UNITE

LABORATORY SET UP AND EQUIPMENT

I.C. **LABORATORY OF BIOCHEMICAL METHODOLOGIES AND EXPERIMENTAL MODELS**

- MODULE: **BIOCHEMICAL METHODOLOGIES**

BIOS-07/A

5 ECTS (CFU)



LABORATORY SET UP AND EQUIPMENT

BY THE END OF THE LESSON, THE STUDENT WILL BE ABLE TO:

- Describe the organization of a biochemistry laboratory (work areas, material flows, clean/dirty pathways).
- Identify basic glassware and plasticware and indicate their correct use (beakers, volumetric flasks, test tubes, pipettes, micropipettes, graduated cylinders, etc.).
- List and explain the function of basic instrumentation (pH meter, balances, centrifuges, stirrers, thermostatic water baths, refrigeration systems, spectrophotometer, etc.).
- Describe the equipment found in a cell culture laboratory (laminar flow hood, co_2 incubators, inverted microscope, etc.) And the fundamental environmental requirements.
- Distinguish between the different biosafety levels (bsl-1 to bsl-4) and the main containment measures in biomolecular laboratories.
- Apply key laboratory safety and conduct protocols (ppe, signage, waste management, accident procedures) in accordance with guidelines.
- Recognize and classify volumetric glassware (classes A, AS, and B; instruments for containing vs. delivering liquids).
- Correctly perform mass and volume measurements using balances, volumetric flasks, pipettes, and graduated cylinders.
- Calculate and use key concentration expressions (molarity, molality, and m/V, m/m, and V/V percentages).
- Design and prepare solutions (from solids and via dilution) by applying the relationships $M=n/V$, $n=m/MM$, and the dilution formula $M_{conc}\,V_{conc}=M_{dil}\,V_{dil}$.
- Identify the main sources of error in volumetric measurements and weighing procedures, and minimize them through good laboratory practices.

EVERYDAY DISCIPLINE

Good Laboratory Practice: the daily rules

No food, drink or cosmetics
Never at the bench — contamination & ingestion risk.

Label everything
Contents, concentration, date, initials, hazard.

Segregate waste
Chemical, biological and sharps never mix.

Read the SDS first
Check the Safety Data Sheet before first use.

Never mouth-pipette
Always use a pipette aid; respect the range.

Decontaminate & tidy
Clean the bench and instruments before leaving.

LAB ORGANISATION

A lab is divided into functional zones

The layout follows a clean-to-dirty flow and keeps desk work separate from wet work.

Write-up / dry area
Desks, notebooks, computers — no reagents here.

Wet bench
Sample prep, reactions, routine analytical work.

Fume-hood / ventilation area
Volatile or toxic chemicals handled under extraction.

Chemical & reagent storage
Segregated by hazard class; flammables in cabinets.

Cold storage
Fridge 4 °C, freezers −20 / −80 °C, cold room.

Waste collection point
Labelled streams for chemical & biological waste.

BUILT-IN SAFETY

Engineering & emergency safety features

Every lab is equipped with these — know where each one is before you start work.

Fume hood & biosafety cabinet
Contain fumes, aerosols and biological material.

Eyewash & safety shower
Flush eyes/skin for 15 min after a spill.

Fire extinguisher & blanket
Right class for the fire; blanket for clothing.

First-aid & spill kits
Immediate response to injury or chemical spill.

Marked emergency exits
Keep routes clear; know the assembly point.

Gas & electrical shut-off
Master valves/switches to isolate services fast.

Locate safety equipment on day one — during an emergency is too late.

PROTECTING YOURSELF

Personal Protective Equipment (PPE)

Your last barrier against chemical, biological and physical hazards.



Lab coat

Buttoned, wrists covered.



Gloves

Nitrile; change when contaminated.



Safety goggles

Splash & impact protection.



Face mask

When aerosols or powders arise.

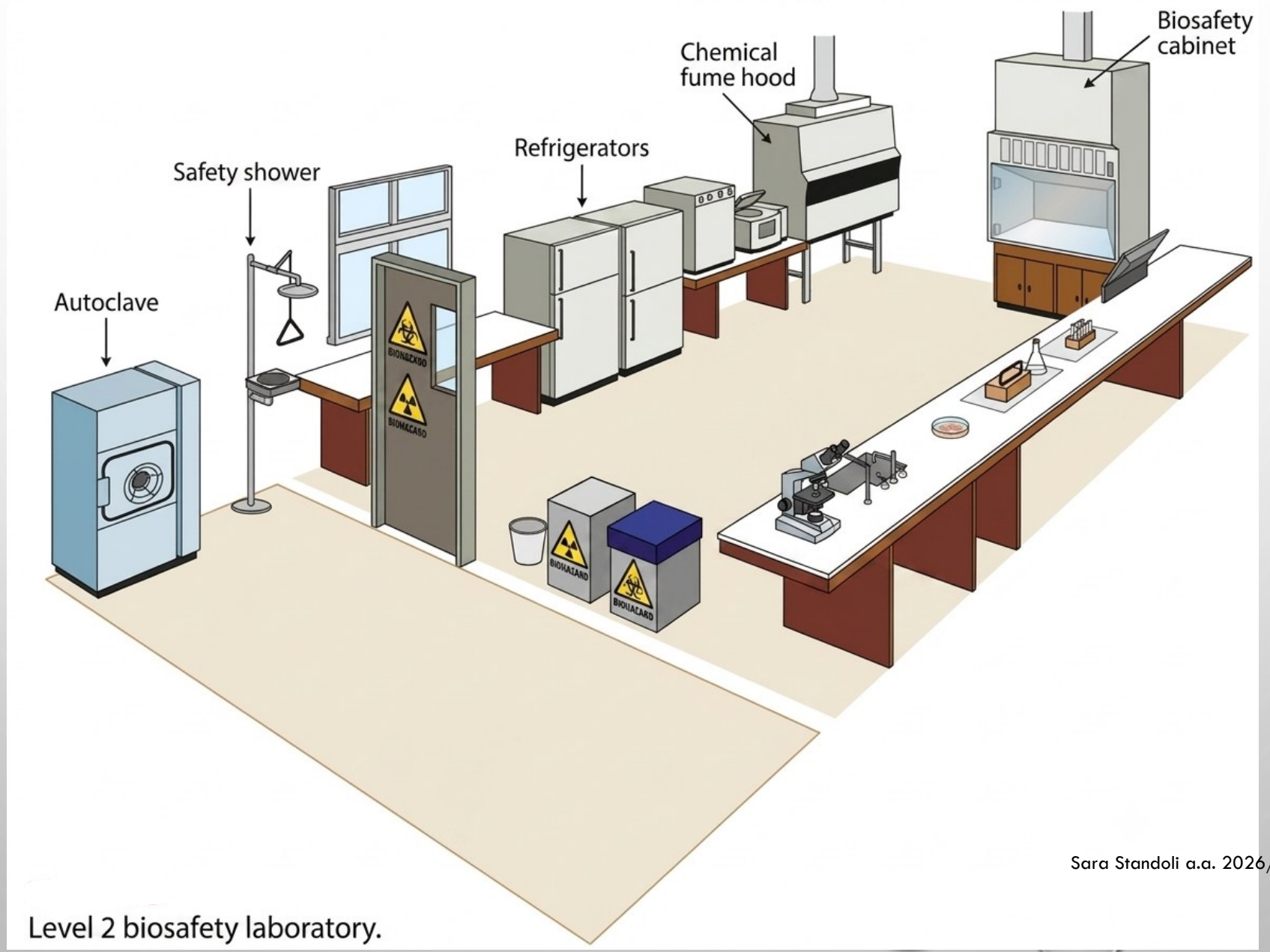


Closed shoes

Full foot cover; tie back hair.



PPE is the LAST line of defence — remove gloves before touching door handles, phones or keyboards.



Level 2 biosafety laboratory.

Biosafety levels: match the lab to the risk – for cell culture

BSL-1	**Well-characterised, non-pathogenic agents** Standard open bench + good lab practice.	*e.g. E. coli K-12*
BSL-2	**Moderate-hazard agents (ingestion / skin)** Biosafety cabinet, restricted access.	*e.g. S. aureus, Salmonella*
BSL-3	**Serious agents transmissible by inhalation** Directional airflow, respirators, sealed room.	*e.g. M. tuberculosis*
BSL-4	**Dangerous, often untreatable agents** Positive-pressure suits, dedicated facility.	*e.g. Ebola, Marburg*

Most 2nd-year teaching work is carried out at BSL-1, occasionally BSL-2.

LABORATORY SET UP AND EQUIPMENT

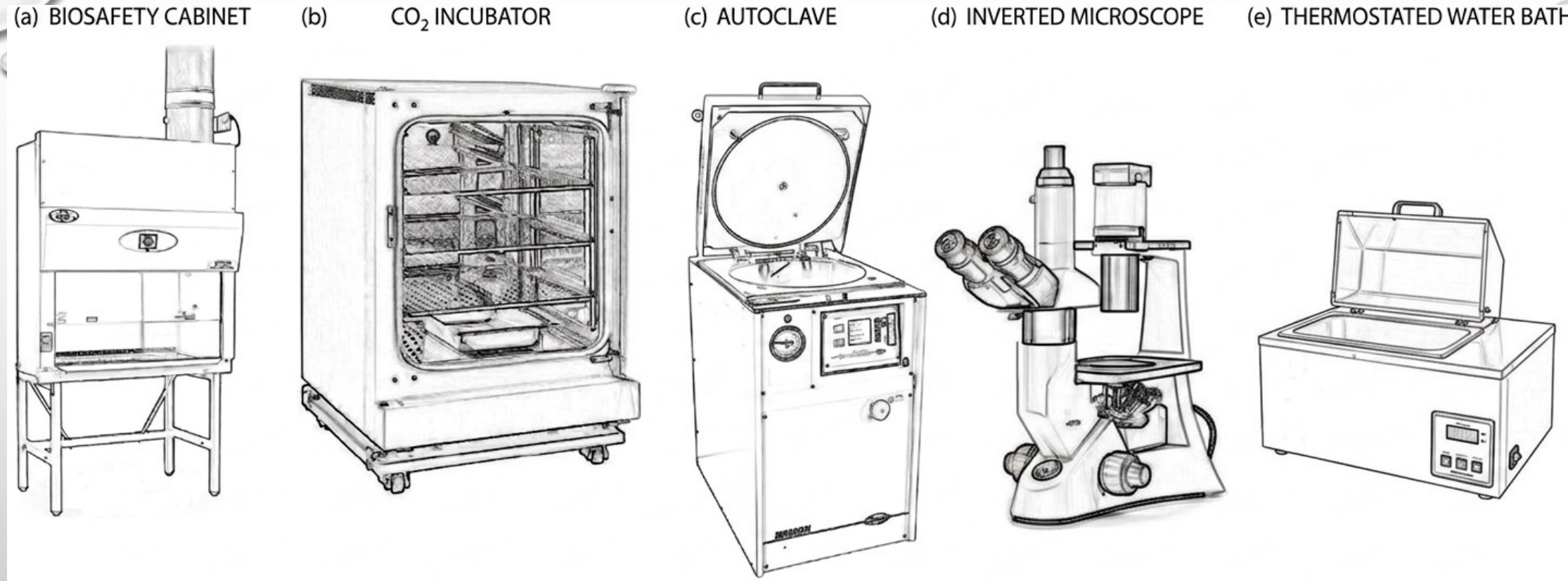


Figure 3.2. Common equipment for a cell biology laboratory:
(a) Biosafety cabinet;
(b) CO_2 incubator; (c) Autoclave;
(d) Inverted microscope;
(e) Thermostated water bath.

Biosafety levels: match the lab to the risk – for cell culture

Cell culture hoods: types & three classes

Laminar-flow hood: pushes HEPA-filtered sterile air over the work area, protecting cultures from the operator — and, in some designs, the operator from the cultures. **Horizontal** units blow air toward the operator (media prep / non-infectious work); **vertical** units — biosafety cabinets — filter air before it exits, for hazardous organisms.

Class I

Operator Strong

Culture Limited

Best for

Low-risk organisms; operator protection only. Air is drawn in past the operator and out through the top of the hood.

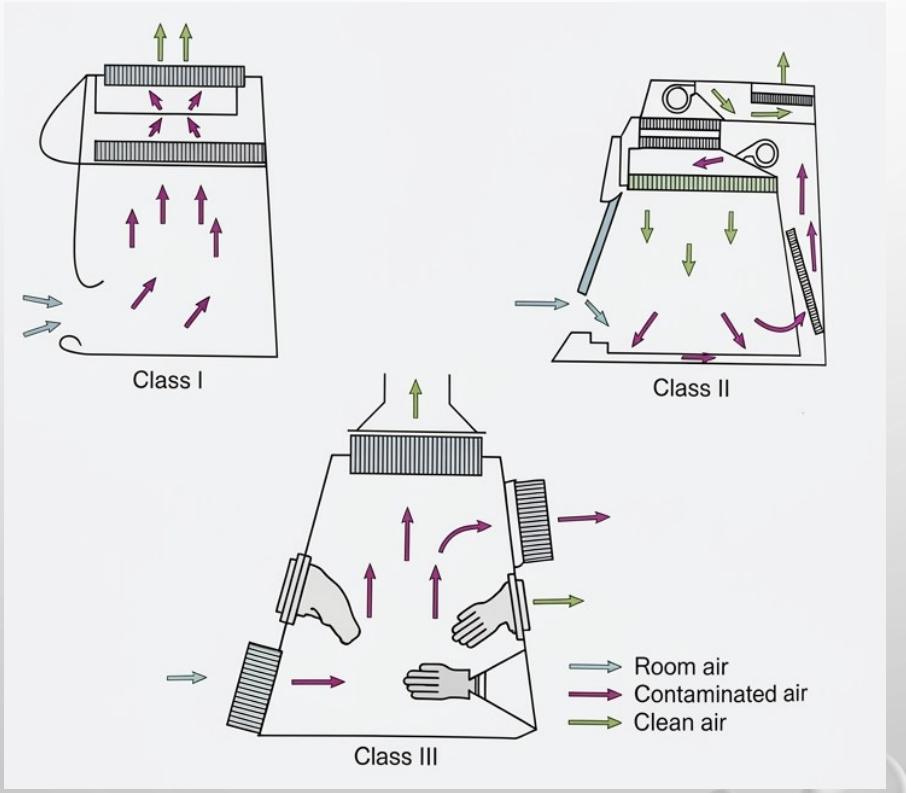
Class III

Operator Maximal

Culture Maximal

Best for

Highly pathogenic organisms and known human pathogens. Fully sealed cabinet operated through two glove ports.



Class II

Most common

Operator Strong

Culture Strong

Best for

Animal cell culture, low–moderate-risk agents. Incoming air is HEPA-filtered and streams down over the culture. Not for high-risk pathogens.

Biosafety levels: match the lab to the risk – for cell culture

Biological agents are classified into four international ***Risk Groups*** based on their infectivity, the severity of the disease they cause, and the availability of treatments or preventive vaccines

Risk Group	Risk Level	Human Disease Risk	Treatment / Prevention	Common Examples
Group 1	Low	Unlikely to cause disease in healthy adults.	Not applicable.	E. coli (non-pathogenic), Bacillus subtilis
Group 2	Moderate	Can cause human disease, but rarely serious; low community risk.	Effective treatments and vaccines often available.	Hepatitis B, HIV, Salmonella
Group 3	High (Individual)	Causes serious or lethal human disease; low to moderate community spread.	Treatments or vaccines may be available.	Tuberculosis (Mycobacterium tuberculosis), Anthrax
Group 4	High (Individual & Community)	Causes severe, often fatal disease; easily transmitted.	Effective treatments or vaccines usually not available.	Ebola virus, Marburg virus, Lassa virus

LABORATORY SET UP AND EQUIPMENT – CELL CULTURE

CO_2 incubators: keeping cells alive



Optimal growth conditions

Constant 37 °C in a humidified atmosphere with 5% (or 10%) CO_2, strictly regulated.

pH control

CO_2 dissolves into the bicarbonate-buffered medium, holding physiological pH 7.2–7.4.

pH buffering: $HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$

Humidity & hygiene

A sterile water tray stops medium evaporating; keep it microbe-free (Roccal-D 1%, copper sulfate, 70% IMS) or use copper-coated units.

Monitoring & gas supply

Check with a gas analyser (Fyrite); a changeover unit auto-switches CO_2 cylinders and an alarm signals a low level.

Other essential cell-culture equipment



Inverted phase-contrast microscope

Visualise living cells; check morphology and spot early signs of stress.



Water bath

Thaw frozen cell stocks and warm media to 37 °C before use.



Centrifuge

Separate cells from culture medium by pelleting.



Culture plasticware

Petri dishes, multi-well plates (6–96 wells) and T-flasks (T-25/75/225) for adherent or suspension cells.



Liquid nitrogen tank

Long term (cryogenic) cell storage (-196°C)



Autoclave

Steam-sterilise media, glassware and waste — typically 121 °C, ~15 psi, 15–20 min.

Other Laboratory Organization - Biochemistry Laboratory

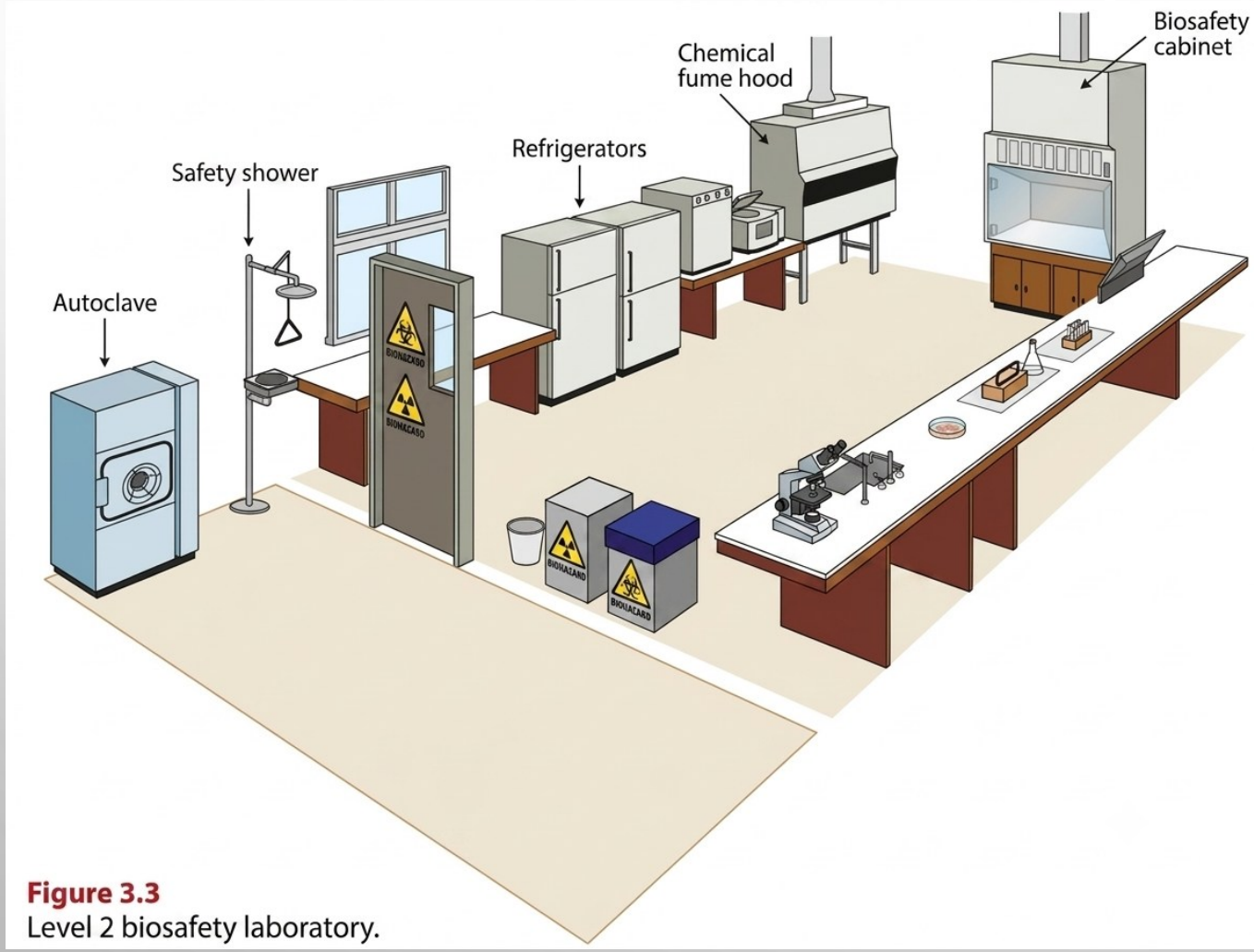


Figure 3.3
Level 2 biosafety laboratory.

- The facility is organized into functional areas (weighing, solution preparation, instrumentation area, etc) designed for the safe execution of experiments involving biomolecules and biological samples.
- Workflow and Materials: an orderly sequence of steps (receipt, storage, use, and disposal of reagents and samples) designed to minimize contamination, experimental errors, and risks to the operator.

Other Laboratory Organization - Biochemistry Laboratory

Basic instrumentation - Core small instrumentation of the bench



pH meter

An instrument that measures the pH of a solution using an electrode sensitive to hydrogen ion concentration; essential for preparing buffers and controlling experimental conditions.



Centrifuge

An instrument that separates mixture components using centrifugal force; it enables the separation of cells, organelles, and protein fractions based on density and size.



Vortex mixer

Fast, thorough tube mixing.



Stirrer / hotplate

Mixing with gentle heating.



Spinner

Centrifuges tubes/qPCR strips or plate at low speed to bring droplets down from the lid and walls to the bottom.



Block Heater

To perform sample controlled incubation temp.



UV-Vis spectrophotometer

An instrument that measures light absorption by a solution at a specific wavelength, allowing for the determination of biomolecule concentrations.

Other Laboratory Organization - Biochemistry Laboratory

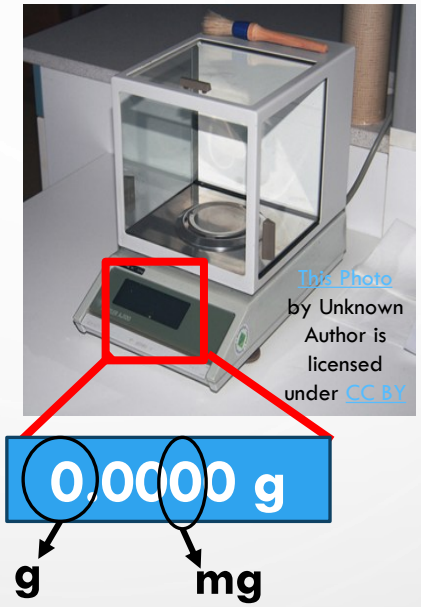
Basic instrumentation - Core small instrumentation of the bench

Technical balance



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Analytical balance



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An instrument for weighing solid reagents with high precision; essential for preparing solutions of known concentration and minimizing quantitative errors. The main characteristics of a balance are its sensitivity and capacity.

- **Technical balances** are sensitive to the nearest **hundredth of a gram;** they are generally used for preparatory measurements, where an error of 0.05 grams is acceptable
- **Analytical balances** are sensitive to the nearest **tenth of a milligram;** they are more sensitive instruments that measure an object's mass with a precision of 0.00005 g (0.05 mg). Moreover, they feature two sliding side doors; when closed, these allow measurements to be taken without weight fluctuations caused by air currents.

Both balances must be levelled using adjustable feet controlled by screws.

Other Laboratory Organization - Biochemistry Laboratory

Basic instrumentation – Refrigeration system



LIQUID NITROGEN



Refrigerators, freezers, and liquid nitrogen used to store reagents, media, sera, and biological samples at controlled temperatures, preserving their stability and activity.

Laboratory Glassware

Classification of glassware

Glassware consists of vessels designed to hold or dispense limited quantities of substances. These containers fall into two main categories:

- **non-graduated**, such as test tubes, Erlenmeyer flasks, separatory funnels, and beakers;
- **graduated**, such as cylinders, pipettes, and volumetric flasks—also known as volumetric instruments—because they have been precisely calibrated by the manufacturer.

Glassware can be manufactured using standard soda-lime glass or, more commonly, borosilicate glass (Borosilicate 3.3)—often known by trade names such as Pyrex® or Schott-Duran®. The latter is highly resistant to temperature fluctuations, thermal shock, and chemical exposure.

For quality certification—in accordance with European DIN (Deutsches Institut für Normung) and ISO (International Organization for Standardization) standards—volumetric instruments must be marked with the letters A, AS, or B, indicating whether the glassware belongs to Class A, AS, or B:

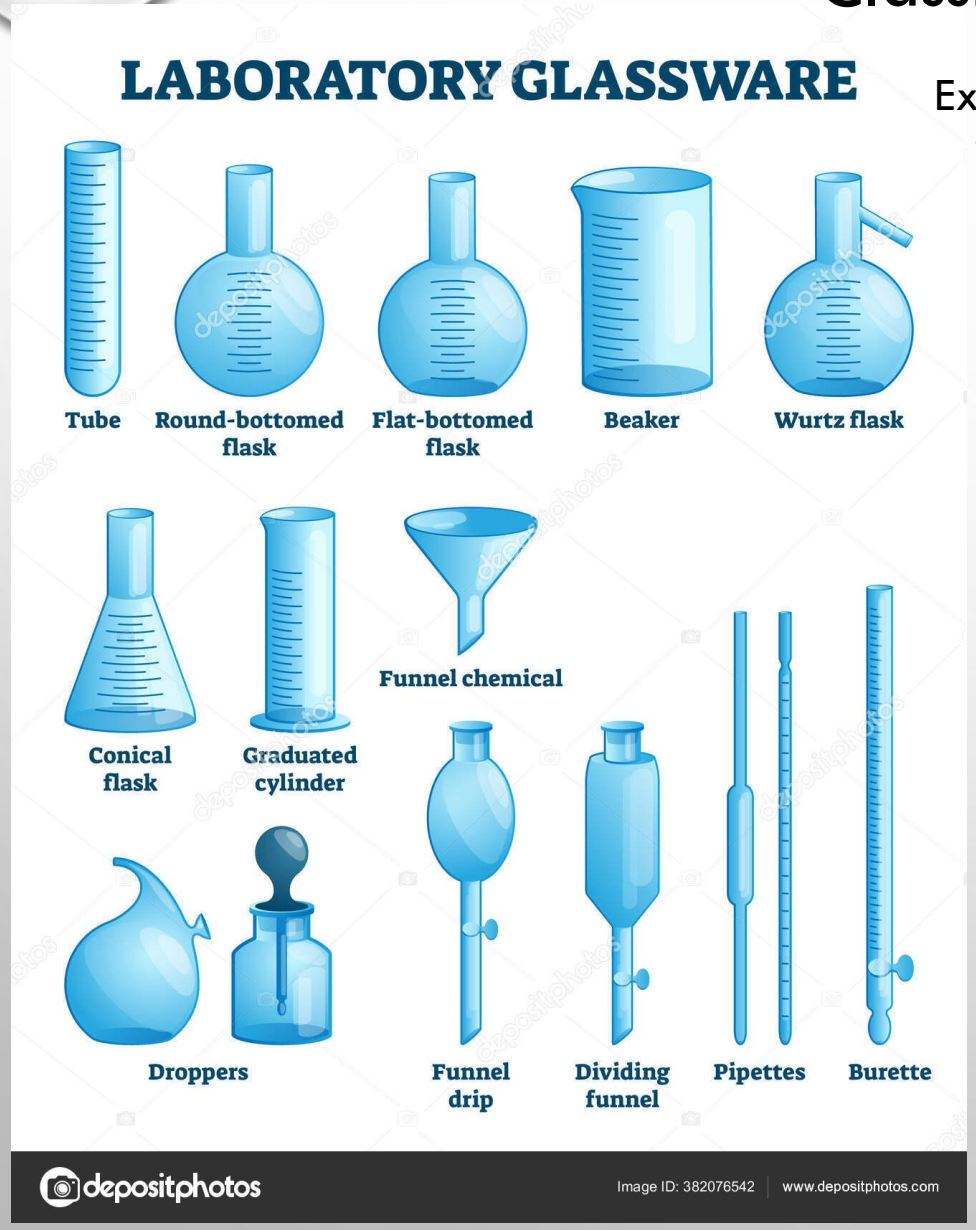
- **Class A and AS** volumetric instruments are calibrated for delivery (i.e., to dispense liquid), meaning ***the transferred volume is always less than the volume contained within the vessel***; this is due to the thin film of liquid that adheres to the container's inner surface. During calibration, delivery and waiting times are determined based on the instrument's class;
- **Class B** volumetric instruments are available in glass or plastic, and their error limits are twice those of Class A/AS instruments (for which no delivery time is specified);

Each volumetric measuring instrument is individually calibrated using double-distilled water by weighing the dispensed liquid.

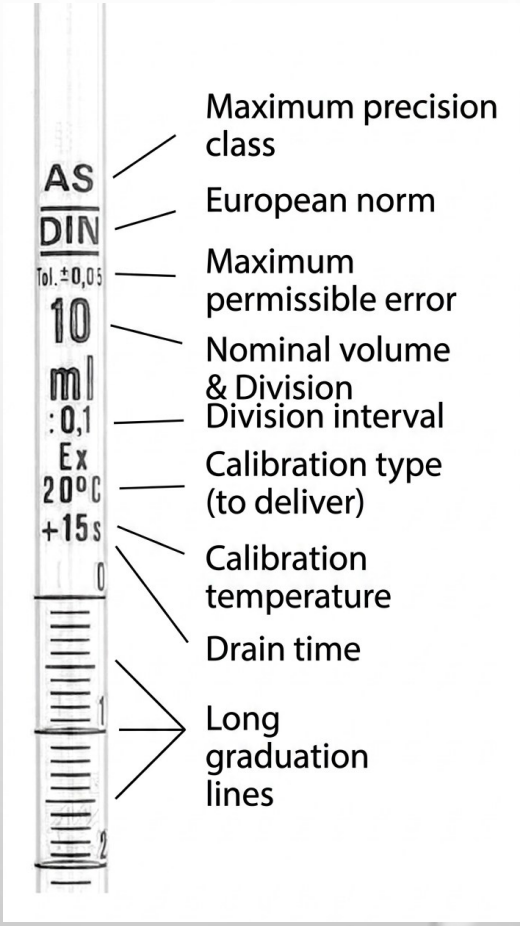
Since the volume of a liquid mass varies with temperature, all volumetric equipment is calibrated by convention at 20°C (errors resulting from the operating temperature remain within the limits permitted by regulations).

Laboratory Glassware

Classification of glassware



Example of information present on a measuring instrument, in this case a serological pipette



Laboratory Glassware

Classification of glassware

Volume (mL)	Error Limits	
	Class A and AS (± mL)	Class B (± mL)
0.5	0.005	0.010
1	0.008	0.015
2	0.010	0.020
5	0.015	0.030
10	0.020	0.040
20	0.030	0.060
25	0.030	0.060
50	0.050	0.100
100	0.080	0.150

Error limits for volumetric instruments, subdivided by precision classes: maximum for class A and AS (the latter used for fast dosing with pipettes and burettes), with a double error limit for class B.

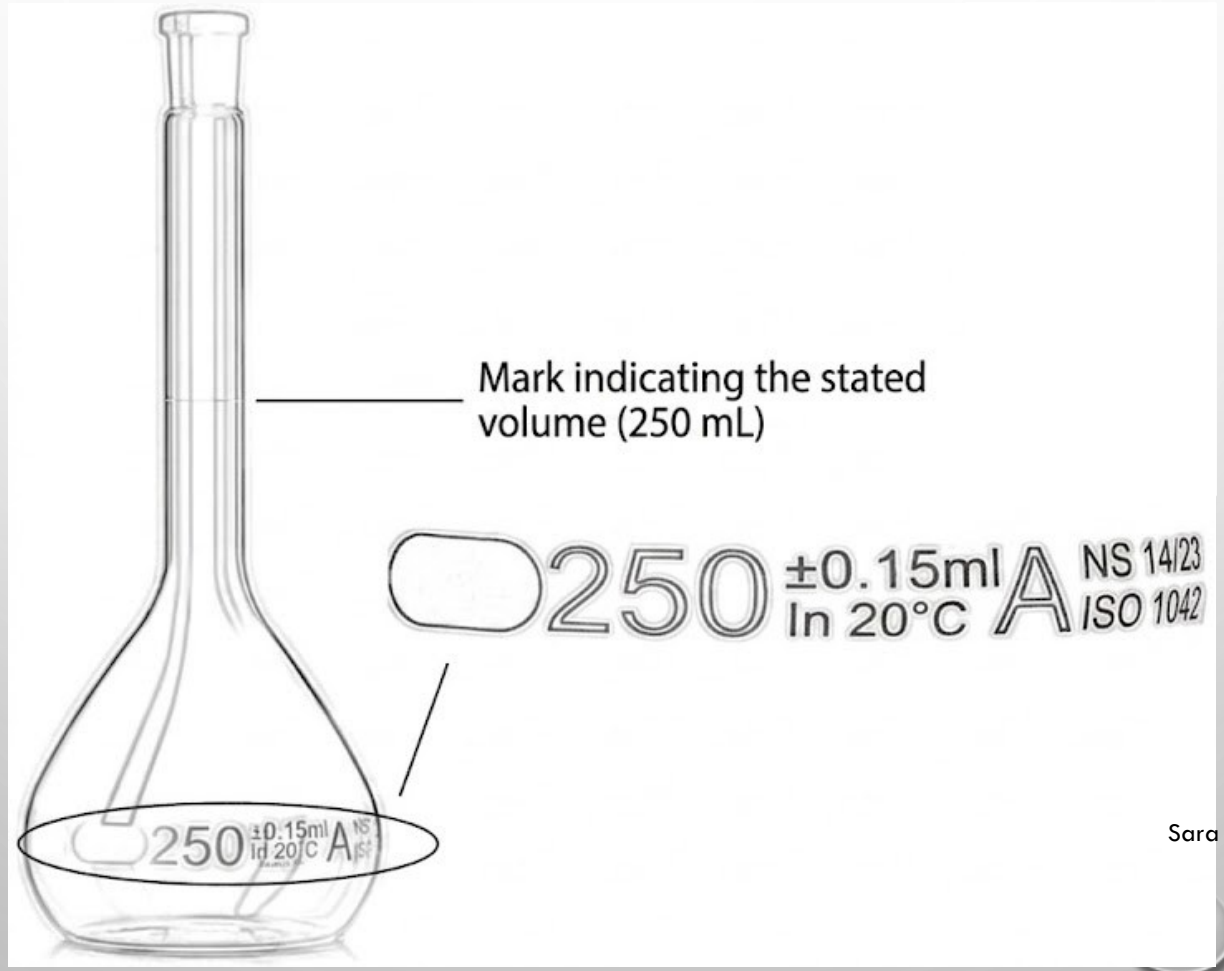
Temperature (°C)	Mass of 1mL (g)
15	0.9979
20	0.9972
25	0.9961
30	0.9948

Mass of 1mL of double-distilled water measured at different temperatures

Laboratory Glassware

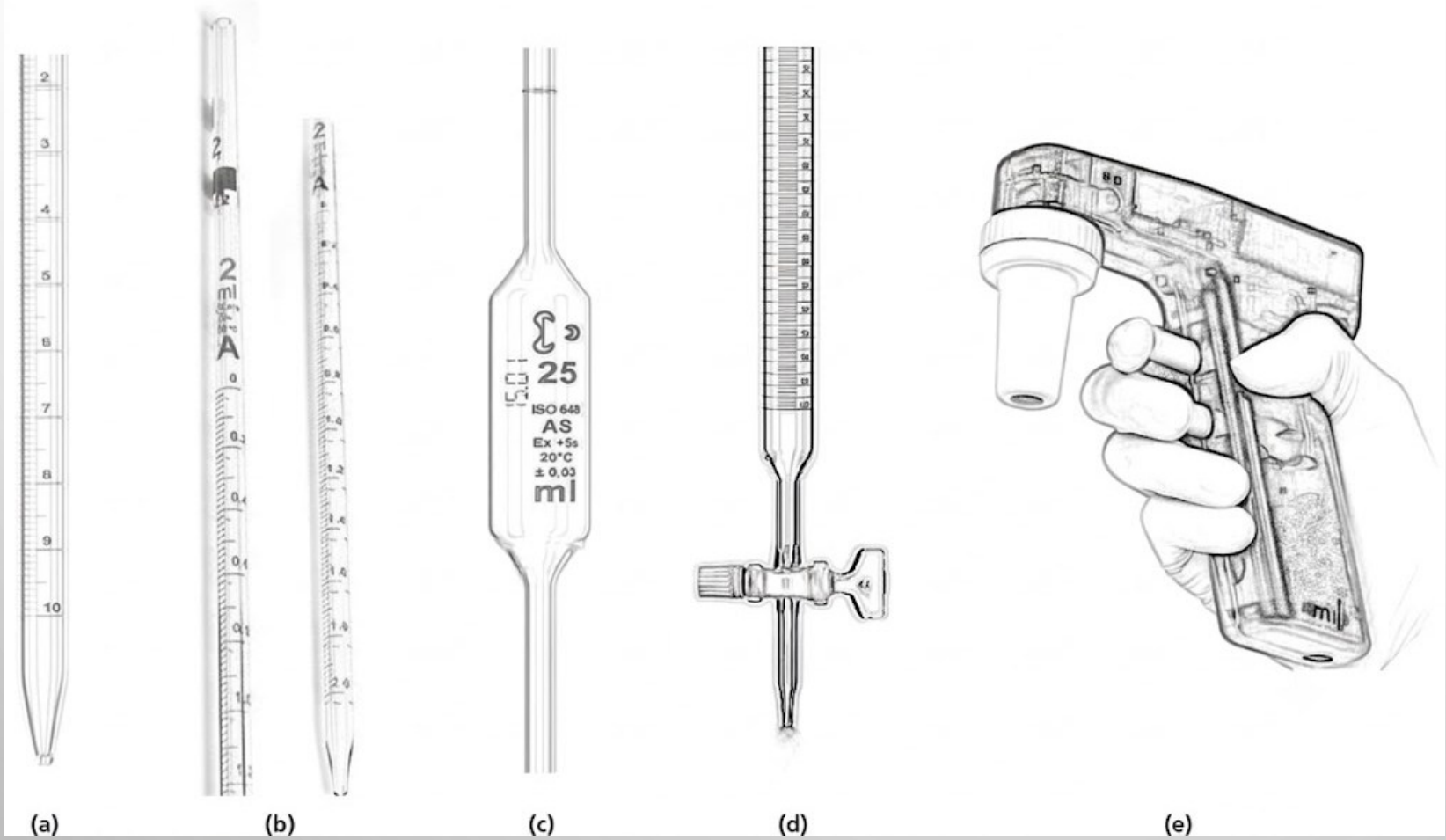
Classification of glassware

General appearance of a volumetric flask. In the enlargement: the stated volume (250mL), the maximum permissible error (±0.15 mL), the calibration temperature (20°C), the container's accuracy class (A), and the ISO standards.



Laboratory Glassware

Classification of glassware



Pipettor and various types of pipettes used in the laboratory:

a) Serological pipette,

b) Mohr pipette,

c) Volumetric pipette,

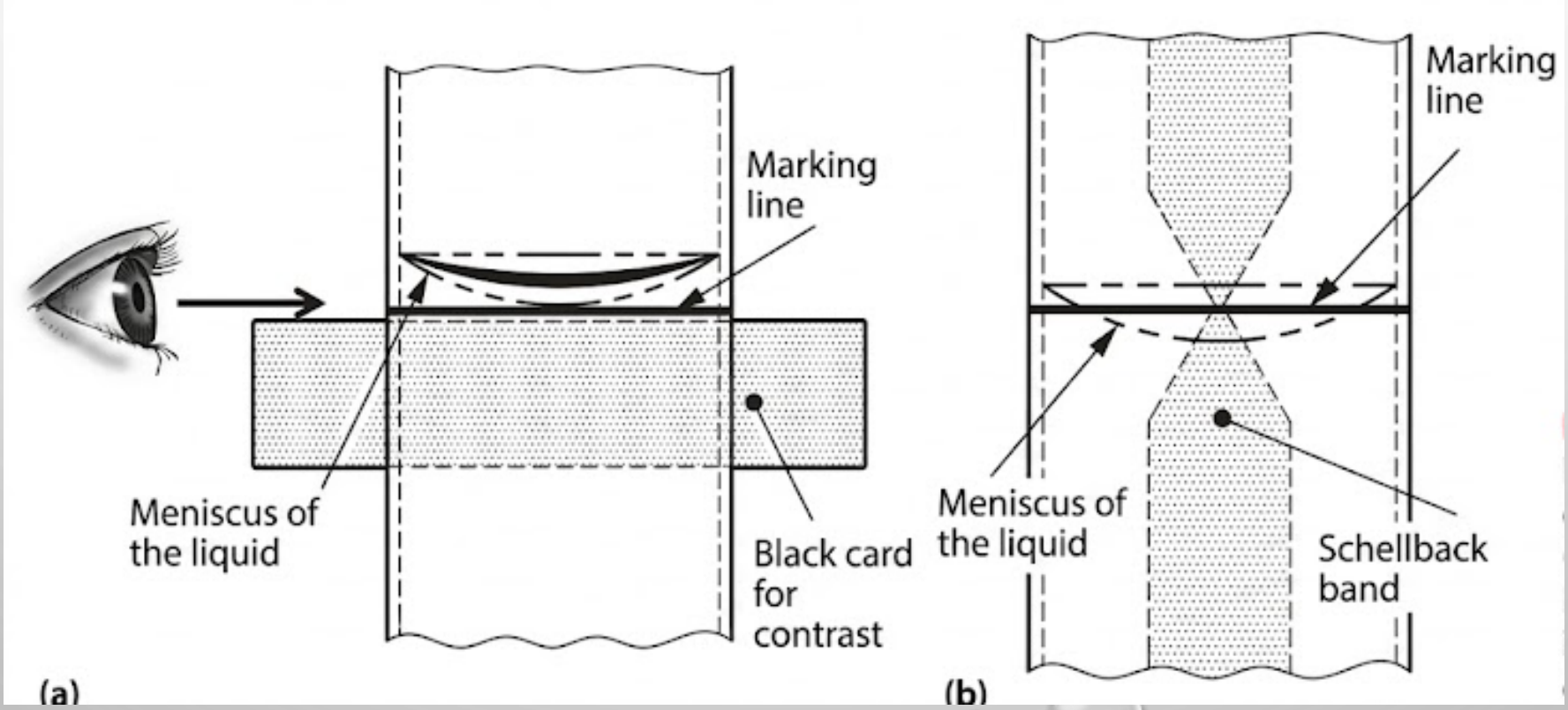
d) Burette,

e) Automatic pipettor

Laboratory Glassware

Classification of glassware – how to measure correctly

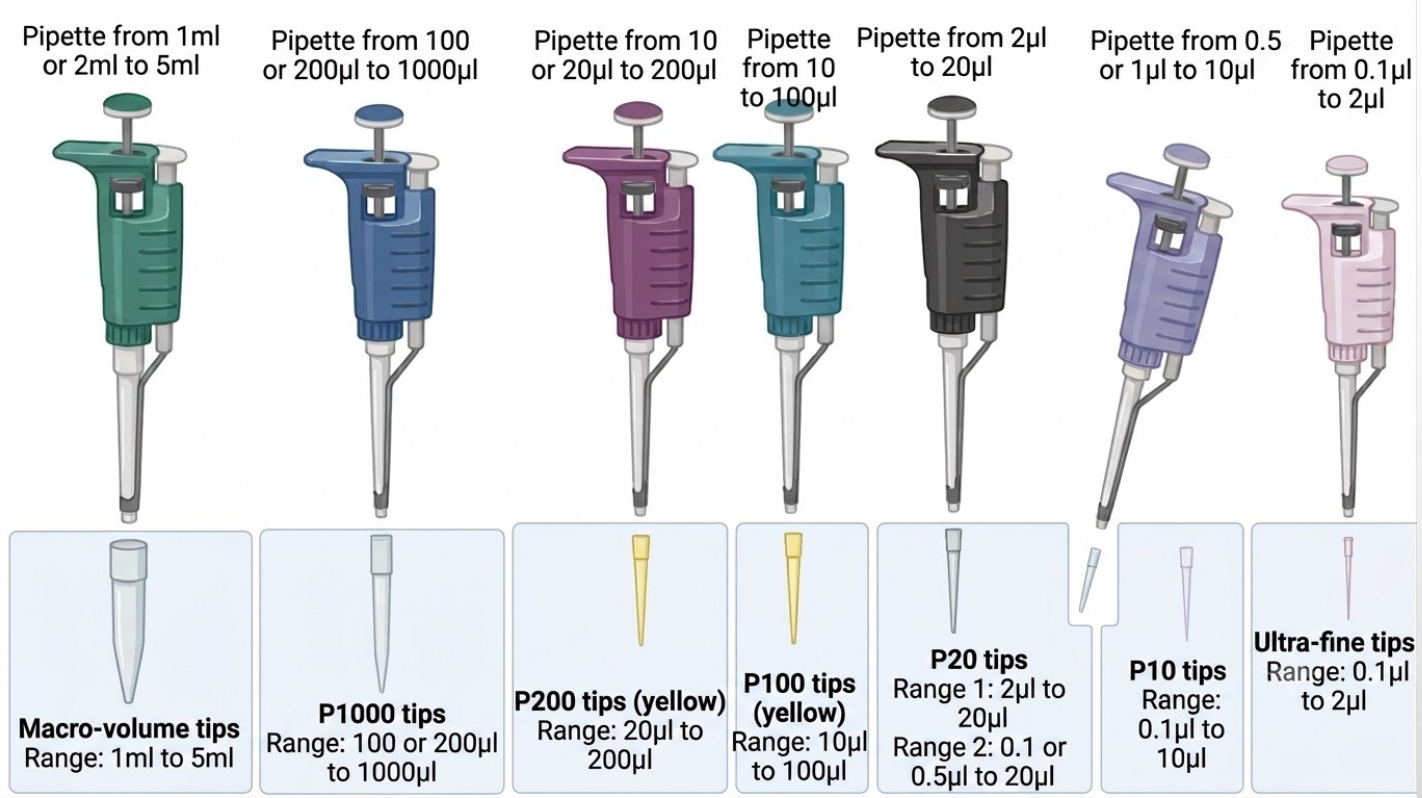
Reading of the meniscus a) in volumetric containers and b) in a burette.



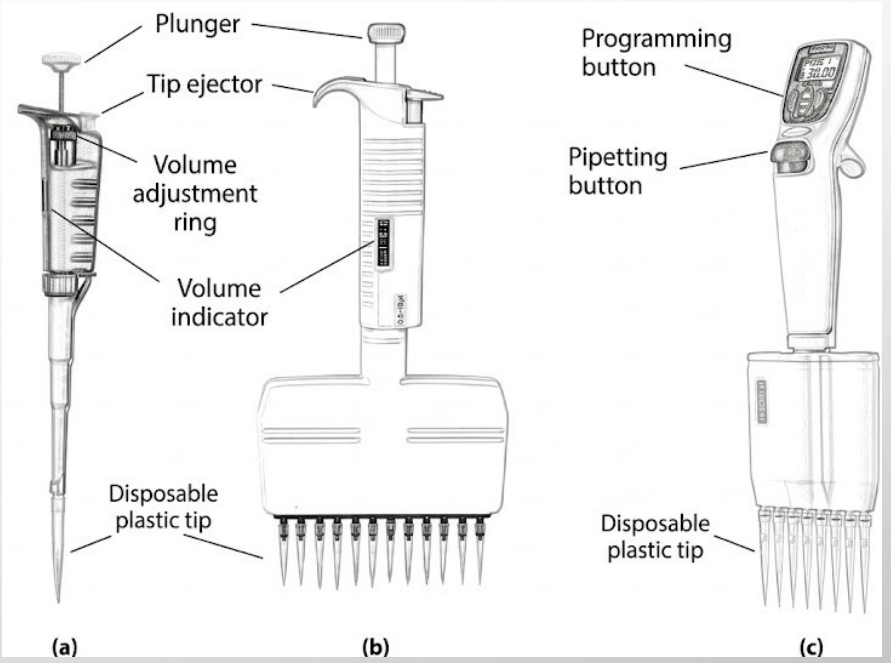
Laboratory Components

Bench Components - Pipettes

Pipettes and pipette tips



Pipettes Elements

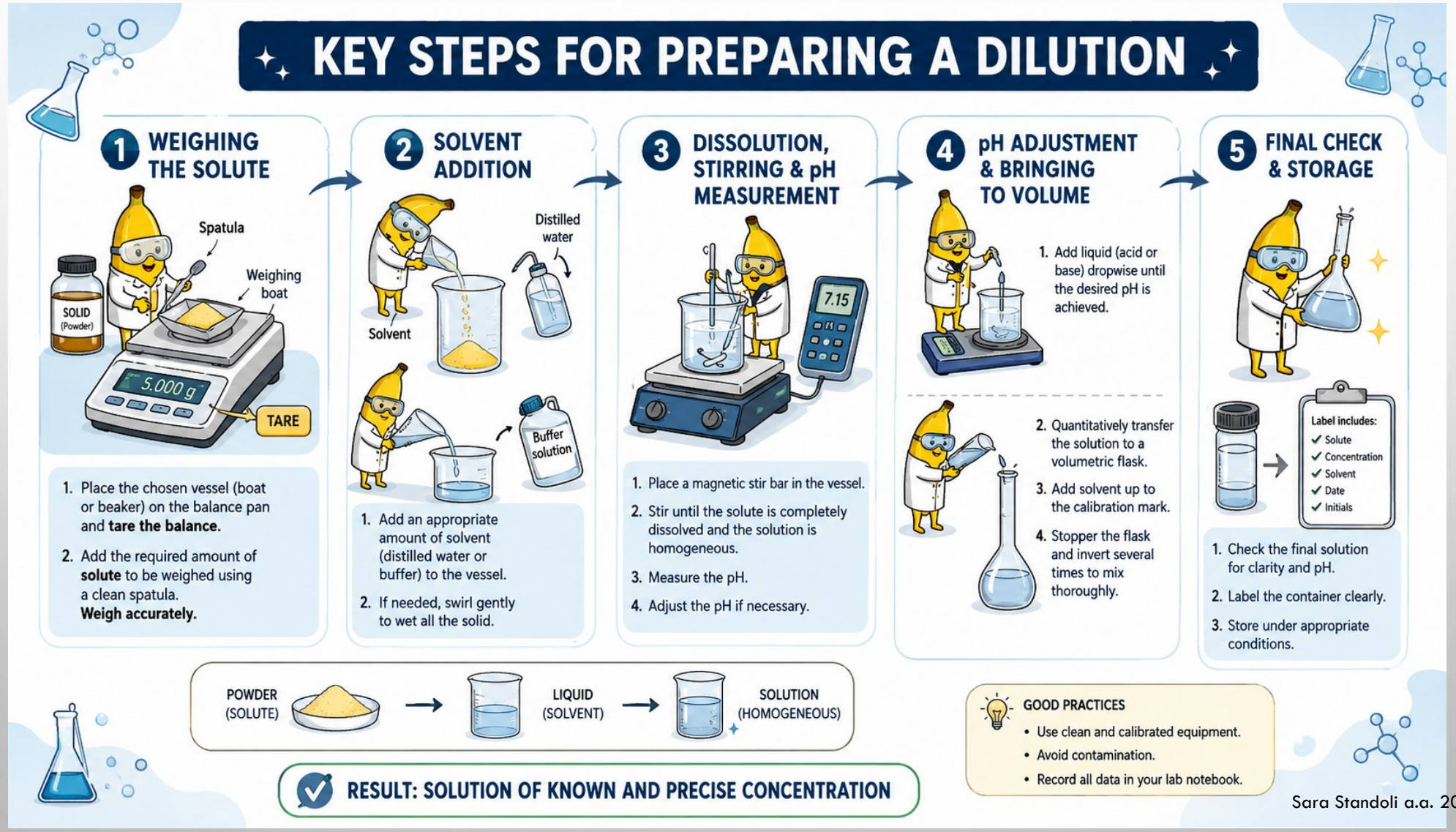


Multichannel

Electronic multichannel

PREPARATION OF SOLUTIONS

CONCENTRATION: the amount of a substance contained in a specific volume or mass (homogeneous mixtures consisting of a solvent and one or more solutes);



REVIEW: CONCENTRATION AND DILUTION FORMULAS

1 Expressing concentration

$M = n / V = m / (MW \cdot V)$

molarity (mol/L)

% w/w = (m solute / m solution) · 100

e.g. 5 g in 100 g → 5%

% w/v = (g solute / mL solution) · 100

e.g. 9 g in 100 mL → 0.9%

% v/v = (V solute / V solution) · 100

for miscible liquids

ppm = mg / L ppb = µg / L

traces and dilute reagents

2 Diluting

$C_1 \cdot V_1 = C_2 \cdot V_2$

the dilution equation

Df = V final / V taken

Df = C initial / C final

e.g. 1 mL into 10 mL → DF = 10 (1:10)

conc. drops 10-fold

Serial dilutions: factors MULTIPLY

1:10 × 1:10 = 1:100

3 Preparing in practice

From a solid:

$m = M \cdot MW \cdot V$

weigh solute, bring to volume

From a liquid stock:

$C_1V_1 = C_2V_2$

aliquot from concentrated stock

Correct for density (d) and purity (%) of reagent

commercial conc. reagents

4 Mixing of multiple solutions of known concentration,

$M = (na + nb)/(Va + Vb)$

na: number of moles of solution a, nb: number of moles of solution b, Va: volume of solution a, Vb: volume of solution b.

Mind the units.

1 L = 1000 mL · 1 g = 1000 mg = 10^6 µg.

Always check that the units cancel before you calculate.

Note: Caution is required when preparing dilutions of strong acids or bases, as they react with water, causing a sudden rise in temperature and a risk of highly corrosive splashes. Therefore, the solute (acid or base) must be added to the water, not the other way around; because the solute is denser than the liquid, it sinks to the bottom of the container, allowing the heat generated to dissipate through the water.