrigerator & Freezer
- Centrifuge and heat block
- Micropippette & tips
- Dedicated lab coats

Courtesy of Miswar Fattah (IACC)

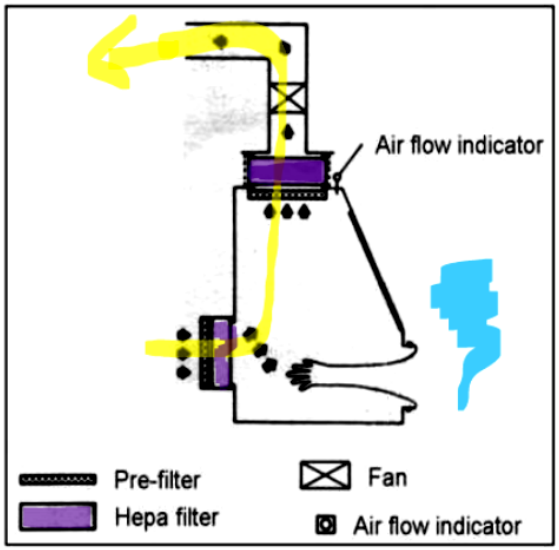
Biosafety Cabinet for Molecular Diagnostics



Class I



Class II



Class III

Courtesy of Miswar Fattah (IACC)

Minimum Instrument in Molecular Laboratory: PCR & Post PCR room

PCR Area (room / lab)
-20°C Freezer (small, about 100 L capacity)
PCR work station / PCR hood. (Two numbers, one for preparation of PCR master mix and other for addition of nucleic acid template) Or A fabricated inoculation hood with UV and fluorescent lights.
Thermocycler / Real time PCR
Micropipettes (2.5 µl, 10 µl and 100 µl capacity) for PCR
Cryobox to hold temperature sensitive chemicals
Post-PCR Area (room / lab)
Microwave oven
Balance
Electrophoresis apparatus (Power pack, gel tank, casting tray, comb)
UV transilluminator or Gel documentation system with a computer
Consumables including chemicals for buffer preparation, agarose, tips, gloves, tissue paper etc.,

Courtesy of Miswar Fattah (IACC)

Additional intruments & equipment

70% ethanol

Sample Refrigerator (-70 °C)

Refrigerator (Reagent)

Laboratory Coat

Vortex Mixer

Pipettes & Filtered Tips

Biohazard Container

2nd Tubes

Biological Safety Cabinet - 2

PCR Hood

Microcentrifuge

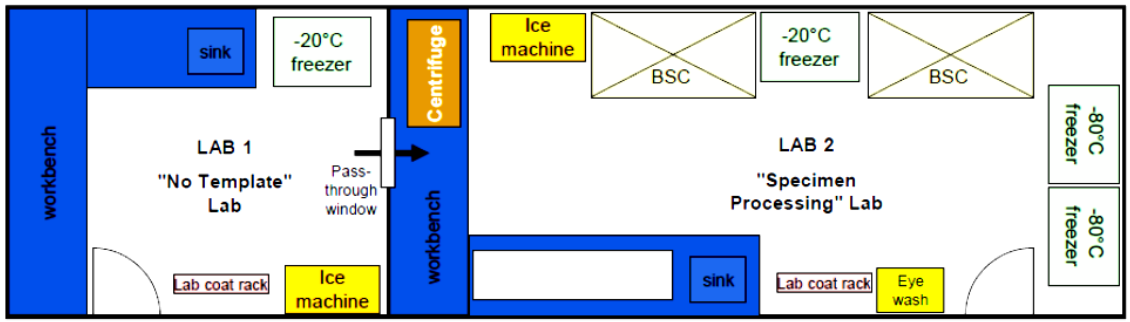
Tubes

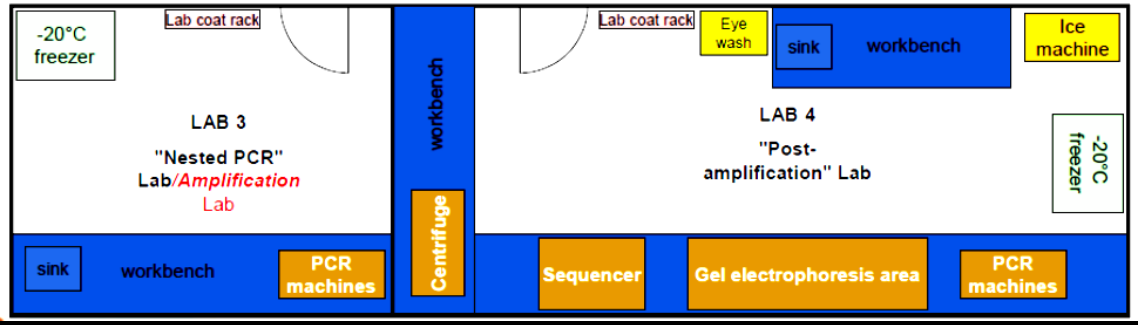
Safety Gloves

Safety Glasses

Courtesy of Miswar Fattah (IACC)

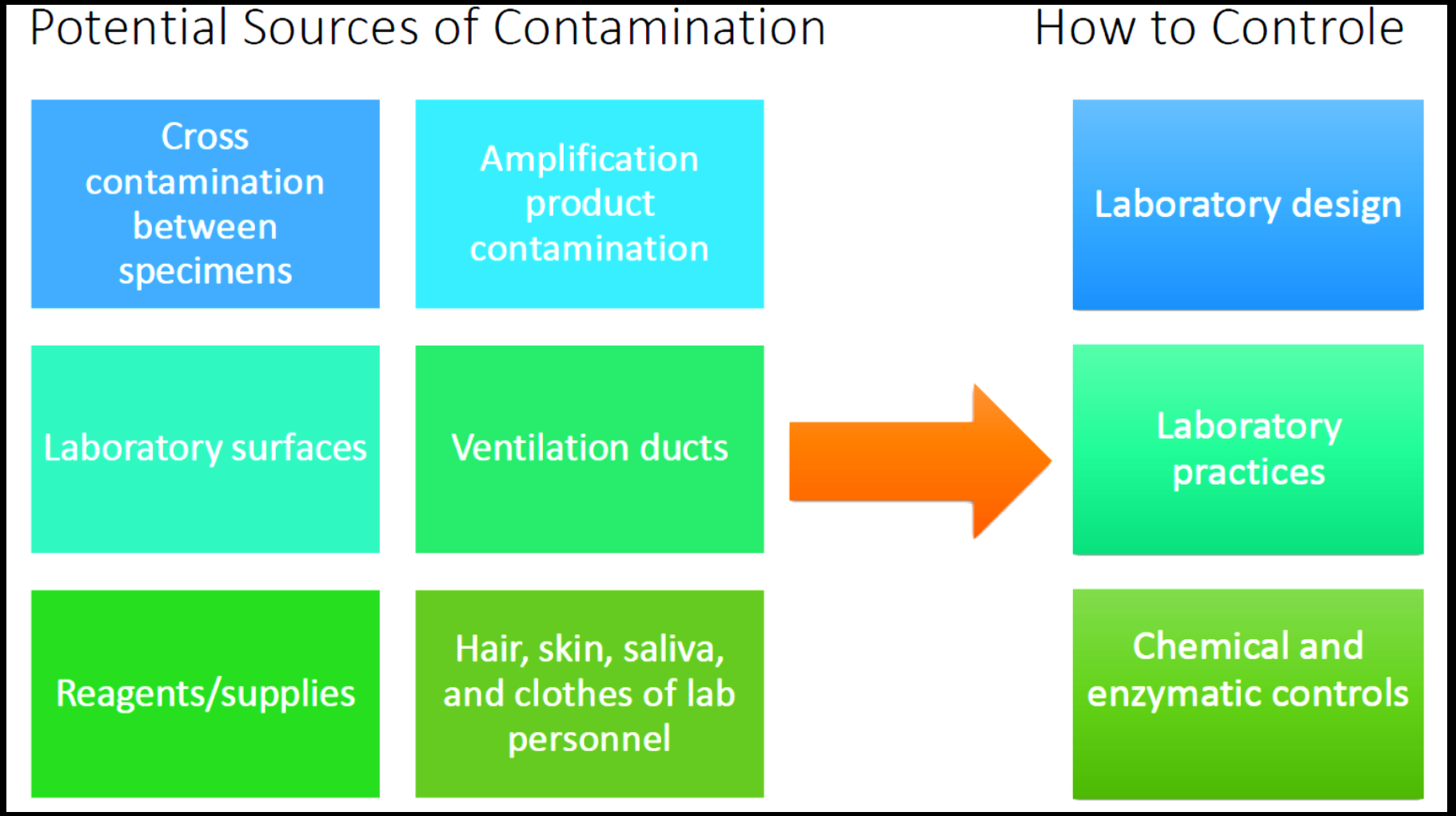
Example position Instrument of a Four-Lab Layout





Pass box

Courtesy of Miswar Fattah (IACC)



Courtesy of Miswar Fattah (IACC)

Difference Molecular lab

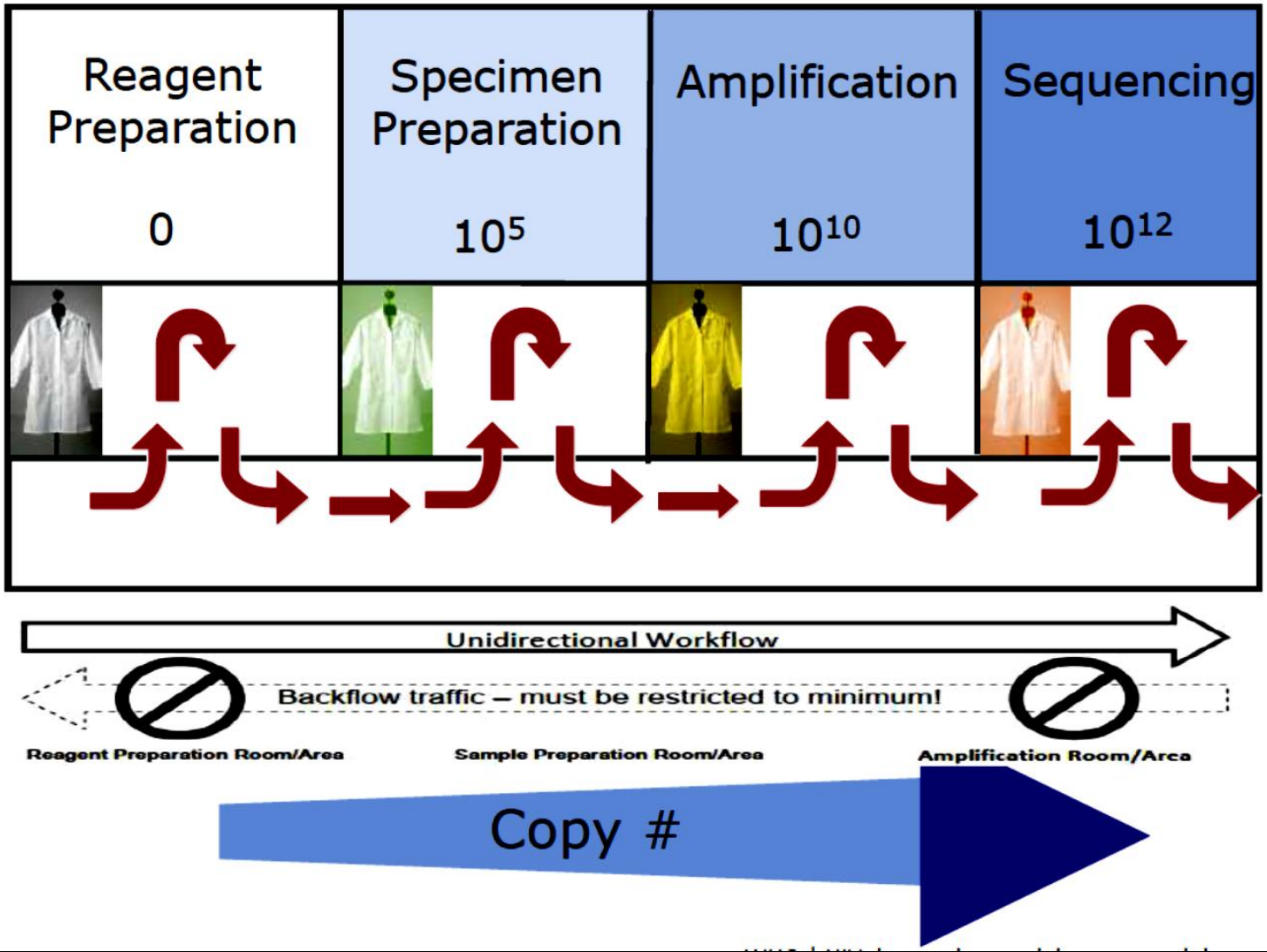
Avoid DNA Contamination

Very low Volume

0,5 uL, 1 uL, 5 uL, 10 uL, total volume 25 -50 uL

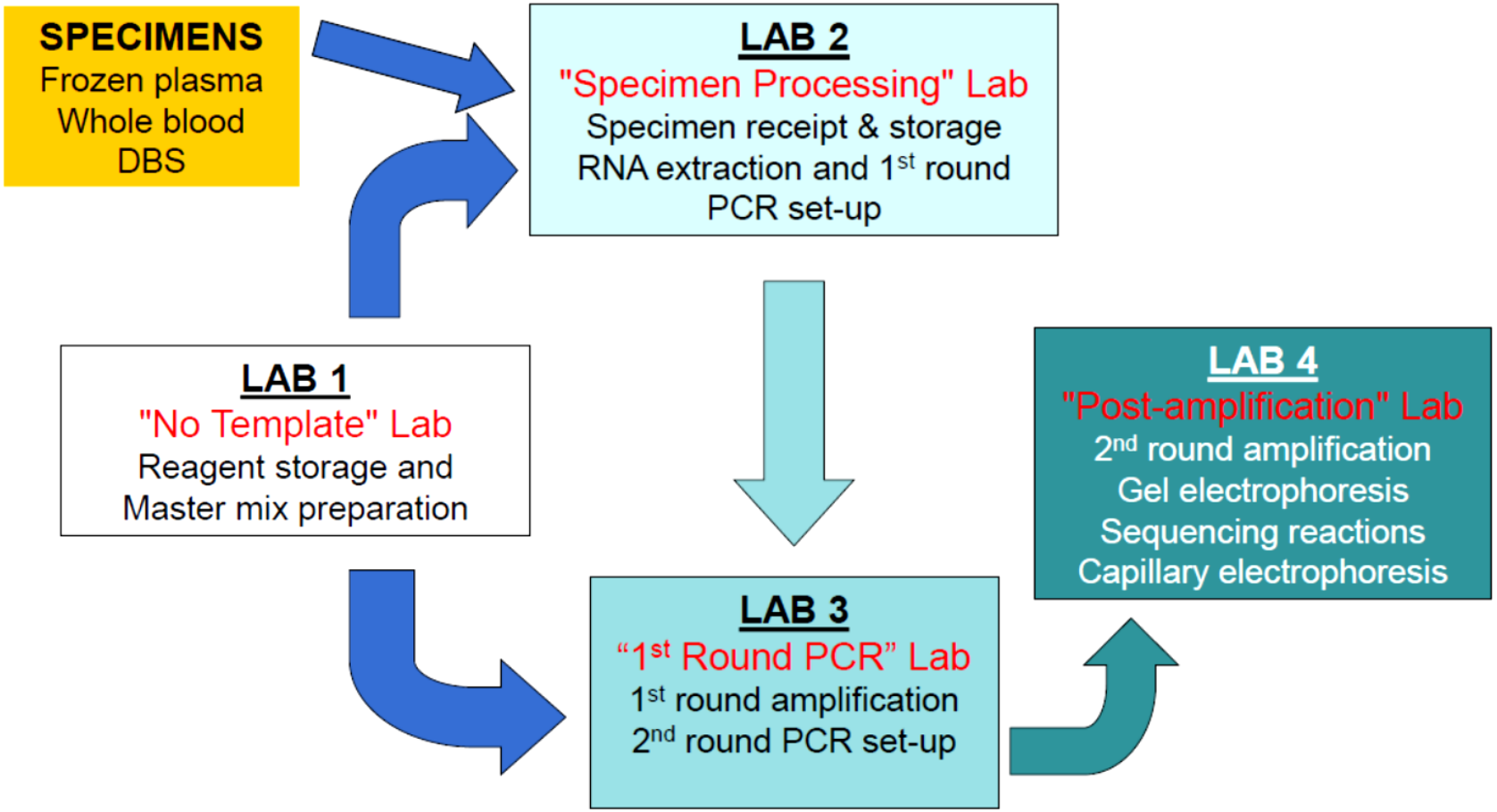
Courtesy of Miswar Fattah (IACC)

Unidirectional workflow



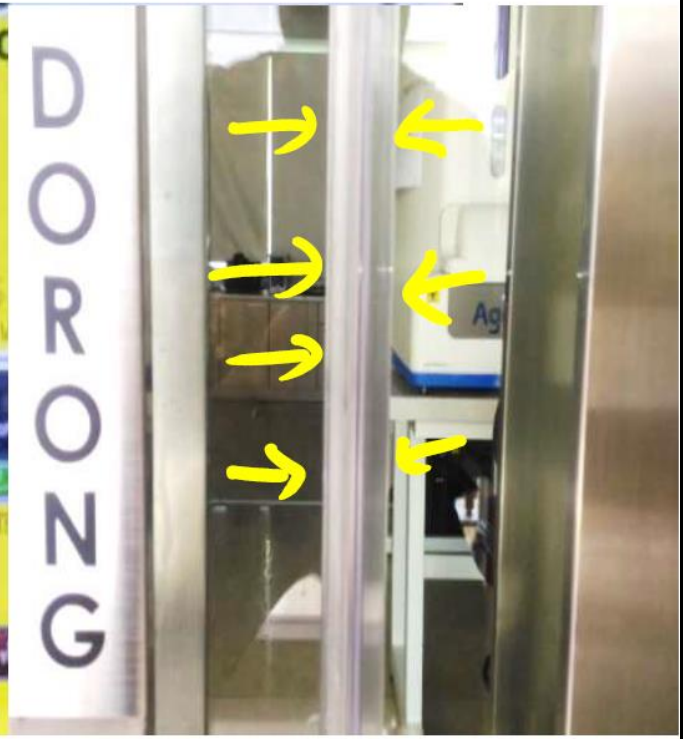
Courtesy of Miswar Fattah (IACC)

Ideal Lab Workflow



Courtesy of Miswar Fattah (IACC)

Pressure & Temperature Controller



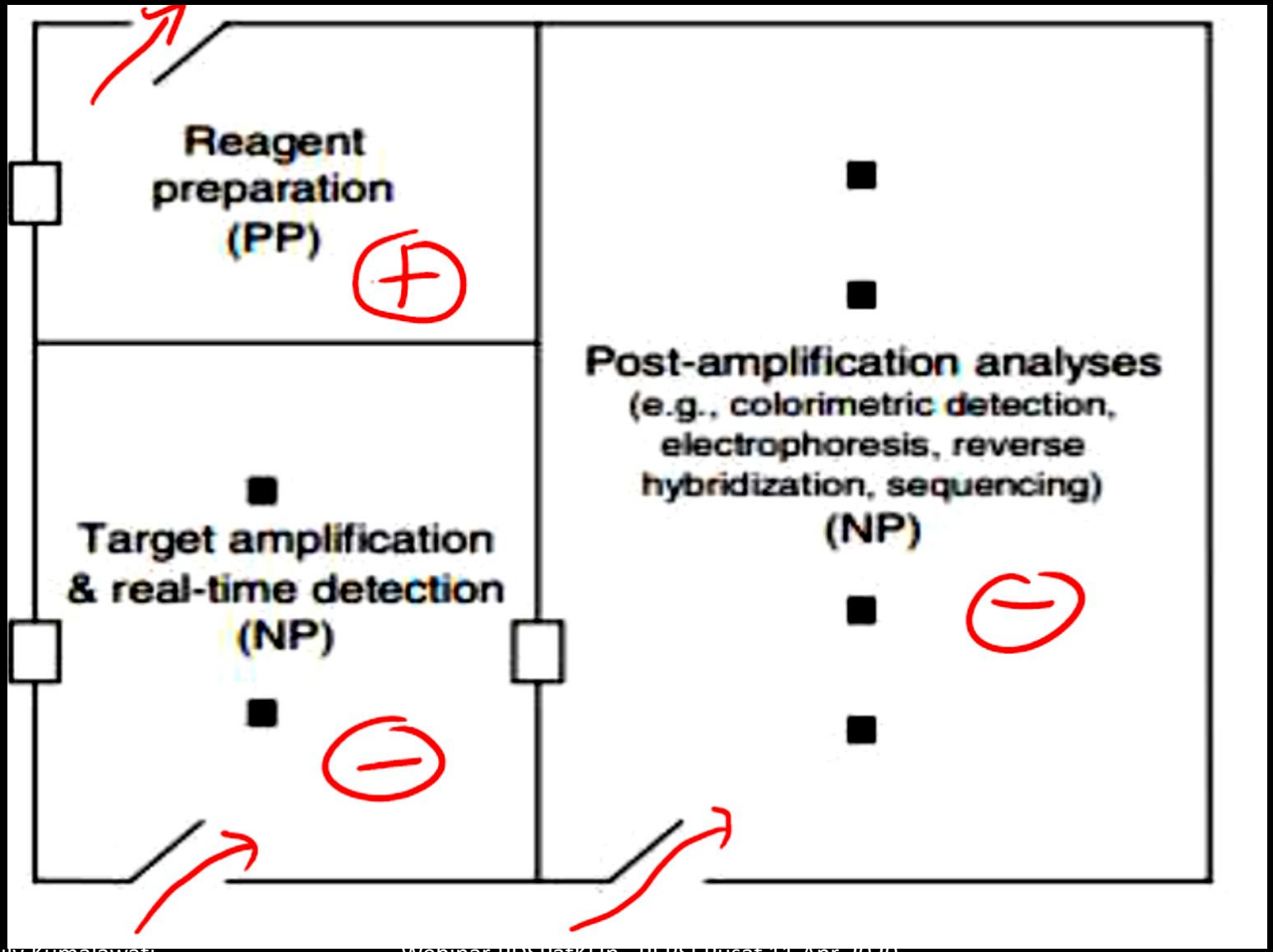
Courtesy of Miswar Fattah (IACC)

UV LAMP and Electronic Timer Switches

Electronic Timer Switches

Courtesy of Miswar Fattah (IACC)

Laboratory air pressure



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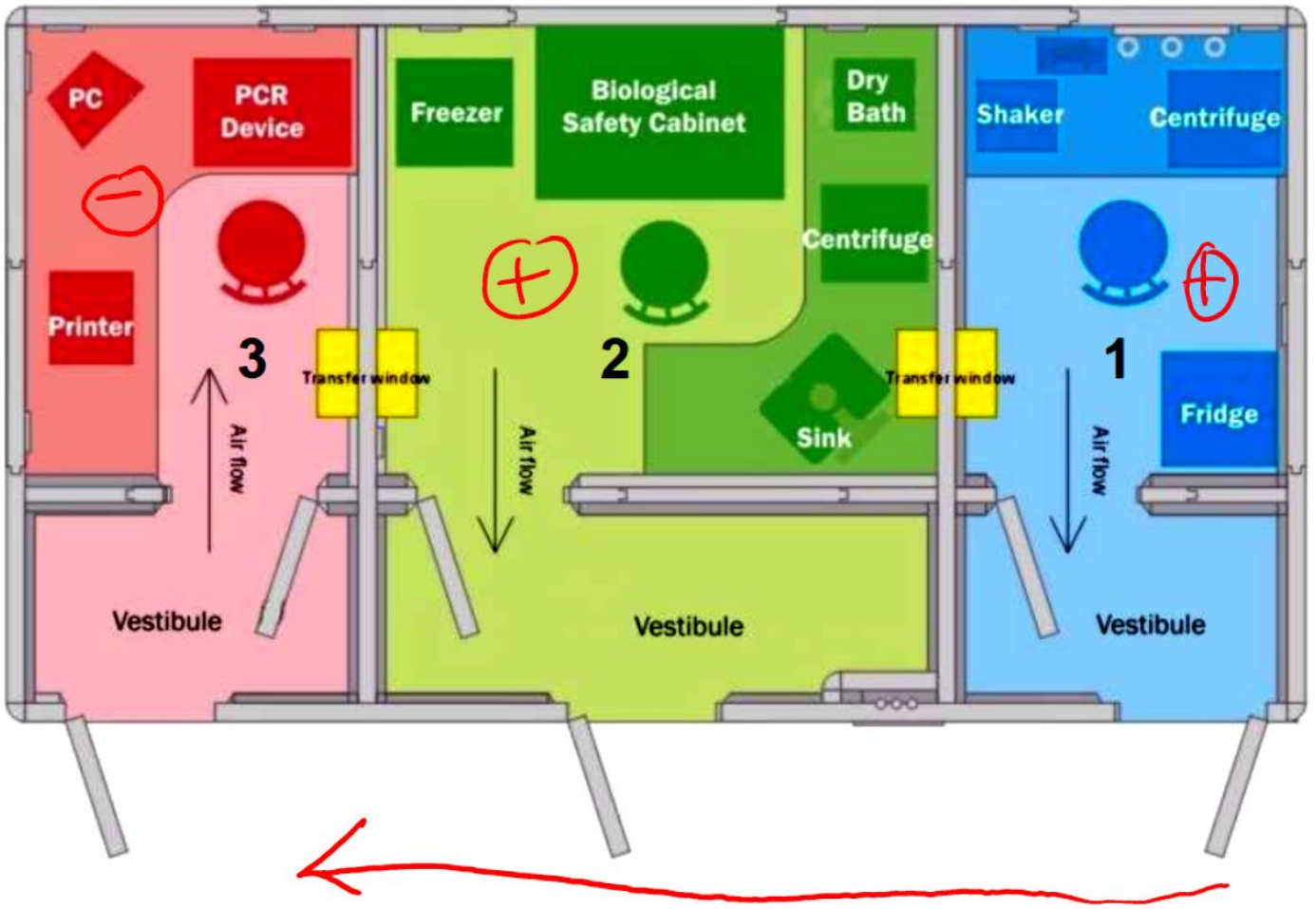
Three-Lab Setup PCR Laboratory



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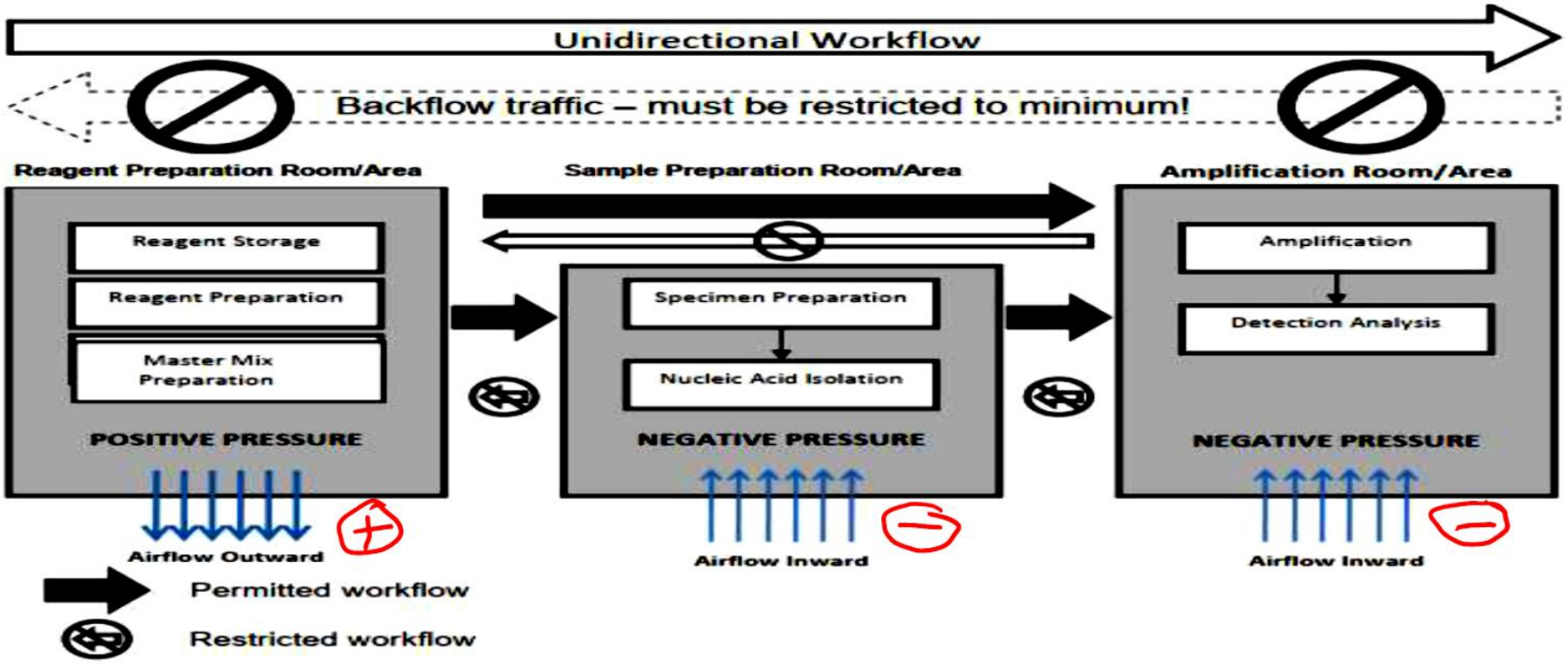
CLSI guideline fro 3 lab setup



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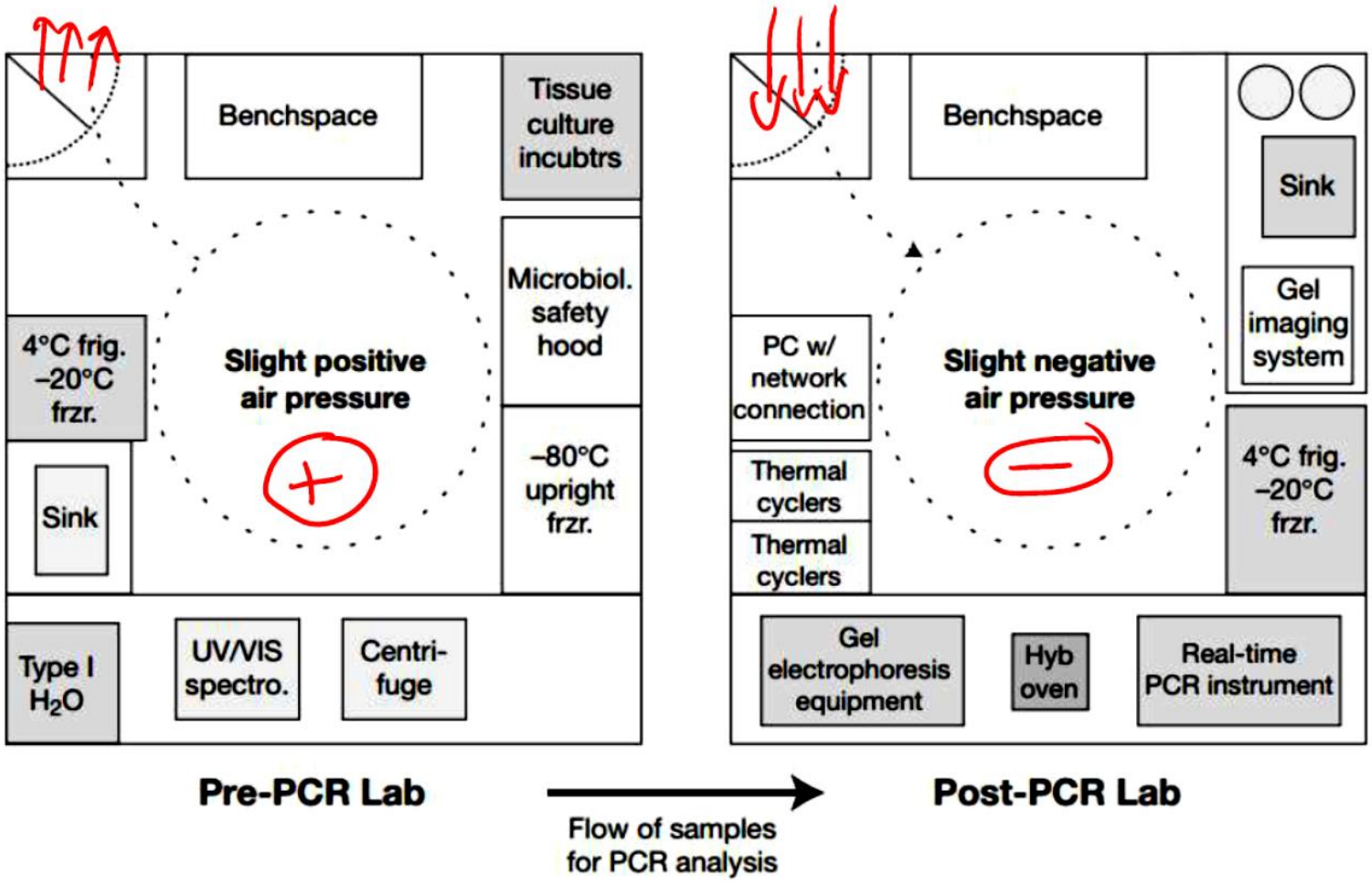
Example 2 lab setup



Courtesy of Miswar Fattah (IACC)

Take home messages

- **Plan carefully**
- **Consider the technologies that are going to be used**
- **Be alert of any hazards**


gracias
je vous remercie
arigato
xie xie
salamat
dank U wel
nandri
Terima Kasih
istuti
kop khun kha
gamsahamnida
Cảm ơn
Thank You
danke schön