Medical Laboratory Service

NTQF **Level III**

Learning Guide #34

Unit of Competence: Perform Standard Calibrations

Module Title: Performing Standard Calibrations

LG Code: **HLT MLS3** M07 **LO1-LG34**

TTLM Code: **HLT MLS3TTLM 0414v1**

Calibration

Calibration is a process that allows the operator to determine if a piece of testing equipment is performing to the degree of

accuracy and precision required. Calibration will vary depending on the equipment being used. For instance, calibration of an optical microscope is very different to that of an analytical balance or a pH meter.

Accuracy relates to the ability of the machine/operator to measure as close as possible to the 'true value' of the measurement (in reality the true value is never achieved as there are variations in all measuring systems).

Precision relates to the ability of the machine/operator to give the same reading when the same thing is measured a number of times. In this instance, weighing the same object ten times in succession should give ten readings that are very close together. (We would not necessarily expect them to be identical, due to variations in the measuring system and conditions).

Note: a piece of equipment can be precise yet be inaccurate.
For example, suppose that the true value for the mass of a standard is 10.1200 g and five consecutive readings of this standard give the following results:

3.3456	3.3467	3.3412	3.3443	3.3457

The balance is precise (as the five measurements are close to each other) BUT the balance is certainly NOT accurate (as the true value is 10.1200 g)!

Precision and Accuracy

The precision of a measurement is the reproducibility of that measurement done under identical conditions.

For example, if every member of a laboratory determined the molarity of the same unknown acid, you might wish to know how precise the measured molarities were. In each case, how much did the measurement deviate from the mean of all the measurements taken?

If the precision is high you can usually state with some confidence that there are not large random or indeterminate errors influencing the results. Indeterminate errors are those which are a natural consequence of the way in which the measurements were made.

Accuracy is the closeness of an experimental value to the true or accepted value. If the true value is unknown, then a highly precise value is usually considered to be accurate, but beware of systematic or determinate errors. These are errors that can be accounted for and (hopefully) avoided.

For example, if all of your titrations were within 0.01 mL of one another, but your burette actually delivered volumes that were out by 10% over the range used, you would have a very precise measurement (few random errors) but not a very accurate one (significant systematic error).

Reference Standards

Reference standards or reference materials may be defined as a material or substance with one or more property values that are sufficiently homogeneous, stable and well established to be used for the calibration of an apparatus, the assessment of a measurement method or for assigning values to materials. Without reference materials or substances there could be no calibration. What would we calibrate the apparatus or method against?

Think of a top loading balance. How is this balance calibrated so that it gives accurate and reproducible (precise) measurements in grams? At some stage in its manufacture and subsequent re-calibration a standard mass must be used to calibrate the balance. This standard mass must be calibrated against another standard mass and so on until finally the standard mass is traceable to the standard kilogram housed under special and secure conditions at an international site.

There are two kinds of chemical reference materials used in chemical and biological laboratories:

1. Pure substances, e.g. 1.00M NaCl, or
2. Matrix Materials, e.g. 3.00mM glucose in human serum.

The benefit of using matrix reference materials is that it gives a better representation of the accuracy and precision of measuring this analyte in this matrix.

There are three main types of reference materials.

1. Standard Reference Materials (SRM): a Certified Reference Material (CRM) issued by the National Institute of Standards and Technology (NIST)
2. Certified Reference Material (CRM): a reference material issued and certified by an organisation generally accepted to be technically competent, for example the National Analytical Reference Laboratory (NARL), and
3. In-house reference material: a material developed by the laboratory for its own internal use.

Reference materials have five main uses in the laboratory:

1. to develop and validate accurate methods of analysis, ensuring traceable measurement results at a working level
2. to verify that test methods are performing according to validated performance levels
3. to calibrate measurement systems
4. to assure the long term integrity of measurement quality assurance programs
5. to use as test materials for inter-laboratory comparisons and proficiency programs.

Reference materials come under the guidelines of the National Measurement Act (NMA), 1999 and the purpose of the NMA can be summarised as:

"That we must ensure that measurements are what they purport to be."

For instance, when you purchase 25L of petrol you want and expect that you will receive 25L +/- a small acceptable variation. You would be very upset if you found that you only received 23L!

Of course all chemical and physical standards must be eventually traceable to international standards based on the SI units and such standards shall be calibrated by a competent body that can provide traceability to a national or international standard of measurement.

The SI base units are:

Physical Quantity	Unit	Symbol
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Luminous intensity	candela	cg
Amount of substance	mole	mol

Other SI units are derived from these seven base units, for instance, **joule** the unit of energy is **kg m^2 s^{-2}**. The other derived units are:

Physical Quantity	Unit	Symbol
Frequency	hertz	Hz
Energy	joule	J
Force	newton	N
Pressure	pascal	Pa
Power	watt	W
Electric charge	coulomb	C
Electric potential difference	volt	V
Electric resistance	ohm	Ω
Electric conductance	siemens	S
Electric capacitance	farad	F
Magnetic flux	weber	Wb
Inductance	henry	H
Magnetic flux density (magnetic induction)	tesla	T

All reference standards in Australia have to be traceable back to SI base unit standard though State, National and ultimately international organisations.

Optical Microscope

As a Laboratory Technician you must be able to use a range of different equipment. This includes the optical microscope. In this section you will learn how to perform safety checks and troubleshoot problems with this equipment. All operations must comply with relevant standards, appropriate procedures and/or enterprise requirements.

There are three tasks you will undertake in this section.

1. Safety and pre-use checks
2. Troubleshooting procedures
3. Documentation and communication

Task 1. Safety and pre-use checks.

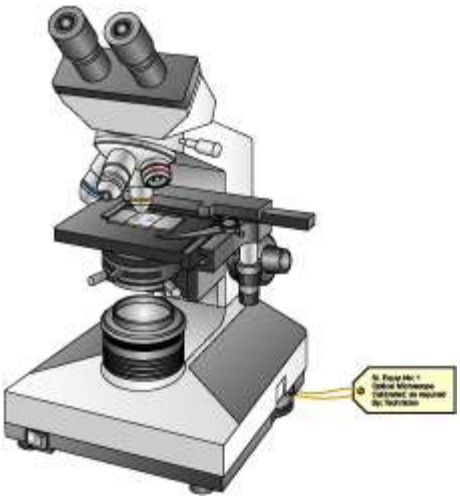
This task has three steps.

1. Introduction to the optical microscope
2. Perform safety and pre-use checks
3. Risk assessment

Step 1. Introduction to the optical microscope

You are responsible for the maintenance and set-up of the optical microscope at your Lab. In order to prolong the service interval and life of the microscope you need to plan your approach to the care and cleaning of it.

Note: Maintenance tasks are of a minor nature and do not include a complete stripping down and re-alignment of the optical systems which should only be performed by a qualified scientific instrument service technician.

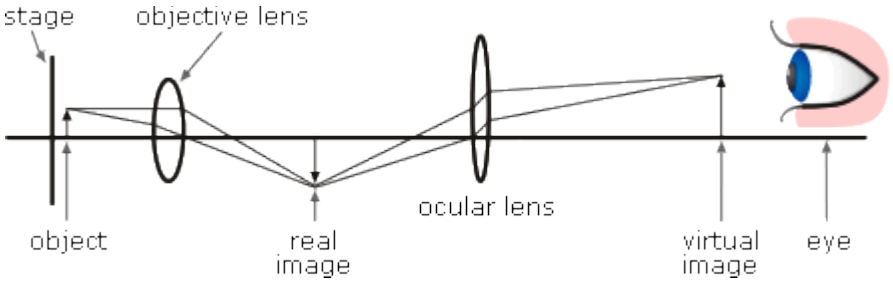


The Microscope

Modern biologists work with fairly small components of living organisms, tissues, cells and biomolecules. To actually see them, an instrument which can magnify (or more properly, make magnified images) is needed. The compound microscope is the instrument designed for this task.

The Compound Microscope

To magnify an image, a magnifier or a lens is needed (a piece of glass which makes everything appear larger). But there is a limit to how far a simple magnifier can make things bigger: that is, about 8-10 fold. Lenses must be added, one behind another (compounded), to increase this magnification. In this manner, we can magnify the image up to 2000 times its size. The classic compound microscope magnifies in two steps: first with an objective lens that produces an enlarged image of the object in a 'real' image plane. This real image is then magnified by the ocular lens or eyepiece to produce the virtual image.



Two convex lenses can form a microscope. The *objective lens* is positioned close to the object to be viewed. It forms an upside-down and magnified image called a *real image* because the light rays actually pass through the place where the image lies. The *ocular lens*, or eyepiece lens, acts as a magnifying glass for this real image. The ocular lens makes the light rays spread more, so that they appear to come from a large inverted image beyond the objective lens. Because light rays do not actually pass through this location, the image is called a *virtual image*.

The most important consideration for image formation with the objective lens other than its **magnification** or power is its **numerical aperture**. This is a number that is directly related to the **resolving power** of the objective. It is a critical aspect in obtaining a useful microscopic image.

Magnification

Magnification is the extent to which the image of the specimen, viewed under the microscope, is enlarged.
Enlargement occurs in two stages:

1. at the objective lens (at this stage it's called the real image)
2. the eyepiece or ocular lens further enlarges the real image (called the virtual image).

Resolving power

Resolving power is the ability to distinguish two objects which are close together as separate objects (that is, the ability to observe detail). Most people can resolve objects that are 0.1 mm apart or greater with their unaided eye. The microscope provides much greater resolution.

Numerical aperture (NA)

The numerical aperture of a lens system is a measure of the light gathering capacity of the lens system and determines its resolving power and depth of field.

Parts of a Microscope

Microscopy is an essential skill in Biology as it enables us to:

- observe objects too small to be seen with the unaided eye
- resolve structural details of very small objects.

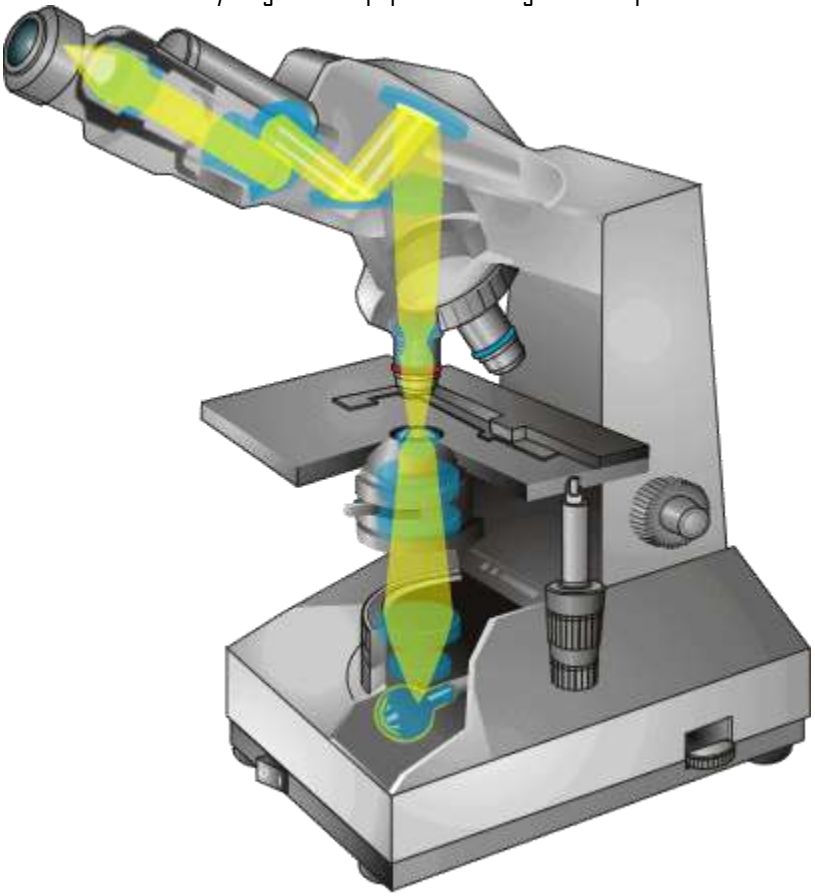
Specimens viewed with the microscope are normally illuminated with transmitted light. This means that the light passes through the specimen to the eye. The specimen must therefore be very thin.

An important part of all biological procedures is the correct use of a standard compound microscope. In order to get the best image possible from different types of microscopes, it is crucial that the light path be set up properly.

The prepared slide is placed on the stage of the microscope for examination. The principal parts of a typical microscope are shown here. Use your mouse to rollover the names of the parts, for information about each part.

Light path through a microscope

Shown in this cutaway diagram of a popular teaching microscope are the internal components and light path.



SOP: Care of an Optical Microscope

1. Location for use: avoid the following conditions - dust, vibration and exposure to high temperature, moisture or direct sunlight.

2. Use the coarse adjustment only with the low power objective.

3. Clean all oculars and objectives with lens paper and 70% ethanol after each use. Use a soft brush to remove dust.

4. Move or transport the microscope with one hand under the base and the other hand gripping the arm.

5. Avoid jarring or bumping the microscope.

6. Use oil each time the oil immersion lens is used. Use immersion oil with the oil immersion objective only.

7. Store the covered microscope in a protected area. Cover with the vinyl cover and store in a place free from moisture, dust and fungus.

8. Since bulbs are expensive and have a limited life, turn the illuminator off when you are done. When replacing a bulb or fuse be sure to turn off the power switch and disconnect the power source cord from the socket.

9. Never attempt to dismantle the microscope, to avoid the possibility of impairing the operational efficiency and accuracy.

10. To maintain the performance of the instrument have the microscope serviced regularly, eg annually.

11. If a microscope is unserviceable, attach an 'Unserviceable' tag to the arm of the microscope and disconnect the power lead. Fill out the Equipment Log and advise the Senior Technician.

Step 2. Perform safety and pre-use checks

Because of the nature of its work a laboratory normally contains many hazards. If these hazards are not properly understood and managed, they have the potential to cause harm to people working in and around the environment.

An optical microscope is a piece of equipment with few inherent safety hazards but safety still needs to be considered prior to using the microscope. Safety hazards include:

- Heavy weight hazard from dropping the heavy microscope onto your foot
- Sharps hazard from broken slides during handling
- Chemical hazard from the stains used on the slides
- Infection hazard from biological samples
- Electrical hazard from frayed or broken wires
- Burns hazard from the light bulb

SOP: Set-up and Pre-use Checks of the Microscope

1. Carefully unpack the microscope from its carrying box or remove the dust cover.

2. Place in a suitable position on the bench at the correct working height for the user. Do not place too close to the edge of the bench.

3. Check the power cord is in good order and plug it in if not already plugged into a power socket.
4. Switch on the power at the socket and on the microscope, check the light bulb is operating.
5. Rotate the objective lens turret to place the 4x objective lens in position over the light source.
6. Clean all refracting surfaces to remove dust and grease. See the 'SOP: Care of an Optical Microscope' for more information.
7. Always fill in the microscope user's book with your name, date, time you used the instrument and note any comments on its performance.

Step 3. Risk assessment

Risk and hazard are two sides of the one coin but they have very different meanings and should not be confused. For more information on risk, hazard and risk assessment read the following study notes.

Risk Assessment

Risk means the likelihood that something will occur and hazard describes the severity of the hazard in human or environmental outcomes.

Risk assessment is a simple technique that allows the laboratory technician to make important decisions about safety in their laboratory. Risk assessment uses two parameters when assessing risk:

1. The **severity** of the hazard should it occur, and
2. The risk (or **likelihood**) that it would occur.

For instance the hazard (severity) of a jet airliner crashing into your lab would be extreme but the likelihood would be very unlikely. This would give an overall risk assessment value that would be low which means that no action needs to be taken to manage the risk.

The following table, and explanatory note explains the risk assessment process more clearly.

How Likely? (Risk)	**How Severe (Hazard)?**			
	No treatment or first aid only	**One - two days off work**	**More than two days off work**	**Killed or disabled**
Very Likely (Could happen frequently)	3	2	1	1
Likely (Could happen occasionally)	4	3	2	1

Unlikely (Will rarely happen)	5	4	3	2
Very Unlikely (Probably will never happen)	6	5	4	3

Manage the hazards as follows:

Rating 1: highest priority - corrective or preventative action needs immediate action

Rating 2: put in place preventative or emergency procedures ASAP

Rating 3: }
Rating 4: } lower priority but you need to plan for these (e.g. paper cut, fall)
Rating 5: }

Rating 6: lowest priority - would be addressed when all other hazards are attended to.

Task 2. Troubleshooting procedures

This task has three steps.

1. Checking the optical microscope
2. Making a decision
3. Routine maintenance

Step 1. Checking the optical microscope

According to the **SOP: Set-up and Pre-use Checks of the Microscope** there are a number of factors to check before using the microscope. These are:

- that the microscope is suitably placed on the bench
- that the power cord is safe, plugged in and turned on
- that the bulb is illuminated when the switch on the microscope is turned on
- that the microscope is clean and free of dust.

You have just set up the microscope and turned it on and the bulb has not illuminated. What would you do now?

Assignment: Troubleshooting the Microscope

You have just turned on the microscope and the bulb has not illuminated. How do you investigate and rectify (if possible) this situation?

1. List all the possible reasons that would cause the bulb to not light and how the problem could be rectified. (You should list between ten and twenty reasons).
2. In what order would you investigate these possible causes?
3. For each of the possible causes is it safe for you to fix, and if not, who is authorised to perform the investigation? This is your hierarchy of control.
4. Who or what else would you consult if the problem were not easily fixed?

Note: the best way to present this answer is in the form of a table. The first possible reason is done for you below.

Possible Problem	Rectified by:	Safe for me to fix?	Who else may be authorised to fix it?	Who or what else may I need to consult?
1. Not plugged in to wall outlet.	Plug into socket.	Yes	Not applicable	Not applicable
2.				
.				
.				
.				

Step 2. Making a decision

Based on the information in Step 1 you should now have some idea of how to proceed. To do this you need to make some decisions about the best way to investigate the lack of illumination in the microscope.

Making a Decision

As a laboratory technician you will be expected to be able to work unsupervised, be proactive in your work and to make decisions on your own regarding workflows, ordering, dealing with faulty equipment, customer service etc.

Using the faulty microscope as an example, the following Note follows the decision making process that you could go through.

Let us assume that you have checked all the simple things such as whether the microscope is turned on and set up correctly.

Decision Number One:
Check the maintenance records for the microscope as this may give you some ideas on how to proceed. The maintenance record is shown below and reveals that the lamp was replaced yesterday and also about three months ago. Is a pattern emerging?

Also decide if a lack of recent regular cleaning has contributed to the problem. You may need to consult with other staff members to determine this.

Decision Number Two:
Check with your staff who replaced the bulb to see if he saw anything unusual when he replaced the bulb yesterday. Did he install the correct bulb following the correct procedures? Maybe the bulb used yesterday was faulty?

Decision Number Three:
Replace the bulb but only after checking that the required spare parts (the bulb and possibly the fuse) are available in the store and that you know how to relace them safely and accurately by accessing The SOP for **Changing a Microscope Bulb or Fuse** before proceeding.

Decision Number Four:
Decide whether you need help or advice after reading the SOP. If so, seek that assistance.

Decision Number Five:
Have a fall-back plan if replacing the bulb does not rectify the problem. For instance:

- try another bulb
- replace the fuse
- send microscope to the manufacturer for repairs. It may still be under warranty.

SL Equipment Number: _____ **Service Log**

Type of Equipment: Optical Microscope

Summary of Records to be kept in this Service Log:

Set-up and Pre-use Checks: Daily - Only discrepancies recorded
Safety Checks: Daily - Only discrepancies recorded
Calibration Checks: Weekly - Details recorded
Supplier Servicing: Quarterly and malfunctions - Details recorded

DATE	EVENT	COMMENTS	BY
3/2/04	Installation after purchase	Microscope Operative	Haben
11/2/04	Cleaning	Microscope OK	Alem
18/2/04	Cleaning	Microscope OK	Letay
25/2/04	Cleaning	Microscope OK	Yihdega
27/2/04	Set-up and pre-use	Replace bulb	Abraha
28/3/04	Cleaning	Microscope OK	Yihdega
29/5/04	Quarterly Service	Microscope Operative	Alem
Yesterday	Set-up and pre-use check	Replace bulb	Letay

Step 3. Routine maintenance

You may have decided that the best way to proceed is to change the bulb. To do this you will need a copy of the following document.

SOP: Changing a Microscope Bulb or Fuse

1. Before replacing the lamp bulb or fuse, be sure to turn off the power switch and disconnect the power cord from the socket.
2. Carefully lay the microscope on its side.
3. Carefully unscrew the base plate underneath the microscope stand and open it.
4. Remove the bulb by pulling gently. Check to see if the wire in the bulb is broken or 'burnt out'.
5. Replace with new bulb of the same wattage.
6. Replace the base plate.
7. When replacing a fuse, keep the microscope in an upright position.
8. Look at the rear of the base stand, where you will see a small plastic cover with 'fuse' written on it.
9. Unscrew the cover carefully, remove it and check whether the fuse is broken with a magnifying device. Replace the fuse if it is broken.

10. Replace the fuse with the same type of fuse. Screw the cover into place.

11. Plug the microscope into the socket and turn the microscope on to check the light.

Task 3. Documentation and communication

This task has two steps.

1. Unserviceable equipment
2. Documentation and communication

Step 1. Unserviceable equipment

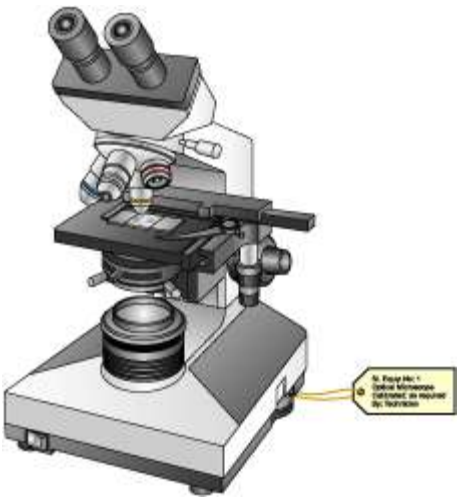
In the laboratory you need to ensure that the equipment that you are about to use is in serviceable condition and is valid to use for the testing of samples. In this case we now know that the microscope is unserviceable.

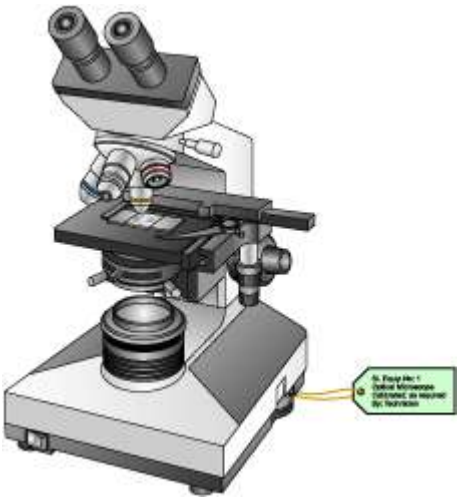
Unserviceable pieces of equipment should not be used and should be removed for repair or replacement. You have decided that the microscope needs to be repaired by the manufacturer. How do you do this and what else needs to be done?

At your lab

- each piece of equipment has its own unique identifying number, called the ***SL Equipment Number***
- a log book is kept for each piece of equipment in which calibration and service information is recorded
- Unserviceable equipment is not used but is removed and tagged for repair or replacement.

You need to identify another microscope to use for your work today. In the equipment storeroom you locate two unused microscopes. Check the calibration tags on each microscope and the Log Book (Service Log) to ensure that the equipment is in a serviceable condition and ready to use. Clicking on the tag will show you the calibration tag and the Log Book (Service Log) for you to check.





Step 2. Documentation and communication

There needs to be some documentation and communication to ensure that all appropriate staff members know of the unserviceable equipment.

Documentation and Communication

There are a number of important factors to consider when a piece of equipment is considered unserviceable.

1. Communication in the laboratory

You need to let other staff members know that the equipment is unserviceable and that it should not be used. This is achieved by:

- filling in the **Service Log** with full details of the problem
- putting an **unserviceable tag** on the piece of equipment and possibly **moving** it to a storage area (**quarantining** the equipment)
- informing your supervisor and/or other staff members verbally of the problem
- discussing the problem at the weekly staff meeting
- Circulating an intra-laboratory memorandum.

You may also need to check with your supervisor or the laboratory manager for authorisation to initiate repairs.

2. Communication with the manufacturer

You will need to contact the manufacturer or distributor and arrange for them to repair the microscope either on-site or off-site. How do you determine who the manufacturer/distributor of the equipment is if the equipment is old and has been in the laboratory for years? Any of the following approaches will be useful.

- Check for labels on the equipment itself
- Check the Service Log for details

- Check with other laboratory staff or your supervisor
- Check with the accounts department - they may have paid an invoice for service
- Check with service companies who provide generic servicing for a range of laboratory equipment, they may be able to help. They will be listed in the telephone directory.

The next step is to communicate with the repairer. This is achieved by following the guidelines below.

- In the first instance, by telephoning the repairer and talking with the service department.
- Before making this phone call be sure you know:
    - The make, model and serial number of the equipment
    - When it was purchased
    - Warranty details (if applicable)
    - Service details (from service log)
    - Details of the problem
    - What you have tried to do to rectify the problem. For instance, 'we changed the fuse and the globe but there was still no light'.
    - What you want done to rectify the problem. For instance, 'have your serviceman call in this week to assess repairs and provide me with a quote'.
- You may need to follow this call up with an email or written documentation
- You may need to record the request for maintenance in your enterprise system.

3. Post Repair Period

You will need to monitor the repair process and:

- liaise with the manufacturer over repair details, warranty details and any costs involved
- determine return date and arrange for return of equipment
- receive the equipment back into the laboratory
- update service log details
- carry out pre-use and safety checks
- place equipment back into service ensuring that any unserviceable tags have been removed
- advise all laboratory and other staff (for instance accounts payable) that the equipment has now been returned and is serviceable again
- Send any documentation to the appropriate department.
- You have now managed the process of arranging for the repair of a piece of faulty equipment by an outside party.

4. Using Records as an Historical Account

The service log records are a vital tool for troubleshooting, warranty claims, maintenance of regular servicing, and in some circumstances achieving and maintaining accreditation or certification by an outside body (for instance ISO19025). The use of service records as an historical account is vital for the continued existence of your laboratory.

You should ensure that:

- records are complete and kept fully up-to-date

- records are legible
- all staff are made aware of the necessity for accurate records and know how to fill them out correctly
- records are checked against maintenance schedules to ensure that all routine servicing and maintenance is carried out
- records are stored in a safe and secure manner.

Maintenance Procedures

Maintenance procedures vary between different classes of equipment. For instance there is no maintenance required for a mercury bulb thermometer, apart from periodic calibration by an authorised agency, but there is a relatively high requirement for maintenance to ensure the continued functioning of a High Performance Liquid Chromatograph (HPLC).

Maintenance procedures are usually sourced from the following:

- the experience of senior laboratory staff
- laboratory generated SOPs
- manufacturer's manuals accompanying the equipment
- outside accreditation or registration authorities
- published scientific papers
- internet-based materials.

Whatever the source, the maintenance procedures are usually formalised into an SOP if one does not exist for a new piece of equipment or modified from an existing SOP for updated equipment.

SOPs are often contained in a Methods Manual or a similar folder. The use of such SOPs for maintenance is supported by a system to train appropriate staff in maintenance procedures and to record all details of maintenance and the associated calibration of equipment after routine maintenance. Often two 'record' documents are used, although sometimes these are combined, called the:

- Maintenance Log
- Calibration Log.

The ethos behind maintenance procedures is that the following always occurs:

- all staff are trained in the importance of routine maintenance
- correct maintenance is carried out by staff authorised and competent to perform this maintenance
- maintenance is carried out in a timely manner
- such maintenance is recorded for future reference
- the results of the maintenance are passed on to other staff members when nessessary
- maintenance that cannot be carried out in-house is passed to authorised service personnel from outside agencies.

SOP: Maintenance Schedules

Background:

1. Maintenance schedules are used in a lab is to formalise and regulate routine maintenance procedures used for analytical equipment.

2. Maintenance schedules shall be appended to (or be located as close as possible to) each piece of equipment, listed in the maintenance log for each piece of equipment and kept in a central manual of maintenance.

3. Details of maintenance performed will be recorded in the maintenance log immediately after such maintenance is performed.

Authorisation:

1. Development of maintenance schedules shall be the responsibility of the Senior Technician who may delegate such tasks but who is responsible for final checking and authorization of each schedule before it is put into action.

- Each maintenance schedule shall be a controlled document with MS number, Version and Date details.
  Details:
  The following details shall be included in the maintenance schedule:
  - equipment name and serial number
  - list of required routine maintenance items with a brief description of each activity
  - a list of intervals for each activity, eg. every seven days, after 100 hours etc
  - a schedule in the form of a calendar or similar document.

Example of a Maintenance Schedule:

Liquid Scintillation Counter – Model LSC X1008, Serial # BD 12987C

MaintenanceActivity	Description	Interval
Clean	Inside of cabinet using a mild detergent	Weekly
DPM Check	Calculate efficiency of detector	Fortnightly
Grease	Grease pulley sprockets	Monthly

Calendar Example
Based on a 30 day month

Day 7 - Clean

Day 14 – Clean, DPM Check

Day 21 – Clean

Day 28 – Clean, DPM Check

Day 30 – Grease

Repeat maintenance schedule for each following month.

Maintenance Records

Maintenance records are an integral part of the management system for a laboratory. They provide an historical record of the following activities associated with the use of a particular piece of equipment.

- Routine maintenance including whether that maintenance was carried out in a timely and regular manner.
- Non-routine maintenance e.g. minor breakdowns or globes/fuses blowing.
- Technical maintenance carried out by an outside agency such as a major service.
- Major breakdowns and repairs.
- Warranty-associated activities.
- Replacement of major components.
- Data for use in evaluating the reliability and running cost of the equipment.
- Data for use in quality audits such as accreditation or registration by outside agencies.

At a minimum, maintenance records must contain the following information.

- Details of the piece of equipment including:
    - name of equipment
    - model and serial number
    - date put into service.
- Dates of all maintenance carried out.
- Name of person performing the maintenance function.
- A comments column.

Maintenance records should be kept for at least the life of the equipment and usually for a number of years after that in case details of the testing performed by the equipment need to be verified in the future.

Staff should be trained in the value and correct use of maintenance records and there should be clear enterprise guidelines of who is authorized to perform such maintenance and who is authorized to fill in the maintenance records.

Step 3. The science behind equipment maintenance

You have previously looked at Maintenance Procedures, Maintenance Schedules and Maintenance Records. In this step you will pull all this information together to produce a Maintenance Plan. **Maintenance Plans** are useful tools for the development of procedures and protocols for the regular maintenance of equipment. They are usually developed as each new piece of equipment is introduced into the laboratory.

Maintenance Plans

Maintenance plans are usually developed when a new item of equipment is introduced into the laboratory.

They are developed for the following reasons:

- to prolong the life of the equipment
- to increase reliability
- to minimise breakdowns
- to ensure that the equipment is available when needed.

When should equipment be serviced?

- On a regular time basis, e.g. every three months.
- On a usage basis, e.g. every 200 hours or 1000 samples.
- According to seasonal workload, e.g. service equipment during slow periods.

What kinds of things are required in a maintenance plan?

- Depending on the equipment a plan may include:
    - cleaning
    - lubrication
    - checks on condition
    - moving parts replacement or service
    - electronic checks.

How should routine maintenance be done?

- According to manufacturer's manual.
- Depending on the technician's own experience.
- Depending on the experience of the other users.
- According to a documented procedure.
- May use a formal checklist of items.

Who should do the maintenance?

Who	Advantages	Disadvantages
Service Agents	• Trained • Competent • Contract basis • No drain on staff time	• Not always available • Cannot respond immediately • Usually expensive
Laboratory Staff	• Have interest in the equipment • Immediately available • Usually cheaper	• Interruption to normal work • Not specially trained • May cause more problems

Where maintenance should be done?

Laboratory	Agent's Workshop
• Preferable	• Sometimes necessary
• Can see what is done	• Has specialised facilities
• Avoids transport costs or damage	• Better environment for repairs
• Less lost time	

Calibration and maintenance of micropipettes

Introduction:

Micropipettes are clinical laboratory equipments that are used for measuring & transferring liquids of certain volume.

Most commonly used pipettes have a piston with a button on top that, when depressed, creates suction for aspiration and dispensing the volume. These pipettes are used with disposable pipette tips. They are manufactured with a one-stop or two-stop pipetting cycle. A one stop pipette is the simplest to use in that depressing the button will push out the air from the pipette tip. A two –stop air displacement pipette is similar except that there are two positions to depress the suction button.

CORRECT OPERATION OF A MICROPIPETTE

Always follow the procedure provided by the vendor for the proper use of a micropipette. It is important to follow the recommendation for removing excess fluid from the outside of the pipette tip. For example, for positive displacement pipettes, the manufacturer often recommends dipping the filled syringe in distilled water to remove excess fluid clinging to the outside. For air displacement pipettes, as shown, the manufacturer recommends carefully wiping the outside of the pipette with a disposable tissue, taking care not to touch the bottom of the pipette tip.

It is also important to fully deliver the fluid according to the manufacturer's recommendations. For positive displacement pipettes, the syringe is rinsed into the receiving or diluting fluid so that the sample will be fully dispensed. For air displacement pipettes, there is a separate step in the dispense cycle that blows the final drop of fluid into the receiving container. In those cases, it is important to dispense the fluid toward the bottom of the receiving container so as to minimize loss by aerosolization or splashes.

1. If a disposable pipette tip is required, position the tip on the pipette.

2. Depress the plunger or suction button. If this is a two-stop pipette, the button is depressed to the upper position, which is generally around the halfway point.

3. While keeping the suction button depressed, place the pipette tip into the fluid to a level that will aspirate fluid and not air bubbles.

4. Slowly release the suction button up so that the fluid is aspirated, without air bubbles, into the pipette tip.

5. Remove the excess fluid from the outside of the pipette tip according to directions.

6. Dispense the fluid into the vessel by pushing the suction button down. If this is a two-stop pipette, the button is depressed all the way to the bottom.

7. For a one-stop pipette, the process is the same except that the button has only one position for aspirating and dispensing the fluid.

Pipetting techniques

A. Forward pipetting:

For standard solutions including buffers, water diluted saline and dilute acids and bases.

More accurate and precise results than reverse pipetting.

Entire volume of liquid aspirated in to the pipette tip is dispensed.

1. Press the operating button to the first stop.

2. Dip the tip into the solution to a depth of 1 cm, and slowly release the operating button. Withdraw the tip from the liquid touching it against the edge of the reservoir to remove excess liquid.

3. Dispense the liquid into the receiving vessel by gently pressing the operating button to the first stop.

After one second, press the operating button to the second stop. This action will empty the tip. Remove the tip from the vessel, sliding it along the wall of the vessel.

4. Release the operating button to the ready position

B. Reverse pipetting:

For high viscosity solutions small volume, buffers with detergent, and solutions that foam easily.

1. Press the operating button to the second stop.

2. Dip the tip into the solution to a depth of 1 cm, and slowly release the operating button. This action will fill the tip. Withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid.

3. Dispense the liquid into the receiving vessel by depressing the operating button gently and steadily to the first stop. Hold the button in this position. Some liquid will remain in the tip, and this should not be dispensed.

4. The liquid remaining in the tip can be pipetted back into the original solution or thrown away with the tip.

5. Release the operating button to the ready position.

Pipette calibration methods

Pipette accuracy and precision must be checked periodically so that only valid pipettes are used.

Methods for pipette calibration

Gravimetric

Spectrophotometer

Titration

A.Gravimetric pipette calibration

Gravimetric analysis of a pipette involves determining the mass of highly pure distilled water delivered by pipette using room temperature water, the resulting mass is converted to volume using a density conversion factor for pure water adjusted to the temperature.

1. Record the barometric pressure and temperature of pure water (type I), since these factors affect weight of substances.

2. Weigh a clean weighing vessel and adjust the scale to tare (zero mass).

3. Adjust the pipette to the desired volume and dispense according to the manufacturers procedure into a previously weighed and tarred vessel.

4. Use the following calculation:

(W2 – W1) x Ft = actual capacity in mL

W2 = weighing vessel after volume is pipetted into it (cover on)

W1 =empty weighing vessel with cover (to 0.1 mg)

Ft =factor to adjust mass to volume based on temperature (At 4^0C, 1.0 g pure H_2O is 1.0 mL)

5. Repeat 10 times and determine the mean volume and standard deviation.

6. Perform calculations for % accuracy and precision. Use the manufacturer's specifications to determine accuracy and precision of the micropipette and to adjust the pipette accordingly.

For example, a valid glass pipette has an accuracy = 99.5 % and precision = 0.5%.

% Accuracy = (actual volume from pipette/expected or nominal volume) x100

% Precision = (standard deviation/mean volume) x100

For an example calculation of pipette precision, let's use these data: n =10,

Pipette expected volume = 100 uL, standard deviation =4, pipette actual or mean

Volume = 98.

% Accuracy = (98/100) x100 = 98%

% Precision = (4/98) x100 = 4.1%

B.Sepctrophotometric pipette calibration

This procedure involves testing colored reagents such as potassium dichromate or p- nitrophenol with known absorbance at certain wavelengths. Since concentration relates to absorbance, volume can be determined from the procedure.

The following spectrophotometric procedure is used to verify calibration of "to deliver" transfer or measuring micropipettes that deliver volumes up to 1000 uL. This is an example of the use of p-nitrophenol in spectrophotometric verification of the calibration of a 10uL pipette.

1. All glassware should be cleaned, rinsed with distilled water, and dried.

2. Reagents required: around 1.0 L of 0.01-mol/L NaOH and 100 mL of 105-mg/dL p-nitro phenol (PNP). Dissolve 105 mg of high-purity PNP in deionized water in a 100-mL volumetric flask. Fill to the mark, mixing thoroughly. Prepare dilutions of the stock PNP. Fill three

250-mL volumetric flasks with 250 mL 0.01-mol/L NaOH so that it reaches the volume mark. Add 1.0 mL of stock PNP to each flask using a different volumetric pipette each time. Mix well.

3. Make test solutions by adding 2.5 mL of 0.01-mol/L NaOH to each of 5 cuvettes. To each cuvette, using the test micropipette and following the manufacturer's direction for operation, add 10 uL of PNP solution. The manufacturer's directions should specify whether this pipette is to be dipped into rinse fluid or wiped with tissue prior to dispensing. Instructions may specify that the pipette is to be rinsed out into the receiving fluid, or to have the remaining drop blown out with the dispensing button followed by a tip discard. These are important aspects to follow consistently as they impact upon accuracy and precision of the pipette. In this example, 2500 uL + 10 uL, or 2510uL, is the presumed total volume of each cuvette. Note that this volume would change depending on the size of the micropipette used.

4. Cover each cuvette and mix by inversion 8 to 10 times.

5. Using distilled water in the reference cuvette, adjust to zero absorbance at 401 nm in a previously warmed up spectrophotometer.

6. Read the absorbance of each cuvette and record the absorbance readings on the attached chart. The presumed correct absorbance (Abs.) reading is 0.550.

7. Calculate the volume in micro liters (uL) delivered by the pipette for each of the 5 tests using the following formula:

(Abs. obtained/0.550) x 0.003984 x2510 = volume delivered by pipette in uL

The dilution of PNP is 1/251 =0.003984 and 2510 is the total volume of each cuvette.

8. Determine the mean, standard deviation, and coefficient of variation (%CV) from the 5 volumes delivered by the pipette.

9. %CV is the measure of precision. Accuracy error is determined as:

([Expected volume – Obtained volume] x 100%)/Expected volume

Accuracy should meet the manufacturer's guidelines or be <1.0 %, whichever is smaller.

Pipette Calibration Form

Pipette Serial #_____

Date	Tech.	Pipette Volume	1st Sample	2nd Sample	3rd Sample	4th Sample	5th Sample	Mean	Standard deviation	%C.V.	% Accuracy

Pipette Verification Schedule **Year:_____**

Pipette #	Volume	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Pipette maintenance :

Pipette cleaning

Cleaning requirements depend on pipette use and the liquid. The chemical compatibility of the pipette should be checked prior to cleaning. When necessary, protective clothing, goggles and disposable gloves should be worn.

Table 1 cleaning guideline

Pipetted liquids	**Cleaning guidelines**
Aqueous solutions and buffers	Open the pipette, rinse the contaminated parts thoroughly with distilled water, and allow to dry.
Acids and alkalis	It is advisable to clean the tip cone and the lower part of the tip ejector with distilled water more frequently if acids or alkalis are handled. Clean as described in "Aqueous solutions and buffers".
Organic solvents	Immerse the contaminated parts in a detergent solution such as Deconex∃ 12 Basic. Rinse thoroughly with distilled water and allow to dry.

Radioactive solutions	Open the pipette and place the contaminated parts in a strong detergent or cleaning solution. Rinse several times with distilled water and allow to dry. Decontamination should always be followed by confirming that radioactivity has been reduced to an acceptable level. All used cleaning materials are radioactive waste and must be disposed of according to regulations.
Proteins	Open the pipette, immerse the parts in a detergent solution, such as Deconex∃ 12 Basic. Rinse well with distilled water and allow to dry.
DNA, RNA	• DNA can be eliminated by immersing pipette parts in at least 3% (w/v) sodium hypochlorite for at least 15 minutes (2, 3). Rinse well with distilled water and allow to dry. • Treat the pipette parts with Thermo Scientific DNA AWAY (Cat. no. 7008 and 7009 according to instructions. • Exposure to ultraviolet (UV) light for 30-60 minutes will further reduce but not completely eliminate DNA contamination on the pipette surface (4). • No special treatment is required to remove RNA because it degrades rapidly and is sensitive to ubiquitous RNases
DNase, Rnase	RNase can be removed by first cleaning the pipette with a detergent solution, followed by thoroughly rinsing with water and then 95% ethanol to speed the drying process. Pipette parts are then soaked in a 3% hydrogen peroxide solution for 10 minutes. Finally, the parts are rinsed thoroughly with DEPC-treated water (5) and allowed to dry. • Treat the pipette parts with Thermo Scientific RNase AWAY (Cat. no. 7006 and 007) according to instructions. • DNase can be destroyed by autoclaving (15 min, 121°C
Viruses, mycoplasma, bacteria and fungi	Ultraviolet (UV) radiation is a practical method for inactivating viruses, mycoplasma, bacteria and fungi. While Finnpipettes are UV resistant, the handles might change color from gray to light yellow. If the inner parts of the pipette are exposed to UV light, make sure that the piston and O-rings are sufficiently lubricated

Pipette maintenance :

Pipette cleaning

Cleaning requirements depend on pipette use and the liquid. The chemical compatibility of the pipette should be checked prior to cleaning. When necessary, protective clothing, goggles and disposable gloves should be worn.

Decontamination & sterilization of micropipette

• Decontamination – Any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.

• Disinfection – A physical or chemical means of killing microorganisms, but not necessarily spores.

• Sterilization – A process that kills and/or removes all classes of microorganisms and spores.

Before assembling the pipette, wipe the piston with 70% ethanol and lubricate with the lubricant that is provided with the pipette. When removing RNase, use a freshly opened ethanol bottle and prepare 70% ethanol in DEPC treated water.

Pipette sterilization

Autoclaving is the simplest sterilization method if all pipette parts tolerate extreme heat. Pipettes should be autoclaved according to the manufacturer's instructions. To achieve sterility, a holding time of at least 20 minutes at 121^{0}C (252^{0}F) is required.

Chemical disinfection and sterilization

Chemical disinfectants or sterilants are used to decontaminate surfaces and equipment if autoclaving is not possible or practical. The choice of a chemical disinfectant or sterilant depends on the microorganisms of concern.

Also, the chemical compatibility of the materials should be taken into account .If the lower tip cone and the tip ejector of a pipette have to be chemically decontaminated; the pipette should be disassembled according to the Instructions for Use.

Common laboratory disinfectants, such as 70% ethanol or 5% sodium hypochlorite, can be used to clean the surface without any effect on antimicrobial treatment.

Conversion Table

Values of the conversion factor Z (µl/mg), as a function of temperature and Pressure, for distilled water.

Temperature °C Air pressure kPa*

80	85	90	95	100	101	105
15.00	1.0017	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020
15.50	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020	1.0021
16.00	1.0019	1.0020	1.0020	1.0021	1.0021	1.0021	1.0022
16.50	1.0020	1.0020	1.0021	1.0021	1.0022	1.0022	1.0022
17.00	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023	1.0023
17.50	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024	1.0024
18.00	1.0022	1.0023	1.0023	1.0024	1.0025	1.0025	1.0025
18.50	1.0023	1.0024	1.0024	1.0025	1.0025	1.0026	1.0026

19.00 1.0024 1.0025 1.0025 1.0026 1.0026 1.0027 1.0027
19.50 1.0025 1.0026 1.0026 1.0027 1.0027 1.0028 1.0028
20.00 1.0026 1.0027 1.0027 1.0028 1.0028 1.0029 1.0029
20.50 1.0027 1.0028 1.0028 1.0029 1.0029 1.0030 1.0030
21.00 1.0028 1.0029 1.0029 1.0030 1.0031 1.0031 1.0031
21.50 1.0030 1.0030 1.0031 1.0031 1.0032 1.0032 1.0032
22.00 1.0031 1.0031 1.0032 1.0032 1.0033 1.0033 1.0033
22.50 1.0032 1.0032 1.0033 1.0033 1.0034 1.0034 1.0034
23.00 1.0033 1.0033 1.0034 1.0034 1.0035 1.0035 1.0036
23.50 1.0034 1.0035 1.0035 1.0036 1.0036 1.0036 1.0037
24.00 1.0035 1.0036 1.0036 1.0037 1.0037 1.0038 1.0038
24.50 1.0037 1.0037 1.0038 1.0038 1.0039 1.0039 1.0039
25.00 1.0038 1.0038 1.0039 1.0039 1.0040 1.0040 1.0040
25.50 1.0039 1.0040 1.0040 1.0041 1.0041 1.0041 1.0042
26.00 1.0040 1.0041 1.0041 1.0042 1.0042 1.0043 1.0043
26.50 1.0042 1.0042 1.0043 1.0043 1.0044 1.0044 1.0044
27.00 1.0043 1.0044 1.0044 1.0045 1.0045 1.0045 1.0046
27.50 1.0045 1.0045 1.0046 1.0046 1.0047 1.0047 1.0047
28.00 1.0046 1.0046 1.0047 1.0047 1.0048 1.0048 1.0048
28.50 1.0047 1.0048 1.0048 1.0049 1.0049 1.0050 1.0050
29.00 1.0049 1.0049 1.0050 1.0050 1.0051 1.0051 1.0051
29.50 1.0050 1.0051 1.0051 1.0052 1.0052 1.0052 1.0053
30.00 1.0052 1.0052 1.0053 1.0053 1.0054 1.0054 1.0054
*1kPa = 10 hPa

Calibration and maintenance of Balance

Introduction

Balances or scales are used in the clinical laboratory to weigh out chemicals in making media or reagents. Balances can also be used to check the function of a pipetteusing the gravimetric method, relating the mass of liquid dispensed by the pipette to its volume. There are two main types of balances: mechanical and electronic.

Mechanical scales work by a fulcrum mechanism, with a more delicate fulcrum for measuring small mass to a high degree of accuracy. Double-pan, single-pan, and analytical mechanical balances are used in laboratory situations. Electronic balances are available that use electromagnetic force to balance the weight by restoring the balance arm back to null position.

The simplest mechanical scale is a double-pan balance. Currently, this type of balance is used in teaching situations but not used very often in the clinical laboratory. It has two pans, and the unknown mass is place in the left pan and standard weights are placed on right pan. It is the least accurate of all balances, since the mass is determined by placing standard weights on the right pan until the center balance needle rests on the zero or mid-point on the analog scale.

Single-pan scales are available in a mechanical format comprising a short arm with a weighing pan and external weights that can be slid onto the long arm to reach a balance. Electronic top-loading balances use a platform on which to place the item to be weighed; the platform is balanced via electromagnetic force. The output is visible on light - emitting diode (LED) or liquid crystal diode (LCD) readout. Single-pan scales are employed in the clinical laboratory for measuring out nutrients when preparing bacteriologic growth media, for measuring out reagents for making histological stains, or for other uses. Although they have a moderate level of accuracy, they are easy to use and versatile and have large capacities of up to 20 kg.

Analytical balances are the most accurate type of balances. Mechanical versions have a single pan with internal weights to balance the unknown mass in the measuring pan. The mechanical analytical balance works by balancing on a very delicate fulcrum. This type of balance is very precise and accurate to 01 mg, but has a smaller capacity than single-pan top-loading balances. The electronic analytical balance works by an electromagnetic coil restoring the balance beam to a balanced position.

It has the capability to determine the mass of the weighing vessel and reset to zero. This feature is called **tare** capability.

Balance maintenance

In order for balances to maintain proper function, periodic maintenance and functional checks must be performed. The balance must be installed on a sturdy counter or table free from vibrations. Prior to each use, the balance should be assessed as level. There is generally a liquid with a bubble within a level marker that can indicate if the balance is level. If the bubble is off-level, leveling screws may be used to adjust the length of each leg of the balance so that the balance can be properly leveled. The balance is level when the bubble is in the center of the level ring. After every use, the weighing pan should be placed in an arrest position so that it won't be attempting to measure mass, and any spilled chemical should be brushed off.

With each use: With the weighing pan secured (arrested) use a clean camel- hairbrush to sweep away remnants of dust or chemicals into a dustpan. Verify that weighing pan is free from dust and fingerprints prior to use.

With each use: Verify placement on a vibration-free bench, in an area free from air drafts, and direct sun. Adjust the lengths of the centering screw legs using the level bubble, as available. Zero the balance with empty pan. Place a weighing vessel or paper on the pan and adjust the tare button to subtract out the mass of the vessel. Always secure the pan in standby mode before adding weight to the balance.

Quarterly: Check the accuracy and precision of the balance by weighing NIST class S weights (50, 100, 200, 500, 2000 and 4000 mg) in triplicate taking care to handle with clean cotton gloves or forceps in order to protect the standardized weights.

Balance calibration

The accuracy of a single-pan balance is then checked with standard weights such as class S for larger masses and class J for smaller masses as determined by the National Institute of Standards and Technology (NIST). For example, an analytical balance at 0.100 g should be accurate to 0.000025 g when checking the class J standard weight.1 The manufacturer's problem-solving guide should be consulted when inaccuracy is determined.

Balance Calibration Verification Worksheet

Laboratory_____ Month_____ Year___ Balance Manufacturer and Model_____ Quarterly Verification		
Mass of NIST Class S 0.050g 0.100 g 0.200 g		
1.	1.	1.
2.	2.	2.
3.	3.	3.
4.	4.	4.
5.	5.	5.
Mean	Mean	Mean
Mass of NIST Class S		

0.500g	2.00 g	4.00 g
1.	1.	1.
2.	2.	2.
3.	3.	3.
4.	4.	4.
5.	5.	5.
Mean	Mean	Mean

Accuracy of Balance: |Expected mass - Obtained mass| x 100% / Expected mass					
0.050 g	0.100 g	0.200g	0.500 g	2.00g	4.00g

Precision of Balance: Standard deviation x 100% / Mean					
0.050 g	0.100 g	0.200g	0.500 g	2.00g	4.00g

Documentation of validity acceptance based on manufacturer's specifications for accuracy and precision.
Date and signature of tech.

Centrifuge calibration and maintenance

Introduction

The purpose of a centrifuge in the clinical laboratory is to separate substances ofdifferent mass or density. *Centrifugal force* is the outward force when a sample isspun at a high rate of speed. A centrifuge speeds up the natural separation that occurs by gravity over time. For example, centrifugation can separate serum quicklyfrom clotted blood or plasma can be separated from anticoagulated blood cells.

A centrifuge can also be used to separate precipitate from supernatant in a solution.

The centrifugal speed is measured in revolutions per minute (rpm). Typical uses for the centrifuge include generating 1200 rpm for 10 to 12 minutes for serum from cells, 12,000 rpm for a microhematocrit, or 100,000 rpm for chylomicron separation.

In addition, the force generated in centrifugation is important because it takes into account the effects of gravity (*g*) during centrifugation. For example, one typically needs to generate 14,000 *g* for a microhematocrit or 178,000 *g* for chylomicron separation. This force is called relative centrifugal force (RCF) and is also known as gravitational force. RCF, in units of *g,* for gravitational force, relates to revolutions per minute based on the size of the centrifuge radius in centimeters. The formula is

$$RCF = 1.12 \times 10^{-5} \times r \times rpm^2$$

The radius, or r, is determined by measuring the radius of the centrifuge arm from the center to the tip of centrifuge arm.

RCF is calculated when comparing two different centrifuges used for the same purpose. This factor is helpful for calibrating revolutions per minute between the two centrifuges so that the correct revolutions per minute can be determined for each centrifuge.

There are three main types of centrifuges: horizontal-head, angle-head, and ultracentrifuges. *Horizontal-head centrifuges* are the swinging bucket type in which the centrifuge tubes are held in a vertical position when not moving but are horizontal when the centrifuge is fully in motion. They generate low speeds only but can produce a tight pellet of precipitate or a clotted cell in the bottom of the tube.This type of centrifuge is recommended for serum separator devices.

An *angle-headcentrifuge* has a fixed 25- to 40-degree angle at which the tubes are held during centrifugation.

The sediment packs at an angle but not as tightly as with a horizontalhead centrifuge. This type of centrifuge is adequate for cell packing, but since the sediment is at formed at an angle and not tightly packed, decantation is not recommended.

The third type of centrifuge is an *ultracentrifuge.* This centrifuge generates the highest speeds. The centrifuge head is held at a fixed angle but generates tight sediment buttons due to the high speed generated. In order to reduce the heat produced by the friction generated by high centrifugal speeds, ultracentrifuges are refrigerated.1 This type of centrifuge is especially useful for lipoprotein separations since refrigeration enhances the separation. Ultracentrifugation is also used in drug-binding assays, such as separating free drug from protein-bound drug, since ultracentrifugation is successful at sedimentation of proteins.

Safety Considerations in Centrifugation

There are some important safety considerations regarding the operation of centrifuges.

One of the most important considerations is to balance the load. If the centrifuge is allowed to generate speed when the load is imbalanced, it can cause serious vibrations that can break the test tubes, spilling the contents within. If the imbalance generates enough force, the centrifuge can fall over and cause serious damage and endanger the people in its vicinity. Another less obvious but equally dangerous safety consideration is the generation of aerosols, or microdroplets of the samples. These microdroplets can be inhaled or enter into mucosal membranes of the mouth, eyes, or nose with possible toxic or infectious disease consequences.

Aerosols can be generated during vigorous shaking, when a vacuum is released, and during centrifugation. However, aerosols can be prevented by keeping the samples covered during centrifugation and by keeping the centrifuge lid covered during operation until the head comes to a complete stop. That way, if a tube breaks during centrifugation, the droplets can be contained within the centrifuge and not sprayed around the room. Several layers of gloves and tweezers may be needed to safely remove broken glass and while wiping up spills in the centrifuge. When opening centrifuged evacuated tubes, it may be necessary to open them in an aerosol containment box or wrap several layers of gauze around the top when opening it to catch any aerosol released with the vacuum.

Maintenance and calibration of centrifuge

Maintenance is also required to keep the centrifuge functioning properly. Keeping the centrifuge clean and checking the brakes are important aspects of maintaining safe operation of the centrifuge. The main aspects of function checked are speed generated and the timing device. The revolutions per minute can be checked with a **tachometer**, a small strobe light with a photo detector that determines how many revolutions per minute are generated by the centrifuge.

Tachometer - device that measures speed in revolutions per minute

Weekly or biweekly depending on usage: Clean interior components with soap and water followed by freshly made 10% v/v bleach solution, including sample buckets. Wearing protective gloves wipe interior sides and bottom taking care when removing broken pieces of glass.

Weekly: Place two equally balanced containers into the centrifuge, cover and operate at the most commonly used speed, listening for unusual vibrations. Check the braking mechanism to ensure a smooth gradual stop.

Monthly: Inspect gasket and check for wear and defects. Inspect cover latch for appropriate seal. Inspect head, head shaft and coupling for evidence of wear, cracks in fitting, corrosion, uneven wear and signs of fatigue. Inspect brushes for wear and replace according to manufacturer's instructions.

Monthly: Check the timer of the centrifuge at 15 minutes, 10 minutes, 5 minutes and 1 minute for the time the centrifuge motor is spinning (reaches the desired RPM until the motor shuts off) using a stopwatch.

Monthly: Check the revolutions per minute at several commonly used speeds including 3000 and 1500 rpms while centrifuging a balanced load (after it has reached stable speed) using a tachometer aimed at the reflective strip viewed through the top of the centrifuge.

Quarterly: Lubricate centrifuge shaft according to manufacturer's instructions, if applicable.

Centrifuge Maintenance Procedure Checklist

Month___ Year_____	Weekly Check balance and brake, Date and sign.	Weekly Clean interior, Date and sign.	Monthly Inspect parts including brushes, Date and sign.	Monthly Timer check for 1, 5, 10, 15 minutes. List times	Monthly RPM check for 1500, 3000. List RPMs	Quarterly Lubrication if necessary, Date and Sign.
Week 1						
Week 2						
Week 3						
Week 4						

Section Introduction

As a Laboratory Technician at your Lab Laboratories you must be able to use a range of different equipment. This includes the spectrophotometer. In this section you will calibrate a virtual spectrophotometer and then measure a number of calibration check samples. All operations must comply with relevant standards, appropriate procedures and/or enterprise requirements.

There are two tasks to do in this section.

1. Spectrophotometer - Perform set-up and calibration checks of laboratory equipment
2. Spectrophotometer - Optimisation of procedures

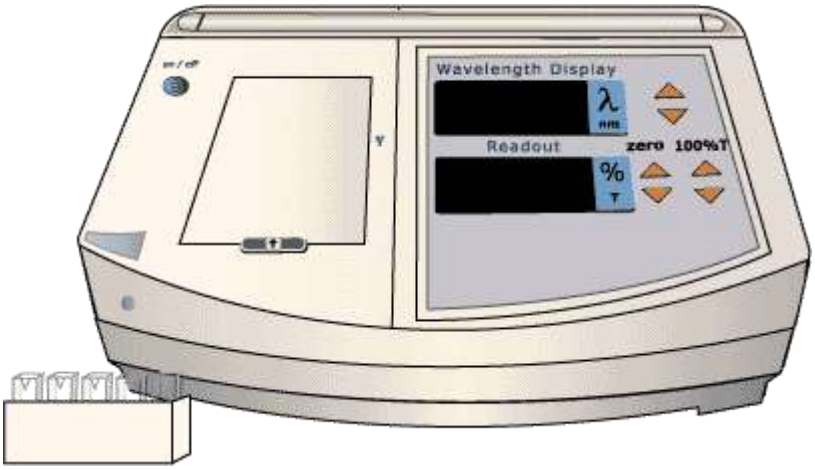
Task 1. Spectrophotometer - Perform set-up and calibration checks of laboratory equipment

This task has three steps.

1. Introduction to the spectrophotometer
2. The importance of maintenance and calibration
3. The importance of calibration records

Step 1. Introduction to the spectrophotometer

As the Laboratory Technician you are responsible for the use of the spectrophotometer at SimuLab. Read the following Study Notes for more information on the use of the spectrophotometer.



The Spectrophotometer

The spectrophotometer is an analytical instrument that measures the changes in a beam of light of a given wavelength that passes through a solution sample held in a special receptacle called a cuvette.

The cuvette is usually 1 cm square with two frosted sides and two clear window sides. The spectrophotometer is calibrated to a light path of 1 cm across the cuvette. The window sides allow the transmission of the beam of light and are calibrated so that the cuvette will only work if placed into the cuvette holder in the correct orientation. A mark on the outside of the cuvette is aligned with a mark on the spectrophotometer to allow correct orientation of the cuvette in the instrument.

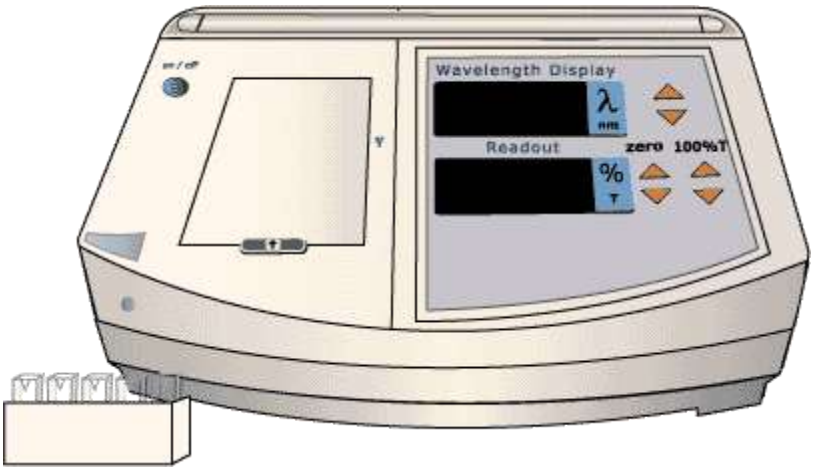
The spectrophotometer is able to use a beam of light in the UV-Visible spectrum that is selected by the operator to suit the requirements of testing. The wavelength of light is measured in nanometres (10^{-9}metre).

The spectrophotometer either measures the amount of light transmitted through a solution (called **transmittance**) or the amount of light absorbed by a solution (called **absorbance** or **extinction**).

At our lab we use transmittance. The spectrophotometer is first zeroed (with no cuvette or sample in the sample holder) then a blank solution (ie the substrate containing the test sample) is used to calibrate the instrument to 100% transmittance. That is, no light of that wavelength is absorbed by the blank solution.

The calibration of the instrument is then checked using Calibration Check Standards to ensure that the instrument has been calibrated correctly and is ready to use to measure unknown samples.

When measuring unknown samples, a reduction in transmission is then directly related to the increase in concentration of the sample of interest.



The important parts of the spectrophotometer (for the operator) are:

On/Off button	turns the machine on and off.
Pilot light	indicates that the machine is turned on.

Wavelength display	indicates the selected wavelength in nm (nanometre).
Digital readout	indicates the % Transmission of the light beam.
Cuvette sample holder	holds the cuvette in a light proof container when the lid is closed. It is important that the lid is closed, as extraneous light from the environment will cause the instrument to misread.

Step 2. The importance of maintenance and calibration

The spectrophotometer is a more complex piece of machinery than the pH meter but it is less complex than analytical equipment such as AAS and HPLC. Nevertheless it still needs to be used and maintained according to the manufacturer's instructions and will malfunction if used incorrectly.

Read the following two SOP's to find out more about the care, set-up and calibration of the spectrophotometer, then undertake the following activity.

SOP: Care and Maintenance of the Spectrophotometer

General:

1. The Metcalfe Model 20 Spectrophotometer has a service contract and is serviced regularly by Metcalfe Servicing Division. For malfunctions and servicing beyond the calibration and routine care items listed below, call Metcalfe Servicing Division on 9555-8888 and quote Service Contract number **SS5632/X**.
2. When the spectrophotometer is unserviceable, attach a 'Spectrophotometer Unserviceable' tag to the front of the spectrophotometer and disconnect the power lead. Annotate the Service Log accordingly and advise the Senior Technician.

Safety Checks - Daily:

1. Check that electrical connections are fully coupled, that cords are not frayed and that there is no liquid on or about the spectrophotometer.
2. **Under NO circumstances is any staff member of SimuLab permitted to open the sealed case of the spectrophotometer or attempt any procedures not listed below**.
3. Unidentified spilt chemicals should be removed with extreme caution whilst wearing standard SimuLab PPE - see cleaning section below.

Cleaning - Daily or as required

1. Carefully clean the sample holder, especially after using corrosive or salt solutions.
2. Mop up any spilt liquids and brush any spilt chemical from the spectrophotometer and adjacent areas.
3. Wash the cuvettes immediately after use (do NOT let the sample dry out in the cuvette). Rinse the cuvettes with deionised

water at least three times, allow them to drain and dry them inverted.

Records

1. Details of all routine maintenance, malfunctions and repairs shall be kept in the Service Log.

SOP: Spectrophotometer - Bacterial Suspension and Browning Enzyme Analysis

Set-up and Pre-use Checks - Daily:

1. Prior to turning the machine on, clean up any spills in and around the sample holder, on the outside of the machine and on the laboratory bench adjacent to the machine.
2. Turn the switch on at the wall.
3. Press the on/off button to turn the machine on. The pilot light should now glow.
4. Allow 20 minutes for the electronics to warm up.
5. Adjust the wavelength (λ) to the appropriate setting by pressing the up or down buttons adjacent to the wavelength display. Set the wavelength to 686 nm (for measurement of bacterial suspensions) or to 590 nm (for measurement of the apple pulp browning enzyme).
6. With the sample holder empty and closed, adjust the digital readout to read zero by pressing the up or down buttons marked Zero.
   The Spectrophotometer is now zeroed.
7. Take a clean and dry cuvette and fill with the Blank solution using a pipette. Always hold the cuvette at the top.
8. Wipe the outside of the cuvette with a tissue to remove fingerprints.
9. Align the reference mark on the outside of the cuvette with the index mark on the sample holder, insert the cuvette into the sample holder and close the lid of the sample holder.
10. Adjust the digital readout to read 100% by pressing the up or down buttons marked 100% T.
11. Repeat steps 6 - 10 until the readings are correct.
    The Spectrophotometer is now calibrated, meaning that the 0% and 100% transmittance have been set and that measurements within this range will be accurate.
12. Repeat steps 7 - 9 to measure samples. Record the reading shown in the digital readout for each sample. Rinse the cuvette between samples.

Calibration Checks - Weekly:

(To check that the calibration is stable and accurate across a range of measurements)

1. Zero and calibrate as above.
2. Measure the % Transmittance of the following sealed standards. Results must fall within the designated ranges. Note that different calibration samples are used for the different listed applications.

Bacterial suspension

Standard	Serial Number	Designated Range % Transmittance @ 686 nm
100%	Met 123/100	99 - 101%
75%	Met 123/75	74 - 76%
50%	Met 123/50	49 - 51%
25%	Met 123/25	23 - 27%
13%	Met 123/13	10 - 16%
0%	Met 123/0	0 - 1%

Browning Enzyme Analysis - Apple Pulp

Standard	Serial Number	Designated Range % Transmittance @ 590 nm
100%	Met 129/100	99 - 101%
75%	Met 129/75	74 - 76%
50%	Met 129/50	49 - 51%

25%	**Met 129/25**	**23 - 27%**
0%	**Met 129/0**	**0 - 1%**

Calibration Fails?

1. Change the lamp - follow instructions in the Users Manual.
2. Repeat set-up and calibration.
3. If calibration still fails contact Metcalfe Servicing Division - machine cannot be adjusted by laboratory staff.

Step 3. The importance of calibration records

Recording the results of calibration is an important step in laboratory procedures. To do this you will need to print the following two documents:

Forms

SL Equipment Number: 107 **Service Log**

Type of Equipment: Spectrophotometer

Summary of Records to be kept in this Service Log:

Set-up and Pre-use Checks: Daily - Only discrepancies recorded

Safety Checks: Daily - Only discrepancies recorded

Calibration Checks: Weekly - Details recorded

Metcalfe Servicing: Quarterly and malfunctions - Details recorded

DATE	EVENT	COMMENTS	BY
3/2/00	Installation	Spectrophotometer Operative	Metcalfe
11/2/00	Calibration	Spectrophotometer OK	Max
18/2/00	Calibration	Spectrophotometer OK	Colin
25/2/00	Calibration	Spectrophotometer OK	Colin
27/2/00	Routine Service	Replaced lamp	Metcalfe
28/2/00	Set-up and Pre-Use	Spectrophotometer not zeroing	Colin
29/2/00	Malfunction Service	Re-aligned lamp	Metcalfe
30/2/00	Calibration	Spectrophotometer OK	Colin
TODAY			

Forms

SL Equipment Number: 107 **Calibration Log**

Type of Equipment: Spectrophotometer

Requirements for a functional spectrophotometer:

Zero: Was a stable zero obtained - Yes/No?

Calibration: Was calibration stable - Yes/No?

Calibration Checks: Standards within % range? Show actual result.

Date	By	Zero Yes/No	Calibⁿ Yes/No	Calibration Checks					
				100 99-101	75 74-75	50 49-51	25 23-27	13 10-16	0 0-1
11/2/00	Alem	Yes	Yes	99	74	51	25	11	0
18/2/00	Haben	Yes	Yes	101	75	49	24	15	0
25/2/00	Letay	Yes	Yes	99	76	50	23	13	1
30/2/00	Letay	Yes	Yes	100	74	51	27	11	0
TODAY									