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Practical laboratory skills for molecular biologists

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Practical laboratory skills for molecular biologists

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Objectives of this guide

Working in any kind of laboratory will require the analyst to possess a basic set of practical skills. Measuring mass or volume, and preparing solutions of known concentration, are likely to be everyday activities. In addition, working in a molecular biology laboratory will entail specific considerations related to the nature of the materials being handled.

Samples analysed in molecular biology laboratories are frequently of biological nature − human or animal tissue − which can pose health risks to the analyst. Frequently, the analyte of interest is DNA or RNA which, with current technologies, will need to be amplified in order to be detected. This amplification step is susceptible to cross-contamination from previously amplified material. Therefore, preventing contamination of the samples and protecting the analyst are critical concerns for molecular biologists.

This guide covers the basic laboratory skills and other measures required to produce valid results in a molecular biology laboratory − results that are not compromised by poor practical technique, possible cross-contamination or non-validated methodology.

This guide is an extension to our “Laboratory skills training handbook”. It is designed to stand alone, but where practical exercises could be useful, it is recommended to refer to both in combination.

1 Working in the laboratory

The laboratory is a potentially hazardous working environment. You may well be using chemicals, biological materials and equipment which, if not handled correctly, could cause you or your colleagues harm. However, with the proper procedures in place, work can be carried out safely. It is essential that you familiarise yourself with the general safety procedures in place in your laboratory and with any special procedures required to carry out a particular test method safely. When working in the laboratory you also need to know how to select an appropriate test method and equipment, and understand the importance of following standard operating procedures. This section covers:

- Health and safety issues
- Selecting a test method and equipment
- Importance of standard operating procedures

1.1 Health and Safety issues

When working in the laboratory you must always:

- Wear suitable eye protection (safety glasses or goggles); this is always important but if using a UV-transilluminator (to view gels), a full PVC visor should be worn
- Wear a laboratory coat: the style and colour of the laboratory coat may vary depending on the specific set-up of the laboratory. 'Howie' style laboratory coats with cuffed wrists are generally recommended for laboratories where DNA/RNA is analysed using amplification techniques to minimise potential carryover of genomic material
- Wear suitable footwear (e.g. do not wear open-toed shoes or sandals)
- Wear gloves: gloves are essential in biological laboratories; both to protect the analyst from hazardous chemicals and biohazards, and to prevent contamination of samples and reagents with biological material from the analyst. There are many types of gloves; a brief summary of properties and intended uses is included in Table 1-1. It is worth noting that the majority of tasks in the majority of laboratories can be carried out wearing natural rubber or nitrile gloves
- Wash your hands on leaving the laboratory

Table 1-1. Considerations when selecting the appropriate type of protective laboratory gloves

Type of glove	When to use	When not to use
Natural rubber (latex) (incidental use)	When handling water based material	When handling solvents In case of allergies
Nitrile (incidental use)	General use In case of latex allergies	When very precise and accurate handling is required as they are relatively thick
Polyvinyl chloride (PVC) (extended contact)	When handling acids	When handling organic solvents
Neoprene (extended contact)	Acids, bases, alcohols, fuels, peroxides, hydrocarbons, and phenols	Poor for halogenated and aromatic hydrocarbons
Butyl (extended contact)	Ketones and esters	Gasoline, aliphatic, aromatic and halogenated hydrocarbons
Polyvinyl alcohol (PVA) (extended contact)	Aromatic and chlorinated solvents	Water based solutions
Fluoro-elastomer (Viton)™ (extended contact)	Chlorinated and aromatic solvents	Ketones

In the laboratory you should never:

- Eat or drink
- Smoke
- Apply cosmetics

You should know the meaning of common warning and hazard signs used in the laboratory. You will see different coloured signs:

- **Blue** signs are mandatory
- **Red** signs are prohibitive (or relate to fire alarms/fire-fighting equipment)
- **Green** signs give safety instructions
- **Yellow** signs give warnings

Some examples of signs you may see in the laboratory are shown in Figure 1-1.

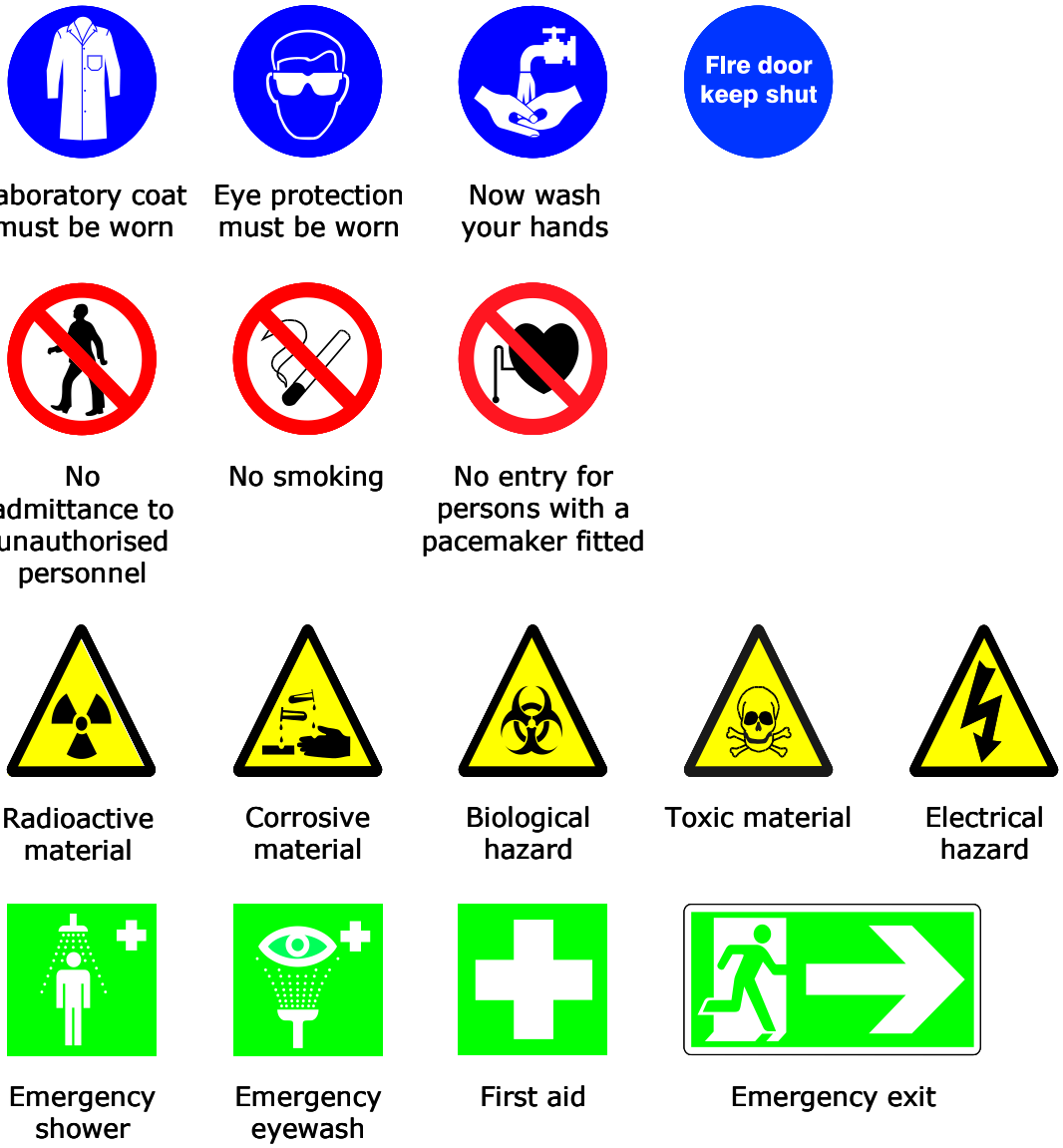


Figure 1-1. Example of laboratory signs

1.1.1 Working with hazardous materials

1.1.1.1 Recognising hazardous substance labelling

Hazardous substances can take many forms, including:

- chemicals
- products/formulations containing hazardous chemicals
- fumes
- dusts
- vapours
- mists
- nanoparticles
- gases and asphyxiating gases
- biological agents.

The packaging of a substance will display symbols indicating the nature of the hazard. The 'orange square' symbols, with which you may be familiar, are being replaced. The European Regulation on classification, labelling and packaging of substances and mixtures (the 'CLP Regulation') adopts the United Nations GHS (Globally Harmonized System) hazard pictograms Both the old and new symbols are displayed in Table 1-2. With the new system, the hazard is not described with one word, but a statement, which needs to be read carefully.

Table 1-2. International hazard symbols

Description	Old pictogram	New pictogram	Hazard type
Exploding Bomb			Explosives
Flame			Flammable substances
Flame Over Circle			Oxidising gases, liquids and solids
Gas Cylinder			Gases under pressure or compressed
Corrosion			Corrosive (to metals, skin, eyes)
Skull and Crossbones			Acute toxicity (oral, dermal, inhalation)

Table continued

Table 1.2 continued

Description	Old pictogram	New pictogram	Hazard type
Exclamation Mark			Health hazard: Acute toxicity (oral, dermal, inhalation) Specific Target Organ Toxicity – Single exposure
Health Hazard			Serious health hazard: Mutagenicity, carcinogenicity, toxicity, aspiration hazard
Environment			Hazardous to the aquatic environment

Hazard symbol labels are fixed to individual packages of hazardous substances to warn people who handle them or use their contents. Pictograms are also incorporated into supply labels giving information about the most significant immediate danger and the emergency action to take. A typical supply label is shown in Figure 1-2.

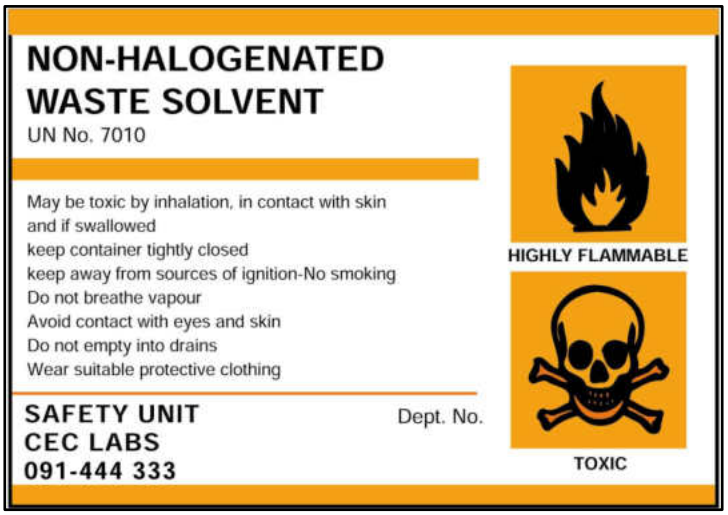


Figure 1-2. Hazard warning label (old style)

1.1.1.2 Control of substances hazardous to health (COSHH).

Employers are required by law to ensure adequate control of exposure of employees in the workplace to hazardous materials that cause ill health. This includes controlling exposure to materials that cause skin diseases and to materials that enter the body through the skin and cause problems elsewhere. The regulations pertaining to this requirement are the Control of Substances Hazardous to Health Regulations 2002 (Amended) (COSHH) and employers and employees are required to comply with its requirements. All parties are therefore required to:

- assess risks
- provide adequate control measures and ensure their use and maintenance
- provide information, instruction and training
- provide health surveillance in appropriate cases

A 'COSHH assessment' must be carried out to identify the hazards and risks associated with substances used in the laboratory.

1.1.1.3 Safety data sheets

Suppliers of chemicals must provide an up to date safety data sheet if a substance is dangerous for supply. Safety data sheets provide information on chemical products that help users of those chemicals to make a risk assessment. They describe the hazards the chemical presents, and give information on handling, storage and emergency measures in case of accident. They will also highlight any specific risks associated with a substance.

The information will also help identify what needs to be done to mitigate any hazards to health or the environment. However, they do not take the place of a COSHH assessment as this has a much broader content. The material safety data sheet is usually provided online with most commercially available products.

1.1.2 Waste disposal

Waste generated in the laboratory needs to be disposed of carefully. Laboratory specific instructions should be in place for each category of waste and should always be followed. In general terms, waste generated in a laboratory should be disposed of as follows:

1.1.2.1 Biohazard waste

This type of waste includes all utensils, containers, tissue paper, etc. that has been in contact with biohazardous material. It also includes sample material. This type of waste is usually segregated in strong bags (yellow coloured) marked with the biohazard symbol. When these bags are full they need to be securely closed with strong ties and are usually collected by specialised waste companies.

1.1.2.2 Sharps

This type of waste includes hypodermic syringe needles, scalpel blades and disposable scalpels, glass capillary pipettes, microscope slides and any other equipment used in the laboratory labelled as sharp. This type of waste is usually collected in yellow bench-top bins labelled with biohazard warning signs. When the sharps bins are filled to the fill line they must be closed and locked and collected by specialised waste companies.

1.1.2.3 Chemical waste

Chemical waste (reagents, reaction products, laboratory-prepared solutions, solvents) need to be disposed of responsibly. Laboratory specific instructions should be in place and followed very carefully. A common procedure for disposal of solvents and other harmful reagents is to decant

into 'Winchester' bottles within a fume hood. These are labelled with the name of the chemical to be disposed of. When full, the bottles will be disposed of by specialised companies.

1.1.2.4 General waste

This type of waste includes paper, containers, plastic ware and other waste that has not been in contact with chemical or biohazardous material. It can and should be disposed of as normal office waste.

1.2 Laboratory environment

Laboratories need to be controlled in a way that is fit for the analytical purpose, does not affect the quality of the process and maintains sample integrity. In laboratories where PCR (polymerase chain reaction) assays are carried out, contamination prevention measures such as spatial separation, biocontainment and traffic flow are important considerations that you need to be aware of.

1.2.1 Spatial separation

Laboratories where DNA and RNA material is routinely amplified and analysed should, where possible, have distinct separate areas for the different stages of the analytical process. However, although this is one of the most widely cited means of controlling cross-contamination it is, in practice, the most difficult to set up and adhere to, usually due to space limitations.

Typically, in a laboratory performing PCR, pre-PCR sample preparation areas should be separate from the PCR set-up areas which, in turn, should be separate from the post-PCR area. A design for a laboratory that complies with the spatial separation guidelines outlined above is illustrated in Figure 1-3.

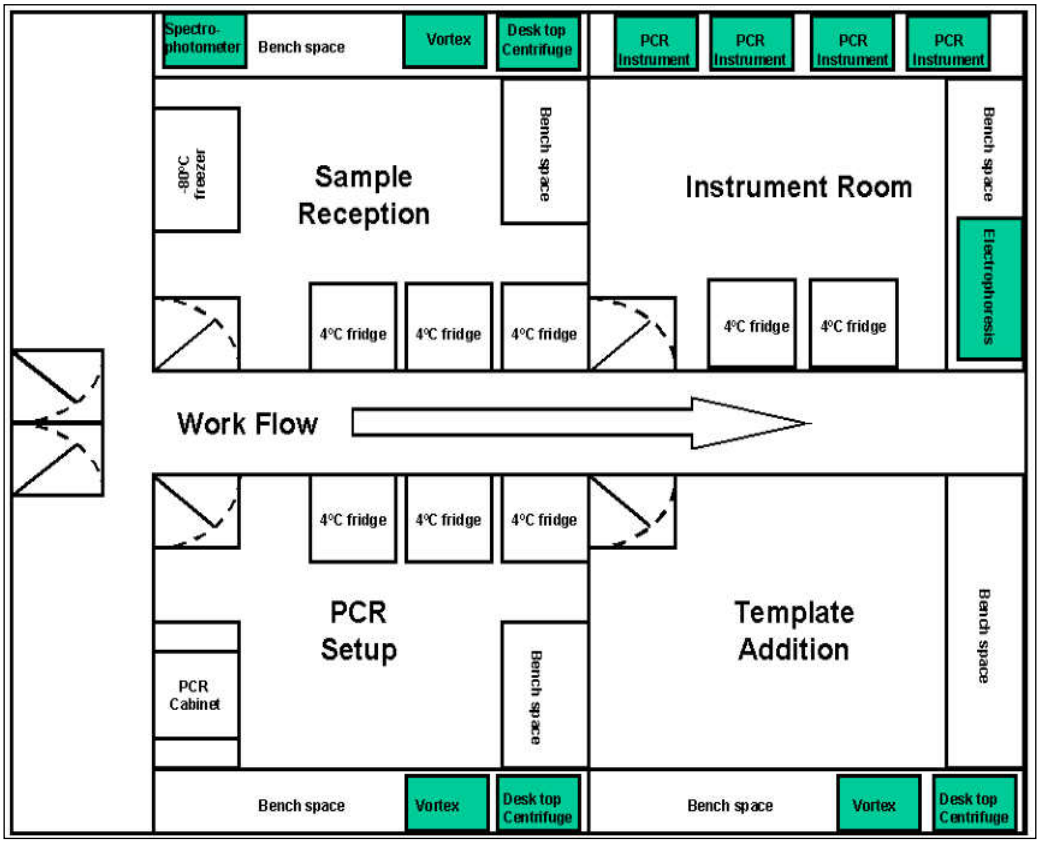


Figure 1-3. An example of a laboratory layout which demonstrates the spatial separation of rooms for pre- and post-PCR procedures

In this type of laboratory, the flow of analysts, samples and reagents should always go in one direction, from the pre-PCR areas (sample reception, PCR set-up and template addition) to the positive, post-PCR areas (instrument room). Where analysts need to return to pre-PCR areas, they may be required to wear additional protective clothing such as hairnets and masks to prevent 'shedding' post-PCR genomic material. This is a common requirement in forensic laboratories analysing biological material.

Consideration should be given to the concentration of template being handled in the pre-PCR area. High copy number nucleic acid template could pose a similar cross-contamination risk as post-PCR material. In cases where the use of high copy number template is unavoidable, it is preferable that the sample(s) be diluted in a room that is separated from all stages of the PCR procedure.

Where laboratories have the space to separate the different stages of the process, colour coding of all equipment, laboratory coats and, in particular, moveable laboratory ware (such as sample racks, storage boxes, pipettes, etc.) is highly desirable to stop items moving between areas. Where specific items need to return to pre-PCR areas from post-PCR areas, they must be decontaminated either by the use of UV radiation, or immersion in ethanol.

Laboratory coat colour coding is used to restrict analyst access to the different areas.

Figure 1-4. Restricted access entrance to laboratory

In practice, space constricted laboratories may not have enough room to provide physical separation, in which case colour coding of equipment, laboratory ware and even furniture is essential to minimise cross-contamination.

You should become aware of the layout of the laboratory and follow very strictly the guidelines referring to equipment and analyst flow around the working areas.

1.2.2 Biocontainment

The term 'biocontainment' is used in relation to laboratory biosafety, and is implemented in laboratories in which the physical containment of organisms or agents is required. Containment is achieved by isolation in environmentally and biologically secure cabinets or rooms, to prevent accidental contamination of workers or release into the surrounding environment. The level of containment varies from restricted access rooms to the use of biosafety cabinets. The level required depends on the types of samples or reagents used and the sensitivity of the assay to be carried out. It serves both to protect the analyst and environment from a hazard, and to minimise the contamination of the samples that are being tested.

There are three types of biosafety cabinets.

1.2.2.1 Class I biosafety cabinet

Open-front negative pressure cabinet, with exhaust air from the cabinet filtered by a high-efficiency particulate air (HEPA) filter. This cabinet provides personal and environmental protection, but not sample protection.

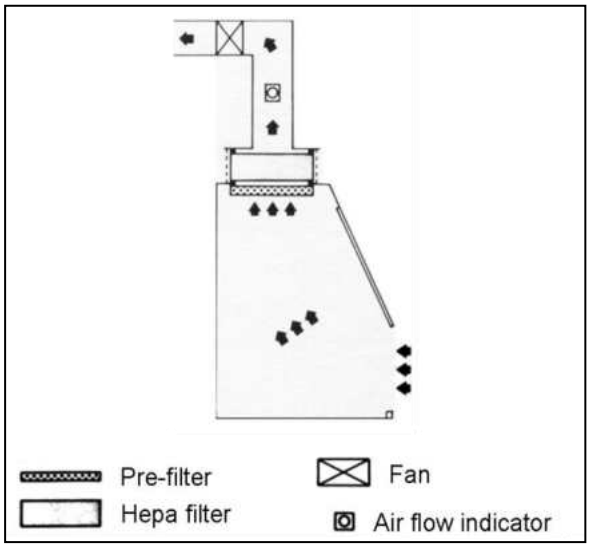


Figure 1-5. Diagrammatic representation of a class I biohazard safety cabinet

1.2.2.2 Class II biosafety cabinet

Open-front ventilated cabinet, with vertical laminar flow. These cabinets provide a HEPA-filtered, re-circulated mass airflow within the work space. The exhaust air from the cabinet is also filtered by HEPA filters and therefore it provides personal, environmental and sample protection.

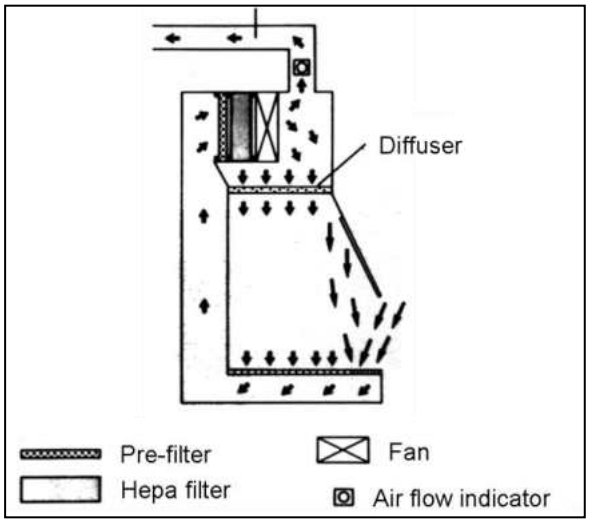


Figure 1-6. Diagrammatic representation of a class II biohazard safety cabinet

1.2.2.3 Class III biosafety cabinet

This is a totally enclosed ventilated cabinet. Operations within the Class III cabinet are conducted through attached rubber gloves.

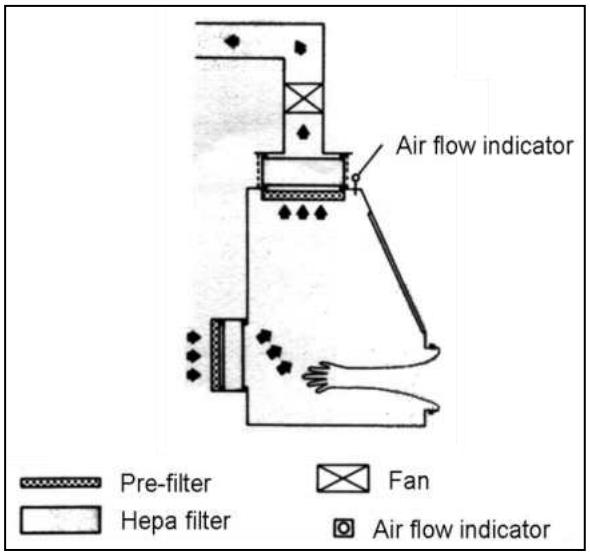


Figure 1-7. Diagrammatic representation of a class III biohazard safety cabinet

Most laboratories use Class II cabinets as they offer protection against most biohazards while also protecting the work in progress.

1.2.3 A clean and clear bench

In any laboratory, working on clean and uncluttered surfaces is important. In a molecular biology laboratory there is particular emphasis on working on surfaces that are very clean, with only the

equipment that is absolutely necessary present. This is to minimise the possibility of cross-contamination from airborne nucleic acid material.

After the experimental work is complete, the work surface should be cleared completely; all the laboratory ware should be cleaned and stored away. The work surface should be wiped with a proprietary solution; in many cases ethanol can be used.

Waste such as pipette tips and used Eppendorf tubes should be stored in a container and disposed of immediately (preferably in a sealed bag) after finishing work.

The plastic ware and equipment (racks, pipettes) used in the experiment should be wiped or soaked with a cleaning solution and, where possible, placed under a UV light.

1.3 Equipment

1.3.1 Equipment selection

The primary requirement when selecting equipment is that it is fit for purpose. The process of acquiring equipment is usually the responsibility of the laboratory manager. It is quite common to find that the laboratory offers different models of equipment for seemingly the same purpose and therefore the analyst will need to ensure that the experimental requirements are served by the equipment specification. For example, the laboratory may have different types of balances, with different precision. The analyst will need to select the type of balance that is best suited to the mass and type of sample to be weighed. This will be discussed in more detail in the sections on measuring mass, measuring volume and preparing solutions.

1.3.2 Log books and maintenance records

All equipment should have an associated log book that contains at least the following information:

- Description of the equipment
- Manufacturer's serial number
- Date of receipt and installation
- Location
- Software and version installed (if relevant)
- Records of updates and maintenance checks
- Faults and resolutions

1.3.3 Equipment ownership

All equipment should have an 'owner', a nominated member of the team who has responsibility for ensuring the equipment is maintained and serviced regularly. The owner should be competent in the operation of the equipment and can be responsible for training other staff. To avoid damage or incorrect use, it is vital for operators to be fully trained and demonstrate competence, before being authorised to use equipment.

1.3.4 Calibration

Equipment used to for analytical measurements should be calibrated using measurement standards and used within the limitations of accuracy and/or capacity specified by the manufacturer. Depending on the complexity of the equipment and the availability of reference materials and calibration standards, the calibration may be performed in-house or by a visiting engineer. In any case, there should be a calibration schedule for the equipment and records showing checks performed.

1.4 Method selection

Regardless of the management standards adhered to or the type of work being carried out, it is important to use methods that are fit for the purpose intended. This applies to all procedures, from those used in the initial sampling, through handling, transport, storage, preparation, analysis and reporting. In ISO/IEC 17025:2005[1] there is a definite hierarchy of methods that should be used, starting with international standards at the top, followed by published methods (peer reviewed publications) and then laboratory developed methods.

When considering the adoption of a new method developed from scratch in-house, a search of the relevant scientific literature is the best place to start. The details published in scientific articles will have been the subject of peer review and can be considered 'state of the art'. These sources of information may assist with gaining further insight into the specifics of a technique. Useful literature search engines for this purpose include PubMed at the NCBI (www.ncbi.nlm.nih.gov/entrez), and ScienceDirect (www.sciencedirect.com).

Unfortunately in many publications key detail is sometimes missing, making duplication of a procedure difficult. If this is the case, a more generalised search of the internet may yield additional useful information but care should be exercised when selecting appropriate search terms. Additional sources of useful information include commercial supplier websites, and laboratory home pages.

Many commercial suppliers offer time and effort saving kits that may enable the replication of the desired technique using validated protocols and reagents. However, these can be expensive and require additional equipment supplied by the vendor. An alternative source of information is therefore laboratory home pages. These are frequently established and maintained by leading researchers in the field but are not regulated to the same degree as the other sources discussed in this section.

Regardless of the source of a method it is essential to ensure that the method has been validated before use (see section 10.4). Even when using published methods that have been previously validated, checks should be carried out within the laboratory to demonstrate that published levels of performance can be achieved (and that they are fit for purpose). Without an adequate validation exercise there is no guarantee that a method will produce reliable or meaningful data. It is important to recognise that if a deviation from the original method occurs, then the method will no longer be validated and extra work will be required to establish that the results obtained are fit for purpose. When performing practical work it is essential to record details of the procedures performed and any deviations from the stipulated protocol in a laboratory notebook (see section 11.2.3).

1.4.1 The importance of standard operating procedures

All test methods should be written up as a clear and unambiguous set of instructions which is often referred to as a *standard operating procedure* (SOP). The aim of using SOPs in the laboratory is to ensure consistent application of test methods. Often analyses will be carried out by a number of different analysts; getting them all to follow the same SOP should improve the comparability of their results. The publication, storage and circulation of SOPs needs to be managed within the laboratory's quality management system to ensure they are kept up to date and are easily available.

[1] ISO/IEC 17025:2005 'General requirements for the competence of testing and calibration laboratories'

2 Measuring volume

The majority of work carried out in a molecular biology laboratory involves the precise measuring of small volumes of liquid reagents and enzymatic suspensions. These include preparing solutions of known concentration (see section 6) and preparing reagents (see section 7). Accurate micro-volumetric measurement, as well as measurement of larger volumes, is therefore a crucial aspect of the work carried out. This section outlines the key points you need to remember to be able to make accurate measurements of volume:

- The different types of equipment available for handling liquids
- Which type of equipment to use
- How to clean the equipment
- How to check the accuracy of the volume contained in or delivered by different items of equipment
- How to use the equipment correctly

2.1 Types of equipment available

2.1.1 Automatic pipettes

Automatic pipettes are available in both fixed volume and variable volume models. They are made out of plastic and metal and the variable volume models are fitted with either a mechanical or electronic volume setting device.

The liquid is confined to a plastic tip, which is readily exchangeable. Automatic pipettes are classified according to the mechanism they employ for aspirating and dispensing liquids. Two mechanisms are commonly employed and pipettes are therefore classed as being either air displacement, or positive displacement.

Figure 2-1. (A): Air displacement automatic pipette, (B) Positive displacement automatic pipette. (Image courtesy of Gilson UK)

2.1.1.1 Air displacement pipettes

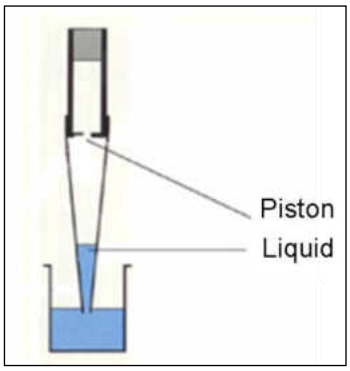
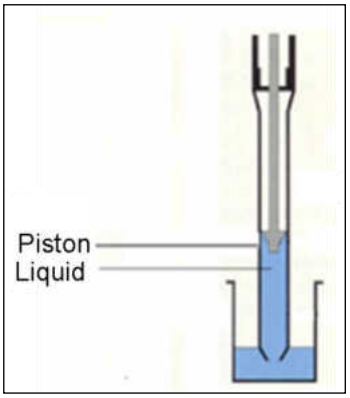


Figure 2-2 shows a schematic of an air displacement automatic pipette. This type of pipette is recommended for use with aqueous solutions and for routine laboratory work. The piston is part of the pipette and moves a cushion of air between itself and the liquid in order to aspirate (draw up) and dispense the liquid. The volume of air displaced is equivalent to the volume of liquid aspirated.

Figure 2-2. Diagrammatic representation of an air displacement pipette

2.1.1.2 Positive displacement pipettes



This type of pipette is usually employed with viscous (thick) and corrosive (damaging) liquids. With these pipettes, the piston is part of the disposable pipette tip and forms a seal in the pipette tip so that the liquid is in direct contact with the piston. See Figure 2-3.

Figure 2-3. Diagrammatic representation of a positive displacement pipette

2.1.1.3 Single channel vs. multi-channel pipettes

Multichannel pipette models provide higher sample throughput when using microwell plates. Adjustable Spacer models, where the space between the tips can be modified, allow the transfer of samples between tubes and different plate formats. Manufacturers generally supply multichannel pipettes in 8 or 12 channel format although 16, 48 and 64 channel pipettes are also available for use with 384 well plates. The volumes that can be dispensed and the tips that are utilised are identical to those employed with single channel pipettes. Examples of single and multichannel air displacement pipettes are shown in Figure 2-4.

Figure 2-4. Examples of single and multichannel air displacement pipettes. (Image courtesy of Gilson UK)

2.1.1.4 Electronic pipettes

With manual pipettes the piston action is accomplished by the user manually depressing and releasing the plunger with their thumb. With electronic pipettes the piston action is controlled by the pipette. The user pushes a button to initiate aspiration and dispensing of the liquid. Electronic pipettes reduce the force needed by the user's thumb and are of value when repetitive pipetting is required. Electronic pipettes often have multiple options, including the mixing and multi-dispensing of liquids. Examples of electronic single and multichannel air displacement pipettes are shown in

Figure **2-5**.

Figure 2-5. Examples of electronic, single and multichannel air displacement pipettes. (Image courtesy of Gilson UK)

2.1.1.5 Pipette components

The most common components of a single channel automatic pipette are illustrated in Figure 2-6.

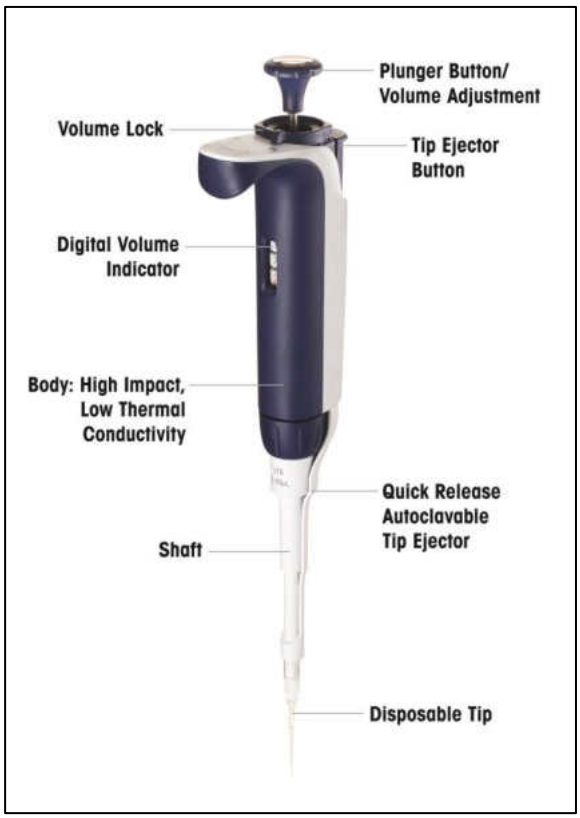


Figure 2-6. Automatic pipette components (Image courtesy of Rainin, a Mettler Toledo Company)

2.1.1.6 Setting the volume

The volume of liquid to be aspirated is set using the volumeter. The dials are normally coloured either black or red to indicate the position of the decimal point, depending on the make and

model. The volume is set by rotating the thumbwheel or the push-button. Maximum accuracy is obtained by setting the volume as follows:

If decreasing the volume setting: Slowly rotate thumb wheel to reach the required setting. Make sure that you do not to overshoot the mark.

If increasing the volume setting: Rotate the thumbwheel past the required value by approximately 1/3 of a turn. Then slowly decrease to reach the volume, making sure not to overshoot the mark.

2.1.1.7 Basic pipetting technique (forward pipetting technique)

When the plunger button is pushed, two stop positions can be felt. The first one is the 'aspirate' position. The second one is the 'purge' position. The basic forward pipetting technique consists of pushing the plunger to the first stop, and then releasing to aspirate the right volume. To dispense, the plunger is pushed slowly to the first stop, and then to the purge position to ensure all the liquid has been dispensed.

2.1.1.8 Reverse pipetting technique

Reverse pipetting is an alternative technique for pipetting viscous liquids, volatile solvents, or when pipetting small volumes <5 microlitres. The technique starts by depressing the push button to the purge position (the second stop), then releasing it to draw the liquid up. At this point there will be too much liquid in the tip. However, when the liquid is dispensed by pushing the piston to the aspirate position (the first stop), the extra liquid is left inside the tip. When using this method, the tip is automatically pre-wetted. The extra liquid also helps when pipetting volatile solvents because some of the solvent will tend to evaporate. Reverse pipetting is preferred over forward pipetting in situations where it is important that air is not introduced from the pipette tip. Forward pipetting involves pushing the plunger to the second stop position, which causes air to 'blow out' the liquid from the pipette tip. This could be problematic in some cases, e.g. when loading the wells of an electrophoresis gel as samples can be 'flushed out' by the air bubble. Forward pipetting can also introduce bubbles into samples which could cause problems for downstream applications. As reverse pipetting does not dispense all of the liquid, air should not be discharged from the pipette tip.

2.1.1.9 Environmental factors which can affecting pipetting.

- Air pressure
- Temperature of air, pipette, tips, and liquids
- Evaporation rates
- Surface tension of liquids
- Relative humidity

2.1.1.10 Considerations for optimal pipette performance

- Select the appropriate pipette for the task. The volume to be pipetted should be within 35% – 100% of the pipette's range
- Check for leaks or any other pipette malfunctions
- Choose the correct size and style of pipette tip
- Pipettes need to be calibrated and serviced regularly (check that the pipette is within its calibration date before use)
- Carry out regular checks on the performance
- Keep the pipette upright when the tip is filled with liquid

- Read the manufacturer's manual to understand the pipette being used
- If the pipette does not function properly, first ensure that the tip is properly fitted on the pipette and that it is set to the correct volume
- If it is still not functioning remove it from use and notify the laboratory manager to have it serviced

2.1.1.11 How to minimise pipetting error

- Allow all liquids and equipment to equilibrate to ambient temperature before beginning work
- Pre-wet the pipette tip by aspirating and dispensing the liquid before aspirating the required volume
- Immerse the tip vertically into the liquid, approximately 2-5 mm below the meniscus, keeping well clear of the container walls and bottom
- Aspirate using a consistent speed, rhythm, and plunger pressure
- Retain the tip in the liquid for 1 second following aspiration and withdraw the tip slowly and smoothly without touching the sides of the container
- To dispense the liquid, touch the pipette tip to the sidewall of the receiving vessel immediately above any liquid already in the vessel
- Place the pipette in an appropriate stand between pipetting cycles to avoid warming the pipette as this can affect the volume of liquid dispensed

2.1.1.12 Types of pipette tip

The pipettor and plastic disposable tip together form a pipetting system. A wide range of tips, of varying quality and cost, are available for both air and positive displacement pipettors from a range of manufacturers. It is important that tips meet the analytical requirements. Depending on the application, tips may need to be certified by the manufacturer as free from DNA, DNase, ATP, Pyrogens, PCR inhibitors and/or trace organics.

Air displacement pipette tips

Air displacement pipette tips are manufactured by a large number of commercial suppliers and are available as filter or non-filter plugged. Use of aerosol resistant filter tips is essential in critical applications such as PCR, forensics and clinical diagnostics to ensure protection of samples and pipettes against cross-contamination. The most common sizes available are: 10, 20, 100, 200, 300, 1000, and 5000 µL volumes and they are generally available in racked or bagged format.

Positive displacement pipette tips

Positive displacement pipette tips are generally supplied pre-racked, and either sterilized or unsterilized. The tips are generally available in 10, 25, 50, 100, 250, and 1000 µL volumes.

2.1.2 Beakers

Beakers are flat-bottomed cylindrically shaped vessels, with graduation marks on the side. Beakers are not meant to be used for accurate volume measurements. They are often used as vessels for transferring and mixing solutions, before they are made up to the required volume in a volumetric flask.

2.1.3 Conical/Erlenmeyer flask

Conical or Erlenmeyer flasks have a flat-bottom, with a cone shaped body and a short neck (see Figure 2-7). They have graduation marks on the side of the vessel, much like a beaker and are also not meant to be used for accurate volume measurements. Conical flasks are used as containers when mixing solutions and when transferring solutions by pipette (e.g. during the preparation of solutions of known concentration, see section 6.2.7).

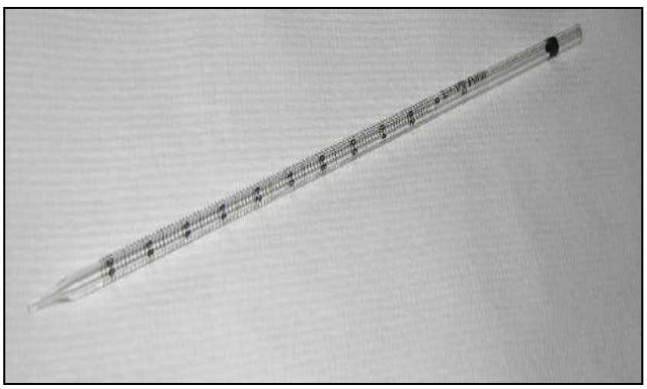
Figure 2-7. Conical flasks

2.1.4 Measuring cylinder

Measuring cylinders, also known as graduated cylinders, are cylindrical glass tubes which are closed at one end and fitted with a support to allow the cylinder to be freestanding (see Figure 2-8). The cylinder has graduations along the length of the tube and the open end is fitted with a pouring spout or a ground glass joint. Measuring cylinders are not accurate enough for quantitative work, but they are useful for approximate volume measurements (e.g. for preparing reagents, see section 7).

Figure 2-8. Measuring cylinder

2.1.5 Glass graduated pipette



Pipettes of this type consist of a long glass tube. The tube is marked with a series of graduations (as shown in Figure 2-9), enabling a range of volumes to be delivered. These pipettes are mainly used for dispensing pre-determined volumes where the highest level of accuracy is not required.

Figure 2-9. Glass graduated pipette

There are two main types of graduated pipettes:

Type 1 delivers a measured volume from the top zero mark to the selected graduation mark;

Type 2 delivers a measured volume from a selected graduation mark to the tip, which forms the zero mark. For this type of pipette, the residual liquid in the tip may need to be expelled, in which case the pipette will be marked 'blow-out'.

2.1.6 Volumetric flasks

Volumetric flasks (also known as graduated flasks) are flat-bottomed, pear-shaped glass vessels with a long narrow neck as shown in Figure 2-10. The neck of the flask has a single etched calibration mark. The top of the neck has a ground glass socket for a stopper, which may be made from polyethylene, polypropylene or glass. When filled to the calibration mark each flask will contain the specified amount of liquid. Volumetric flasks should comply with BS EN ISO 1042:2000[2]. The main use of a volumetric flask is in the preparation of a solution of known concentration (see section 6).

Figure 2-10. Volumetric flask

2.2 Markings on equipment used for volumetric measurements

In addition to the graduation and calibration marks, glassware that is used for making volume measurements will carry other markings that are relevant to particular applications (see

Figure 2-11). You should be aware of these markings and should check equipment before use.

[2] BS EN ISO 1042:2000 'Laboratory glassware – One-mark volumetric flasks'

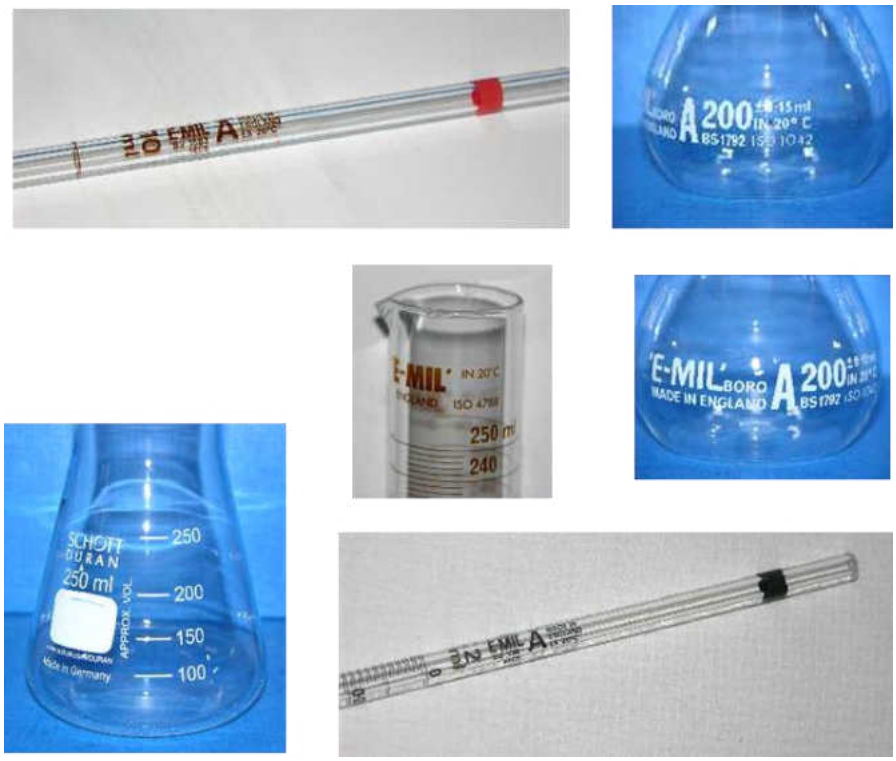


Figure 2-11. Illustrations of markings on volumetric glassware

Table 2-1. Markings on volumetric glassware

Marking	Information
Volume, with units	Stated volume of the equipment.
Temperature	Temperature at which the stated volume applies (usually 20 °C).
Class A or Class B	Class of glassware – defines the tolerance of the stated volume (see section 2.3).
Identification number	Identifies a specific item – is included on any certificate of calibration for the item (see Figure 2-12).
BS/ISO/DIN number	Indicates the item complies with the stated British (BS), international (ISO) or German (DIN) standard.
Seconds	The liquid delivery time for a pipette.
Ex or In	Indicates the item either delivers (Ex) or contains (In) the stated volume.
Blow-out	Indicates (for pipettes) that the residual liquid in the tip is part of the stated volume and must be blown out.
Trade name or material	Type of glass, e.g. Pyrex, borosilicate.
Colour code	Coloured band on stem (for pipette) that indicates the capacity.

2.3 Selecting a suitable piece of equipment

Your choice of equipment will depend on the level of accuracy needed to carry out the measurement you require. For graduated items of glassware, such as graduated pipettes and measuring cylinders, you should choose an appropriate sized item for the volume that you are measuring. For example, the capacity of a graduated pipette should not be more than twice the volume you want to measure.

Many items of volumetric glassware are commercially available in two classes – Class A and Class B. The distinction between the two is based on tolerance limits.

Table 2-2 shows typical tolerances for the graduations of Class A and Class B volumetric glassware such as pipette and volumetric flasks. It is worth noting that under the British Standard there is no Class A or Class B classification for measuring cylinders; just one level of accuracy. However suppliers are able to import measuring cylinders of Class A and B levels under, for example, the DIN (Deutsches Institut für Normung) standard system.

Generally, for volumetric glassware, Class A tolerances are about half those of Class B. The stated tolerance is a useful indication of the uncertainty in the measured volume, making it easier to judge which type and class of equipment is required for a particular task. Class A is generally used for quantitative work where a higher degree of accuracy is required.

Table 2-2. Tolerances for Class A and Class B volumetric glassware

Equipment	Nominal volume /mL	Graduations /mL (Class A/B)*	Tolerance /mL	
			Class A	Class B
Graduated pipette	1	0.01	0.006	0.01
	5	0.05	0.03	0.05
	10	0.01/0.1	0.05	0.1
	25	0.02/0.2	0.1	0.2
Volumetric flask	5	-	0.025	0.04
	10	-	0.025	0.04
	50	-	0.06	0.12
	250	-	0.15	0.3
	1000	-	0.4	0.8
Measuring cylinder	5	0.1	0.05	0.1
	10	0.2	0.10	0.2
	25	0.5	0.15	0.5
	100	1.0	0.5	1.00
	500	5.0	1.5	5.00
	2000	20.0	6.00	20.0

* For all glassware except graduated pipettes, the graduation marks are the same for both Class A and B items.

For graduated pipettes, the graduation marks in the table have been noted for Class A first followed by Class B (Class A/Class B).

Automatic pipettes, Class A pipettes, graduated pipettes and volumetric flasks are also available with individual calibration certificates. Figure 2-12 shows a typical calibration certificate.

Certificate of Calibration

Certificate No. 3750246007
Issued by STARLAB (UK) Ltd
Issue Date: 03-Sep-2012
Approved signatories
Andy Hall ☑
David Liberty ☐
Denise Fane de Salis ☐ Signature:
Calibrated by : David Liberty
Calibration date : 03-Sep-2012

4296

Accepted 17/9/2012

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Customer : LGC - Teddington
Address : Queens Road, Teddington, TW11 0LY

Pipette model : Gilson, Pipetman
Volume : 2.00 to 20.00 µl UKAS ISO 3 Star 10 Reading, 1 Channel (2, 10, 20 µl)
Serial no. : M23786M
In use at : Molecular Biology 516
Site service : Yes
Calibration Type : As returned Tip Type : Tip One

Calibration Method:
The device has been calibrated gravimetrically in general accordance with ISO 8655 and Starlab procedure SP106. The calibration was carried out using pure water which complies with BS 3978:1987 (ISO3696:1987). A disposable tip was kept for the whole of the test. The balance used for the test was calibrated using weights manufactured to OIML Class E2 and traceable to National Standards.

Water Temperature :	23.60 °C			
Air Temperature :	23.70 °C			
Air Pressure :	1,026.3 mbar			
Humidity :	58.00 %RH			
Z Factor :	1.0036 µl/mg Distilled water			
Balance details :	Balance	Manufacturer	Model	SerialNumber
	1	Mettler	XP26	B026047566

UKAS is one of the signatories to the multilateral agreement of the European co-operation for accreditation (EA) for the mutual recognition of calibration certificates issued by accredited laboratories.

This certificate is issued in accordance with the laboratory accreditation requirements of the United Kingdom Accreditation Service. It provides traceability of the measurement to recognised national standards, and to units of measurement realised at the National Physical Laboratory or other recognised national standards laboratories. This certificate may not be reproduced other than in full, except with the prior written approval of the issuing laboratory.

Figure 2-12. Typical calibration certificate for an automatic pipette

Table 2-3 shows typical manufacturers' data for the tolerances for volume measurements made using automatic pipettes. Automatic pipettes are not covered by the 'class' system used for glassware. You should consult the manufacturer's information for the exact specification for a particular pipette.

Table 2-3. Typical tolerances for automatic pipettes

Nominal volume	Tolerance
1 μL	± 0.025 μL
10 μL	± 0.1 μL
100 μL	± 0.8 μL
1000 μL (1 mL)	± 8 μL (± 0.008 mL)
10000 μL (10 mL)	± 60 μL (± 0.06 mL)

2.4 Cleaning and maintenance of volumetric equipment

You should check the condition of all volumetric equipment before use. Take special care when using equipment such as glass pipettes, as the tips can be easily damaged if these items are not stored correctly.

Glassware should be clean and free from dust. To test if an item is clean:

- Allow water to drain from the item: the absence of discrete droplets of water on the interior surface indicates a clean, dust free interior
- If droplets of water remain on the interior surface of the item, the glassware should be cleaned prior to use. How to clean and dry glassware:
    - clean with a suitable detergent such as Teepol® or Decon® 90
    - thoroughly rinse with water
    - allow to drain at room temperature, or rinse with acetone and then allow to drain or apply a gentle stream of clean dry air

Volumetric glassware should not be washed in a dishwasher at high temperatures or dried by heating in an oven as this may affect the accuracy of the graduation marks.

2.4.1 Special considerations for cleaning glassware used for RNA analysis

RNA is extremely vulnerable to degradation by RNase enzymes, which can be found either in samples or in the processing environment. RNases are small thermostable enzymes found throughout nature. RNases are also found on the surface of human skin, where they are thought to play a role in defence against retroviruses.

It is therefore of paramount importance that gloves are worn throughout any RNA experiment, and that any glass or plastic ware that has possibly been touched with bare hands is treated prior to use.

Where possible, the use of RNase free glassware or disposable plastic ware is recommended. RNases are extremely stable enzymes and cannot be eliminated by the use of conventional cleaning methods such as autoclave. Dry heating of glassware is effective, and glassware may be rendered RNase free by heating to 250 °C for 4 hours, or alternatively 200 °C for 24 hours (but see note in 2.4 regarding accuracy of graduation marks).

2.5 Checking the accuracy of volumetric equipment

For certain applications, when the uncertainty associated with the volume being measured needs to be minimised, it is necessary to establish the accuracy of the equipment by formal calibration, rather than to rely on the quoted tolerances shown in Table 2-2.

Formal calibration entails gravimetrically measuring the quantity of water delivered by, or contained in, the equipment. The volume can then be calculated from a simple calculation using the density of water.

Formal calibration is a complex operation, so laboratories often have volumetric equipment calibrated by a third-party that is accredited to carry out such work (e.g. accredited by the United Kingdom Accreditation Service (UKAS)). A full record of the calibration should be obtained along with a formal certificate of calibration, specifically identifying the item of equipment to which it refers (see Figure 2-12).

As a result of a formal calibration, any bias associated with a graduation mark will be detected and appropriate corrections can then be made when the equipment is used for routine work. Any bias should not exceed the manufacturer's tolerance for a particular item of equipment.

Calibrated volumetric equipment that is in regular use should be re-calibrated at specified intervals as damage, misuse or general wear and tear may affect the validity of the calibration. Automatic pipettes should be serviced and calibrated regularly (typically annually) by a recognised supplier of such equipment who is also accredited to carry out calibrations.

For those items of volumetric equipment that have not undergone a formal calibration procedure, it is useful to carry out spot checks, at regular intervals, to confirm that the accuracy is suitable for the application. Follow the check list below to carry out an accuracy check on an item of volumetric glassware or an automatic pipette:

- Select a suitable balance, bearing in mind the volume of liquid to be dispensed (see section 3.3 for information on selecting a suitable balance). For volumes above 200 mL a 2-figure top-pan balance may be used but for smaller volumes an analytical balance or micro balance will be required
- The accuracy of the volume delivered by or contained in the equipment should be gravimetrically determined using distilled or deionised water (for information on the correct use of a balance see section 3.3.1)
- The item to be checked must be clean and dust free (if necessary it can be cleaned as outlined in section 2.4)
- The water used for the accuracy check needs to equilibrate to ambient temperature and this temperature should be recorded to the nearest degree centigrade
- The item is filled to the required graduation mark
- For pipettes the volume of water is discharged into a pre-weighed clean dry vessel. The vessel is reweighed and the mass of the water delivered by the pipette is determined
- For volumetric flasks the mass of the water contained in the flask is determined by weighing the empty, dry flask and then weighing again after it has been filled to the graduation mark
- The recorded mass of water is converted to a volume by dividing by the density of water at the appropriate temperature (see Table 2-4)
- If the difference between the stated volume for the item and the measured volume is more than the quoted tolerance, the procedure needs to be repeated. If it fails again then do not use the item and consult your supervisor/laboratory manager

- For pipettes, both automatic and glass, repeatability (see section 10.4.1) can be checked by carrying out replicate determinations as outlined above, then calculating the mean and standard deviation of the volume delivered and comparing these to the manufacturer's specification
- Items capable of delivering different volumes (i.e. graduated glass pipettes, and variable volume automatic pipettes) should have their calibration checked when delivering a range of volumes

Table 2-4. Density of water (based on weighing in air) at different temperatures

Temperature (°C)	Density (g mL^{-1})	Temperature (°C)	Density (g mL^{-1})
16	0.99789	21	0.99694
17	0.99772	22	0.99672
18	0.99754	23	0.99649
19	0.99735	24	0.99624
20	0.99715	25	0.99599

2.6 Checklists for making measurements of volume

When using the volumetric glassware discussed in this section, it is very important to read the meniscus correctly. Figure 2-13 shows three examples of how the meniscus could be viewed. Both (a) and (c) are incorrect; viewing the meniscus from below or above will introduce parallax error. Figure (b) shows the correct position for reading the meniscus accurately. The meniscus should always be viewed at eye level.

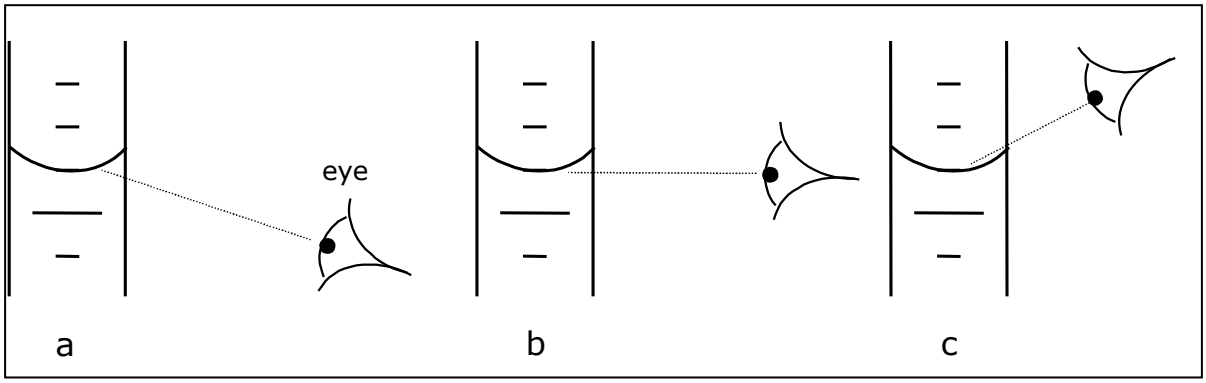


Figure 2-13. Reading a meniscus

Table 2-5 summarises the key points to remember when using an automatic pipette.

Table 2-5. Dos and don'ts for using an automatic pipette

Do	Don't
Select a pipette with the correct capacity (consult the manufacturer's instructions).	
Check the pipette is clean and undamaged.	Use a pipette that is dirty or damaged.
Check the pipette is within its calibration date.	Use a pipette that is out of calibration.
Carry out an accuracy check if required (see section 2.5).	
Select the correct tip for the type of auto pipette and volume required.	
Check that the correct volume has been set when using a variable volume auto pipette.	
Use the appropriate pipetting technique (forward vs. reverse pipetting).	
Hold the pipette vertically and immerse the tip to the required depth in the liquid to be pipetted.	Hold the pipette at an angle while filling the tip.
Pre-wet the tip by aspirating the required volume.	Use a pipette tip that has not been pre-wetted.
Remove excess liquid from the outside of the tip before dispensing the liquid.	Leave droplets of the liquid on the outside of the tip.
Aspirate the liquid at a steady rate.	Operate the piston mechanism too rapidly.
Dispense the liquid with the pipette tip touching the inside of the receiving vessel at an angle.	
Pipette further portions of the same liquid with the same tip.	Reuse a tip if any droplets of the previous portion of liquid remain in the pipette.
Re-wet the tip if a different volume is selected (but make sure that the new volume is within the capacity of the tip).	
Change tip if you need to pipette a different liquid.	Reuse a tip if a different liquid is to be pipetted.
Keep the pipette upright if the tip is filled with liquid.	Place a filled pipette on its side or invert it as liquid may enter the mechanism, causing damage and contamination.
Clean the pipette and store upright in a pipette stand or carousel.	Leave pipettes dirty and incorrectly stored.

Table 2-6 summarises the key points to remember when using a measuring cylinder.

Table 2-6. Dos and don’ts for using a measuring cylinder

Do	Don’t
Select an appropriate sized measuring cylinder for the volume to be measured.	Use a measuring cylinder that is too large or too small.
Check the measuring cylinder is clean and undamaged.	Use a measuring cylinder that is dirty or damaged.
Check for faded graduation marks.	Use a measuring cylinder with faded graduation marks.
Make sure the liquid is at ambient temperature.	Measure liquids that are significantly above or below ambient temperatures.
Rinse the measuring cylinder with distilled/deionised water followed by the liquid to be measured.	
Dispose of the rinse solution immediately.	
Line up the bottom of the meniscus with the graduation mark, making sure that your eyes are level with the calibration mark (see Figure 2-13).	View the graduation mark from above or below when judging the position of the meniscus (see Figure 2-13).
Clean the measuring cylinder after use by rinsing it with water and then drying it.	
Store measuring cylinders in a clean dust free environment at ambient temperature and away from corrosive substances.	

Table 2-7 summarises the key points to remember when using a glass pipette.

Table 2-7. Dos and don'ts for using a glass pipette

Do	Don't
Check the pipette is clean and undamaged.	Use a pipette that is dirty or damaged.
Check the calibration mark (or marks) is not faded.	Use a pipette with a faded calibration mark(s).
Fill the pipette using a suitable pipette filler.	Fill a pipette by mouth.
Transfer liquid to be pipetted to a clean dry beaker or conical flask.	Pipette directly from the stock bottle of the liquid being pipetted.
Make sure the liquid is at ambient temperature.	Pipette liquids that are at temperatures significantly above or below the calibration temperature of the pipette.
Rinse the pipette with distilled/deionised water followed by the liquid you want to dispense.	
Dispose of the rinse solution immediately.	
Check the length of time it takes the liquid to drain from the pipette.	Use a pipette with a delivery time that differs significantly from the expected time marked on the pipette.
Carry out an accuracy check if required (see section 2.5).	
Check that the pipette does not drip when filled.	
Make sure that the tip of the pipette stays below the surface of liquid when filling.	Raise the tip of the pipette above the level of the liquid when filling.
Fill the pipette to just above the calibration line then remove the pipette from the liquid before adjusting the level of liquid so that the bottom of the meniscus is in line with the calibration mark, making sure that the pipette is vertical and that your eyes are level with the calibration line (see Figure 2-13).	View the calibration line from above or below when judging the position of the meniscus (see Figure 2-13).
Check for air bubbles after filling.	
Wipe the outside of the pipette after filling and before dispensing the liquid.	Forget to wipe the outside of the pipette before dispensing the liquid.
Dispense the liquid with the pipette tip touching the inside of the receiving vessel at an angle.	
Let the liquid drain from the pipette under gravity (unless stated otherwise).	Expel the small amount of liquid left in the tip of the pipette after emptying (unless the pipette is marked blow-out).
Clean and dry the pipette and store in a clean dust free environment at ambient temperature and away from corrosive substances, making sure that it cannot roll.	Leave pipettes dirty and incorrectly stored.

Table 2-8 summarises the key points to remember when using a volumetric flask.

Table 2-8. Dos and don'ts for using a volumetric flask

Do	Don't
Select a flask of the required capacity.	Use a flask of the incorrect capacity (take care if flasks of similar volume are available, e.g. 200 mL and 250 mL; ideally these should not be stored together).
Check the flask is clean and undamaged.	Use a flask that is dirty or damaged.
Check that the calibration mark and other markings are not faded.	Use a flask with a faded calibration mark.
Carry out an accuracy check if required (see section 2.5).	
Make sure the solution is at ambient temperature before making up to volume.	Prepare solutions at temperatures that are significantly above or below the calibration temperature of the flask.
Ensure that all solids have dissolved before making a solution up to volume.	
Line up the bottom of the meniscus with the calibration line, making sure that the flask is vertical and that your eyes are level with the calibration mark (see Figure 2-13).	View the calibration line from above or below when judging the position of the meniscus (see Figure 2-13).
Avoid holding the bulb end of the flask to minimise any warming of the solution.	Hold the flask by the bulb end as this may cause the solution to warm.
Ensure you use the correct type of stopper for the flask.	
Ensure that stoppers are 'matched' to particular flasks if a stoppered flask is to be weighed.	
Rinse the flask with water and dry it after use.	
Store flasks in a clean dust free environment at ambient temperature and away from corrosive substances.	

3 Measuring mass

Measurements of mass are usually carried out less frequently than measurements of volume in a biology laboratory. However, there will be situations where you will be required to weigh accurately a specified amount of material. This could be weighing a portion of the sample required for analysis, or a chemical needed to prepare a solution of known concentration (see section 6). This section outlines the key points you need to know to make accurate measurements of mass:

- The different types of balance available for making mass measurements
- Which type of balance to use
- How to check the accuracy of the balance
- How to use the balance correctly

3.1 Mass versus weight

Both 'mass' and 'weight' are used interchangeably in general conversation, however they have very different meanings.

Mass is the amount of material in an object and does not change with the environment in which the object is located. Weight is a force arising from the interaction of the mass with the earth's gravitational field, which varies with location.

Balances are used to determine the mass of an object on the basis of its weight; the downward force the object exerts on the balance pan. Ideally, balances should be placed on shock absorbing surfaces, such as vibration damping tables. This is especially important when using micro or analytical balances.

3.2 Types of balance available

There are three main types of balance used in a laboratory and they vary in their weighing capabilities, maximum capacity and readabilities.

3.2.1 Micro balance

This balance typically has a readability of 0.001 mg (i.e. 0.000001 g, also written as $1x10^{-6}$ g) and is normally used for weighing quantities of less than 0.1 g. The typical maximum capacity of a balance like this is 10 g. Micro balances are enclosed in a see-through casing with doors so dust does not collect and air currents in the room do not affect the delicate balance (see Figure 3-1).

Figure 3-1. Micro balance

3.2.2 Analytical balance

An analytical balance (shown in Figure 3-2) has a readability of between 0.01 mg and 1 mg (five to three decimal places); it is normally used for weighing quantities of 0.1 to 100 g. Analytical balances are available with different maximum capacities, for example, 150 g and 500 g. Analytical balances, like micro balances, are inside a see-through enclosure with doors so dust does not collect and air currents in the room do not affect the balance.

Figure 3-2. 4-figure analytical balance

3.2.3 Top-pan balance

This balance is capable of weighing quantities in excess of 1 kg with a typical readability of 0.01 to 1 g (depending on the actual balance used), and is often used for applications such as preparing large quantities of reagents and weighing bulk samples. Like the analytical balance, top-pan balances are available with different maximum capacities, normally of between 1 and 30 kg. A top-pan balance is not usually enclosed. It is normally open to the environment of the laboratory so the effect of draughts and air currents needs to be considered, especially when weighing relatively small amounts of material.

Figure 3-3. 2-figure top-pan balance

3.3 Selecting a suitable balance

The choice of balance will depend on the amount of material that you need to weigh and the accuracy required in the weighing. You will therefore need to consider both the readability and the capacity of the balances available in your laboratory. You should choose a balance suitable for the measurement you are making. A rule of thumb is to ensure that weighings are recorded with a minimum of four significant figures although more may be required for certain applications. For example, 0.01234 g (a readability of five decimal places) and 12.34 g (two decimal places) is likely to be acceptable but values of 0.012 g (three decimal places) or 12 g may give insufficient information. The capacity of the balance is also important, for example, when weighing a mass of 2 kg it is better to use a balance with a maximum capacity of 5 kg than a balance with a capacity of 50 kg. It is important to note that a balance should not normally be used at >95% of its capacity. When selecting a balance remember to take into account the mass of the weighing vessel as well as the mass of material to be weighed.

Table 3-1 outlines some suggestions of balances that may be used for routine measurements of different masses. The suggestions in the table will give weighings with at least five significant figures which will be more than adequate for most routine laboratory operations.

Table 3-1. Balance selection for a particular weighing operation

Mass to be weighed / g	Recommended balance	Readability
0.01	Micro (6-figure)	0.000 001 g
0.1	Analytical (5-figure)	0.000 01 g
1	Analytical (4-figure)	0.0001 g
10	Analytical (4-figure)	0.0001 g
100	Top-pan (2-figure)	0.01 g
1000	Top-pan (1-figure)	0.1 g
10 000	Top-pan (0-figure)	1 g

3.3.1 Checking the balance accuracy and set up: balance location

You should pay attention to the manufacturer's recommendations regarding the environmental requirements for the location of the balance in the laboratory. You should also be aware of the important factors outlined in Table 3-2.

Table 3-2. Factors to consider when choosing the location for a balance

Factor	Advice
Draughts	The balance should be situated in a draught free location away from doors, windows, passers-by and other equipment. Air conditioning can also cause unwanted draughts.
Vibrations	For micro and analytical balances, a solid bench top of stone or slate at least 40 mm thick, free standing and isolated from any other work apart from weighing is preferred. All balances are subject to vibrations and this arrangement should help to minimise them. However, minimising the effect of vibrations is not so critical for top-pan balances which can be used on a laboratory bench top.
Level surface	The balance must be mounted on a level surface. The feet of the balance should be adjusted when the balance is set up, to ensure that it is level. Many balances have an in-built spirit level and this should be checked each time before the balance is used.
Cleanliness	The balance should be located in a clean area free from dust, water and chemical splashes, corrosive substances, organic vapours and aerosols.
Temperature	Temperature fluctuations can cause gradients in the balance mechanism, so the ambient temperature should be stable to within ±3 °C.
Humidity	Humidity is not a major concern, as it should be relatively stable in a laboratory environment, provided condensation does not appear on components of the balance. If it does, you should assess the laboratory environment immediately.
Magnetic fields	A magnetic field could cause permanent changes in the response of the balance and should be avoided. Therefore, do not locate the balance near equipment which may generate a strong magnetic field.
Electrical interference	Balances should remain on at all times. However, electronic balances are sometimes subject to electrical interference. If this becomes a problem a stable power supply (e.g. filter plug) should be used.

3.3.2 Calibration and accuracy

Once you have selected a particular balance for a specific task, you should check that it has been correctly calibrated for the mass range required. This is easily done by reviewing the calibration certificate for the balance and checking that the interval since the last calibration is acceptable. The quality management system for your laboratory should prescribe the interval between full calibrations. As a guide, 12 months is adequate for most purposes.

Full balance calibration will provide readings that are traceable to the national standard of mass (the kilogram) held in the UK by NPL (National Physical Laboratory). Through the national standard held at NPL the measurements are also traceable to the international standard kilogram, held at the International Bureau of Weights and Measures (BIPM) in Paris. Therefore, all measurements of mass carried out on a suitably calibrated balance in the UK will be comparable with readings obtained on properly calibrated balances in other laboratories in the UK and overseas.

The calibration process should be carried out by a body with suitable accreditation for such work (e.g. accredited by UKAS). The procedure should include examinations and tests of various aspects of balance performance, such as repeatability, linearity, zero and tare mechanisms and eccentric or off-centre loading. Adjustments will be made as appropriate, to ensure that the readings are within specification. A record of the balance calibration should be kept and filed.

In between full calibrations, accuracy checks on all laboratory balances should take place either on a regular basis (daily, weekly, monthly) or before use, depending on your laboratory's requirements. Such checks need not be as extensive as a full calibration. Accuracy checks are most effectively done by placing (using forceps) calibrated weights of known value on the balance pan and comparing the measured mass to the true value. As long as the difference does not exceed a given critical value, the balance is believed to be performing correctly. The criteria for assessing balance accuracy are usually based on knowledge of what the balance should be able to achieve. You should record results of the accuracy checks in a suitable log book/file. Before you use a balance you should ensure that the required accuracy checks have been carried out and that the balance performance is acceptable. If the performance is not acceptable the balance should not be used and the problem should be reported to your laboratory manager/supervisor.

3.3.3 Correct use of balances for different applications

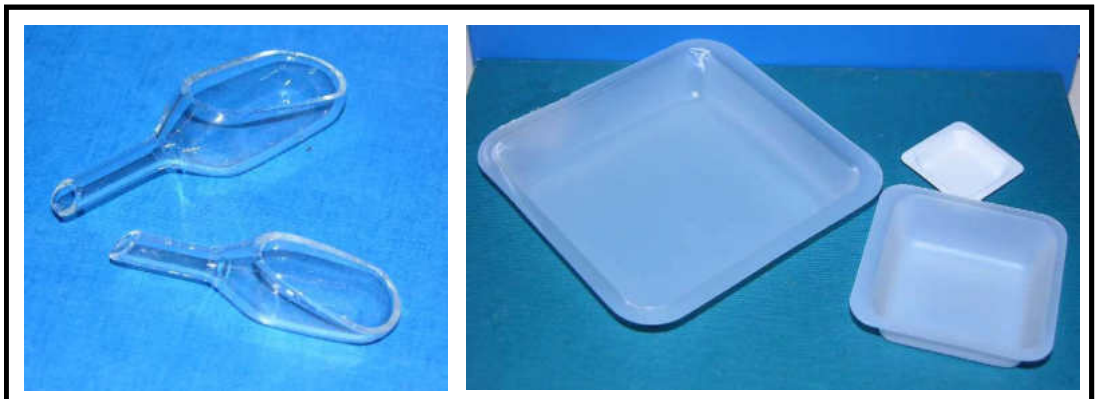
When weighing out a substance you should always use a suitable container. The empty container should be clean, dry and free from dust. It also helps if the container is of a design that assists transfer of the weighed substance into the vessel or apparatus subsequently required for the analysis. Remember that the size and mass of the container, in relation to the amount of material that will be weighed out, also needs to be considered. For example, if you needed to weigh 1 g of material you should use a small weighing boat or weighing funnel rather than a beaker, which may weigh 50 to 100 g.

Table 3-3 summarises some types of container that can be used for weighing different substances.

Table 3-3. Containers and types of substance to be weighed

Substance	Type of container	Extra information
Solid substance	Weighing paper or boat*, beaker, flask or bottle, weighing funnel *	
Liquid	Weighing boat*, beaker, flask, bottle or Eppendorf tube (small volumes)	
Volatile liquid	Stoppered weighing bottle or flask	Container must have a well-fitting stopper to minimise losses through evaporation.
Hygroscopic substance	Stoppered weighing bottle or flask	A stoppered container is used to minimise pick-up of moisture from the air, such substances may be dried in a desiccator prior to weighing.
Toxic substance (Including Category 1 & 2 biohazard samples)	Closed container, e.g. weighing bottle or flask	Toxic substances must be weighed in a closed container. If possible this should be carried out in a fume hood/cupboard.

*As shown in Figure 3-4



a) Weighing funnels b) Weighing boats

Figure 3-4. Illustration of different weighing containers

The effects of static electricity can be a problem when weighing dry, finely divided materials. Static electricity can make powders difficult handle as it causes the particles to 'float' about. This, in turn, causes balance readings to drift and can introduce systematic errors. Antistatic weighing boats and antistatic 'guns' are available which can help to reduce the problem.

Once you have selected a suitable balance and weighing container, Table 3-4 outlines the operations required to weigh the substance correctly. It is important to note that when carrying out accurate weighings, the items being weighed should be handled as little as possible and

gloves should always be worn. In addition, items should always be at ambient temperature when weighed.

There are two main approaches to weighing:

- Weighing by difference: used if you need to transfer the substance into another vessel before continuing with the assay
- Using the tare facility: allows the substance to be weighed directly into the vessel used for the analytical procedure so no transfer is necessary

Table 3-4. How to weigh a substance

Operation	Action
Setting up the balance	Plug in the power supply and switch on at least 20 min before use to allow the balance to 'warm up' (it is, however, recommended that the balance is left on in the stand-by mode). Check the balance is level and adjust if necessary. Gently clean the balance pan with a brush to remove any dust or loose particulate matter.
Checking the accuracy of the balance	Set the balance to read zero and check that zero is displayed. Carry out an accuracy check (see section 3.3.2). Check that the zero is displayed. Ensure any calibrated weights used for the accuracy check are returned to their storage box.
Weighing by difference	Place a suitable empty container on the centre of the balance pan. Close the balance door and wait for a stable reading then record the mass (M_0). Remove the container from the balance and transfer the substance to be weighed into the container. Replace the container on the balance pan, close the balance door, wait for a stable reading then record the mass (M_1). Remove the container from the balance pan. If the substance is transferred quantitatively (i.e. by washing with water or a solvent), the mass of the substance is $M_1 - M_0$. If the substance is transferred 'dry' to another vessel, once the transfer has been carried out, return the container to the balance pan, close the door, wait for the reading to stabilise, then record the mass (M_2). The mass of the substance transferred is $M_1 - M_2$.

Table continued

Table 3-4. continued

Operation	Action
Using the tare facility	Place the empty container on the centre of the balance pan, close the door and press the tare button. Wait for the reading to stabilise at zero. Remove the container from the balance and transfer the substance to be weighed into the container. Replace the container on the balance pan, close the door and wait for the reading to stabilise. Record the reading. This is the mass of the substance in the container.
Tidying up after weighing	Once you have finished the weighing process, gently clean the balance pan with a brush, collect any debris and discard. Close the balance door and leave in stand-by mode. Clean any debris from around the balance bench. Report any problems encountered with the balance to your laboratory manager/supervisor.

3.3.4 Checklist for making measurements of mass

Table 3-5 summarises the key points to remember when making measurements of mass.

Table 3-5. Dos and don'ts for measuring mass

Do	Don't
Wear gloves.	
Check the balance is switched on. If not, switch it on and leave to equilibrate for 20 min.	Use a balance that has not had time to warm-up.
Check the balance is level with the in-built spirit level or a separate level.	Use a balance that is not level.
Clean the balance pan and surrounding area before use.	Use a balance that has dust or loose particulate matter on or around the balance pan.
Check that the balance has been calibrated within a reasonable time period (e.g. the last 12 months).	Use an uncalibrated balance.
Check the zero reading.	
Check the accuracy of the balance.	Use a balance that has not had its accuracy checked.

Table continued

Table 3-5 continued

Do	Don't
Use a suitable container to weigh out the material of interest.	Use an unsuitable weighing container (e.g. a beaker when weighing small amounts of material, or an open container when weighing a volatile liquid, see Table 3-3 in section 3.3.3).
Check items are at ambient temperature before they are weighed.	Weigh items that are hotter or colder than ambient temperature.
Weigh by difference if the material needs to be transferred into another vessel.	
Use the tare facility if the sample can be weighed directly into the vessel used for further analytical work.	Use the tare facility if weighing by difference.
Close the doors (if working with a micro or analytical balance) and let the reading stabilise before recording it.	Take readings without closing the balance doors and waiting for the balance to stabilise.
Clean up the balance and surrounding area after use.	Leave the balance dirty for the next user.
Leave the balance in stand-by mode.	Switch off the balance.

4 Quantification of nucleic acid material

In molecular biology laboratories, quantification of nucleic acids is commonly performed to determine the average concentrations of DNA or RNA present as well as to determine their purity. There are several methods in common use to establish the concentration of a solution of nucleic acids, including spectrophotometric quantification and UV fluorescence in presence of a DNA dye.

4.1 Measurement of nucleic acid concentration using UV spectroscopy

Nucleic acids absorb ultraviolet light in a defined manner. Maximal absorbance of UV light is observed at a wavelength of 260 nm, with the amount of light absorbed being directly proportional to the nucleic acid concentration in the sample.

By employing the Beer-Lambert Law it is possible to relate the amount of light absorbed to the concentration of the absorbing molecule.

Beer-Lambert Law: $A = \varepsilon c l$

Where A is the absorbance (also known as optical density), ε is the molar absorptivity (also known as the extinction coefficient), c is the concentration of the solution and l is the path length. The path length is usually fixed at 1 cm by the spectrometer, but this assumption should be checked against the instrument specification.

At a wavelength of 260 nm, the average extinction coefficients are:

- for double-stranded DNA : 0.020 $(\mu g/mL)^{-1}$ cm^{-1}
- for single-stranded DNA: 0.027 $(\mu g/mL)^{-1}$ cm^{-1}
- for single-stranded RNA and for short single-stranded oligonucleotides: 0.025 $(\mu g/mL)^{-1}$ cm^{-1}

An optical density (or 'OD') of 1 therefore corresponds to a concentration of 50 µg/mL for double-stranded DNA. This method of calculation is valid up to an OD of at least 2, however a more accurate extinction coefficient may be needed for the quantification of oligonucleotides. The average extinction coefficient for DNA and RNA assumes an equal distribution of all four bases, each of which has a different extinction coefficient. For shorter oligonucleotides of a known base composition, a more accurate coefficient can be calculated by taking into account the exact proportions of each of the bases.[3]

It is important to consider that spectrophotometric analysis of nucleic acids will allow estimation of different nucleic acid species but does not discriminate between them by absorbance, as all nucleic acids absorb at 260 nm. Therefore, measurement of an RNA sample, for example, will be affected by the presence of contaminating DNA.

4.1.1 Practical considerations of measuring nucleic acid concentrations by UV spectrometry

Although in theory UV determination of nucleic acid concentration is a simple process, it can be fraught with problems in practice. The following factors should be considered.

4.1.1.1 Cuvettes

The cuvettes should be of good quality and transparent in the UV region. Take care to handle cuvettes only on the non-optical surfaces.

[3] Keer JT and Birch L, Essentials of Nucleic Acid Analysis: A Robust Approach, RSC, 2008, ISBN 978-0-85404-367-5, p85

Cuvettes should be prepared for use by rinsing with 95% ethanol followed by pure water and wiped dry with lint-free paper tissue.

4.1.1.2 Sample preparation

The nucleic acid in the samples needs to be fully dissolved. Any particulate matter should be removed by centrifugation or further purifying of the sample (see section 9).

4.1.1.3 Reference blank

To avoid measurement bias a reference blank should be used to correct for any absorbance of the diluent. This is a cuvette containing an identical solution to the cuvette that is being measured, except the nucleic acid component is excluded.

4.1.1.4 Sample concentration

Measurement error has an increased effect on absorbance measurements at low concentrations; therefore higher concentrations are preferred. Absorbance readings equal to or below 0.01, which corresponds to approximately 0.5 µg/mL, are not considered reliable, although the instrument specification should be checked.

4.1.1.5 Sample purity

As mentioned previously it is important to remember that spectrophotometric analysis of nucleic acids does not discriminate between different species by absorbance, as all nucleic acids absorb at 260 nm..

Furthermore, nucleic acid samples are frequently contaminated with other molecules (e.g. proteins, organic compounds, etc.) which coincidently also absorb UV light. The ratio of absorbances at 260 and 280 nm (A_{260}:A_{280}) is commonly used in the assessment of nucleic acid sample purity. For pure DNA, A_{260}:A_{280} is ~1.8 and for pure RNA A_{260}:A_{280} is ~2.

Contamination with proteins

Contamination of protein solutions by nucleic acids has a significant effect on the A_{260}:A_{280} ratio, since proteins (in particular, the aromatic amino acids) absorb light at 280 nm. The reverse, however, is not true. It takes a large amount of protein contamination to significantly affect the A_{260}:A_{280} ratio of a nucleic acid solution. The A_{260}:A_{280} ratio therefore has high sensitivity for nucleic acid contamination in protein solutions but lacks sensitivity for protein contamination in nucleic acid solutions (see Table 4-1 and Table 4-2).

Table 4-1. Impact of nucleic acid contamination on the A_{260}:A_{280} ratio for protein

% protein	% nucleic acid	A_{260}:A_{280}
100	0	0.57
90	10	1.32
70	30	1.73

Table 4-2. Impact of protein contamination on the A_{260}:A_{280} ratio for nucleic acid

% nucleic acid	% protein	A_{260}:A_{280}
100	0	2.00
90	10	1.98
70	30	1.94

This difference is due to the much higher extinction coefficient of nucleic acids at 260 nm and 280 nm, compared to proteins. Because of this, even for relatively high concentrations of protein, the protein contributes relatively little to the absorbance at 260 nm and 280 nm. While the protein contamination cannot be reliably assessed using an A_{260}:A_{280} ratio, this also means that it contributes little error to DNA quantity estimation.

Contamination with phenol

Phenol is commonly used in nucleic acid purification and can significantly impact on quantification estimates. Phenol has an absorbance maximum of 264 nm but also absorbs strongly at 260 nm and 280 nm, exhibiting an A_{260}:A_{280} ratio of 1.2.

Contamination with thiocyanates

Absorption at 230 nm can be the result of phenolate, thiocyanates, or contamination with other organic compounds. For a pure RNA sample, the A_{230}:A_{260}:A_{280} ratio should be around 1:2:1, and for a pure DNA sample it should be around 1:1.8:1.

Contamination with particulates

Absorption at 330 nm and higher wavelengths indicates contamination of the sample with particulates which tend to cause scattering of light in the visible range. The absorbance at these wavelengths in a pure nucleic acid sample should be zero.

4.2 Fluorescent based systems

The use of fluorescent dyes to quantify nucleic acids has become a common alternative to absorbance spectrophotometry. Fluorescent methods for quantification of nucleic acids depend on the measurement of changes in the fluorescence characteristics of small molecules or dyes upon binding to or intercalating with nucleic acid. Although more expensive than absorbance spectrophotometry, fluorescence-based quantification is more sensitive and often specific for the nucleic acid of interest. Since fluorometers measure fluorescence in relative rather than absolute units, the measurement is first calibrated with a known concentration of a standard nucleic acid solution with characteristics similar to the sample to be measured. Following calibration, a single measurement can establish the concentration of nucleic acid in the solution, but typically a standard curve will be required to ascertain the linearity of the assay over the range measured.

An overview of the common fluorescent dyes used to detect and quantify nucleic acids is given in Table 4-3, and specific details concerning their general properties are outlined in the text below.

Table 4-3. Properties of common dyes used for the quantification of nucleic acids

Dye	Excitation /Emission (nm)	Specificity	Sensitivity	Adverse performance influencing factors	Advantages
DAPI	360/465	A-T		Detergents, polyphosphates	Inexpensive
Hoeschst 33258	360/465	A-T of dsDNA	10 ng/mL	pH, SDS	Inexpensive Sensitive
PicoGreen	485/535	All dsDNA	250 ng/mL	None demonstrated	Sensitive
RiboGreen	485/535	G of all nucleic acids	200 pg/mL	Presence of any DNA	Quantification of RNA
OliGreen	485/535	T of all nucleic acids	100 pg/mL	Presence of DNA or RNA	Quantification of ssDNA Sensitive
Cyanine Dyes (e.g. YO-YO-1)	Various	dsDNA, some single stranded oligonucleotides	0.5 to 2.5 ng/mL	Salt, ethanol, SDS, some variants show base sensitivity	Inexpensive, Sensitive
Ethidium bromide	520/605	dsDNA and RNA	2 ng band, agarose gel	Degrades in presence of sodium nitrate and hypophosphorous acid. Must be stored in absence of light	Inexpensive, Sensitive
Sybr Green I	485/535	dsDNA	25 pg band, agarose gel	Must be stored in absence of light	Inexpensive, non-mutagenic

4.2.1 DAPI

The chemical 4', 6-diamidino-2-phenylindole is generally referred to as DAPI. The compound exhibits a high specificity for DNA, and is categorised as a minor-groove binder. It forms a fluorescent complex only when bound to the minor groove of AT rich sequences of DNA although it can form non-fluorescent intercalative complexes in other regions such as GC sequences or ssDNA. Although other minor-groove binding dyes (including the Hoechst dyes) exhibit a greater overall fluorescence yield compared to DAPI, it still demonstrates a near 20 fold increase in fluorescence when bound to dsDNA.

4.2.2 Hoechst dyes

These are a group of minor-groove binding bisbenzimide compounds. They demonstrate a specificity for AT rich regions although they can also display more complex DNA affinities. Hoechst dyes are generally excited by light in the near UV range (360 nm), and emit light in the blue range (460 nm). The most common variation used for DNA detection and quantification is the Hoechst 33258 dye which exhibits a strong selectivity for dsDNA.

4.2.3 PicoGreen

PicoGreen is a fluorescent dye developed and patented by Molecular Probes. This dye exhibits a strong increase in fluorescence (>1000 times) when bound to dsDNA, but does not show a significant increase of fluorescence in the presence of proteins, carbohydrates, ssDNA, RNA or free nucleotides. The dye also lacks specificity for AT or GC regions so that it can be used on DNA from any source. PicoGreen has been shown to be able to detect DNA down to 250 pg/mL, and has been demonstrated to have an assay linearity which extends over 4 orders of magnitude.

4.2.4 RiboGreen

RiboGreen can be used to detect both RNA and DNA (ssDNA and dsDNA). By employing two dye concentrations, three orders of magnitude of sensitivity are possible, starting at 1 ng/mL. RiboGreen demonstrates some base selectivity, with a 60% decrease in fluorescence in the presence of poly (G) fragments and virtually no fluorescence in poly (U) or poly (C) fragments.

4.2.5 OliGreen

OliGreen is highly sensitive and is capable of detecting as little as 100 pg/mL of ssDNA. It does, however, show significant base selectivity for thymine, with little or no selectivity for adenine, cytosine, or guanine. OliGreen also exhibits significant increase in fluorescence in the presence of dsDNA and RNA.

4.2.6 Cyanine Dyes

This class of fluorochromes includes the TOTO and YOYO family of dyes (e.g. dimeric cyanine dyes), ethidium bromide and Sybr Green. Dimeric cyanine dyes can be used to quantify dsDNA, ssDNA, and RNA. Cyanine dyes are not as sensitive as the PicoGreen group of compounds as they only show a linear range of response over two orders of magnitude with a limit of sensitivity of 0.5 ng/mL. Applications of these dyes include:

YOYO-1 for quantifying oligonucleotides and PCR products

YO-PRO-1 for quantifying dsDNA in solution (reported sensitivity of 2.5 ng/mL)

Additional cyanine dyes and their applications include: Ethidium bromide for quantifying DNA (in conjunction with agarose gel), and Sybr Green for quantifying nucleic acids. This dye can also be employed in real time PCR (qPCR) reactions for DNA quantification.

4.3 Gel electrophoresis

Agarose gel electrophoresis is a widely used procedure for the determination of the concentration and size of nucleic acid molecules. Common applications include the analysis of PCR and restriction enzyme digestion products and the separation of nucleic acid fragments for subsequent extraction and purification. Preparing the gels for this technique is a relatively easy task, but there are a few pitfalls that should be avoided to ensure reliable results.

4.3.1 Practical tips for making and loading agarose gels

Instructions on how to prepare the gel will be specific to the purpose of the analysis and the materials available to the laboratory. Ensure you follow the laboratory operating procedures. These additional points should be followed:

- A designated area should be established for the preparation of agarose gels with equipment (e.g. pipettes) that is used solely for the purpose of preparing gels. Always wear gloves when working with agarose gels and take care to dispose of them after the work is finished

- Ethidium bromide (EtBr) is commonly used as a nucleic acid staining agent. Extreme caution should be exercised when working with ethidium bromide as it is toxic and a mutagen. If adding ethidium bromide to dissolved agarose that has been recently prepared, it is important to wait until the agarose has cooled as ethidium bromide will aerosolise if added to the agarose when very hot, posing a health hazard if breathed in. Pouring the gel when too hot can also damage the gel apparatus and cause it to leak
- As an alternative to adding ethidium bromide to the agarose gel before pouring it, it is also possible for gels to be 'post-stained' by placing the gel in a container filled with enough buffer and ethidium bromide to cover the gel which is then placed on a shaker. The ethidium bromide containing buffer mixture can be used more than once, reducing the amount of ethidium bromide used in multiple experiments. This method also reduces the amount of handling of the ethidium bromide stock solution, which is preferential due to its highly toxic and mutagenic nature
- When pouring agarose gels, avoid creating bubbles as they will impede the flow of the current through the gel. Bubbles can be removed by pricking with a pipette tip
- After the gel has set, avoid pulling out the comb too quickly as this can create holes that can cause the sample to leak from the wells
- When mixing samples with loading dye, a simple and quick option is to mix the sample and loading dye together on the surface of a strip of parafilm. Droplets of loading dye can be made on the surface of the parafilm which can then be mixed with sample by pipetting up and down before loading onto the wells of the gel
- In order to make an accurate assessment of nucleic acid band size, it is important that the sample lane is in close proximity to the ladder. If all lanes of a gel are being used, it is therefore advisable to run ladder at both ends of the gel, and possibly also one in the centre of the gel, depending on the number of lanes
- When loading gels, one hand can be used to support the hand holding the pipette to help guide the tip into the well and keep it steady
- Forward pipetting samples and pushing past the first stop position when loading a gel can sometimes cause samples to be flushed out of the wells. It is therefore preferable for samples to be loaded either by forward pipetting without pushing past the first stop position or alternatively by reverse pipetting (see notes in section 2.1.1.8)
- Insert pipette tips into wells at an angle to avoid puncturing holes in the bottom of the wells

5 Measuring pH

Measurements of pH are frequently made in the laboratory. Sometimes the measurement forms part of a test method (e.g. the pH of a reagent or a sample has to be adjusted to a particular value). In other cases, the pH of the sample itself may be required to determine whether it meets a particular specification. This section covers:

- Definition of pH
- Equipment for measuring pH
- Choosing a suitable electrode
- Care of electrodes
- Calibration of pH meters
- Measuring the pH of test samples

5.1 What is pH?

The pH scale gives a measure of the acidity or alkalinity of aqueous solutions at a specified temperature (usually 20 °C or 25 °C). The pH scale is continuous, from pH=0 (very acidic) to pH=14 (very alkaline). Table 5-1 gives the approximate pH values for a range of materials.

Table 5-1. Typical pH values

pH	Acidity/alkalinity	Examples
0	Very acidic	1 mol L^{-1} hydrochloric acid
1		Battery acid
2		Stomach acid
3		Vinegar
4	Slightly acidic	Tomato juice
5		
6		Saliva
7	Neutral	Pure water
8		
9		Milk of magnesia
10	Slightly alkaline	
11		
12		Household ammonia
13		
14	Very alkaline	1 mol L^{-1} sodium hydroxide

The pH value of a solution is a measure of the activity of the hydrogen ion (a_{H^+}) in that solution:

$$pH = \log_{10} \frac{1}{a_{H^+}} = -\log_{10} a_{H^+}$$

Note that the pH scale is a log scale so the hydrogen ion activity in a solution with pH=5 is ten times higher than it is in a solution with pH=6.

The activity, a_{H^+}, and concentration of hydrogen ions, c_{H^+}, are related via an activity coefficient, γ:

$$a_{H^+} = \gamma \times c_{H^+}$$

Obtaining a value for the activity coefficient is not straightforward so measurements of pH are not normally used to determine concentration values. You should consider pH measurements as a convenient way of making comparative measurements of acidity.

5.2 Equipment for measuring pH

There are two main approaches, depending on the accuracy required:

- pH paper
- pH meter

5.2.1 pH paper

pH papers are strips of paper which are impregnated with compounds that will undergo specific colour changes at particular pH values. pH sticks are also available which have different indicator papers sealed along the length of the stick. pH papers/sticks provide a quick way of getting a semi-quantitative pH measurement for liquids. There are numerous different types of paper available, depending on the pH range of interest and the discrimination between pH values required. Some examples are shown in Table 5-2.

Table 5-2. Examples of pH papers/sticks

Product	pH range	Colour change
Litmus red paper	5 to 8	red to blue
Litmus blue paper	5 to 8	blue to red
Phenolphthalein paper	8.5 to 10	colourless to red
Indicator paper	2 to 12 in 2 unit steps	-
Indicator paper	0.5 to 5 in 0.5 unit steps	-
Indicator strip	0 to 14 in 1 unit steps	-
Indicator strip	1.7 to 3.8 in 0.3 unit steps	-

In all cases the pH is measured by dipping the paper/stick in the sample and comparing the colour of the paper with the reference chart supplied with the product. As there is an element of judgement involved, the readings can be somewhat subjective. Depending on the type of paper used it is possible to measure pH to an accuracy of between 0.3 and 1 pH units.

5.2.2 pH meter

When you require an accurate reading of pH you should use an electronic pH meter. The pH is usually determined by electrochemical measurements. The potential of a pH electrode immersed in the sample is measured with respect to a reference electrode (which is also in contact with the sample) using a pH meter. The pH electrode responds only to hydrogen ions present in the sample, even if there are other positive ions present. The response of the reference electrode is independent of the hydrogen ion concentration.

pH meters give a direct reading of pH either via a moving needle on a graduated scale or (more commonly) a digital display. Meters routinely used in the laboratory give readings to 0.1 or 0.01 pH units. Some of the controls you may find on a pH meter are shown in Table 5-3.

Table 5-3. pH meter controls

Control	Function
Set buffer	Adjusts the reading to display the pH of the standard buffer solution being measured to calibrate the pH meter (see section 5.4
Temperature	pH measurements are temperature dependent so the temperature of the solution being measured must be defined (some meters have an in-built facility to measure the temperature and adjust the reading – this ensures that measurements are not affected by temperature fluctuations).
Slope	Adjusts the meter reading to the pH of a second buffer solution (after using the 'set buffer' function for the first buffer). The meter should not require a large adjustment – if it does this could indicate a fault with the electrode (see section 5.40).

The response of the pH electrode is calibrated using standard aqueous buffer solutions which have known reference pH values. The pH meter reading is adjusted to give the correct pH reading for the reference solution (see section 5.4 for information on calibration).

A pH meter may appear easy to use. However, reliable results will only be obtained if the equipment is set up, maintained and used correctly. The rest of this section discusses the correct use of electronic pH meters.

5.2.3 Choosing a suitable electrode

To make a pH measurement two electrodes are required – the pH electrode (also known as the indicator electrode) and the reference electrode. Most pH measurements of aqueous solutions are made using a *combination electrode* in which both electrodes are contained in a single unit. In this type of electrode the reference electrode usually surrounds the glass pH electrode.

The choice of electrode will depend on the nature of the sample you are analysing. Manufacturers' websites will help you to select a suitable electrode for your application.

5.2.4 Glass electrode

This is the most common type of pH electrode. The end of the electrode which is immersed in the sample consists of a bulb shaped glass membrane. The bulb is filled with an acid solution such as 0.1 mol L^{-1} hydrochloric acid. A conducting wire such as silver wire coated with silver chloride is immersed in the liquid in the bulb. The other end of the wire forms a terminal at the other end of the electrode to the bulb. When the bulb comes into contact with an aqueous solution the potential developed is proportional to the hydrogen ion activity.

5.2.5 Reference electrode

The potential of the reference electrode must be independent of the hydrogen ion concentration of the solution being measured. Two main types of reference electrode are used:

- Silver/silver chloride (Ag/AgCl) electrode – a silver wire coated with silver chloride
- Calomel electrode (Hg/Hg_2Cl_2) – mercury in contact with mercury (I) chloride.

The reference electrode is filled with a suitable electrolyte solution, usually potassium chloride (KCl), and fitted with a semi-porous plug so that an electrochemical contact is established with the sample solution (a liquid junction). This 'single junction' configuration is the most common configuration of a reference electrode although others are available for certain specialist applications. 'Double-junction' electrodes, containing a potassium chloride solution in an inner reservoir and a second electrolyte in an outer reservoir, are also available.

The single junction Ag/AgCl electrode is the most widely used reference electrode for routine measurements. However, this is not suitable for all types of measurement and the choice of reference electrode will depend on the sample being measured. For example, the calomel electrode is recommended for samples containing proteins, sulfides, heavy metal ions or tris buffer as these compounds can react with silver.

5.2.6 Aqueous solutions

The factors that influence the choice of electrode when analysing aqueous solutions are summarised in Table 5-4.

Table 5-4. Choice of electrode for analysing aqueous solutions

Factor	Type of electrode
pH range	
• pH range 1 to 10	General purpose glass electrode and Ag/AgCl reference electrode.
• pH>10	General purpose glass electrodes give low readings in strongly alkaline solutions due to interference from sodium, lithium and (to a lesser extent) potassium ions. Referred to as the 'alkaline error'. Special glass may be required to minimise interferences.
• pH<1	General purpose glass electrodes give high readings for strongly acidic solutions. Referred to as the 'acid error'. Consult manufacturers' guidelines to identify suitable electrodes.
Ionic strength of solution	
• High ionic strength (>0.1 mol L^{-1})	May require modified reference electrode configuration.
• Very low ionic strength	May require a modified reference electrode configuration to avoid contamination of the sample by the electrolyte.

Table continued

Table 5-5. continued

Factor	Type of electrode
Sample composition	
• Sample contains components that react with silver (e.g. waters containing sulfur compounds or biological buffers)	Do not use Ag/AgCl chloride reference electrode. Calomel reference electrode recommended.
• Sample contains components that will react with potassium chloride	Do not use potassium chloride as the electrolyte.
Sample temperature	
	Calomel electrode cannot withstand temperatures >60 °C. Ag/AgCl electrode can be used at elevated temperatures.
Accuracy required for your application	
	Electrodes available capable of reading to between 0.01 and 0.1 pH units.

5.2.7 Non-aqueous solutions

The pH scale is defined in terms of hydrogen activity in an aqueous solution. However, pH measurements can be made for non-aqueous liquids to compare samples of similar composition. Special electrodes are often required for non-aqueous liquids. Key requirements of electrodes for non-aqueous liquids include:

- A low resistance glass membrane
- A 'double junction' reference electrode rather than the porous-plug single junction electrode described previously
- In some cases a reference electrode containing lithium chloride rather than potassium chloride is required (LiCl is soluble in many organic liquids whereas KCl has limited solubility)

Consult manufacturers' websites for information on specific applications.

5.2.8 Viscous samples/samples containing solids

Measuring the pH of very viscous samples or samples with high solids content (e.g. suspensions, slurries, sludges, emulsions) can cause problems. Special electrodes are often required and manufacturers' information should be consulted to identify suitable electrodes. Options for these types of sample include:

- A modified liquid junction in the reference electrode to prevent it becoming blocked/contaminated (e.g. double junction, sleeve or open junction electrode)
- Separate pH and reference electrodes (i.e. an electrode pair rather than a combined electrode)
- An electrode that can be easily cleaned

5.3 Care of electrodes

To obtain reliable pH measurements it is essential that the electrodes are properly stored and maintained. This section covers the main points to consider but you should also consult the manufacturer's instructions for information on storage and maintenance of particular electrodes. The key points are summarised in Table 5-6.

Table 5-6. Storage and cleaning of pH electrodes

Requirement	Action
Do not let the electrode membrane dry out.	Store in an appropriate storage solution: • pH 7 buffer solution for a glass pH electrode • reference electrode filling solution (e.g. KCl) for a reference electrode • mixture of reference electrode solution and a buffer to maintain a suitable pH for a combination electrode Storage solutions are commercially available.
	Immerse the electrode(s) in sufficient storage solution to cover the glass membrane and the liquid junction.
	If the membrane has dried out, rehydrate by placing in the storage solution for at least 12 hours.
	Do not store electrodes in distilled water – this will dilute the reference electrode solution.
Check the level of the solution in the reference electrode.	From time to time the electrolyte solution in the reference electrode will require topping-up.
	When the electrode is not being used ensure the filling hole in the reference electrode is covered (but it should be uncovered when in use to ensure that the electrolyte flows properly through the liquid junction).

Table continued

Table 5-5 continued

Requirement	Action
Regularly check electrode for build-up of salt crystals and deposits on the membrane and liquid junction.	Always clean electrodes before use by rinsing with a gentle jet of distilled water and allowing to drain (electrodes can be blotted dry with lint-free paper but do not rub or wipe electrodes as this may cause damage).
	More rigorous cleaning procedure: • Soak electrode for 30 min in 0.1 mol L^{-1} hydrochloric acid or nitric acid. Rinse with distilled water. Drain and refill reference electrode. Immerse electrode in storage solution or pH 7 buffer for at least 1 hour, ensuring membrane and liquid junction are covered. If deposits have formed on the membrane special cleaning solutions may be required: • Protein deposits: soak in 1% pepsin in 0.1 mol L^{-1} hydrochloric acid for 15 min • Inorganic deposits: soak in 0.1 mol L^{-1} tetrasodium EDTA solution for 15 min • Grease and oil: rinse with mild detergent:methanol solution (1:10) After any of these three cleaning procedures follow the acid soaking procedure described above.
Long term storage of the electrode.	Empty the reference electrode, rinse with deionised water, fill with fresh electrolyte solution and ensure that the filling hole is securely covered.
	Cover the glass membrane and liquid junction with a cap containing a few drops of the electrode storage solution.
	Prior to use, remove the cap and immerse the electrode in storage solution for at least 12 hours.

5.4 Calibration of pH meters

Calibration is required to match the reading given by the meter to the response of the electrode to solutions of a particular pH. This is achieved by measuring buffer solutions with known pH values.

There are a number of options when purchasing buffers for calibration of pH meters:

- Ready-prepared solutions with documented pH values at specified temperatures
    - each solution has a lot-specific pH value, usually quoted with a typical uncertainty of ±0.01 or ±0.02 pH units
    - different pH solutions are available as different coloured solutions to aid identification

- Tablets, sachets and concentrates that you make up in the laboratory to give a solution of the required pH
    - preparation instructions must be followed carefully
- Primary pH standards
    - high purity salts used to prepare buffer solutions (e.g. a solution of potassium hydrogen phthalate with a concentration of 10.13 g L^{-1} has a pH of 4.00 at 20 °C)

Key points to remember when carrying out a calibration:

- Use at least two buffer solutions which bracket the pH of the test samples
- Make sure that the buffer solutions are within their expiry date
    - for buffers prepared in-house the 'shelf life' should be established
- Make sure that the buffer solutions have been stored correctly and are free from contamination, sediment or mould
- Transfer the required amount of the buffer solution to a small, clean dry beaker
    - close the buffer solution bottle immediately after transferring the required amount of solution
    - never return unused buffer to the buffer solution bottle
    - never immerse the electrode in the buffer solution bottle
- Ensure the buffer solution and samples are at ambient temperature before making measurements;
- If the pH meter does not have a temperature sensing probe, record the ambient temperature and set the temperature control on the meter to this value;
- Immerse the electrode in the first buffer solution (in a beaker)
    - make sure the electrode is held vertically and that the bulb and liquid junction are covered by the solution;
- Stir the buffer with a magnetic stirrer – do not stir solutions with the electrode
- The optimum stirring rate will depend on the viscosity of the liquid being measured
    - choose a stirring rate that provides a homogeneous solution without forming a vortex (to avoid drawing CO_2 into the liquid from the atmosphere)
    - use the same stirring rate when calibrating the pH meter as when making pH measurements
- When the meter reading has stabilised (to within about ±0.02 pH units) use the 'set-buffer' control to adjust the display to the pH of the buffer solution
- Discard the buffer and refill the beaker with the same buffer solution
    - if the reading is not within ±0.02 of the reference value re-adjust the 'set buffer' control
- Repeat the procedure until two successive readings agree to within ±0.02 pH units
    - if this is not achieved after the second or third reading you should investigate the problem
- Transfer the second buffer to a clean dry beaker
- Rinse the electrode with water followed by the second buffer solution and allow it to drain/blot dry with lint-free paper (do not rub or wipe electrode)
- Stir the buffer with a magnetic stirrer

- Immerse the electrode in the buffer solution and record the reading once it has stabilised to with about ±0.02 pH units
- The reading should not differ significantly from the reference value for the buffer but if necessary, adjust the reading using the 'slope' control
- If a large adjustment is required (>±0.3 pH units) this could indicate that the 'slope' value of the electrode is significantly different from the expected theoretical value
    - some meters will give a warning if the slope falls outside a specified range
    - a poor slope value can indicate a problem with the electrode – investigate the cause before using the electrode to measure test samples

Note that some pH meters have an *auto-calibration* function which means that the meter will select the correct pH value for the buffer from a pre-programmed list. This function will also take into account temperature effects. You should always follow the manufacturer's instructions when using an auto-calibration function.

5.5 Measuring the pH of a liquid

The procedure for measuring the pH of a liquid is as follows:

1. Ensure that the pH meter has been calibrated

2. Ensure that the liquid being measured is at ambient temperature (this should be the same as the temperature of the buffer solutions during the calibration procedure)

3. Transfer a portion of the liquid to a clean dry beaker

4. Rinse the electrode with distilled water followed by the liquid being measured and allow it to drain/blot dry with lint-free paper (do not rub or wipe electrode)

    - Immerse the electrode in the liquid following the procedure for the first buffer solution described in section 5.4stir the liquid being measured at the same speed as used when stirring the buffer solutions during calibration of the pH meter

5. Discard the liquid and repeat the measurement on a second portion. Depending on the nature of the liquid and the type of electrode, duplicate readings should agree to within between ±0.02 and ±0.1 pH units

6. Before measuring other liquids always rinse the electrode with distilled water followed by some of the liquid to be measured and allow the electrode to drain/blot dry with lint-free paper

7. If you are making a large number of measurements, re-measure at least one of the buffer solutions at regular intervals (e.g. every ten samples)

8. When you have finished your measurements discard all used buffer solutions, rinse the electrode with distilled water and store as described in section 5.3

5.6 Checklist for making pH measurements using a pH meter

Table 5-7 summarises the key points to remember when making pH measurements using a pH meter.

Table 5-7. Dos and don’ts for making pH measurements

Do	Don’t
Select a suitable electrode for the liquids to be measured (see section 5.2.3).	
Ensure that the electrode is clean and undamaged (see section 5.3).	Use an electrode that has dried out without rehydrating it.
Check the level of the electrolyte in the reference electrode.	Use an electrode containing insufficient electrolyte.
Rinse the electrode with distilled water and the liquid to be measured before use and allow to drain.	
Dry electrodes by blotting with lint-free paper.	Dry electrodes by wiping or rubbing.
Calibrate the pH meter with at least two buffer solutions with a pH range that brackets the pH of the liquids being measured.	Use a pH meter without calibrating it.
Check that buffer solutions are within their expiry date, have been stored correctly and are free from mould or sediment.	Use buffer solutions that have passed their expiry date or appear to be contaminated.
Discard any unused buffer solutions.	Return unused buffer solutions to the stock bottle.
Transfer the liquid to be measured to a clean dry beaker.	Place the electrode directly in the buffer or sample container.
Ensure that liquids to be measured are at ambient temperature prior to measurement.	
Record the ambient temperature and set the temperature control (if the pH meter does not have automatic temperature compensation).	
Make sure the filling hole in the reference electrode is uncovered.	Use the electrode with the filling hole covered.
Immerse the electrode to a sufficient depth in the liquid being measured – the glass membrane and liquid junction should be covered.	
Support the electrode vertically in the solution.	Hold or support the electrode at an angle.

Table continued

Table 5-7 continued

Do	Don't
Stir the solution at the appropriate speed with a magnetic stirrer while measuring the pH.	Stir the liquid with the electrode. Stir the liquid too vigorously (i.e. avoid the formation of a vortex).
Make sure reading has stabilised before recording the pH.	
Rinse the electrode with distilled water after use.	
Store electrodes in an appropriate solution (see section 5.3).	Store electrodes in distilled water.
	Allow glass electrodes to dry out.

6 Preparing solutions of known concentration

Solutions of known concentration are used widely in the laboratory. In some cases it may be possible to purchase a suitable solution which has the required concentration quoted with the required degree of certainty. However, many test methods require the preparation of solutions of a specified concentration. These solutions are often required for calibration purposes (see section 6.1) so preparing them correctly is a critical stage in the method. It is therefore important for you to be able to prepare accurately solutions of specified concentrations. This section covers:

- Uses of solutions of known concentration
- Calculating the concentration of solutions
- Selecting a suitable material to prepare a solution
- Practical steps in preparing solutions of known concentration
- Labelling and storage of solutions

To be able to prepare solutions correctly you need to be able to make accurate measurements of mass and volume – make sure you have reviewed the relevant sections in section 2 (Measuring volume) and section 3 (Measuring mass).

6.1 When are solutions of known concentration used?

Solutions of known concentration have a number of uses:

- To calibrate an instrument such as a UV spectrophotometer (calibration is required to relate the response of the instrument to the amount of the analyte present)
- To evaluate the performance of test methods both during method validation and in on-going quality control when the method is in routine use (see Chapter 10)
- To train staff and evaluate their performance

In all of the above examples it is important that the concentration of the solution is known with a high degree of certainty. In the first example the stated concentration of the solution is used in the determination of the concentration of the analyte in test samples. If the actual concentration of the solution differs from the stated value (because the solution has been prepared incorrectly, for example) then the results for test samples will be in error. In the other examples, the stated concentration of the solution is being used to make a judgement about the performance of the method and/or the analyst. If the actual concentration differs from the stated value then you may conclude, incorrectly, that there is a problem with the method or the way in which the analyst has applied the method.

Different terms are used to describe solutions of known concentration, depending on the context in which they are being used. Some commonly used terms are listed in Table 6-1. Some of these terms are formally defined in the International Vocabulary of Metrology[4] or other documents[5].

[4] International vocabulary of metrology – Basic and general concepts and associated terms (VIM), JCGM 200:2012, 3rd edition (2008 version with minor corrections)

[5] IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). XML on-line corrected version: http://goldbook.iupac.org (2006-) created by M. Nic, J. Jirat, B. Kosata; updates compiled by A. Jenkins. ISBN 0-9678550-9-8. doi:10.1351/goldbook.

Table 6-1. Terms used to describe solutions of known concentration

Terminology	Usage
Measurement standard	'Realisation of the definition of a given quantity, with stated quantity value and associated measurement uncertainty, used as a reference'* Examples: A Standard buffer solution with a pH of 7.072 with an associated standard measurement uncertainty of 0.006 Reference material providing quantity values with measurement uncertainties for the mass concentration of each of ten different proteins
Standard solution	A solution of accurately known concentration†
Stock standard solution	Solution used for preparation of working standard solutions and/or calibration solutions, containing the analyte(s) of interest at a certified concentration(s) traceable to national standards
Working (measurement) standard	'Measurement standard that is used routinely to calibrate or verify measuring instruments or measuring systems'*
Calibration standard/solution	Solution prepared from a secondary standard and/or stock solutions and used to calibrate the response of the instrument with respect to analyte concentration
Calibrator	'Measurement standard used in calibration'* This term is only used in certain sectors, for example medical laboratories
Quality control standard/solution/material	Solution used to check the performance of a test method or analytical instrument
*Definition from VIM[4] †Definition from IUPAC 'Gold Book'[5]	

The terminology can be confusing so make sure you are clear about the meaning of any terms used in the standard operating procedures/method protocols in your laboratory.

6.2 Calculating the concentration of solutions

To be able to prepare solutions of known concentration you need to be able to:

- Calculate the concentration of solutions from known masses and volumes
- Work out the amount of material and volume of solvent required to prepare a solution of a specified concentration

One of the key concepts in calculating the concentrations of solutions is the concept of *the mole*.

6.2.1 Amount of substance: The mole

The internationally agreed (SI) unit for measurements of mass is the kilogram. In chemistry, the SI unit for the amount of a substance is the mole (the symbol used to represent the mole is 'mol'). 1 mol of any substance will contain the same number of particles (e.g. atoms or molecules). The number of particles is the *Avogadro constant* which is equal to 6.022×10^{23} and is the number of atoms in 12 g of carbon 12 (^{12}C). So, 1 mol of carbon will contain the same number of atoms as 1 mol of sodium which will be the same as the number of molecules in 1 mol of water. 1 mol of a material is therefore the amount of the material which contains the same number of particles as 12 g of carbon 12.

6.2.2 Converting from moles to mass

In the laboratory we measure out portions of solid chemicals by weighing. We therefore need to be able to convert from moles to units of mass. If you know that you need 0.5 mol of a chemical to prepare a solution, how do you determine how much material to weigh out?

We already know that 1 mol of carbon 12 weighs 12 g. The masses of all other elements are calculated relative to the mass of carbon. These masses are known as *relative atomic masses* and can readily be found in textbooks. The relative atomic mass of sodium, for example, is 23 so sodium atoms are approximately twice as heavy as carbon atoms.

The mass of 1 mol of a material is known as the *molar mass*. The molar mass of carbon is therefore 12 g mol^{-1} and the molar mass of sodium is 23 g mol^{-1}. To calculate the molar mass of a compound simply add the molar masses of the elements present. For example, the molar mass of sodium hydroxide (NaOH) is 40 g mol^{-1} (Na = 23 g mol^{-1}, O = 16 g mol^{-1}, H = 1 g mol^{-1} (using molar masses rounded to the nearest integer)). The molar mass of water is 18 g mol^{-1}.

- To convert from mol to g *multiply* by the molar mass
- To convert from g to mol *divide* by the molar mass

Examples

1) How many moles of Tris-HCl are contained in 50 g?

The chemical formula for Tris-HCl is $NH_2C(CH_2OH)_3 \cdot HCl$. The relevant molar masses (rounded to two decimal places) are shown below.

Cl = 35.45 g mol^{-1} C = 12.01 g mol^{-1} O = 16.00 g mol^{-1}

H = 1.01 g mol^{-1} N = 14.00 g mol^{-1}

The molar mass is therefore:

$35.45 + (4 \times 12.01) + (3 \times 16.00) + (12 \times 1.01) + 14.00 = 157.61$ g mol^{-1}

If 1 mol of $NH_2C(CH_2OH)_3 \cdot HCl$ weighs 157.61 g then 50 g will contain:

$$\frac{50}{157.61} = 0.317 \text{ mol}$$

2) It has been determined via a titration experiment that a solution contains 0.053 mol of sodium chloride (NaCl). How much does 0.053 mol of sodium chloride weigh?

The molar masses of sodium ions and chloride ions are 22.99 g mol^{-1} and 35.45 g mol^{-1}, respectively (rounded to two decimal places). The molar mass of sodium chloride is therefore 22.99 + 35.45 = 58.44 g mol^{-1}.

0.053 mol of sodium chloride would therefore weigh:

0.053 x 58.44 = 3.10 g

6.2.3 Concentrations expressed in mol L^{-1}

A 1 molar solution contains 1 mol of a substance in 1 L of solution. Note that it is 1 L of solution *not* 1 L of solvent. A 0.5 molar solution contains 0.5 mol of the substance in 1 L of solution.

1 L of a 1 molar solution of sodium chloride therefore contains 1 mol of sodium chloride. The molar mass of sodium chloride is 58.44 g mol^{-1}. To prepare 1 L of a 1 molar sodium chloride solution you would need to weigh out 58.44 g of sodium chloride, dissolve it in water and make the volume up to 1 L (see Figure 6-1 for the correct procedure). But what if you don't want 1 L of solution?

To work out how much material you need to weigh out to prepare V mL of a solution with a concentration of C mol L^{-1} use the equation:

$$m = \frac{C}{1000} \times V \times M$$

where m is the amount of material required (g), C is the required concentration (mol L^{-1}), V is the volume of solution required (mL) and M is the molar mass (g mol^{-1}) of the substance. (Note: the factor of 1000 is required to convert the concentration, C, from mol L^{-1} to mol mL^{-1}.)

To calculate the concentration of a solution in mol L^{-1} if you know the mass of the material and the volume of the solution:

$$C = \frac{m}{M \times V} \times 1000$$

(Note: the factor of 1000 is required to obtain the concentration, C, in units of mol L^{-1} rather than mol mL^{-1}.)

Example

How much material would you need to weigh out to prepare 500 mL of a 0.5 mol L^{-1} solution of potassium nitrate (KNO_3)?

M is 101.10 g mol^{-1} (the relevant molar masses are K = 39.10 g mol^{-1}, N = 14.00 g mol^{-1}, O = 16.00 g mol^{-1})

C is 0.5 mol L^{-1}

V is 500 mL

$$m = \frac{0.5}{1000} \times 500 \times 101.10 = 25.28\,\text{g potassiumnitrate}$$

Sometimes you will need to know how many moles of a compound are present in a particular volume of a solution of known concentration.

To calculate the number of moles (x) in V mL of a solution with a concentration of C mol L^{-1}:

$$x = \frac{C}{1000} \times V$$

Example

How many moles of potassium hydrogen phthalate are present in 25 mL if the concentration is 0.1 mol L^{-1}?

C = 0.1 mol L^{-1} and V = 25 mL, so:

$$x = \frac{0.1}{1000} \times 25 = 0.0025 \text{ mol of potassiumhydrogenphathalate}$$

6.2.4 Concentrations expressed in units of mass/volume (m/v)

The concentrations of solutions are also frequently expressed in units of mass/volume such as g L^{-1} (grams per litre) or mg L^{-1} (milligrams per litre). The process of working out how much material is required to prepare a particular volume of a solution with a specified concentration is similar to that used for solutions expressed in mol L^{-1}.

To prepare V mL of a solution with a concentration of C g L^{-1} the amount of material required (m, in grams) is:

$$m = \frac{C}{1000} \times V$$

(Note that if C is expressed in mg L^{-1} then m will be expressed in mg.)

Example

How much sodium hydroxide (NaOH) would you need to weigh out to prepare 500 mL of a solution with a concentration of 4 g L^{-1}?

$$m = \frac{4}{1000} \times 500 = 2 \text{ g}$$

It is also straightforward to convert between concentrations in mol L^{-1} and g L^{-1}:

- To convert from mol L^{-1} to g L^{-1} multiply by the molar mass
- To convert from g L^{-1} to mol L^{-1} divide by the molar mass

So, given that the molar mass of sodium hydroxide is 40.00 g mol^{-1}, a solution with a concentration of 4 g L^{-1} is the same as a solution with a concentration of 4/40 = 0.1 mol L^{-1}.

In some test methods, the concentrations of solutions are expressed in terms of one particular component of a compound (e.g. a particular ion). For example, in a method for the determination of nitrite in water samples, the concentrations of the calibration standards are expressed in terms

of mg nitrite per L. To prepare these standards you need to identify a suitable source of nitrite ions (e.g. sodium nitrite) and dissolve an appropriate amount of the material in the required volume of water. You can calculate how much nitrite is required using the equation given above. For example, to prepare 1 L of a solution with a nitrite concentration of 1000 mg L^{-1} you would need 1000 mg (i.e. 1 g) of nitrite. If you are using sodium nitrite to provide the nitrite ions you need to work out how much to weigh out.

The formula of sodium nitrite is $NaNO_2$ so its molar mass is 68.9953 g mol^{-1}. The molar mass of nitrite ions (NO_2^-) is 46.0055 g mol^{-1}. If you weighed out 1 g of sodium nitrite you would only have 46.0055/68.9953 = 0.6668 g nitrite. To obtain 1 g of nitrite you therefore need to weigh out 68.9953/46.0055 = 1.4997 g sodium nitrite.

The general calculation for determining the mass of the compound required ($m_{compound}$) can be written:

$$m_{compound} = \frac{M_{compound}}{n \times M_{ion}} \times m_{ion}$$

where $M_{compound}$ and M_{ion} are the molar masses of the compound and the ion, respectively, n is the number of the ion of interest present in the formula of the compound and m_{ion} is the mass of the ion required. (In the case of nitrite ions obtained from $NaNO_2$, n=1.)

6.2.5 Obtaining the required concentration by dilution

The practical steps in preparing a solution of known concentration by dilution are shown in Figure 6-2. Given a starting solution (sometimes called the stock solution) of known concentration, how do you decide how much of that solution to take and what volume to dilute it to? To do this you must calculate the 'dilution factor'. The dilution factor is the ratio of the concentration of the stock solution to the concentration of the diluted solution.

If a solution with a concentration of 100 mg L^{-1} is prepared from a stock solution with a concentration of 1000 mg L^{-1} the dilution factor is 1000/100 = 10. If the diluted solution has a concentration of 10 mg L^{-1} the dilution factor is 100.

Once you know the dilution factor you must select a volume of the stock solution and a final volume of the diluted solution which will give you the required dilution factor. For example, to get from a concentration of 1000 mg L^{-1} to 100 mg L^{-1} you could take 10 mL of the stock solution and dilute it to 100 mL, following the procedure shown in Figure 6-2. Alternatively, if you only required a smaller volume of the diluted solution, say 25 mL, you would need to dilute 2.5 mL of the stock solution.

If you decide on the volume of the diluted solution that you need, the equation for calculating the volume of the stock solution (V_{stock}) that you need to dilute is:

$$V_{stock} = \frac{C_{dil}}{C_{stock}} \times V_{dil}$$

where V_{dil} is the final volume of the diluted solution, C_{stock} is the concentration of the stock solution and C_{dil} is the required concentration of the diluted solution.

Sometimes it is possible to achieve the required concentration of the diluted solution in a single step. For example, a dilution factor of ten, as in the example above won't cause any problems. But what if you needed to prepare 100 mL of a solution with a concentration of 0.5 mg L^{-1} from a commercially supplied standard solution with a concentration of 1000 mg L^{-1}? This would require you to transfer 0.5 mL of the stock solution into a 100 mL volumetric flask. Table 2-2 illustrates that as the volume being measured decreases the uncertainty in the volume delivered increases.

For example, the tolerance for a Class A 1 mL pipette is 0.008 mL (0.8%) compared to a tolerance of 0.02 mL for a Class A 10 mL pipette (0.2%). It can also be more difficult to measure small volumes accurately which increases the uncertainty further. It may therefore be necessary to carry out more than one dilution to reach the desired concentration. However, each dilution step will introduce uncertainties. You will therefore need to devise a plan which involves the minimum number of dilutions while achieving an acceptable level of uncertainty in the concentration of the diluted solution. In some cases, when the dilution factor is large, a smaller uncertainty will be achieved if one or two intermediate dilutions are carried out compared to a single dilution involving the transfer of a small volume of the stock solution.

6.2.6 Selecting a suitable material to prepare the solution

If you consult a chemical supplier's catalogue to source a particular compound you will see that there are often a number of different 'grades' of material. The grade relates to the purity of the material and how well it has been characterised by the supplier. When preparing a solution of known concentration it is important to select a material with a suitable purity. The uncertainty associated with the purity of the material will contribute to the uncertainty in the concentration of the solution. If the solution is being used for calibration purposes, the uncertainty in its concentration will contribute to the uncertainty in the final result obtained for test samples. It is therefore good practice to use material with the highest purity available (i.e. as close to 100% as possible). Generally, the higher the purity the more expensive a material will be (see Table 6-2).

Table 6-2. Characteristics of different 'grades' of materials

Grade	Stated purity	Information
Specified laboratory reagent/general purpose reagent	>97%	Information on a limited number of possible impurities. Information not stated for individual lots of material.
Analytical reagent	>99%	Information on a wider range of possible impurities. Analytical information for individual lots of material quoted for some impurities.

The analytical reagent would be the best choice as it has a higher purity and has been characterised more thoroughly. If you require a higher level of certainty than can be achieved using an analytical reagent it may be necessary to use a certified reference material (CRM). A CRM is a material that has been characterised very rigorously for a particular property (e.g. its purity). It is accompanied by a certificate which states the value for the property of interest plus an estimate of the uncertainty associated with the value. When calculating the concentration of a solution it will be necessary to take account of the purity of the material used as this will contribute to the uncertainty in the concentration of the solution.

6.2.7 Practical steps in preparing solutions of known concentration

Section 6.2 explained how to calculate the masses and volumes required to prepare a solution of known concentration. This section describes the practical procedures for preparing a solution. Solutions of known concentration can be prepared in a number of different ways depending on the nature of the analyte and/or the concentration required:

- Weighing out a solid material of known purity, dissolving it in a suitable solvent and diluting to the required volume
- Weighing out a liquid of known purity, dissolving it in a suitable solvent and diluting to the required volume
- Diluting a solution previously prepared in the laboratory

- Diluting a solution from a chemical supplier

Remember to record all masses and volumes used in the preparation of solutions in a laboratory workbook, and to show how you calculated the concentration of the solution.

The procedure for preparing a solution by dissolving a solid material is shown in Figure 6-1. The procedure for preparing a solution by dilution of a more concentrated solution (either prepared in the laboratory or from a chemical supplier) is shown in Figure 6-2.

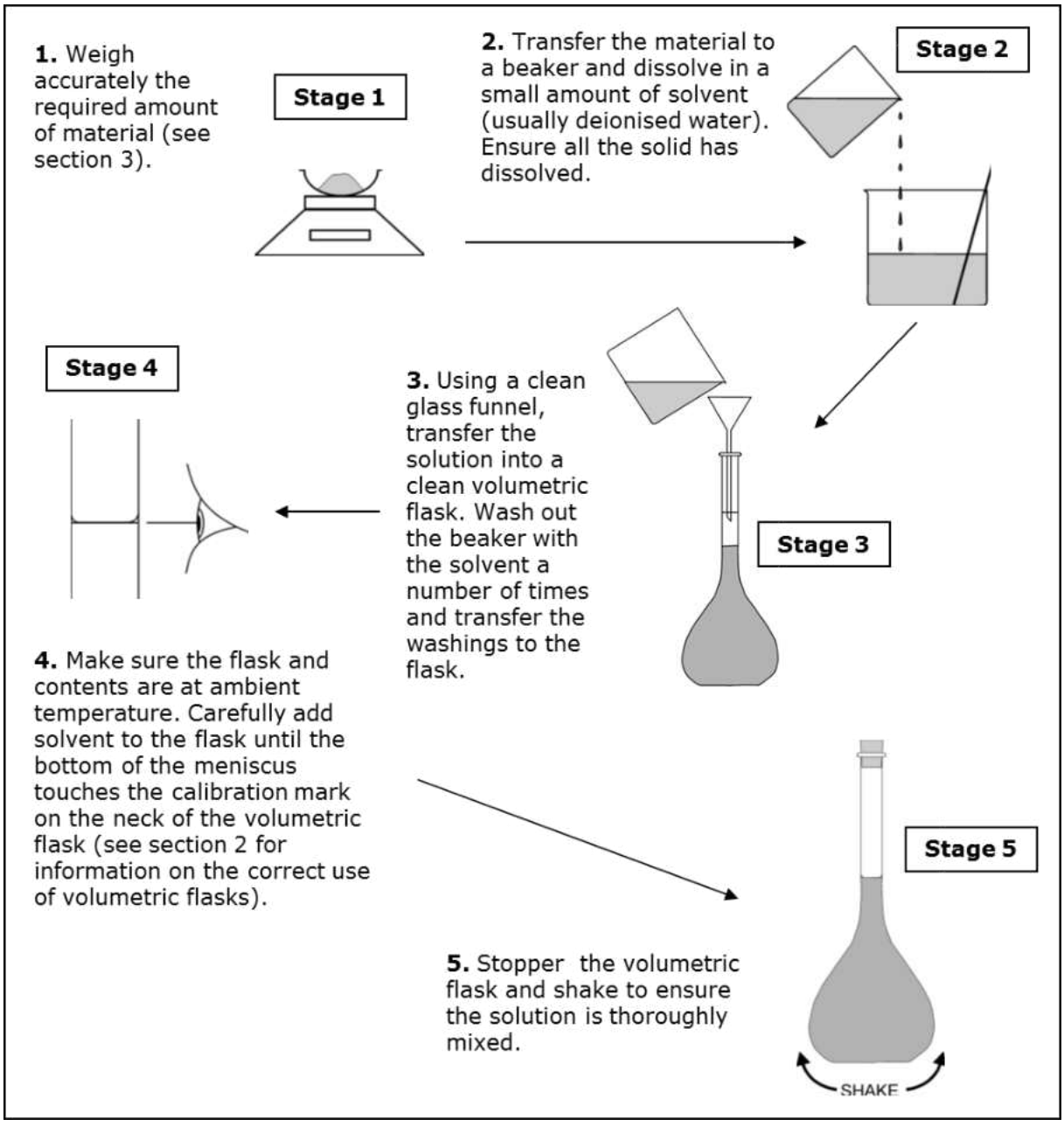


Figure 6-1. Procedure for preparing a solution of known concentration from a known amount of a solid material

Note that in some cases the solute may be a liquid rather than solid. The procedure is very similar to that shown in Figure 6-1. The required amount of the liquid is weighed accurately (see section 3.3.3). The liquid is then transferred directly to the volumetric flask containing some of the solvent.

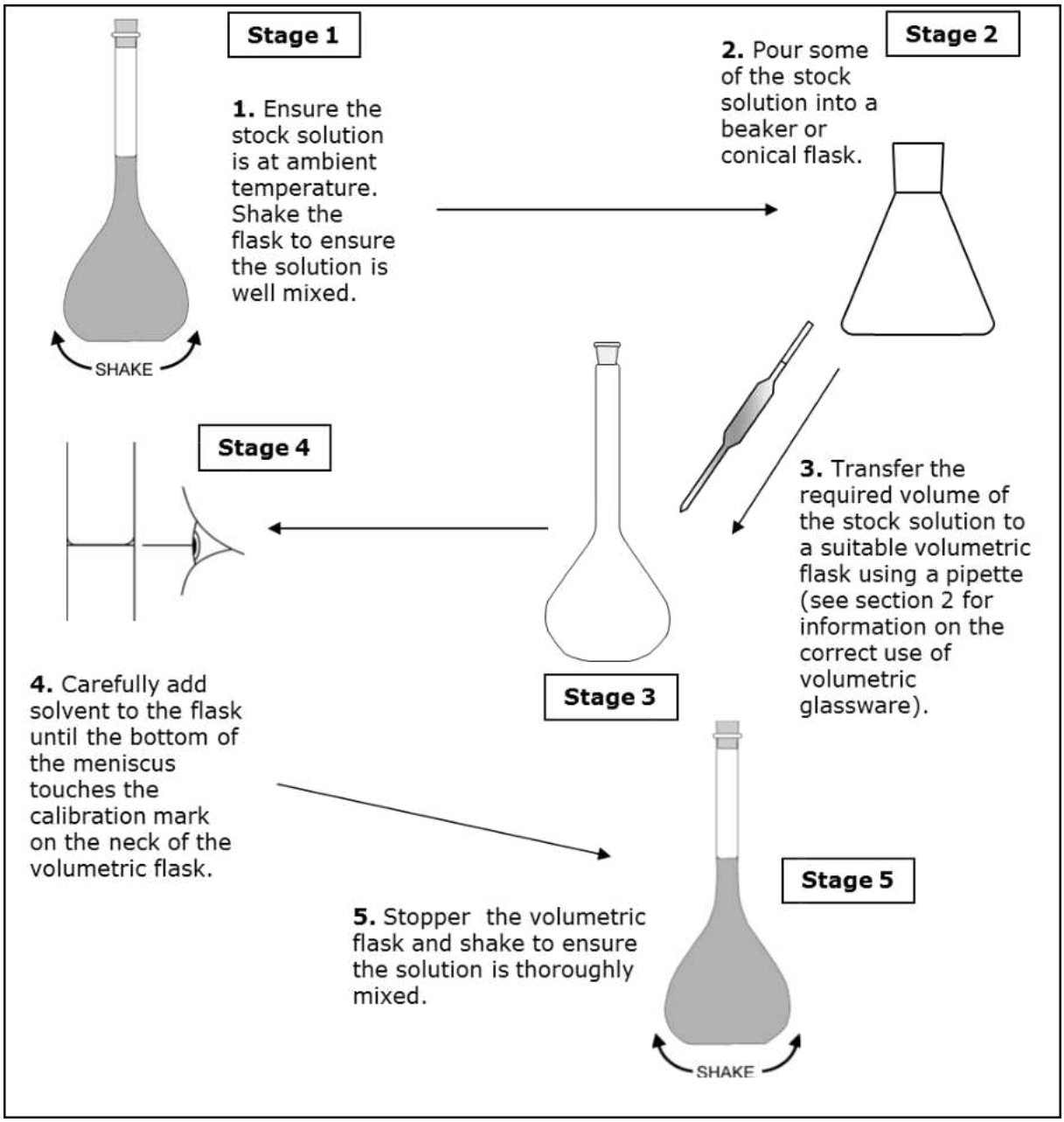


Figure 6-2. Procedure for preparing a solution of known concentration by dilution

6.3 Labelling and storage of solutions

Once you have prepared the solution you need to think about how you will store it and how it will be identified in the future. Remember the following key points:

- Solutions should not be stored in volumetric flasks – transfer them to a suitable container for storage
- Ensure that solutions are stored correctly. Some solutions will need to be stored in a refrigerator while others may be light-sensitive and need to be stored in amber bottles
- All solutions should be clearly labelled with the following information:
    - the name and concentration of the solution
    - date of preparation
    - name of analyst
    - review or expiry date

    - hazard information (if appropriate)
- The label must be securely attached to the container and be written in water insoluble ink

In some cases, particularly where volatile solvents are used, it is useful to check for any changes in the mass of the solution during storage. After the solution has been prepared, it is transferred to a suitable container and the mass of the sealed container and the solution is recorded. Prior to an aliquot of the solution being used, the container is re-weighed. The mass should not be significantly different from that recorded prior to the solution being stored. After the required volume of the solution has been transferred from the storage container, the solution is reweighed before being returned to storage. If a significant change in mass is observed after the solution has been stored then it should not be used.

6.4 Checklist for preparing solutions of known concentration

Table 6-3 summarises the key points to remember when preparing solutions of known concentration.

Table 6-3. Dos and don'ts for preparing solutions of known concentration

Do	Don't
Preparing a solution by dissolving a solid material	
Use a material with a suitable purity (grade) and ensure that it has been stored correctly and is within its expiry date.	Use material that appears to have been stored incorrectly or that has passed its expiry date.
Use a clean, dry spatula to transfer the required amount of material.	Return unused material to the original bottle.
Make sure you have correctly calculated the amount of material required.	
Weigh accurately the required amount of material (see section 3.3.3 for information on making accurate measurements of mass).	
Ensure that all glassware used is clean, dust free and undamaged.	Use dirty glassware or glassware that is damaged and/or has faded graduation marks.
Transfer the material to a beaker and dissolve in a small amount of solvent.	Transfer the material directly to the volumetric flask.
Make sure that material has dissolved completely.	
Use a small glass funnel to transfer the solution from the beaker to the volumetric flask.	
Rinse the beaker with solvent and transfer the rinsings to the volumetric flask.	

Table continued

Table 6-3 continued

Do	Don't
Make sure that the solution is at ambient temperature before making the solution up to the calibration mark with solvent (see section 2.6 for information on the correct use of volumetric flasks).	Make the solution up to the calibration mark if its temperature is significantly different from the ambient temperature. (Note that if the ambient temperature is significantly different from the calibration temperature of the glassware used this will increase the uncertainty in the concentration of the solution.)
Make sure the solution is mixed thoroughly before use.	
Preparing solutions by dilution of a stock solution	
Make sure the stock solution is at room temperature.	Use a stock solution straight from the refrigerator.
Plan a dilution scheme to minimise the uncertainty in the concentration of the diluted solution.	Carry out large dilutions in a single step.
Ensure that all glassware used is clean, dust free and undamaged.	Use dirty glassware or glassware that is damaged and/or has faded graduation marks.
Ensure the stock solution is well mixed before use.	
Transfer the stock solution to a beaker or conical flask for pipetting (see section 2.6 for information on the correct use of pipettes).	Pipette directly from the stock solution bottle/flask. Return unused solution to the stock bottle.
Make sure the diluted solution is well mixed before use.	
All solutions	
Transfer the solution to a suitable container for storage.	Store solutions in volumetric flasks.
Clearly label containers with: the name and concentration of the solution; date of preparation; name of analyst; review or expiry data; hazard labels (if appropriate).	
Store solutions correctly (e.g. in a refrigerator if necessary).	

6.5 Special considerations when preparing solutions for working with RNA

Solutions which must be rendered RNase free should be prepared from components reserved specifically for work with RNA. RNase free solutions may then be treated in several ways, the most common of which is the use of DEPC (diethylpyrocarbamate). DEPC irreversibly inhibits RNase, and may then be removed by autoclaving. (NOTE: DEPC is highly toxic and volatile. It must be used only in a fume hood.) The limitations of DEPC are that solutions must be heated to remove it.

DEPC also reacts with amines. Therefore to decontaminate heat labile, RNA containing, or TRIS buffered solutions, alternative methods must be employed. Autoclaving is not sufficient to destroy RNases present in solutions (see notes on preparing glassware for RNA analysis in section 2.4.1).

Samples containing RNA are frequently decontaminated during extraction by treatment with guanidinium salts. Alternatively, small sample volumes containing Tris buffers can be protected by the addition of RNase inhibitors such as RNasin, a 40 kb protein or vanadyl ribonucleosides, which inhibit RNase by binding to it.

7 Preparing reagent solutions

Section 6 explained how to prepare solutions when it is important that the concentration of the solution is known with a high degree of certainty (e.g. when a solution is used for calibration). There are often other solutions which are used when carrying out a test method that aren't involved directly in the determination of the concentration of the analyte in test samples. These solutions are known as *reagent solutions*. This section covers:

- Calculating the concentration of reagent solutions
- Preparing reagent solutions
- Storage and labelling of reagent solutions

7.1 Calculating the concentration of reagent solutions

There are many different types of reagent solution used in the laboratory. These include aqueous solutions of various compounds (e.g. sodium hydroxide solution) and mixtures of liquids (e.g. buffer solutions used for PCR reactions).

In some cases it will be possible to buy a suitable solution from a chemical supplier but frequently you will have to prepare reagent solutions yourself. Depending on the solution, this may involve weighing out a suitable mass of material and dissolving it in an appropriate volume of solvent, or mixing appropriate volumes of two or more liquids. Since reagent solutions are not involved with the calibration of test methods, their concentrations do not generally need to be known with the same degree of certainty as standard solutions. In other words, a larger uncertainty in the concentration of reagent solutions is usually acceptable compared to standard solutions used for calibration.

7.2 Solution concentrations expressed as %w/v or %v/v

'% w/v' is shorthand for 'percent weight by volume'. It is the number of grams of solute in 100 mL of the solution. Note that the term '%m/v', which is shorthand for 'percent mass by volume', is also used. To prepare 1 L of a reagent solution with a concentration of 10% w/v you would therefore need to dissolve 100 g of material in 1 L of solvent.

Although these terms are in common use in laboratories, ISO and IUPAC do not recommend their use. The preference is to give the appropriate unit ratio to avoid any ambiguity (e.g. 0.1 %w/v is equivalent to 1 g L^{-1}). However, as you may well come across these terms in the laboratory, they are covered here.

To calculate how much material you need to weigh out (in grams), use the equation:

$$w = \frac{C}{100} \times V$$

where C is the concentration expressed as %w/v and V is the volume of solution required.

'%/v/v' is the expression used when a known volume of liquid solute is made up to a specified volume. It is therefore the number of mL of the solute in 100 mL of solution.

Some method protocols may describe the composition of a mixture of liquids as follows:

'acetonitrile and water mixture, 75/25 v/v'

This simply specifies the relative volumes of the two liquids that are required. In this case 75 mL of acetonitrile plus 25 mL of water (or 750 mL acetonitrile plus 250 mL water) would be required. The concentrations of such solutions are approximate so it is acceptable to measure the volumes of the liquids using a measuring cylinder.

7.3 Solution concentrations expressed as mol L^{-1}

The calculations required to prepare solutions when the concentration is expressed in mol L^{-1} are discussed in detail in section 6.2.

7.4 Preparing reagent solutions by dilution

As with standard solutions you may need to prepare a reagent solution by diluting an existing solution. The principles are the same as described in section 6.2. However, if an approximate concentration is acceptable, measuring cylinders can be used rather than a pipette and a volumetric flask.

For example, if you needed to prepare 500 mL of approximately 0.1 mol L^{-1} sodium hydroxide from a 1 mol L^{-1} stock solution you would need to measure out:

$$\frac{0.1}{1} \times 500 = 50\,\text{mL of } 1\,\text{mol L}^{-1}\text{ stock solution.}$$

To prepare the solution you could measure out 50 mL of the 1 mol L^{-1} solution using a measuring cylinder and add 450 mL of water to give a total volume of 500 mL. Remember that this approach is much less accurate than the procedure described in Figure 6-1 and should never be used for the preparation of standard solutions.

7.5 Preparing reagent solutions

The basic procedures for preparing reagent solutions are the same as those outlined in Figure 6-1 and Figure 6-2. The difference is that if a larger uncertainty in the concentration is tolerable, the solution can be prepared using equipment that has a lower level of accuracy. For example, a top pan balance rather than an analytical balance could be used and volumes could be measured using measuring cylinders rather than pipettes and volumetric flasks.

It may also be acceptable to use materials of a 'lower' grade than those used for preparing standard solutions, for example a general purpose reagent rather than an analytical reagent. However, you should check that any impurities present in lower grade materials won't interfere with the analysis you are undertaking.

It is important to be clear about the level of accuracy required. Often this can be determined from the method protocol. Statements such as, 'prepare approximately 10% w/v sodium hydroxide solution', 'prepare a water/acetonitrile mixture 50/50 v/v' indicate that a high level of accuracy is not required.

7.6 Labelling and storage of reagent solutions

- All reagents should be stored in a suitable container and under suitable environmental conditions (e.g. stored in a refrigerator if temperature sensitive or protected from light if light-sensitive)
- All reagent containers must be clearly labelled with the following:
    - the name and concentration of the solution
    - date of preparation
    - name of analyst
    - review or expiry date
    - hazard information (if appropriate)
- The label must be securely attached to the container and be written in water insoluble ink

7.7 Checklist for preparing reagent solutions

Table 7-1 summarises the key points to remember when preparing reagent solutions.

Table 7-1. Dos and don'ts for preparing reagent solutions

Do	Don't
Use a material with a suitable purity (grade) and ensure that it has been stored correctly and is within its expiry date.	Use material that appears to have been stored incorrectly or that has passed its expiry date.
Use a clean, dry spatula to transfer the required amount of material.	Return unused material to the original bottle.
Make sure you have correctly calculated the amount of material required.	
Weigh the amount of material required using a balance with a suitable accuracy (a top pan balance is acceptable for preparing solutions of approximate concentration).	
Ensure that all glassware used is clean, dust free and undamaged.	Use dirty glassware or glassware that is damaged and/or has faded graduation marks.
Choose glassware that will measure volumes with the required level of accuracy (measuring cylinders are acceptable for preparing solutions of approximate concentration).	
Make sure the solution is mixed thoroughly before use.	
Transfer the reagent solution to a suitable container for storage.	Store solutions in volumetric flasks.
Clearly label containers with: the name and concentration of the solution; date of preparation; name of analyst; review or expiry data; hazard labels (if appropriate).	
Store solutions correctly (e.g. in a refrigerator if necessary).	

8 Preparing master mixes and setting up multiple reactions: reagent and sample tracking considerations

In most testing or research laboratories samples are commonly analysed in run batches. Analysing 24, 36, 96 or even more samples at the same time can be standard procedure for many laboratories. Therefore it is often practical to prepare 'master mixes' – solutions that can be aliquoted into individual reaction tubes or wells on microplates. These master mixes are mixes with a relatively high number of reagents that need to be combined in small volumes.

Once the master mix is aliquoted out, the samples can be added to the individual tubes or wells. It is essential to use reagent and sample tracking systems to ensure that each addition is performed correctly.

We will use the example of setting up a PCR to illustrate best practice in reagent and sample tracking.

8.1 Keeping track of reagents when setting up a PCR assay

Although increasingly the reagents needed to set up PCR reactions are obtained in kits ready to use, there will be times where master mixes need to be prepared from scratch. Very commonly, these reagents are stored in small aliquots. When preparing the master mix, great care has to be taken to avoid contamination with nucleic acid material from the samples or from the analyst. The PCR master mix is commonly a mixture of:

- PCR buffer
- dNTPs
- Oligonucleotides (very often referred to as primers, there will be at least two but it can be many more if the analysis requires a multiplex reaction)
- PCR enzyme
- Water

You need to ensure that as reagents are added to the master mix, they are checked off against a list. Since the individual volumes of the reagents can be small, you cannot rely on a visual check to know whether a particular component has been added or not.

Table 8-1 is an example of a PCR reaction set up checklist. A typical reaction could be 25-100 µL in volume, however with current technologies the reaction volumes are becoming smaller, and are increasingly pre-set up in reaction arrays. The reaction set up list in Table 8-1 is for PCR reactions that are 25 µL in volume. It is good practice to add a few extra reactions to the final number, as it is likely that some volume will be lost when aliquoting into the reaction tubes. In the example in the table the number of reactions required is 36, but the number has been rounded to 40 to compensate for aliquoting losses.

Table 8-1. PCR reaction set up list (preparing a master mix)

Reagent (concentration)	Final conc	Vol per reaction (µL)	Vol for 40 reactions (µL)	Batch number	Added?
10X PCR buffer (including $MgCl_2$)	1X	2.5	100	#11223	√
dNTPs (1.25 mM each)	0.2 mM each	4	160	#1134	√
Primer 1 (10 µM)	0.4 µM	1	40	#1113- 66	√
Primer 2 (10 µM)	0.4 µM	1	40	#1113-67	√
PCR Enzyme – Taq Polymerase, 5 U $µL^{-1}$	1.25 units	0.25	25	# 122	√
Water	-	11.25	450	Aliquot	√
DNA (sample vol to be added to each tube)	-	5	-	-	√

8.2 Checklist for preparing the master mix solution

The master mix formula will vary depending on the objective of the experiment. The volumes required to make up the master mix are likely to be very small and the number of reagents will usually be at least six but is often more. You will need to ensure that:

- At any given time only one tube of reagent or final master mix container is open
- A method is employed to identify clearly which reagents have been added already and which haven't, as it will be difficult to know by observing the volume of the master mix
    - frequently this is done by rotating the tubes once they have been used, so the cap points in a different direction or by transferring the used tube to a different rack
- The added reagent is always checked off in the reagent log to ensure that sample tracking is complete
- When performing qPCR with a probe, the tube is shielded from light in order to prevent a loss of fluorescence
- Molecular biology grade water is used for master mix preparation – this is essential
- All reagents are thoroughly defrosted prior to use
- A few additional reactions (5-10%) are added to the final number to compensate for volume lost when aliquoting

8.3 Keeping track of samples

Once the master mix has been prepared, it can be aliquoted into the reaction tubes or wells using automatic pipettes (single or multichannel).

The addition of sample extract to the individual reaction tubes/wells is a critical step as it can be compromised by the risk of both contamination and of sample mix-up. To minimise the risk of

contamination, you should ensure that only one tube is open at any one time. To minimise the risk of sample mix-up, adding the same sample to more than one tube, or adding two samples to one tube, a clear sample tracking system must be in place. One common way is to use multiple racks:

- Sample rack A: a rack for the samples to be processed
- Sample rack B: a rack with processed samples
- Reaction tube rack A: rack with PCR tubes with master mix
- Reaction tube rack B: rack with PCR tubes, master mix and added sample
- Working rack: rack holding the sample tube and PCR tube being processed

As samples are processed tubes are moved from pre-processing racks (A) to post-processing racks (B) via the working rack. Only one sample tube and one reaction tube can be placed in the working rack at any one time.

Sample tracking is enhanced by checking sample identification numbers against a sample list or grid as they are processed.

9 Centrifugation

This section outlines the things that you will need to know when using a centrifuge:

- When to use a centrifuge
- The difference between revolutions per minute (rpm) and g (acceleration due to gravity)
- The different types of centrifuge available
- How to use a centrifuge correctly

9.1 What is centrifugation and when is it used?

Centrifugation is a separation process which uses the action of centripetal (centre seeking) force to promote accelerated settling of particles in a solid/liquid mixture. Particles in a solution are separated according to their size and shape, the density and viscosity of the medium and rotor speed. Two particles of different masses will separate at different rates in response to gravity (inertia).

Protocols for centrifugation typically specify the amount of acceleration to be applied to the sample rather than specifying the rotational speed such as revolutions per minute (rpm). However, in chemical analysis rpm are frequently quoted. The acceleration is often quoted in multiples of *g*, the acceleration due to gravity. This distinction is important because two centrifuge rotors with different diameters running at the same rotational speed will subject samples to different accelerations. The higher the rotational speed, the higher the *g* force exerted on the solid phase and the faster the solids will accumulate at the bottom of the centrifuge tube. Acceleration can be calculated as the product of the radius of the rotor and the square of the angular velocity (see section 9.2). The speed and time of the run will depend on the type of centrifuge that is being used and the nature of the samples.

Centrifugation is an important mechanical means of separating the components of a mixture and is a widely applied technique for general use, for example to separate liquids from solids, drainage of liquids from solid particles and stratification of liquids according to density. It is utilised widely in both chemical and biological analysis. In biological analysis centrifugation is commonly used in extraction and clean-up protocols for DNA analysis and cell culture. It is also used in food science, during sample treatment, to separate insoluble components such as fats and oils (in meat products) from the liquid phase extraction solvent.

9.2 rpm versus g

As discussed in section 9.1, it is important to understand the difference between the speed of a centrifuge in revolutions per minute (rpm) and the acceleration or force applied in multiples of *g*. The following equations show their relationship.

$$RCF = 0.000\ 011\ 18 \times r \times \omega^2$$

$$\omega = \sqrt{\frac{RCF}{0.000\,01118 \times r}}$$

Where: RCF is the relative centrifugal force or number of *g*, i.e. acceleration

ω is the speed in revolutions per minute

r is the centrifugation rotor radius in cm

9.3 Different types of centrifuge

There are many different types of centrifuge (some are shown in Figure 9-1), here they have been categorised by speed and capacity.

9.3.1 Ultracentrifuge

a. Bench top
b. Floor standing (see Figure 9-1a)

9.3.2 High-speed

c. Micro (see Figure 9-1b)
d. Bench top
e. Floor standing

9.3.3 Low-speed

f. Small
g. Bench top (see Figure 9-1c)
h. Floor standing

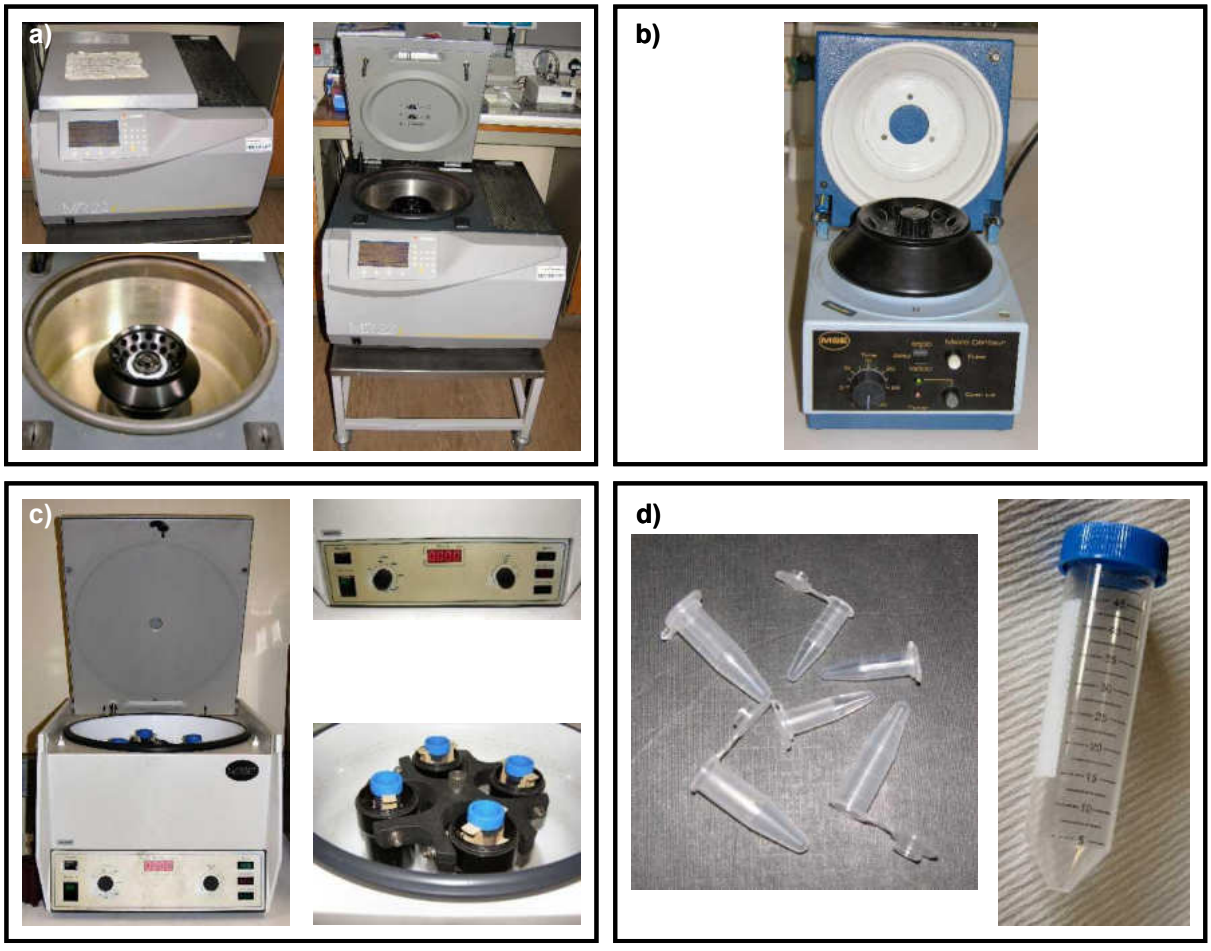


Figure 9-1. Different types of centrifuge, (a) to (c), and centrifuge tubes (d)

9.3.4 Rotors

The two main types of centrifuge rotor are a fixed angle rotor (where the sample is held at a specific fixed angle) or swinging bucket mechanisms (where the sample swings out on a pivot). Other rotors are available for particular applications – consult manufacturers' websites for details.

Fixed angle rotors generally achieve separation more quickly, as the substance under centrifugation will have an increased relative centrifugal force applied to it for a given rotor speed and radius. One other significant advantage is that fixed rotor centrifuges have very few moving parts and therefore have virtually no major mechanical failures.

Swinging bucket rotors (also known as horizontal rotors), although slower to achieve separation than the fixed rotor mechanism, have advantages due to their centrifugal action. Unlike the fixed rotor mechanisms, particles for separation have to travel the full distance of the centrifuge tube and are not forced against the side of the tube. Therefore particles separated in this way will stay intact. The only major disadvantage of this type of rotor is that they have a number of moving parts which are prone to failure with extended use.

Table 9-1 outlines the differences between the main types of centrifuge and summarises some of the more common applications. This has been collated using information from a number of different manufacturers. When selecting a suitable centrifuge you will need to consider its capacity and the speed/force required. For the majority of centrifuges both fixed angle and swinging bucket rotors can be used, depending on the application and the capacity required. Centrifuges generate heat due to the friction of the spinning rotor. Many models (especially those that operate at the highest speeds) are therefore available with a refrigeration unit. Refrigeration mechanisms maintain low temperatures (typically 4 °C at maximum speed) to protect heat sensitive samples and heat labile compounds. Refrigerated centrifuges are commonly used in the isolation of DNA, RNA and protein cell viruses. Some models are also available with a heating element.

Table 9-1. Examples of centrifuge specifications and applications

Type of centrifuge	Typical specifications	Applications
Ultracentrifuge		
a) Bench top	Maximum RCF (x *g*): 280 000 – 1 019 000 Typical capacity: 20 – 108 mL (e.g. 8 tubes each with a capacity of 13.5 mL)	• Final stage isolation of sub-cellular organelles; protein precipitation and proteomics; virus isolation; genetic analysis such as DNA, RNA and plasmid preps; lipoprotein work • Equilibrium centrifugation • Liquid-liquid extraction
b) Floor standing	Maximum speed (rpm): 50 000 – 100 000 Maximum RCF (x *g*): 302 000 – 802 000 Typical capacity: 50 – 500 mL (e.g. 12 tubes x 40 mL or 8 tubes x 7 mL)	

Table continued

Table 9-1 continued

Type of centrifuge	Typical specifications	Applications
High-speed		
a) Micro	Maximum speed (rpm): 13 000 – 14 000 Maximum RCF (x *g*): 12 000 – 18 000 Typical capacity: 12/24 tubes x 1.5 – 2 mL	• Fast pelleting • PCR post-reaction clean-up • Cell culture • Plasma and general purpose separations • DNA sample preparation • Sub-cellular fractionation and protein identification • Liquid-liquid extraction of emulsions
b) Bench top	Maximum speed (rpm): 10 000 – 30 000 Maximum RCF (x *g*): 17 700 – 100 000 Typical capacity: 4 tubes x 100 mL, 24 tubes x 0.5 mL or 10 x microplates (1 – 10 µL per well)	• Separation or preparation of sub-cellular components, proteins, precipitates, viruses nucleic acids, mammalian/insect cells and blood components • Liquid-liquid extraction of emulsions • Solvent extraction
c) Floor standing	Maximum speed (rpm): 20 000 Maximum RCF(x *g*): 42 037 Maximum capacity: 6 tubes x 290 mL	
Low-speed		
a) Small	Maximum speed (rpm): 5000 Maximum RCF (x *g*): 2500 Typical capacity: 10 tubes x 20 mL	
b) Bench top	Maximum speed (rpm): 6000 Maximum RCF (x *g*): 4750 Typical capacity: 4 tubes x 50 mL or 8 tubes x 15 mL	• Separation of emulsions and suspended matter
c) Floor standing	Maximum speed (rpm): 8000 Maximum RCF (x *g*): 9320 Typical capacity: 4 tubes x 750 mL	• Liquid/solid extractions • High weight precipitates

9.4 Correct operation of a centrifuge: safety and quality issues

The safe use of a centrifuge is important for both the operator and the instrument. Centrifuges should be serviced regularly (at least every 12 months) by an expert service engineer to identify any problems, and to check the rotors for corrosion and strain.

It is also important to ensure that the centrifuge is operated in such a way as to minimise any possibility of cross-contamination of samples. The key issues to consider are discussed in the following sections.

9.4.1 Types of container

It is important to use the correct type of container for a particular rotor. Follow the manufacturer's guidelines on which containers are most suitable for the different types of rotor. Centrifuges have adaptable rotor plates and buckets to take different tubes and plates. Tube capacities range from 50 mL to 0.5 mL.

In chemical assays tubes of between 15 – 40 mL are typically used to centrifuge samples but volumes of up to 250 mL are not uncommon. With biological/clinical techniques the volumes will often be much smaller. Therefore, the sample may be spun in 0.5 – 1.5 mL tubes, or even in a 384 well plate (each well can contain up to 5 μL of sample). Figure 9-1 d) shows some examples of containers used in centrifugation.

9.4.2 Operating a centrifuge

When using a centrifuge it is very important that you follow the correct protocol. You must always consult the manufacturer's instruction manual before use. Table 9-2 summarises the step by step actions needed to carry out the correct operation of a centrifuge.

Table 9-2. Operating a centrifuge

Operation	Action
Open the lid	The lid of the centrifuge can only be lifted if the centrifuge is plugged in and the rotor has stopped moving. Otherwise, for safety, the lid will remain in the locked position.
Load the centrifuge rotor	The centrifuge rotor should be loaded with appropriate sample tubes and balanced as discussed in section 9.4.3.
Close the lid	Once the centrifuge rotor has been loaded, close the lid and push down firmly so that it 'clicks'. This indicates that it is locked (often a light will illuminate to show the lid is locked).
Set the speed	Different centrifuges will have different speed setting mechanisms. Some will have digital dials that you use to adjust the speed to the required rpm, others will have set speeds which can be selected as appropriate.

Table continued

Table 9-2 continued

Operation	Action
Set the run time	As with the speed settings, different types of centrifuge will have different mechanisms for setting the run time. A specific time can be selected using digital buttons or pre-selected options. Many centrifuges also have the option to 'pulse' for a few seconds by holding the pulse button down.
Press start	Once both the speed and time have been set, the start button can be pressed to begin centrifugation. The centrifuge will spin at the specified speed until the set time has elapsed.
Wait for process to finish	Once the centrifuge has spun for the allocated time, the rotor will slow down and stop. Many centrifuges will display 'end' when the run has finished.
Open lid and remove tubes	The lid light will be illuminated so as to alert the operator that the run has finished and the rotor is stationary. The lid locking mechanism will then unlock and the lid can be lifted and the sample tubes removed.

9.4.3 Balancing the load

The load in the centrifuge must be carefully balanced. Small differences in the mass of the load at different positions on the rotor can result in a large unbalanced force when the rotor is at high speed. This force can put strain on the equipment, which eventually can lead to failure of the rotor. This will cause serious damage to the equipment and may lead to personal injury. Therefore, the minimum number of centrifuge tubes you can use is two as this will allow the load to be evenly balanced. All tubes should contain approximately the same amount of liquid. If you need to centrifuge more than two tubes they should be evenly spaced around the rotor plate so as to keep the centrifuge balanced. If you do not have sufficient samples to balance the rotor you should use 'balance tubes' containing a liquid similar to the sample (or of similar density). If using a swinging bucket rotor, the buckets should be balanced. This is easily achieved by matching the weight of each bucket using balance tubes containing water and/or adding additional solvent to sample tubes. Figure 9-2 shows some examples of balanced rotors with different numbers of samples.

Some centrifuges are fitted with 'imbalance detectors' and will shut down if an unbalanced rotor is detected. The main symptoms of an imbalance are excessive noise and vibration, and possibly movement of the centrifuge. If an imbalance does occur, stop the run immediately and rebalance the rotor plate or buckets before repeating the run.

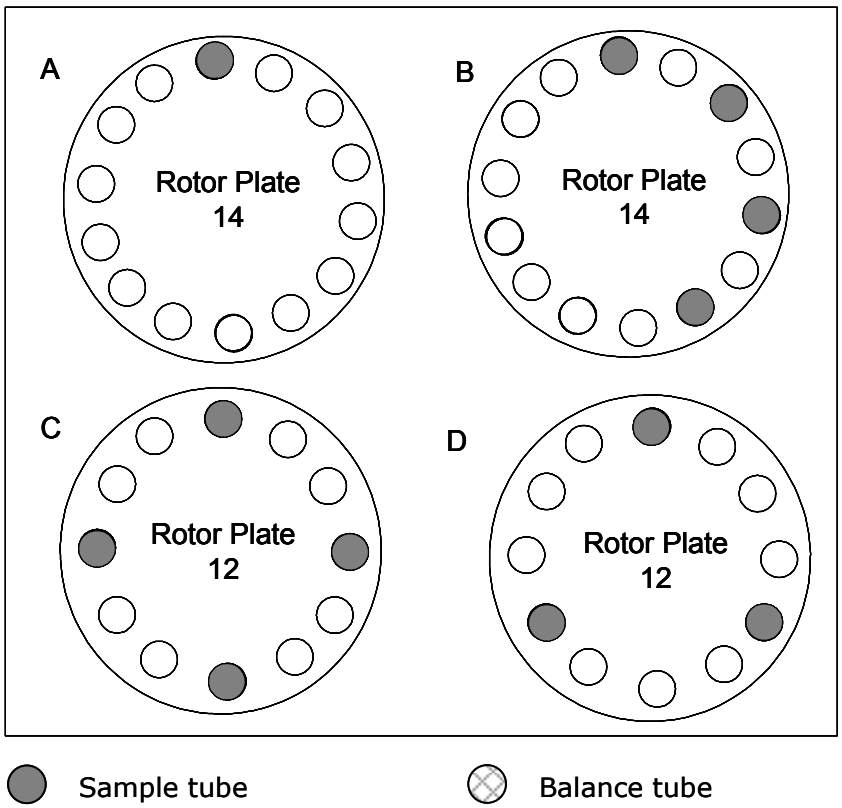


Figure 9-2. Some examples of balanced rotor plates

A) Represents a balanced 14 position rotor plate with one sample and one balance tube. B) Shows a 14 position rotor plate with four samples and three balance tubes. C) Represents a 12 position rotor plate with four balanced samples. D) illustrates a 12 position rotor with three balanced samples.

9.4.4 Touching moving parts

Centrifuge rotors should never be touched while they are moving, as they can cause serious injury. Modern centrifuges have safety features that prevent accidental contact with the rotor, e.g. locking lids (see table 9.2).

9.4.5 Spills

Liquid that has spilled in or on the centrifuge should be wiped up immediately, this will help prevent corrosion or damage to the rotor and other parts of the centrifuge. You shouldn't clean the rotor with a wire brush or any corrosive (e.g. alkaline) detergents as this may cause damage to the mechanism.

It is also essential to keep centrifuges clean to avoid any possibility of cross-contamination of samples.

9.4.6 Corrosive/infectious materials

Extra care should be taken when using corrosive or infectious material in a centrifuge. If an infectious material is being used, tubes should be sterilised immediately after use and if spillages occur, the rotor should be autoclaved.

9.5 Checklist for using a centrifuge

Table 9-3 summarises the key points to remember when using a centrifuge.

Table 9-3. Dos and don'ts for centrifugation

Do	Don't
Ensure that the correct rotor is in place and properly fitted.	
Make sure the rotor is correctly balanced.	Use an unbalanced rotor. Use a swinging bucket rotor with missing buckets.
Check that the centrifuge tubes are undamaged otherwise they may break under the force of centrifugation. Use suitable sample tubes for the rotor you have chosen.	
If there are insufficient samples to balance the rotor, use 'balance tubes'.	Overload the centrifuge.
Fill the sample tubes correctly.	Use tubes with too much or too little sample.
For swinging bucket rotors use bucket lids/covers if available.	
Make sure that the centrifuge lid is closed and locked.	Try to use the rotor if the lid is not fully closed.
Set the speed and time you require to centrifuge your samples.	
Stop the centrifuge immediately if there is excessive noise or vibration.	Leave a centrifuge running if it is making excessive noise and vibrating.
Wait until the centrifuge has reached its full operating speed and you are sure that it is operating correctly before you leave it.	Leave the centrifuge before you are sure it is operating correctly.
Wait for the centrifugation process to stop completely before opening the lid.	Open the lid while the rotor is still running. Touch moving parts while the centrifuge/rotor is working.
Remove samples carefully so as to not disturb any particles.	
Clean up any spills or leaks in the centrifuge rotor.	Leave spilled substances to cause corrosion to the rotor or contamination of future samples.
Leave the lid open for a short time after use if the centrifuge has been heated or cooled to avoid condensation.	
Record the centrifuge usage – rotors are subject to significant stresses and may need to have a reduced maximum speed as they age and eventually be retired.	

10 Quality management

This guide describes the steps you should take when carrying out some key laboratory operations to help make sure that the results you produce in the laboratory are reliable. Sound practical skills are a key part of ensuring the quality of analytical results. But what do we actually mean when we talk about 'quality' or 'quality assurance'? In addition to staff training, what other activities should laboratories carry out to ensure the quality of the results they produce? This section provides a brief introduction to a number of key topics:

- Definition of quality and why it is important
- Quality management, quality assurance and quality control
- Method validation
- Precision, bias, accuracy and other method performance parameters

10.1 Definition of quality

A dictionary definition of quality is, 'the degree of excellence of a thing.' In relation to a company offering a service or manufacturing a product, the concept of quality is about providing customers with a service or item that meets their needs, i.e. it is 'fit for purpose'. Laboratories can be considered to be both offering a service (the ability to carry out particular tests) and delivering a product (the results obtained from the analysis of test samples). The measurements you make in the laboratory will always have a customer – the person that will use the data. Your customers may be external to your organisation or they may be people from other departments within your organisation – both are equally important. Measurements are always made for a reason; the customer will use the measurement results to help them solve a problem or answer a particular question. It is important to understand why you are carrying out analyses and to know what the data produced will be used for. Without this information, it is impossible to judge whether results are fit for purpose as you do not know what the purpose is.

Measurement results are considered fit for purpose if the measurement uncertainty (see section 10.4.7) is acceptable. In some situations (e.g. the analysis of amount of viral RNA in a patient sample by RT-PCR) an uncertainty of 10 or 20% might be acceptable and the customer would still be able to use the data. In another situation – the analysis of GMOs in wheat for example – such a large uncertainty would be unacceptable as there is an enforceable limit food manufacturers have to comply with.

However, knowing the measurement uncertainty and demonstrating that it is acceptable is not the whole story. To be of use to the customer results also need to be reported within a reasonable timescale. If the customer needs the measurement results before they can release a batch of a product onto the market they may not want (or be able) to wait several weeks for the data.

Laboratories need to have systems in place to give them confidence that the results they deliver to their customers on a daily basis are fit for purpose – it cannot be left to chance! This is where the concepts of quality management, quality assurance and quality control come into play.

10.2 Quality management, quality assurance and quality control

You may have come across the term 'quality management system'. A quality management system is a set of procedures put in place by an organisation to ensure that staff have the facilities, equipment and resources to carry out their work effectively. A quality management system will include quality assurance and quality control activities. The terms 'quality control' and 'quality assurance' are often used interchangeably but they actually have quite different meanings.

- **Quality control** is a planned programme of activities designed to ensure that the product (e.g. analytical data) is fit for purpose. Quality control is what you do on a day to day

basis to ensure that laboratory systems are working correctly and that results produced are of the required quality.

- **Quality assurance** is a planned set of documented activities designed to ensure that the quality control programme is carried out effectively, to demonstrate that it has been done and that it is appropriate. If quality control is what you do then quality assurance tells you how to do it and provides the infrastructure within your organisation to enable it to be done.

Many organisations base their quality management system on international standards such as ISO/IEC 17025:2005 and have the system audited by a third-party. This enables the organisation to demonstrate to their customers that they take quality seriously and have sound systems in place (see section 10.3).

Day to day quality control activities include:

- Analysis of blanks
    - check for contamination or interferences
    - check for unintended amplification products
- Analysis of positive controls
    - check method is working consistently
    - assess method efficiency
    - check for inhibitory factors
- Analysis of standards and reference materials
    - calibration of instruments
- Analysis of QC samples
    - check the method is working consistently
    - plot QC results on control charts
- Replicate analysis of samples
    - gives greater confidence in the result
- Participation in proficiency testing schemes
    - an independent check of laboratory performance

When thinking about the reasons for carrying out quality control and quality assurance, it is worth remembering that it usually costs less to prevent a problem than it does to correct it!

10.3 International quality standards

There a number of international standards available that cover the quality of service that organisations offer to their customers. The standards most commonly used in laboratories are:

- ISO 9001:2015, Quality management systems – Requirements
- ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories
- ISO 15189:2012, Medical laboratories – Particular requirements for quality and competence
- Good Laboratory Practice Regulation

Different standards are appropriate for different organisations, depending on the nature of their work; some laboratories work to more than one standard. The main features of the standards are summarised below.

10.3.1 Standard ISO 9001:2015

- A general standard that applies to all organisations
- Specifies the requirements for a quality management system where the organisation has to:
    - demonstrate its ability consistently to provide a service that meets customer and applicable regulatory requirements
    - aim to enhance customer satisfaction through the effective application of the system, including processes for continual improvement of the system
- Does not test the competence of organisations (e.g. the ability of a laboratory to produce reliable test results) but checks how the organisation controls its processes
- Organisations are certified by a third-party (e.g. BSI) against the requirements of the standard

10.3.2 ISO/IEC 17025:2005

- Applies to all laboratories carrying out tests and/or calibrations
- Laboratories have to operate a quality management system that meets the principles of ISO 9001
- Tests the competence of the laboratory and its staff to carry out particular tests/calibrations
- Laboratories are accredited to the requirements of the standard by the national accreditation body (e.g. UKAS in the UK)
- Accreditation is usually awarded for a particular combination of analyte, matrix and test method (the 'scope' of the accreditation)

10.3.3 ISO 15189:2012

- Developed to cover the specific needs of medical laboratories
- Incorporates the requirements of ISO 9001 and ISO/IEC 17025
- Effectively a customised version of ISO/IEC 17025 for medical laboratories
- Accreditation is carried out by the national accreditation body

10.3.4 Good Laboratory Practice (GLP)

- Legal requirement that regulatory studies undertaken to demonstrate the health or environmental safety of new chemical or biological substances must be conducted in compliance with the principles of GLP
- The principles set out a quality management system dealing with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported
    - there must to be sufficient information available for the study to be reconstructed at a future date
- Relates to a study and not specific tests

- Each country has a monitoring authority that assesses studies to ensure they meet the requirements of the GLP principles (e.g. UK GLP Monitoring Authority which is part of the Medicines and Healthcare products Regulatory Agency (MHRA))

An organisation may be accredited to ISO/IEC 17025 for a number of its test methods but may also be certified to ISO 9001 for the 'non-technical' areas of its business (e.g. finance, human resources) and the technical areas not covered by ISO/IEC 17025. This ensures that all areas of the organisation are working under the same management system.

10.4 Method validation

The validation process typically entails understanding the reason the measurements are being made, and producing data to demonstrate that the method performance is fit for purpose. Ideally, the validation study will need to cover all the steps in the method, from sampling and sample storage through to data analysis, to ensure evaluation of all parameters that may influence the result.

The actual level of assessment and validation that is undertaken will depend on the intended use of the method and the importance of the data produced. There are many performance parameters that may potentially be investigated for a particular method; hence, choosing the characteristics to be investigated is an essential part of the validation process. For quantitative work, evaluating random error (precision) and systematic error (method bias) is crucial to achieve accurate measurement results. Unless working at trace levels, establishing the limit of detection (LoD) and quantification (LoQ) may not be needed, although it is important to know the range over which the method response is linear. For example, a well optimised qPCR assay, can be precise over a large dynamic range, but it is important to confirm this empirically.

Performance parameters are briefly listed in the following section; their inclusion in the validation study will need to be evaluated at the method validation planning stage.

10.4.1 Precision

Precision is defined as the closeness of agreement between independent test/measurement results obtained under stipulated conditions. Measurements for precision estimates should be performed on identical samples, and expressed as the standard deviation or relative standard deviation of the results obtained from the precision study.

The conditions under which the repeated measurements are made will determine the type of precision estimate obtained. Three common types are repeatability, reproducibility and intermediate precision. It should also be noted that all the steps required to make a measurement will contribute to the random error and may also contribute to the systematic error. Consequently for the most accurate measurements, the whole process from sampling to data analysis needs to be considered.

10.4.2 Bias

Bias is a measure of the trueness of a result and is caused by systematic errors, rather than the random errors which influence the precision of results.

Bias is calculated as the difference between the observed averaged value from the study and the accepted reference value of the test sample, and is often expressed as a percentage difference from the expected reference value.

10.4.3 Ruggedness

Over time experimental parameters will vary to some extent, with a possible effect on the method performance. Ruggedness or robustness testing helps identify those parameters which have a significant effect on the performance of a method and provides useful information on how closely such parameters need to be controlled. A rugged or robust method is one that exhibits a

performance unaffected by changes in the experimental parameters within the defined control limits.

To evaluate ruggedness, experiments which deliberately and systematically introduce known changes to parameters are designed, and the effect assessed. This approach can be streamlined and simplified using experimental design tools such as the 'Plackett-Burman' design.

Parameters identified as having a significant effect require further study to set suitable control limits.

10.4.4 Selectivity (Specificity)

Selectivity is the extent to which the method can be used to determine the target analyte without interferences from other components of similar behaviour. It is recommended that the method is assessed for its selectivity by performing measurements on samples which contain the target analyte and known impurities to ensure performance is not compromised at any stage.

10.4.5 Detection limit (Sensitivity)

It must be noted that while in biological analysis 'sensitivity' is used to describe the lowest concentration of the target analyte that can be measured, it has a different definition in other fields so it is important to follow specific sector guidelines where available.

Sensitivity is often determined as part of the method validation and gives an indication of the lower operating limits of the method. Most experiments to determine detection limits require a replicated sequence of experiments on low-concentration samples, blanks (negative controls) or low-concentration spiked materials or standards.

10.4.6 Working range and linearity

The working range is the interval between the upper and the lower concentrations of an analyte over which the method performance has been determined to be acceptable. The term linearity is frequently linked with the working range of the method and refers to the ability of a method to give a response that is directly proportional to the concentration of the analyte.

In order to establish the working range of a method it is necessary to study the response of standards (or where standards are not available, in-house samples or spiked samples) whose concentrations span the range of interest.

Evaluation of linearity can be performed visually and is supported with objective regression data.

10.4.7 Measurement uncertainty

Measurement uncertainty can be defined as an estimated range of values within which the true value of the measurement resides. The range of values gives an indication of the reliability of a measurement result.

Experiments performed during method validation often provide information which can be used in evaluating measurement uncertainty. In the assessment, all possible sources of variability in the measurement process are considered and evaluated to create an uncertainty budget. The uncertainty estimate will therefore include the effect of both random and systematic errors.

The experimental result can then be reported as $x \pm y$, where x is the reported measured value and $\pm y$ is the degree of uncertainty associated with the measurement result.

10.5 Sample handling

There are a number of factors that need to be considered when handling samples, as changes in integrity, poor labelling and inappropriate sampling and preparation procedures may significantly affect the results of any analysis.

10.5.1 Chain of custody

In order to ensure traceability to source, receipt of a sample should be recorded in an appropriate registration system. Sample registers should contain information that includes a unique identifier (sample number) for the sample as well as a description and the date of receipt. Laboratories that have a very high throughput of samples usually employ an electronic Laboratory Information Management System (LIMS) but as a minimum there should be a paper-based record. Once a sample has been entered into the registry, the unique identifier number should be used to follow the sample through any analysis process and for reporting of the results subsequently.

10.5.2 Labelling a sample

Once a sample number has been generated, the original sample and any consequent subsamples need to be labelled at all times. As a minimum the sample needs to be labelled with their unique identifier but it could also have the following information:

- Date received
- Initials of booking-in operator
- Required storage conditions
- Expiry date (by which it will need to be disposed)

10.5.3 Special considerations when labelling samples and subsamples

When labelling a sample or subsamples, you need to ensure that the information will not fade/disappear or be easily overlooked:

- Use secure labels that will not become unstuck if the sample is frozen
- Use permanent ink on the labels and/or tube which will not wash off easily
- When labelling tubes label the lid for quick identification on a rack but also label the side of the tube. This is so the identity of the sample can be confirmed at the point of analysis if required

10.5.4 Sample and reagent storage

Once a sample has been received by the laboratory, every effort should be made to keep the sample in a suitable and stable environment. There are often specific requirements for storage of forensic, microbiological and radiochemical samples. In all cases the laboratory should store the samples in such a way as to avoid degradation or contamination.

When performing DNA extractions the temperature at which the sample has been stored can greatly affect the sample quality and extraction yield. Freezing samples can prevent further growth of contaminating micro-organisms, whilst naturally occurring autolysis and DNA degradation by endogenous and exogenous enzymes can also be abated by reduced temperature.

Samples requiring long term storage should be maintained in an environment in which their integrity is known to be maintained and inherent enzyme activity is arrested. Any more than the minimum of required freeze-thawing cycles should be avoided, as this may induce cell or DNA breakdown.

During the isolation or manipulation of nucleic acids, degradation can occur as a result of microbial or enzymatic contamination of the buffers and reagents employed. To reduce the occurrence of this, and to prolong the shelf life, solutions are routinely autoclaved or, where this is not compatible, filter sterilized in order to remove sources of contamination. The isolation and manipulation of RNA requires the adoption of additional measures due to the resilient nature of the majority of RNases (see section 6.5).

10.6 Archiving

Samples and data may both need to be stored for a period of time after an initial analysis has been performed and reported. In all cases, if samples, data or paperwork need to be archived, a system for filing will need to be in place so that locating the item and retrieving it are possible at a later date.

The environment of the storage facility may need to be controlled to ensure that no degradation occurs and this may include control of temperature, humidity and light. The requirements of Good Laboratory Practice (GLP) for archiving material are more stringent than ISO 9001 and ISO/IEC 17025. However, discussion of these requirements is beyond the scope of the current section and the reader is referred to reference 6.

10.6.1 Electronic data

Copying (backing up) electronic data onto servers, external drives, or other media, ensures that there will be a copy of the data even if the laboratory computers fail. Care needs to be taken that a suitable media type is used for the storage and that the data will be retrievable after a significant time has elapsed. The manufacturer's warranty length will also affect the ability to retrieve the data in the future. There is no point in storing data for 20 years if the manufacturer warranty only guarantees retrieval for 10 years. It is also important to note that different media types and different makes of the same media type may all have different life spans.

In order to maximise the likelihood of retrieval, at least two copies of the data should be stored. It is also sensible that these copies be stored in physically separate places, preferably in a fire safe environment. Ideally one copy should be stored off-site, but if this is not possible, they should, at least, be stored in separate parts of a building.

Consideration should be given to the software used to store the data. Programs change considerably over time so thought should be given to the formatting of the stored data. For example it may be wise to store the file in tab-delimited format, which should be readable by numerous programs, rather than as a format specific to an individual program.

Off-site electronic data storage has become a large commercial concern. There exist a large number of companies who for a fee will copy and archive data off-site for external clients. This places responsibility for data maintenance with a third party, but at the same time raises questions of data security.

[6] Good Laboratory Practice, OECD principles and Guidance for Compliance Monitoring, 1st edition, OECD, 2006, ISBN 9264012826

11 Laboratory notebooks

11.1 Aim

The work undertaken in the laboratory needs to be thoroughly documented for a wide range of reasons. In most cases, results will need to be independently verified by a peer or supervisor, and therefore, access to the methodology and calculations used to arrive at them needs to be detailed and easily accessible. Where results are not acceptable, contested or replicated, the analyst needs to provide clear detail of the work carried out so it can be repeated. Furthermore, to support any resultant patent application it is necessary to ensure that proof of conception/invention and subsequent diligence in taking ideas forwards into practice be recorded in a permanent, unambiguous and provable manner.

In practical terms this requires that the researcher should employ and maintain some form of record taking in order to catalogue the work they are undertaking. In most instances this will take the form of a standard laboratory notebook. Electronic methods are becoming increasingly popular although there are traceability issues associated with these forms of record keeping.

11.2 The laboratory notebook

The notebook should be of bound construction and contain numbered pages. All notebooks should contain an indexing sheet and a list of authorised notebook signatories at the front.

11.2.1 Data entries

- Ideally data should be entered into the notebook as the work is being performed, but definitely no later than 1 week after the experiments or analysis have been performed
- Entries should be legibly written in permanent pen or, if handwriting is illegible, typed-up using a word processing package, then printed and attached to the notebook (see section 11.2.6 for information on adding papers to a notebook)
- Non-permanent markers such as pencil should not be used
- Any mistakes or corrections should be indicated by striking through with a single line. The original script should still be legible. The dated and initialled correction should be entered along with an explanation.
    - correction fluid, such as Tippex, must not be used
- Pages should not be removed from the notebook under any circumstances
- All blank spaces in the notebook (full or part pages) must be crossed out in pen, initialled and dated to prevent any additions at a later date.
- The notebook must have a table of contents
- Experimental procedure
    - All experiments recorded in the laboratory notebook should be dated and have a descriptive title and the initials of the analyst
    - Start each new experiment on a new page of the notebook

11.2.2 Aims

The purpose of the work should be described at the outset.

11.2.3 Methods

A narrative description of what was done must be noted in detail in laboratory notebooks. Cross reference entries where appropriate for maximum clarity. For example, if using sample identifiers,

enter the book and page number where the sample description and coding explanation can be found. Particular attention should be paid to recording the following information as appropriate:

- A reference for the method, e.g. a paper, manufacturer's protocol, standard operating procedure, previously used method, etc.
- Any modifications to the noted method and reasons for such modifications
- If a procedure is repeated a number of times, reference must be made to the original experiment
- Volume, concentration and lot number of sample/reagents/materials used
- Internal standards employed
- Experimental conditions such as time experiment run, temperature etc.
- Equipment and model used, plus instrument settings, including software version
- Location of electronically stored raw data – computer, path and file name
- Data manipulation used
- Define acronyms, abbreviations or jargon the first time they are used

11.2.4 Results

- These include raw data and analysed data
- Links to electronically stored data must be noted
- All relevant gel photos, graphs, pictures etc. must be attached and adequate labelling and explanations given
- Computer programs used for data analysis should be referenced
- Ensure all calculations can be followed through and understood by other personnel

11.2.5 Conclusions

These can be brief, but should detail your interpretation of results from the experiment and what you intend to do next in light of these and maybe other results.

11.2.6 Attachments

- Attachments should be limited to a single layer per page
- Only glue or tape should be used to make attachments and placed on at least three edges of the item being attached
- Attachments must not be stapled into laboratory notebooks
- Any items stuck into the book should be initialled and dated across the point of attachment
- All attachments should be legible

11.2.7 Signatories and Dates

Designated signatories (Principal Investigators, Senior Scientists etc.) must sign the notebook signature list in the front of the laboratory notebook.

For work which may be required to support a patent:

- Minimum patentability standards require each notebook page be signed and dated by the person doing the work and a corroborating witness (no later than one week after the work has been entered)
- A corroborating witness must be an unbiased non-inventor who preferably witnessed performance of the work
- If the primary signatory is not involved in routine day-to-day checking of the notebook, then he/she must carry out a periodic review every 30 pages or every month, which comes first

For non-patentable work:

- Laboratory notebooks should be checked and signed by the primary signatory once a month or when a reasonable number of pages have been completed
- Other signatories can be used to check specific items within a laboratory notebook (nominated individuals as noted in official files), for example calculations

12 Further reading

All equipment in the laboratory should have a set of instructions detailing best practice operation and in most cases, a manufacturer's manual. Additionally, most equipment manufacturers will have online resources that are accessible free of charge on their website. In the first instance, refer to these instructions, manuals and web resources for further guidance on how to use and operate laboratory equipment.

Additionally there are publications and web resources offering generic guidance for molecular biologists working in the laboratory.

12.1 Publications

Laboratory skills training handbook, LGC, C Bailey and V Barwick (2007) ISBN 978 0 948926 259

Essentials of Nucleic Acid Analysis: A Robust Approach, RSC Publishing, Jacquie T Keer and Lyndsey Birch (Eds) (2008) ISBN 987 0 85404 367 5

Good practice guide for the application of quantitative PCR (qPCR), LGC, T Nolan, J Huggett, E Sanchez (2013)

12.2 Websites

http://www.lgcgroup.com/our-science/national-measurement-institute/publications-and-resources: This section of the LGC website contains links to a range of guides and resources developed with funding from the National Measurement System to assist those making chemical and biological measurements.

http://bitesizebio.com: Community based website with basic skills resources and access to a global audience.

https://www.neb.com/tools-and-resources: Website by New England Biolabs providing various tools and resources including protocol selection tools, troubleshooting, tutorials, and a video library.

https://www.thermofisher.com/uk/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library.html: website by ThermoFisher Scientific that provides a library of molecular biology resources, including articles and web tools.

http://www.biocompare.com: Price comparison website that enables a comparison of equipment and reagents available from a number of manufacturers.

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