GUIDELINES FOR MAINTENANCE OF EQUIPMENT IN A
CLINICAL CHEMISTRY LABORATORY

WORLD HEALTH ORGANISATION,

MINISTRY OF HEALTH

AND

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SRI LANKA

GUIDELINES FOR MAINTENANCE OF EQUIPMENT IN A CLINICAL CHEMISTRY LABORATORY

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PREFACE

An element of good laboratory performance is the proper functioning of laboratory equipment. The use of an instrument may be limited by inadequate maintenance. All the major equipment in the laboratory should have a maintenance programme. The more complex an instrument is, the more the user will depend on the support of a supplier for its maintenance. It is therefore pertinent to foresee the extent of costs that need to be included for the use and maintenance of an instrument and to consider its lifespan as well as its workload. The quality of laboratory test results depends on among other factors on the performance of equipment. The proper functioning of equipment needs careful operation and preventive maintenance. Early detection of malfunctions and appropriate corrective measures will prevent unexpected costs, breakdown of services, deterioration of quality and credibility of the laboratory. The purpose of this manual is to address some of the fore mentioned issues and offer guidelines for achieving greater levels of quality and functionality in selection, operation and maintenance of laboratory equipment.

GENERAL INTRODUCTION

The first step in optimal use of equipment is the appropriate selection of tests to suit the needs of the population served by the clinical chemistry laboratory. To ensure optimal use of a piece of equipment a maintenance programme should be introduced, and aid to this programme is the preparation of the manual for maintenance of equipment. The equipment provided must be appropriate to the test to be performed and be in good working order. Staff, operating equipment should be competent to do so and may be required to demonstrate this competence. Operating manuals for equipment should be readily available and staff handling such equipment must be able to check the critical operating characteristics and should do so at intervals appropriate to the equipment and its work load. Regular service checks, records of calibration, preventive care and maintenance of each item should be kept for the life of the equipment.
In this manual the maintenance of commonly available equipment in a clinical chemistry laboratory are included. Each equipment is described with regard to the principle, operating procedure and maintenance. Practical and simple calibration procedures for relevant equipment are included. However the operating manual should be consulted for specific requirements for each equipment.

Service manual should be available only to be used by competent biomedical staff. The main objective of this manual is to provide guidelines to identify the faulty performances of the equipment by the technical staff and identification of such faults in early stages will prevent major repair costs. Rectification of the faulty performances should be carried out by the qualified biomedical engineering staff.

A comprehensive training should be provided to the technical staff when purchasing expensive laboratory equipment. It is recommended that pre marketing and post marketing evaluations be performed on each equipment at the time of purchasing. A service agreement at least for five years should be established at the end of the guarantee period.

The manual contains guidelines for writing up specifications for common equipment that are used in the laboratory. The specific, critical requirements are highlighted for each equipment. The procedure for assessment of quotations supplied by the local agents is included in this manual to guide the laboratory staff to obtain the cost effective, best equipment. A format of a maintenance log book for critical equipment is also introduced.

1. GUIDELINES FOR SELECTION OF LABORATORY EQUIPMENT

1. Consider the requirement of the equipment

TO UPGRADE AN EXISTING LABORATORY
Introduce a new test
Replace non functioning or inadequate equipment
Improve performance of an existing test

TO ESTABLISH A NEW LABORATORY
To determine the type of tests required, consult the clinical staff and other laboratory users. The tests should be relevant, cost effective and reliable. Consult the health authorities regarding the local requirement, funding and other factors (personnel, equipment, reagents, consumables, and training of staff) which contribute to an uninterrupted service.

2. Analyse in detail the conditions and resources:

FUNDS:
Government funds through the ministry of health
Donor agency (donor requirements should be compatible with the policies of the government

INFRASTRUCTURE FACILITIES:
Buildings and space
Power supply/generator facility
Water supply
Drainage and disposal of waste

RESOURCES / ENVIRONMENTAL CONDITIONS
Availability of reagents, consumables and stationary
Conditions of the existing equipment:
Comparability
Reliability
Function
Requirements of the new equipment with regard to maintenance, availability of spare parts and back up system
Availability of personnel and technical training
Supervision of the performance of the equipment and service
Type of sample and mode of transport to the laboratory

3. A systematic, comprehensive cost analysis is recommended on yearly basis for a given test over a given instrument considering the following factors.
   Workload
   Acquisition costs (capital investment: purchase, lease or rental)
   Man power needs and costs
   Preventive maintenance costs
   Reagents and disposable items costs
   Service contract costs
   Cost adjustments: effect of inflation on reagent and disposable item costs

4. Organize meetings with health authorities, clinicians and laboratory staff regarding the relevant factors for each category

5. A comprehensive technical analysis of the instrument should be carried out with the assistance of relevant technical experts.

DURABILITY IN LOCAL CONDITIONS

The instrument should withstand the local environmental conditions such as humidity, dust, drought, low or high temperatures. The electronics of the instrument should be protected against humidity during the manufacturing process.

ENVIRONMENTAL CONDITIONS

Some instruments need special environmental conditions such as an air conditioned area.

LOCATION OF THE EQUIPMENT

Availability of the building/floor/ bench area with regards to the suitability and safety should be considered.
Any other physical requirement recommended by the manufacturer.

SAFETY OF THE INSTRUMENT

Consider the risks and dangers associated with the operation of the equipment to the technical staff and local community.
Consider the safety requirements which are mandatory during operation of the equipment
The manufacturer should be able to install and provide advice on maintenance to ensure safe performance.

POWER, GAS AND WATER SUPPLY

POWER SUPPLY: Availability of constant and reliable power supply should be considered.
The power requirements of the equipment in relation to the availability of electricity to the laboratory
A voltage stabilizer/uninterrupted power supply should be purchased with the equipment.
If the equipment is battery powered, the type of battery, rechargeable/ non- rechargeable and replacement should be considered.

GAS SUPPLY: Type of gas: pipe borne or cylinders (capacity of cylinders): hazardous nature of the gas: flammable gas should be stored out side the building:

WATER SUPPLY: Type of water available (hard/soft): Special requirements of water for the instrument (Type 1, 2 reagent grade water): The additional equipment required to produce the specified type of water should be considered. e.g. deionizer and spare parts, consumables

SUSTAINABILITY
The instrument, replacement spare parts, consumables, supplies and reagents should be in the manufacturing line for at least for another 10 years.
The cost of repairs, replacements and consumables should be considered.
During the guarantee period an agreement should be made in writing that the instrument be replaced due to faults in the manufacturing process.
A service agreement should be available for the next five years.

REAGENTS, CONSUMABLES AND DISPOSABLE ITEMS
The instrument may be an open/closed system with regards to reagents. A comprehensive study should be carried out regarding the availability of reagents, costs, and requirements for storage (refrigeration, cold rooms) stability and the mechanism for continuous supply.

ACCESSORIES
The additional requirements for the functioning of the equipment should be considered. (Computer and printer)

TRAINING REQUIREMENT
Consider the adequacy of the level of training and expertise of the laboratory staff and decide on any additional training requirements to operate and maintain the equipment. The installation, operation and service manuals should be provided with the equipment.

SPARE PARTS

The manufacturer should provide a list of essential spare parts and a guide to the life expectancy of the spare parts. The local agent should demonstrate the capacity to service and maintain the equipment.

The number of spare parts that should be ordered depends on the life expectancy of the part, the cost, the ability to fit the part by the local staff or agent and the reliability of storage of the spare part.

The spare parts are expensive and needs to be imported from the manufacturer. There fore maximum relevant information should be given such as manufacturer's name, country of origin, model or type, serial number, a description of the part and the voltage, wattage of electrical parts.

MAINTENANCE

Make a request from the manufacturer/local agent to provide staff training in preventive maintenance and handling of trouble shooting. The local agent should provide immediate remedy to overcome a sudden breakdown of equipment.

Study the manufacturer's guarantee with regards to the period, spare parts, repair and replacement. Any equipment faults due to manufacturing process should be replaced during the warranty period. Study the hidden terms and conditions laid down by the local agent regarding maintenance.

A service agreement should be established at the end of the warranty period. The local agent should specify the services in detail that will be offered during the service contract.

TECHNICAL REQUIREMENTS OF THE INSTRUMENT

The proper selection of the equipment depends on the specifications of the critical elements of the proposed instrument. (e.g. band width of a spectrophotometer is one of the critical elements in deciding on the type of spectrophotometer to be purchased)

All the critical factors should be written when submitting the specifications for an instrument.

A detailed study/survey should be carried out on the instruments available in the market. The local agents should be contacted to obtain the brochures to note the technical details of the equipment. The manufacturers are likely to comment only on the favourable features not the limitations of the instrument. Therefore manufacturer's specifications should not be taken as a guide to define your requirements. Request a list of customers who have purchased similar equipment. You may visit these laboratories to observe the operation, calibration, quality control practices, maintenance and views on after sales service. It is recommended to request a demonstration of the functioning of the equipment in your own setting where possible. (E.g. pipettes, pH meter, balance, spectrophotometer etc) Equipment data specification sheets should be prepared by the laboratory and submitted at the requisitioning

to ensure that the purchasing officer can justify the expenditure and has sufficient information to buy the items correctly.

IN GENERAL A SPECIFICATION SHEET INCLUDES THE FOLLOWING:

Name of the Institution
Description of the service

Name of the equipment
Model (if applicable only)
Value of equipment

Intended use
Technical specifications
Requirements for preventive maintenance
Reagents, consumables, disposable items
Guarantee period
Availability of spare parts
Service contract
Power/gas/water requirement
Voltage stabilizer/UPS
Training requirement
The unit price/total price with and without taxes should be requested from the local agents
Include the phrase "complete functioning unit of ……." in the specifications so that the local agent is required to mention any other requirements and costs for such facilities.

2. EVALUATION OF A QUOTATION FOR EQUIPMENT IN A LABORATORY

A request should be made to the local supplier/manufacturer to submit a quotation for the goods or services. At least three such requests should be made, in order to obtain the best equipment for the best price. A comprehensive data specification sheet should accompany such a request. The supplier will prepare the document providing the details of equipment and the prices. The end users will evaluate the quotations. The evaluation procedures may differ depending on your local requirements. The following procedure is recommended.

- Appoint a team for the evaluation. The team should consist of members of staff who are competent in technical procedures. The person who intends to operate the equipment should be included in the team. The chairperson should ensure that the confidentiality of the process is maintained by all the members.

- Each member of the team is responsible for the final decision. Preferably each member should be given copies of the documents to evaluate individually. This will provide the opportunity to study the quotations thoroughly to make the correct selection. Then the team should meet with the chairperson and a final selection should be made on credible scientific basis.(All the copies of the documents should be returned to the head of the institution along with the evaluation report)

- Write down each feature of the specifications of the instrument and match with the features given in the quotations. Include the prices clearly. Make the best selections with regards to suitability, reliability timely delivery and cost. Once the technical validation is fulfilled consider the cost of the equipment and other services.

- It is important to consider the availability and reliability of the after sales service.

- You should justify your acceptance and rejection. You may be required to give your valid reasons to reject the low cost equipment and accept the one with a higher price.

At this point one cannot alter/add/delete the specifications and requirements.

- If none is satisfied you may be able to request fresh quotations from different suppliers.

- The evaluation report should be submitted by placing the date and signatures of the evaluation team.

- A copy should be retained in the laboratory for future references and to ensure the delivery of the selected equipment and services. The head of the unit or the chairperson should ensure the confidentiality of the retained copy.

- A tender is also a request for costs. Generally a tender is for a large order, over a certain value. Therefore strict procedures are adhered in evaluating the tenders.

- Both processes will be subjected to annual audit by the government and all records should be available for inspection.

- Therefore you are required to have a sound knowledge of the local procedures (country, province, institution,) pertaining to evaluation of equipment.

- An example of data evaluation sheet for a quotation is given below

DATA EVALUATION SHEET FOR A QUOTATION

Quotation reference number
Name of the unit/hospital (*Dept. of Biochemistry,M.R.I)*
Evaluation of quotations submitted for (*name of the equipment)*
Date of receipt of quotations
Date of submission of the evaluation report

Members of the evaluation team Signature

1.
2.
3.

	Quotation 1	Quotation 2	Quotation 3
Name of the item			
Purpose			
Model			
Manufacturer			
Country of origin			
Assembled Manufacturer/local			
Local agent			
Critical technical feature 1			
2			
3			
General requirement 1			
2			
3			
Accessories			
Spare parts			
Warranty			
Service contract			
Availability of manuals			
Training			
Maintenance			

	Quotation 1	Quotation 2	Quotation 3
Unit price			
Total price			
Unit price with taxes			
Total price with taxes			
Costs in the ascending order	2 higher price	1 lowest price	3 highest price
Technical requirements Satisfactory/ unsatisfactory			
General requirements Satisfactory/unsatisfactory			
Accepted/rejected			
Reasons			

Comments:

GUIDELINES TO BE FOLLOWED ON RECEIPT OF NEW EQUPIMENT IN THE LABORATORY

- The supplier should inform the laboratory the expected date and time of the delivery of goods. The delivery should preferably be done on a working day of the week, few hours before the closure of the laboratory.

- Follow the institutional rules/regulations regarding the receipt of laboratory equipment. Documentation at the stores/office should be made before the item is delivered to the laboratory.

- Inspect the packing for any damages in the presence of the representative of the local agent. If any damages are suspected, notify the local agent and relevant authorities.

- If the packing is intact and no damage to the equipment is anticipated, unpack the equipment carefully. Retain all the packaging, labels, supports and booklets.

- Check the instrument for damages. If it appears damaged inform the supplier in writing through the head of the unit and institution.

- Request the technical staff of the local agent to assemble and to perform a "test run" using the installation and operation manuals.

- Check whether the delivered instrument meets the specifications of the quotation. Any discrepancies should be notified to the supplier through the head of the unit and institution. All the operational procedures should be stopped until the acquisition of the correct instrument.

- If the correct instrument is delivered read and follow the installation, operational and service manuals.

- The technical representative of the local agent should be able to demonstrate a satisfactory test run.

- The training of the technical staff of the laboratory should be carried out under the supervision of the technical staff of the company.

- Follow the relevant procedure for equipment evaluation.(e.g. a spectrophotometer should be calibrated and test run of quality control material for each test should be performed: A balance should be calibrated for weighing)

- Check whether the other requirements are fulfilled according to the quotation.(supply of accessories, spare parts, consumables, reagents)

- Read the conditions of the warranty.

- Once the user is satisfied with the performance of the equipment and other relevant conditions, approval for payments should be given to the head of the institution through the head of the unit.

- The inventory holder of the laboratory should make the documentation in the inventory and the inventory number should be clearly written and pasted on to the instrument.

- Maintain the log book with regards to the conditions at installation and preventive maintenance. (e.g.; records of calibration) Any repairs and replacements should be entered in the book.

- A separate record should be maintained to include the entries of the users. (Name of the user, date, time and trouble shooting and corrective measures should be recorded.)

- A standard operating procedure for the equipment should be prepared and be readily available for reference by the users.

- The operational procedure in brief should be available at the bench/site of the instrument.

- Only the authorised trained personnel should use the instrument. Any other persons should obtain permission from the inventory holder and should operate the instrument under the supervision of trained personnel.

3. STANDARD OPERATING PROCEDURES FOR EQUIPMENT AND INSTRUMENTS

Operating manuals for equipment should be readily available and staff handling such equipment must be able to check the critical operating characteristics and should do so at intervals appropriate to the equipment and its workload.

1. Name of the instrument
2. Purpose
3. Principle - A brief description of the principle is sufficient.
4. Specimen type
5. Operating procedure - A stepwise detailed procedure is required.
6. Any special precautions to be observed should be clearly indicated
7. Special safety considerations
8. Procedure for preventive maintenance
9. Job assignments and personnel for maintenance.
10. Surveillance of maintenance procedures.
11. Service requirements
12. Service intervals and remainder system.
13. Authorized personnel for operation and maintenance.
14. The SOP for operating procedure should be retained in a master file with a copy at the site of the instrument.
15. The SOP should be authorized by a technical staff member and the head of the unit along with the signatures and date. Any amendments should be carried out only by the above personnel, again with signature and date.

THE MAINTENANCE LOG BOOK

1. Name of the instrument
2. Model
3. Serial number
4. Inventory number should be pasted on the instrument.
5. Date of purchase
6. Manufacturer
7. local agent – address/ telephone /email
8. Performance of the instrument at installation
9. Preventive maintenance records (daily, monthly and 6 month intervals)
10. Supervisor's observation of preventive maintenance
11. Repair or replacement dates
12. Nature of the repair
13. Records of calibration at installation and follow up

DATASHEET TO BE USED BY THE SUPERVISOR

Name of the Institution				
Description				
Type of Instrument				
Name of the equipment				
Model				
Value of equipment				
Country of manufacture				
Date of manufacture				
Name of manufacturer				
Agent / Local Agent				
Contact Number				
Date of receipt				
Date of installation				
Original condition				
Current condition				
No. of repairs				
Last date of repair				
Nature of repair / cost				
Availability of manual				
Tests performed by the equipment				
Evaluation of performance				
Infrastructure required				

Maintained by:

4. SPECTROPHOTOMETER

The clinical chemistry determinations are based on measurements of light absorption. The photometer and spectrophotometer are optical instruments for the measurement of light absorption.

BEER – LAMBERT'S LAW

The Beer-Lambert's law states that when a monochromatic light traverses a solution, the concentration of the dissolved substance is directly proportional to the amount of radiant energy absorbed or inversely proportional to the logarithm of the transmitted radiant energy. If the concentration of a solution is constant and the path length through the solution that the light must traverse is doubled, the effect on the absorbance is the same as doubling the concentration, since twice as many absorbing molecules are now present in the radiant energy path. Thus the absorbance is also directly proportional to the path length of the radiant energy through the cell (cuvette).

The mathematical relationship that connects absorbance of radiant energy, concentration of a solution and path length is shown by Beer – Lambert law

$$A = abc$$

A is absorbance; *a*, absorptivity; *b*, light path of the solution in centimetres and *c*, concentration of the substance of interest.

The equation forms the basis of quantitative analysis by absorption photometry. Absorbance values have no units. The absorptivity is proportionality constant related to the chemical nature of a solute and has units that are reciprocal of those for *b* and *c*.

When *c* is expressed in moles per litre and *b* is expressed in centimetres, the symbol ε called the molar absorptivity, is used in place of *a* and is a constant for a given compound at a given wavelength under specified conditions of solvent, pH, temperature and so on. It has units of L/mole.cm. The higher the molar absorptivity, the higher is the absorbance for the same mass concentration of two compounds. Therefore in selecting a chromogen for spectrophotometric methods, one should use the chromogen with a higher molar absorptivity, which will impart a greater sensitivity to the measurement.

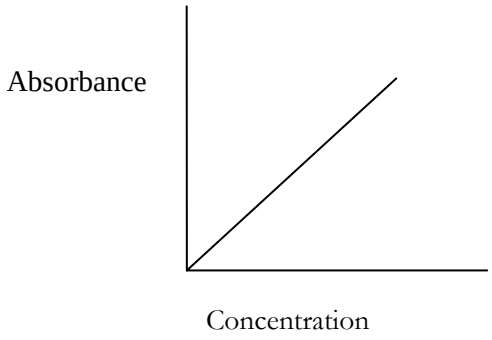
Once a chromogen is proved to follow Beer –Lambert's law at a specific wavelength, that is a linear plot of absorbance versus concentration, with a zero intercept, the concentration of an unknown solution can be determined by measurement of its absorbance and interpolation of its concentration from the graph of the standards. In contrast, when % of transmittance is plotted versus concentration [on liner graph paper], a curvilinear relationship is obtained. Because of the liner relationship between absorbance and concentration it is

possible to relate unknown concentrations to a single standard by a simple proportional equation. Therefore

$$\frac{\text{Absorbance of the standard}}{\text{Absorbance of the unknown solution}} = \frac{\text{Concentration of the standard}}{\text{Concentration of the unknown solution}}$$

$$\text{Concentration of the unknown solution} = \frac{\text{Absorbance of the unknown solution} \times \text{Concentration of the Standard}}{\text{Absorbance of the standard}}$$

The above equation is valid only if the chromogen obeys the Beer's law and both standard and unknown are measured in the same cell. The concentration range over which a chromogen obeys Beer –Lambert's law must be determined for each set of analytical conditions. A calibration graph is drawn with the absorbance versus concentration.



Relationship of absorbance to concentration

Beer-Lambert's law is an ideal mathematical relationship that contains several limitations. Deviations from Beer's law that is variations from the linearity of the absorbance versus concentration curve occur when

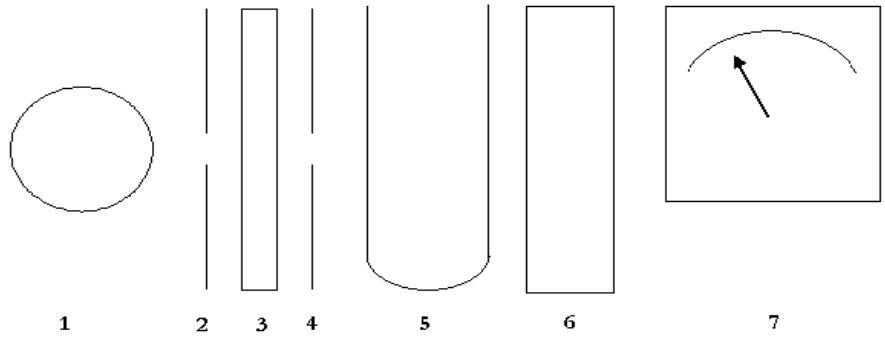
1. Highly elevated concentrations are measured.
2. Incident radiant energy is not monochromatic
3. The solvent absorption is significant compared with the solute absorbance
4. Radiant energy is transmitted by other mechanisms [stray light].
5. The sides of the cell are not parallel.

If two or more chemical species are absorbing the wavelength of incident radiant energy, each with a different absorptivity or if the absorbance of a fluorescent solution is being measured Beer- Lambert's law will not be followed.

4.1 INSTRUMENTATION

PHOTOMETER AND SPECTROPHOTOMETER

The major components of a spectrophotometer are shown in the diagram below. The apparatus can be divided into seven basic components;[1] a stable source of radiant energy, [2] an entrance slit to focus the light ,[3]a wavelength selector[4]an exit slit to focus the light [5] a device to hold the transparent container[cuvette] , which contains the solution to be measured ,[6]a radiant energy detector. [7] A device to read the electrical signal generated by the detector.



If a filter is used as the wavelength selector, monochromatic light at only discrete wavelength is available, and the instrument is called a **photometer**. If a monochromator is used (a prism or grating) as the wavelength selector, the instrument can provide monochromatic light over a continuous range of wavelengths and is called a **spectrometer** or **spectrophotometer.** Spectrophotometers can be **single beam** instruments with a single cuvette holder or **double –beam** instruments with two cuvette holders, one for the sample and the other for the blank; or reference sample .Advantages of the double –beam instrument include the capability of making simultaneous corrections for changes in light intensity, grating efficiency, slit width variation and it is particularly useful for obtaining spectral curves.

SOURCES OF RADIANT ENERGY

Tungsten – filament lamp is used as the source of a continuous spectrum of radiant energy from 360 to 950 nm, Tungsten iodide lamps are often used as sources of visible and near ultraviolet radiant energy. The tungsten halide filaments are longer lasting, produce more light at shorter wavelengths and emit a higher intensity radiant energy than tungsten filaments do. Hydrogen and deuterium discharge lamps emit a continuous spectrum and are used for the ultraviolet region of the spectrum [220 to 360 nm.] The deuterium lamp has more intensity than the hydrogen lamp does. The amount of light emitted from a light source is not constant over a continuous range of wave lengths. A typical lamp has a complex transmittance spectrum with maxima and minima. One must take care in choosing a lamp for a particular analysis, since the amount of light emitted at the desired wavelength may be too little or too much.

WAVELENGTH SELECTORS

Isolation of the required wavelength or range of wavelengths can be accomplished by use of a filter or monochromator .Filters are the simplest devices, consisting of only a material that selectively transmits the desired wavelengths and absorbs all other wavelengths .In a monochromator radiant energy from the source lamp is dispersed by a **grating or prism** into a spectrum from which the desired wavelength is isolated by mechanical slits.

FILTERS

There are two types of [1] those with selective transmission characteristics including glass and Wratten filters and [2] those based on the principle of interference [interference filters] The Wratten filter consists of coloured gelatin between clear glass plates; glass filters are composed of one or more layers of coloured glass; Both types of filters transmit more radiant energy in some parts of the spectrum than in others.

Interference filters work on a different principle. When radiant energy strikes the thin film, some is reflected from the front surface, but some of the radiant energy that penetrates the film is reflected by the surface on the other side. The latter rays of radiant energy have now travelled farther than the first by a distance two times the film thickness. If the two reflected rays are in phase, their resultant intensity is doubled, whereas, if they are out of phase, they destroy each other. Therefore when white light strikes the film, some reflected wavelengths will be augmented and some destroyed resulting in colours.

MONOCHROMATORS

Monochromators can give a much narrower range of wavelength than filters can and are easily adjustable over a wide spectral range .The dispersing element may be a prism or a grating.

Dispersion by a prism is non linear, becoming less linear at longer wavelengths [over 550 nm]. Therefore to certify wavelength calibration ,one must check three different wavelengths .Prisms give only one order of emerging spectrum and thus provide higher optical efficiency, since the entire incident energy is distributed over the single emerging spectrum. A grating consists of a large number of parallel equally spaced lines ruled on a surface .Dispersion by a grating is linear, therefore only two different wavelengths must be checked to certify the wavelength accuracy.

BAND PASS

Except for laser optical devices, the light obtained by a wavelength selector is not truly monochromatic [that is of a single wavelength] but consist of a range of wavelengths. The degree of monochromicity is defined by the following terms. Band pass is that range of wavelengths that passes through the exit slit of the wavelength-selecting device. The nominal wavelength of this light beam is the wavelength at which the peak intensity of light occurs. For a wavelength selector such as a filter or a monochromator whose entrance and exit slits are of equal

width, the nominal wavelength is the middle wavelength of the emerging spectrum.

The range of wavelengths obtained by a filter producing a symmetrical spectrum is usually noted by its half-band width [or half- band pass]. This describes the wavelengths obtained between the two sides of the transmittance spectrum at a transmittance equal to one half the peak transmittance. For monochromators the degree of monochromicity is described by the nominal band width, which corresponds to those wavelengths that are cantered about the peak wavelengths and transmit 75% of the total radiant energy present in the emerging beam of light. For monochromators with variable exit slits, the band pass will also vary.

SLITS

There are two types of slits present in monochromators. The first, at the entrance, focuses the light on the grating or prism where it can be dispersed with a minimum of stray light .The second slit at the exit, determines the band width of light that will be selected from the dispersed spectrum. By increasing the width of the exit slit, the band width of the emerging light is broadened with a resultant increase in energy intensity but a decrease in spectral purity. In diffraction-grating monochromators the exit slit may be of fixed width, resulting a constant band pass .In contrast prism monochromators have variable exit slits. The purpose of both slits in filter photometers is to make the light parallel and reduce stray radiation.

CUVETTES

The receptacle in which a sample is placed for spectrophotometric or photometric measurement is called a cuvette or cell. Glass cuvettes are satisfactory for use in the range of 320 to 950 nm For measurements below 320 nm it is necessary to use quartz[silica]cells .Such cells can be used at higher wavelengths also. Cuvettes with a square cross section and with a circular cross section [that is test tubes]are available, Greater accuracy is achieved by square cuvettes with parallel sides made of optical glass. Although cuvettes usually have internal dimensions[that is path lengths] of 1 cm, cuvettes with other dimensions are available. Macro cuvettes (with 1 cm path length and 2 ml volume), micro cuvettes (with 1 cm path length and 1 ml volume) are used in clinical chemistry determinations.

DETECTORS

BARRIER LAYER [PHOTOVOLTAIC] CELLS.

Barrier layer cells are detectors consisting of a plate of copper or iron on which is a semi conducting layer of cuprous oxide or selenium is placed. This layer is covered by a light transmitting layer of metal that serves as a collector electrode. As illumination passes through the transparent electrode to the semi conducting layer an electron flow is induced in the semi conducting layer and this flow can be sensed by an ammeter, These detectors are rugged relatively inexpensive and sensitive from the ultraviolet region up to about 1000 nm No external power is required and the photocurrent produced is essentially directly proportional to the radiant energy intensity. Barrier layer cells exhibit the fatigue effect which means that on illumination, the current rises above the apparent equilibrium value and then gradually decreases.

PHOTOMULTIPLIER TUBES

A photomultiplier tube is an electron tube that is capable of significantly amplifying a current. The cathode is made of a light-sensitive metal that can absorb radiant energy and emit electrons in proportion to the radiant energy that strikes the surface of the light sensitive metal. These surfaces vary in their response to light of different energies [wavelengths] and so also in the sensitivity of the photomultiplier tube. The electrons produced by the first stage go to a secondary surface, where each electron produces between four and six additional electrons .Each of the electrons from the second stage goes on to another stage, again producing four to six electrons. As many as 15 stages [or dynodes] are present in today's photomultiplier tubes. Photomultiplier tubes have rapid response times. Do not show as much fatigue as other detection and are very sensitive.

PHOTODIODE

Photodiodes are semiconductors that change their charged voltage [usually 5 V] upon being struck by light. The voltage change is converted to current and is measured A photodiode array is a two dimensional matrix composed of hundreds of thin semiconductors spaced very closely together. Light from the instrument is dispersed by either a grating or prism onto the photodiode array. Each position or diode on the array is calibrated to correspond to a specific wavelength. Each diode is scanned and the resultant electronic change is calculated to be proportional to absorption. The entire spectrum is essentially recorded within milliseconds.

4.2 PERFORMANCE OF THE INSTRUMENT

The sensitivity of response of a spectrophotometer is a combination of lamp output, efficiency of the filter or monochromator in the transmission of light, and response of the photomultiplier. As these factors are all functions of wavelength it is clear that the instrument must be reset when one changes wavelengths. This resetting most often takes the form of adjustment of the blank solution to read zero absorbance by changing the photomultiplier gain.

SELECTION OF OPTIMUM CONDITIONS AND LIMITATIONS

When one is establishing a new spectrophotometric procedure it is important to record the absorption spectrum of the material in relation to either water or a reagent blank, depending on the actual method of analysis. The optimum wavelength for a specific analysis will depend on several factors, including the absorption maxima of the chromogen, the slope of the absorption peak and the absorption spectra of possible interfering chromogens. As a general rule for wavelength selections are based on three criteria. (1) Choose an absorption peak with the greatest possible molar absorptivity (2) Choose a relatively broad peak (3) Choose a peak that is as far as possible from the absorption peaks of commonly interfering chromogens.

4.3 CALIBRATION OF SPECTROPHOTOMETER (A practical and simple method)

4.3.1 CALIBRATION OF SPECTROPHOTOMETER – UV RANGE

Wavelength and Photometric Checks Using Liquid Solutions of Substances with known Absorption Characteristics - Acid Potassium Dichromate

REAGENTS

1. Sulphuric acid 5 mmol/L

Calculation of volume of concentrated sulphuric acid to be added.

(Molecular weight = 98.08 g, Specific Gravity =1.84)

1 M solution	= 98.08 g/L
1mmol	= 0.09808 g/L
5mmol	= 0.4904 g/L
Weight of 1 ml	= 1.84 g
Required volume (ml) of conc. sulphuric acid	= $\frac{0.4904}{1.84}$ = 0.2665 = 0.27 ml

Add about 800 ml of distilled water in to a 1 litre beaker Keep the beaker in a basin of water. Carefully add 0.27 ml of conc. sulphuric acid into the beaker. Allow it to cool Mix and transfer in to a 1 litre volumetric flask Mix well. Adjust the final volume to 1 litre. Mix well. Transfer into a brown bottle. (Observe the precautions in handling corrosive acids)

2. Potassium dichromate, 50 mg/L (The analytical grade chemical is recommended. Good quality general purpose reagents from a reputed manufacturer may be used if the analytical grade is not available.).

Dry a portion of Potassium dichromate in a hot air oven at 80 to 90 ^{0}C for 3 to 4 h and then cool in a desiccator. Carefully weigh out 50 mg and transfer quantitatively with 5mmol/L sulphuric acid to a well washed 1 Litre volumetric flask. Make up to the mark with sulphuric acid and mix thoroughly. This solution is stable for a year but may show layering. Therefore mix thoroughly before use if it has been standing for a time Thoroughly wash two silica cuvettes and check that they are matched when filled with sulphuric acid. If not it is preferable to check further cuvettes until a matched pair is found but if this is not possible it will not affect the wavelength check. An accurate absorbance assessment can be obtained by reversing the solutions in the cuvettes and repeating the readings as indicated below

WAVELENGTH CHECK

Rinse one cuvette with the dichromate solution, refill and read against the acid blank at 5 nm intervals from 370 nm downwards. From 355 nm read at 1 nm intervals to define the flat peak stretching from 352 to 348 nm. Extend the interval until 260 nm is reached and then again read at 1 nm intervals to 255 nm to identify the second peak.

Expected result : The solution should show peaks at 350 and 257 nm

ABSORBANCE (EXTINCTION) CHECK

Having established that the wavelength characteristics are correct, take three absorbance readings against the blank at each peak wavelength (350 and 257 nm) zeroing the instrument each time with the acid solution (If a null-point reading is made, move the absorbance setting away from its previous position initially) Rinse the cells and reverse the solutions, repeat the readings in triplicate and take the mean of all six readings for each peak. This eliminates errors due to unmatched cuvettes if these were not matched originally.

Expected result: The mean absorbance should be 0.535 ± 0.005 at 350 nm and 0.720 at 257 nm.

LINEARITY CHECK

Potassium Dichromate solution 200mg/L

Carefully weight out 200 mg of dried potassium dichromate [as given above] and transfer quantitatively with sulphuric acid in to a well-washed 1 litre volumetric flask Make up to the mark with sulphuric acid and mix thoroughly.

Sulphuric acid 5 mmol/L

Dilute 200 mg/L potassium dichromate solution with sulphuric acid 5 mmol/l according to the table given below:

200 mg/L Potassium Dichromate	5 mmol/L Sulphuric Acid	Concentration
1.0ml	9.0ml	20 mg/L
3.0ml	7.0ml	60 mg/L
5.0ml	5.0ml	100 mg/L
7.0ml	3.0ml	140 mg/L
9.0ml	1.0ml	180 mg/L

Take the spectrophotometer readings at 350 nm against the acid blank. Plot the absorbance readings against the concentrations on a graph paper and check the linearity.

STRAY LIGHT

Stray light can cause significant departures from Beer's law, with resultant loss of photometric accuracy particularly with higher absorbance values in the UV region. It is defined as unwanted radiation energy sensed at the detector.

MEASUREMENT OF STRAY LIGHT:

Set the wavelength of the spectrophotometer to 340 nm and set the reference or blank [100% transmission)] with distilled water in the sample compartment. Place the cuvette containing Sodium nitrite 5 g /100ml in the cuvette compartment. Sodium nitrite acts as a blocking filter, absorbing all incident radiation at the wavelength selected but transmitting virtually all of the radiation at longer wavelengths. Therefore any transmission recorded at 340 nm will be a direct measurement of the stray light of the instrument.

Expected result: stray light should be < 0. 1%

An instrument malfunction is indicated whenever the amount of stray radiation exceeds 1%

4.3.2 CALIBRATION OF SPECTROPHOTOMETER – VISIBLE RANGE

(A simplified procedure is described, extracted from W.HO /LAB/89.2)

PHOTOMETRIC LINEARITY
EQUIPMENT AND MATERIALS
Spectrophotometer

Matched cuvettes

Centrifuge

HiCN stock solution

Potassium ferricyanide

Potassium cyanide

Potassium dihydrogen Phosphate (KH_2PO4) anhydrous

Carbon tetrachloride

FERRICYANIDE/CYANIDE REAGENT
Weigh and dissolve in 800 ml of distilled or deionised water in a 1 litre volumetric flask: 200 mg of potassium ferricyanide, 50 mg of potassium cyanide (Note: Highly poisonous by ingestion or inhalation; Handle with extreme care), and 140 mg of potassium monobasic phosphate (anhydrous). Add 1 ml of Tween 20. Dilute with deionised or distilled water to 1000 ml. The pH of the reagent should be between 7.0 and 7.4; this should be checked with a pH meter, if possible. The absorbance at 540 nm read against distilled water blank should not exceed 0.002 A; the colour is pale yellow. If the reagent is stored between 4 and 25^0 C in stoppered borosilicate bottles, in the dark, it will keep for at least two months. It should not be frozen.

PREPARATION OF STOCK HiCN
A 0.5 ml aliquot of whole blood with a Hb concentration between 135 and 145 g/l is added to about 20 ml of ferricyanide/cyandide reagent and about 0.5 ml of carbon tetrachloride. Mix well. Stand the solution for an hour with occasional mixing. Divide the solution evenly into two 15 ml centrifuge tubes and centrifuge at 2500 g for 10 minutes. The clear solution is decanted from any solid residue and from CCl_4 into a 100 ml volumetric flask and diluted to the mark with ferricyanide/cyanide reagent. The absorbance measured at 540 nm should lie between 0.450 and 0.500 A; if necessary, the entire solution may be diluted with reagent.

PREPARATION OF HiCN DILUTIONS.

Using 5ml and 10 ml class A, TD ("to deliver") pipettes (15 and 20 ml pipettes may also be used) and ferricyanide reagent as a diluent, prepare dilutions of the above stock HiCN by pipetting the volumes below into separate containers:

Solutions	Stock HiCN/ml	Ferricyanide Reagent/ml	Hb% Calibrated Spectro:	Abs of Calibrated Spectro:	Abs of Candidate Spectro:
1	25	-			
2	20	5			
3	15	10			
4	10	15			
5	5	20			

PROCEDURE

- Zero the instrument with the reagent blank(Ferricyanide reagent)
- Measure the Hb concentration and corresponding absorbances of all five solutions from a calibrated spectrophotometer
- Measure the absorbance of each solution from the candidate spectrophotometer
- Plot a graph using the Hb concentration/absorbances obtained from the calibrated spectrophotometer Vs absorbances of the candidate spectrophotometer
- A linear response through the origin is expected.

PHOTOMETRIC PRECISION

A complex procedure available for the determination of imprecision in W.H.O LAB 89.2 (The details of an example available at the Department of Biochemistry.)

SELECTION OF THE WAVELENGTH/FILTER

- Analyse one mid-range standard (e.g. 2.5mmol/l standard for calcium) solution and the reagent blank by the given method. Measure the absorbances of these solutions against a distilled water blank, using each wavelength/filter in turn. Note the readings.
- Select the wavelength/filter which gives the largest difference between the standard and reagent blank readings. Establish a linear calibration graph and determine the upper limit of the linearity.

CARE AND MAINTENANCE OF SPECTROPHOTOMETERS

Set the instrument up on a level bench where it will be free from vibrations and not in direct sunlight

Instrument should be protected from dust with a cover.

Manufacturer's instructions should be adhered during installation, operation and maintenance.

Standard operating procedures should be prepared including the use cuvettes, operation of the instrument and waste disposal. Records of maintenance and absorbance readings of blank and standard for each analyte should be documented. A decrease in absorbance readings is one of the early signs of deterioration of lamp energy.

Calibrations are recommended at installation, 6 month intervals and following a repair or replacement (e.g. replacement of a lamp). Any deviations in calibration should be rectified by competent biomedical technical staff.

THE FACTS TO BE CONSIDERED FOR DOCUMENTATION OF SPECIFICATIONS.

Type of instrument (colorimeter/ spectrophotometer) depends on the range of tests done at the laboratory.

Type of spectrophotometer (visible only, uv- visible, with or without recording facility, with or with out temperature control) depends on the type of tests (absorbance, kinetic or scanning measurements)

Band width is an important determinant of the nature of the monochromatic light obtained for measurements.

Accessories (cuvettes, stabilizer/ups) and spare parts (lamps and fuses) should be included.

Available power supply should be documented.

Facilities required during warranty period should be requested. A service agreement should be signed at the end of warranty period.

5. FLAME PHOTOMETER

Flame emission photometry is most commonly used for the quantitative measurement of sodium and potassium in body fluids. Lithium, although present in serum at very low concentrations may also be measured in connection with the therapeutic use of lithium salts in the treatment of some psychiatric disorders.

Atoms of many metallic elements, when given sufficient energy such as that supplied by a hot flame, will emit this energy at wavelengths characteristic for the element. A specific amount or quantum of thermal energy is absorbed by an orbital electron. The electrons, being unstable in this high-energy (exited) state, release their excess energy as photons of a particular wavelength as they change from the excited to their previous or ground state. If the energy is dissipated as light, the light may consist of one or more than one energy level and therefore of different wavelengths. These line spectra are characteristic for each element. Sodium, for example, emits energy primarily at 589 nm, along with other; much less intense emissions (refer the figure below.)). The wavelength to be used for the measurement of an element depends on the selection of a line of sufficient intensity to provide adequate sensitivity as well as freedom from other interfering lines at or near the selected wavelength.

Alkali metals are comparatively easy to excite in the flame of an ordinary laboratory burner. Lithium produces a red, sodium, a yellow, potassium a violet, rubidium a red, and magnesium a blue color in a flame. These colors are characteristic of the metal atoms that are present as cations in solution. Under constant and controlled conditions, the light intensity of the characteristic wavelength produced by each of the atoms is directly proportional to the number of atoms that are emitting energy, which in turn is directly proportional to the concentration of the substance of interest in the sample. Thus, flame photometry lends itself well to direct concentration measurement of some metals.
Other cations, such as calcium, are less easily excited in the ordinary flame. In these cases, the amount of light given off may not always provide adequate sensitivity for analysis by flame emission methods. The sensitivity can be improved slightly by using higher-temperature flames. Of the more easily excited alkali metals like sodium, only 1 to 5 % of those atoms present in solution become excited in a flame. Even with this small percentage of excited atoms, the method has adequate sensitivity for measurement of alkali metals for most bio analytical applications. Most other metal ions are not as easily excited in a flame, and flame emission methods are not as applicable to their measurement.

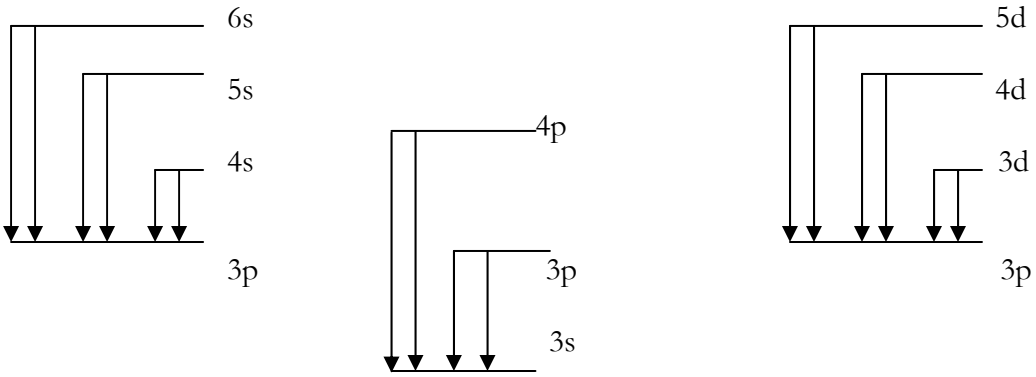


Figure 1

Schematic diagram showing energy levels for certain lines of the sodium spectrum. The major doublet at 589 nm (shown in heavy lines) results when the excited valence electron returns from the 3p orbital to the ground state 3s orbital

5.1 COMPONENTS OF FLAME PHOTOMETERS

Figure 2 shows a schematic diagram of the basic parts of a flame photometer. A cylinder of compressed gas and a two-stage pressure regulator are required. High-pressure tubing must be used to lead the gases to the flame. An atomizer is needed to spray the sample as fine droplets into the flame. The monochromator, entrance and exit slits, and detectors are similar to those discussed previously for spectrophotometers. In effect, the light source for the spectrophotometer has been replaced with an atomizer-flame combination, and one is measuring emission of light rather than absorption.

Various combinations of gasses and oxidants have been proposed and are being used in flame photometry. These include acetylene and oxygen for the hottest flame, and natural gas, acetylene, and propane in combination with either oxygen or compressed air. The choice of flame depends largely on the temperature desired; for sodium and potassium determinations, a propane- compressed air flame appears entirely adequate. Typical flame temperatures are shown in the table below

The atomizer and the flame are critical components in a flame photometer. The atomizer provides a means of drawing the sample through the aspirator and converting it into a fine mist, which then enters the flame. This can be done by passing a gas of high velocity over the upper outlet of a capillary tube, the lower end of which is inserted into the sample. Liquid is then drawn up into a chamber and dispersed into small droplets. The larger droplets settle to the bottom and go to waste. The most important variable in the flame itself is the temperature. Frequent calibration of flame photometers is essential because thermal changes do occur and affect the response of the instrument. In addition, temperature changes affect the output of photocell detectors; for this reason, a period of warm-up, with aspiration of distilled/ deionised water and calibrators, is required before measurements are taken, in order to establish thermal equilibrium for the flame and the atomizer chamber.

Ideally, monochromators in flame photometers should be of higher quality than those found in absorption spectrophotometers. When nonionic materials are burned, light of varying wavelength is given off. This is known as *continuous emission* and will be added to the *line emission* of the element being measured. For this reason, the narrowest band path that is achievable should be used to eliminate as much of the extraneous, continuous emission as possible, but still permit a maximum of the line emission to pass through to the detector. The detectors used in flame photometers operate by the same principle and in the same way as those described for spectrophotometers.

In recent years, the argon inductively coupled plasma (ICP) torch has become commercially available as an excitation source for emission spectrophotometry. With this source, argon ions are inductively coupled to a radiofrequency generator that serves as the means to excite ions and molecules to energy states that will produce light emission.

A common design involves three concentric glass or quartz tubes mounted with a radiofrequency (RF) coil wrapped around the outermost tube. Liquid sample is aspirated up the innermost tube. With the middle tube containing the argon gas fed in an upward direction. An RF generator is set to produce a frequency at 27 MHz. At this frequency, the argon gas is ionized, and the electron rich plasma in the outermost tube inductively reacts with the magnetic field created by the RF coil. These reactions produce a flame like torch that forms near the top of the concentric tubes. The temperature in the flame reaches 5000 to 9000 ^{0} C and allows dissociation of many of the chemical complexes that cause inaccuracies in flame photometry. This transfer of energy into the sample then allows many of the chemical species in the torch to lose energy in the form of emitted light and provide high resolution of the emitted lines in a quality spectrometer.

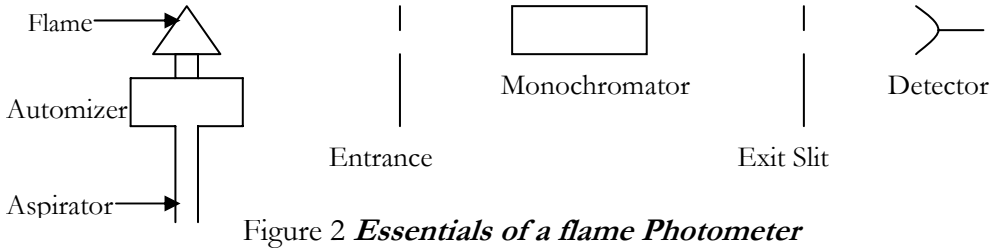


Figure 2 ***Essentials of a flame Photometer***

Flame Temperatures for Various Gas Mixtures

Gas Mixture	Flame Temperature ^{0}C
Natural gas – air	1840
Propane – air	1925
Hydrogen – air	2115
Acetylene – air	2250
Hydrogen – Oxygen	2700
Natural gas – Oxygen	2800
Propane – Oxygen	2850
Acetylene - Oxygen	3110

5.2 DIRECT AND INTERNAL STANDARD FLAME PHOTOMETRY

In some of the instruments of earlier designs, calibrating solutions of sodium or potassium were atomized or aspirated directly into the flame to provide a series of meter readings against which an unknown solution could be compared. This approach, referred to as the direct reading method, presents certain problems:

1. Minor fluctuations in air or gas pressure cause unstable response in the instrument and lead to errors
2. Separate analyses and sometimes separate dilutions must be made for sodium and potassium.
3. The potassium signal is enhanced by the sodium concentration in the specimen.

The latter effect known as *mutual excitation*, results from the transfer of energy from an excited sodium atom to a potassium atom. Consequently, more potassium atoms are excited and light emission is increased. Ideally, then the concentration of sodium and potassium in the calibrators should closely approximate those in the unknown, a situation that is difficult to achieve when analyzing a sample such as urine in which these electrolytes show wide variation in concentration.

In the internal standard method, lithium or cesium is added to all calibrators, blanks, and unknowns in equal concentrations. Lithium has a high-emission intensity, is normally absent from biological fluids, and emits at a wavelength sufficiently removed from that of sodium or potassium to permit spectral isolation. The flame photometer makes a comparison of the emission of the desired element (sodium or potassium) with the emission of the reference lithium element. By measuring the ratios of emissions in this way, small variations in atomization rates, flames stability, and solution viscosity are compensated for. Lithium does not function as a calibrator under these conditions but as a reference element. Variable concentrations of sodium and potassium, in the lithium diluent, must be used to establish calibration curves or to verify linearity of response.

Lithium also acts as a radiation buffer to minimize the effects of mutual excitation. The final working concentration of lithium is so high, compared with that of either sodium or potassium, that the same percentage of potassium becomes excited regardless of the sodium concentration in the sample. Serum lithium concentrations in patients receiving lithium salts are maintained at approximately 1 mmol/L. This amount will produce no significant change in final lithium concentrations in samples containing lithium in the diluent.

A setting agent is frequently recommended for inclusion in calibrators and sample dilutions. This minimizes changes in atomizer flow rates due to differences in viscosity of the samples. Viscosity effects are further reduced by diluting samples 100 to 200 fold.

5.3 CALIBRATION

- Chemicals used for calibration should be of analytical grade
- All the glassware should be thoroughly cleaned and finally rinsed with deionised or good quality distilled water.
- Volumetric flask and pipettes should be of grade A

REAGENT PREPARATION

Sodium chloride and Potassium chloride should be dried separately in an oven at 100^0 C for four hours and after drying, the chemicals should be kept in separate desiccators to attain room temperature.

A. Stock Sodium Standard Solution 1000 mmol/L (Stock A)

Weigh out accurately 58.455 g of dried sodium chloride in a beaker and transfer in to a 1 litre volumetric flask with deionised/distilled water. Rinse the beaker with deionised/distilled water and transfer into the volumetric flask. Make sure that all the chemicals are transferred into the flask. Mix well to dissolve the chemical. Finally make up to the mark with deionised/distilled water. Mix well. Transfer in to a clean polypropylene bottle.(Prepare about 200 ml of the solution)

B. Stock Potassium Solution 100 mmol/L (Stock B)

Weigh out accurately 7.456 g of dried potassium chloride in a beaker and transfer in to a 1 litre volumetric flask with deionised/ distilled water. Rinse the beaker with deionised/distilled water and transfer into the volumetric flask. Make sure that all the chemicals are transferred into the flask. Mix well to dissolve the chemical. Finally make up to the mark with deionised/distilled water. Mix well. Transfer in to a clean polypropylene bottle.(Prepare about 100 ml.of the solution)

C. Diluent Concentrate

Dilute 1 ml of 'Corning 460/405 diluent concentrate' to 1000 ml with deionised/distilled water.

CALIBRATION

Preparation of working standard series

Pipette stocks solution A and B into six 100 ml volumetric flasks as follows

	1	**2**	**3**	**4**	**5**	**6**
Sodium concentration mmol/l	110	120	130	140	150	160
Potassium concentration mmol/l	2	3	4	5	6	8
Stock A (ml)	11	12	13	14	15	16
Stock B (ml)	2	3	4	5	6	8
Make up to 100 ml with distilled water. Mix well						

An example of the calculation to prepare 100 ml of working standard solution of Sodium 110 mmol/l and Potassium 2 mmol/l is as follows

C1V1 = C2V2

C1 =1000 mmol/l
C2 =110 mmol/l
V2 =100 ml

1000 x V1 =110 x 100
V1 =11 ml

C1V1 =C2V2

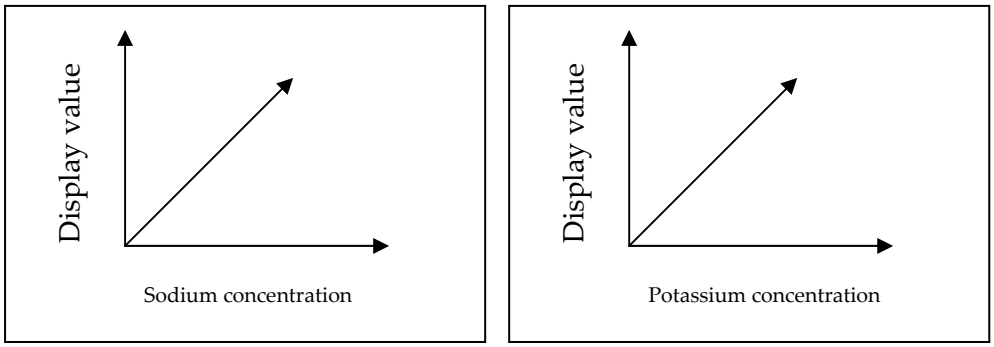
C1 =100 mmol/l
C2 =2 mmol/l
V2 =100 ml

100 x V1 = 2 x 100
V1 = 2 ml

Add 11 ml of stock A solution and 2ml of stock B solution into a 100 ml volumetric flask and make up to 100 ml with deionised/distilled water

PROCEDURE TO PREPARE THE CALIBRATION GRAPH

- 0.1 ml of each working standard should be added to 19.9 ml of diluent in separate containers
- Each diluted working standard solution should be aspirated into the flame photometer for 20 seconds (starting with the lowest concentration to avoid carry over) again allowing 10 seconds between measurement and observe the readings.
- Plot the graph using the concentration Vs readings and observe the linearity.



5.4 OPERATING PROCEDURE

The details of the operation procedure may vary from one instrument to another. Following steps are related to 'Corning 410' clinical model flame photometer, which is available in most hospital laboratories. Follow the manufacturer's manual.

1. ***Sample dilution***: Dilute each serum, quality control sample and working standard solution 1:200 with working diluent concentrate. Into 50 ml conical flasks pipette 19.9 ml of working diluent concentrate and add 0.1 ml of working standard solution/quality control sample/ patient's serum and mix well.
2. Turn on the fuel supply at source
3. Depress the 'power' switch to switch on the instrument 410. The 'power on' LED will be illuminated, the air compressor will start an ignition cycle will commence.
4. If the flame on LED is not illuminated at the end of the ignition cycle, (Refer the operator's manual available with the instrument) Check that the air pressure gauge indicates a reading between 11 and 13 psig. If it does not, lower the air regulator locking ring and adjust the regulator for a reading of 12 psig on the air pressure gauge. Raise the locking ring to lock the air regulator adjuster.
5. Set the filter selector to the required position. Non luminous blue flame with distinct cones can be seen, if does not; adjust the fuel to get distinct blue cone flame.
6. Insert the Nebulizer inlet tube in a beaker containing approximately 100 ml of diluent and allow 15 minutes for the operating temperature to stabilize. This will ensure a stable burner temperature when solutions are aspirated, after the warm up period.

7. While aspirating the diluent, adjust the 'blank' control so that the display reads zero
8. Aspirate a pre diluted standard solution
9. Allow 20 seconds for a stable reading and then adjust 'coarse' and 'fine' controls for a convenient reading (if a 140 mmol/l Sodium standard is aspirated, set the display to 140)
10. Carefully adjust the 'fuel' control for a maximum reading on the display, ensuring that only small adjustments are made, with a pause of several seconds between adjustments.
11. Remove the standard solution, wait 10 seconds, then aspirate a blank solution of diluent for 20 seconds. Adjust the 'blank' control for a zero reading. Remove the blank solution and wait 10 seconds.
12. Repeat steps 8, 9 and 11 until the blank reading is zero (within ± 0.2) and the calibration reading is within ± 1%.
13. Aspirate each of the unknown solutions for 20 seconds, then note the readings in mmol/l
14. Check the calibration frequently
15. When analyzing large batches of samples, recheck instrument calibration every 10 samples with a single standard solution.

NOTE:

- Always use the same batch of diluent for the blank, dilution of samples, quality control material and standards.

- Any difficulty in obtaining a maximum sodium reading should be rectified by opening the inspection flap and adjusting the 'fuel' control until the flame just starts to lift off the burner. Then turn the 'fuel' control back, counter clockwise, until the cones of the flame are on the burner. Close the flap and proceed with paragraph 11.

PRECAUTIONS AND LIMITATIONS:

1. A diluent recommended by the manufacturer of the instrument should be used. Deionised or high quality distilled water should be used to prepare the diluent. Deionised or distilled water must be free from contaminative elements (bacteria or moulds can cause inaccuracies by interrupting or blocking the flow of sample through the nebulizer. Always use the same batch of diluent for the blank and the dilution of samples and standards.
2. Dilute the sera with care. Good quality calibrated pipette or a sensitive diluter must be used. Use the same pipette or dilution equipment for both standards and samples.
3. Accuracy of the results depends on the accuracy and purity of the calibration standard. Always use accurately prepared standards.
4. Both the accuracy and precision of results depends on maintenance and adherence to operating instructions provided by the manufacturer. Careful cleaning of the atomizer-burner, cleanliness of sample containers, the aspirating systems, proper adjustment of flame size, (blue flame with distinct cone) aspiration rate, and geometry of the flame and uniform entry of atomized, diluted sample into the flame are also critical for accuracy and precision. Thermal equilibrium must be established before analysis of unknown samples. Warm up

period is necessary because the initial evaporation of water in the flame decreases the temperature of the burner and the entire burner chamber.

5. Safety: Propane is highly inflammable and potentially explosive and commonly supplied as a liquid under pressure in a cylinder for use with the instrument. Cylinder should never be subjected to heat or mechanical shock. Leakage of propane from the tank, instrument fittings or from valves may be detected with the aid of a soap solution.

6. Site conditions:
   - Never install the flame photometer beneath overhanging cupboards. There must be at least 1 metre of clear space above the chimney.
   - The environment must be clean and free from dust
   - The instrument must be placed on a strong, level work top, free from vibration
   - Avoid the instrument to direct sunlight or draughts

ACCURACY CHECK

- Set the instrument using Corning standard solution (Sodium 140 mmol/l and potassium 5.00 mmol/l)
- Use an assayed (Specimens with stated values) normal and pathological range quality control samples. (process as for patients' samples)
- Observe the readings and calculate the percentage of inaccuracy using the readings

PRECISION CHECK

- Quality Control sample(Sodium around 140 mmol/l and potassium 5.0 mmol/l)or an accurately prepared standard solution is diluted as in the given procedure can be used
- Results obtained from 20 replicates of the same sample, aspirating sample for 20 seconds, then 10 seconds of air.
- Na (140 mmol/l) better than 1.5 % CV
- K (5.0 mmol/l) better than 1.5 % CV

5.5 MAINTENANCE (CIBA-CORNING 410)

GENERAL

The daily, weekly, monthly and six-monthly maintenance should be carried out by the technical staff/biomedical engineering staff...

Maintenance of the air compressor, dilutor and chart recorder, if used, refer the instructions supplied with the equipment.

WARNING

The cleaning of component parts of the instrument such as the nebulizer, mixing chamber, burner parts, drain tubing and drain vessels should be carried out by using tubing disinfectant. The waste should be treated with caution as it may contain pathogenic organisms.

DAILY MAINTENANCE

(PERFORMED BY LABORATORY TECHNICAL STAFF)

Equipment required: **none**

1. Empty waste container, if used.
2. Check air line for condensation, and drain if necessary
3. Check 'U' tube is filled with deionised water.

WEEKLY MAINTENANCE

Equipment required: 10 ml beaker; stop watch; nebulizer cleaning wire; cleaning solution or deproteinizing solution for clinical applications; stainless steel nebulizer tube and sleeve, or polythene inlet tube, as required.

1. Carry out daily maintenance procedure
2. Deproteinising the system
3. Check the operation of the Nebulizer (only by Biomedical Engineering staff)

MONTHLY MAINTENANCE

Equipment required: As for weekly maintenance

1. Carry out daily and weekly maintenance procedures.
2. Check the constant head drain, mixing chamber, 'U' tube and drain tube and clean if necessary. Check that the 'U' tube is refilled with deionised water on reassembly.

SIX-MONTHLY MAINTENANCE (BY BIOMEDICAL ENGINEERING STAFF)

Equipment required: As for weekly maintenance, plus the following; 'U' tube and tube; methanol; tissues; soft lint free cloth; cotton buds.

1. Carry out the daily and weekly Maintenance procedures.
2. Clean the mixing chamber, burner tube, burner and constant head drain
3. Replace stainless steel Nebulizer tube and sleeve, (or polythene Nebulizer tube if fitted), 'U' tube and drain tube.
4. Clean the optical filters and the glass chimney
5. Check air and fuel tubing and connectors for leaks, using a soap solution.

NEBULIZER MAINTENANCE (SHOULD BE CARRIED OUT BY COMPETENT BIOMEDICAL ENGINEERING STAFF)

Equipment required : Stop watch; 10 ml beaker; cleaning wire; cleaning solution or deproteinising solution for clinical applications; stainless steel Nebulizer tube and sleeve, or polythene inlet tube, as required,

OPERATIONAL CHECK

1. Turn off the fuel supply at source.
2. Switch on the 410 (and the air compressor, if it is not connected to the "compressor" socket on the rear of the instrument). Check that the "flame on" LED is off.
3. Fill the beaker with deionised water, and weigh it.
4. Present the 10 ml beaker of deionised water to the Nebulizer tube for an accurately timed minute.
5. Reweigh the beaker and calculate the aspiration rate. If it is between 2 and 6 ml/minute, no further action is required. If it is too low, continue with paragraph 6. If it is too high contact your Corning Distributor. Do not attempt to adjust the Nebulizer, as the capillary position is fixed during manufacture, and is not adjustable.

CLEANING THE NEBULIZER

6. Release the Nebulizer retainer and withdraw the Nebulizer
7. Remove the Nebulizer inlet tube and sleeve, from the Nebulizer. Pass a cleaning wire through the Nebulizer inlet tube and the sleeve.
8. Inspect the end of the Nebulizer capillary and remove any blockages.
9. Insert a cleaning wire into the Nebulizer capillary.
10. Refit the Nebulizer inlet tube and sleeve to the Nebulizer. If difficulty is experienced in cleaning the inlet tube and sleeve, fit new parts. Recheck the aspiration rate, paragraph 3, 4 and 5.
11. If Nebulizer operation is still outside the specification, unscrew the air line connector and remove the Nebulizer inlet tube and sleeve from the Nebulizer.
12. Soak the Nebulizer in a 1 in 100 dilution of cleaning solution or deproteinising solution for clinical applications, agitating it periodically.
13. Rinse thoroughly in deionised water and shake dry.
14. Refit the Nebulizer inlet tube and sleeve and the air line. Recheck aspiration rate.
15. If Nebulizer operation is still unsatisfactory, fit a new Nebulizer. Do not attempt to adjust the Nebulizer, as the capillary position is fixed during manufacture, and is not adjustable.
16. Fit the constant head drain to the spring clip and position it so that the lip on the drain is resting on the spring clip.
17. Connect the fuel tubing to the mixing chamber and cap. Connect the 'U' tube between the mixing chamber end cap and constant head drain. Fit the drain tube to the constant head drain.
18. Fit the Nebulizer into the end cap and position the retainer to lock it.
19. Use a wash bottle to fill the 'U' tube with deionised water. Sufficient water should be used to completely fill the 'U' tube and purge it of air.

CLEANING THE OPTICAL FILTERS AND GLASS CHIMNEY (SHOULD BE CARRIED OUT BY COMPETENT BIOMEDICAL ENGINEERING STAFF)

Equipment required: Soft, lint free cloth; methanol; cotton buds; tissues; cleaning solution.

1. Check that the "flame on" LED is off, fuel is turned off at source, and 410 is switched off.

Warning: Do not proceed until all the parts within the chimney are at a safe handling temperature

2. Lift off the chimney cap from the chimney assembly. Lift out the glass chimney.
3. Move the filter selector to the top position, and push the lever upwards and to the right. This will disengage the filter selector from the stop. Lift the filter selector out of the chimney.
4. Position the filter selector on the lint free cloth so that the colored sides of the filters are uppermost and the thumb grip is at the top, as shown in figure 3

Caution: Handle the optical filters only by the edges, "never" the faces.

5. Using a soft probe, (cotton bud or similar), that will not scratch the filters, push out the first filter. Examine the filter edges and note the wavelength on figure 3, alongside the appropriate space. Remove the remaining two filters and mark the wavelengths on figure 3
6. Carefully wipe each filter with a cotton bud soaked in methanol.
7. Turn the filter selector over so that the large holes and filter retainers are uppermost.
8. Refer to figure 3 and carefully replace the filters, mirrored sides upward, into their respective positions.
9. If a filter is being changed note the new wavelength on figure 3. Also place the self-adhesive label (supplied with the filter) in the correct position over the filter selector label already in place on the front of the chimney assembly. The "top" filter, Na in figure 3, is the "bottom" position on the filter selector label.
10. Refit the filter selector into position inside the chimney and slide it fully in. Check that all three positions can be selected.
11. Soak the glass chimney in a 1 in 100 dilution of cleaning solution, agitating it periodically.
12. Rinse the glass chimney thoroughly in deionised water and dry with clean tissues.
13. Replace the glass chimney "with the clear strip at the bottom". Make sure the glass chimney is seated correctly and refit the chimney cap on top of the chimney.

VOLTAGE SELECTION AND FUSE REPLACEMENT (SHOULD BE CARRIED OUT BY COMPETENT BIOMEDICAL ENGINEERING STAFF))

Equipment required ;fuses.

Warning: For continued protection against fire hazard use only the same type and rating of fuse that was fitted originally to the 410 refer to instrument rear panel.

1. Check that the "flame on" LED is off, fuel is turned off at source, and 410 are switched off.
2. Disconnect the a.c. supply lead form the a.c. supply socket.
3. Disconnect the a.c. supply lead from the "power" connector on the rear panel.
4. Open the voltage selector compartment and withdraw the voltage selector, item 1. To replace fuses continue with paragraph 5; to change the operating voltage continue with paragraph 7.
5. Slide out the two fuse holders, item 2, and remove the fuses.
6. Replace any burned out fuses and slide in the fuse holders.
7. Replace the voltage selector so that the required voltage setting is visible through the window of the compartment cover. Close the compartment cover and refit the a.c supply lead.

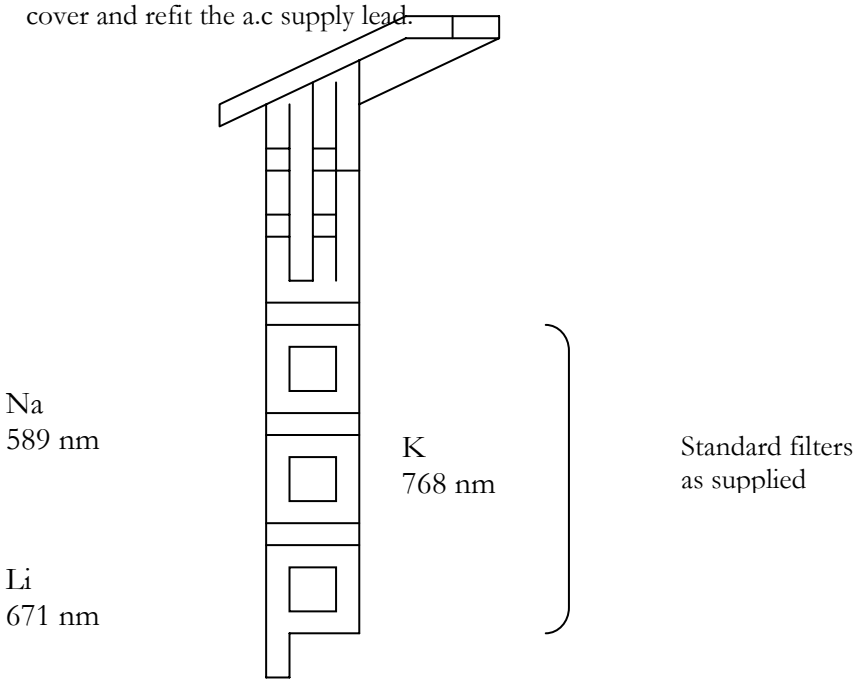


Figure 3

DEPROTEINISING OR DISINFECTING PROCEDURE

NOTE: To deproteinise the system use deproteinising solution, and to disinfect the system use tubing disinfectant.

Equipment required: Deproteinising solution or tubing disinfectant

1. Light the flame
2. Present a beaker of deproteinising solution or tubing disinfectant to the Nebulizer for 10 minutes
3. After 10 minutes have elapsed. Replace the beaker of solution with a beaker of deionised water. Flush the system for two minutes.
4. Shutdown the instrument

6. THE MICROSCOPE

The microscope magnifies the image of objects that are invisible to the unaided human eye

A compound light microscope is the commonest type used in clinical laboratories. It consists of two lens systems to magnify the image. Each lens has a different magnifying power. A compound light microscope may have a single eye piece (monocular) or two eye pieces (binocular)

6.1 TYPES OF MICROSCOPY

LIGHT MICROSCOPY

Uses a beam of light to view specimens

BRIGHT FIELD MICROSCOPY

The field of view is brightly lit so that organisms and other structures are visible against it because of their different densities. Single stain or differential staining may be used depending on the properties of different structures and organisms.

DARK FIELD MICROSCOPY

The field of view is dark and the organisms are illuminated. A special condenser is used which causes light to reflect from the specimen at an angle.

PHASE-CONTRAST MICROSCOPY

Phase- contrast microscopy is a contrast enhancing optical technique that can be utilised to produce high contrast images of transparent specimens, such as living cells, micro organisms and many other structures.
Special condensers and objectives are used to alter the phase relationships of the light passing through the object and that passing around it

FLUORESCENCE MICROSCOPY

In fluorescence microscopy specimens are stained with fluorochromes / flurochrome complexes. Light of high energy or short wavelengths (from halogen lamps or mercury vapour lamps) is then used to excite molecules within the specimen or dye molecule attached to it. These excited molecules emit light of different wavelengths often of brilliant colours.

ELECTRON MICROSCOPY

Electron microscopes use a beam of highly energetic electrons to examine objects on a very fine scale. This examination can yield the following information.
TOPOGRAPHY: Surface features of an object, its texture; direct relation between these features and material properties.
MORPHOLOGY: The shape and size of the particles making up the object, direct relation between these structures and material properties.

COMPOSITION: The elements and compounds that the object is composed of and the relative amounts of them; direct relationship between composition and material properties
CRYSTALLOGRAPHIC INFORMATION: How the atoms are arranged in the object; direct relation between the arrangements and material properties.

6.2 PARTS OF A LIGHT MICROSCOPE

The main parts of the microscope are the eye-pieces, microscope tube, nose piece, objective, mechanical stage, condenser, coarse and fine focusing knobs, and light source.

EYE-PIECES

The specimen is viewed through the eye-piece. It has a lens which magnifies the image formed by the objective. The magnifying power of the eye-piece is in the range 5x - 20x. A movable pointer may be attached to the inside of the eye-piece.

In binocular microscopes, the two eye-pieces can be moved closer or farther apart to adjust for the distance between the eyes by pulling pushing motion or by moving a knurled ring.

MICROSCOPE TUBE

The microscope tube is attached on top of the arm. It can be of the monocular or binocular type. It supports the eye-piece on the upper end.

MECHANICAL TUBE LENGTH

Mechanical tube length is the distance between the place where the objective is inserted and the top of the draw-tube into which the eye-pieces fit

In modern microscopes it is not tubular; it contains prisms that bend the light coming up, thus providing a comfortable viewing angle. In a binocular tube, the light is also split and sent to both eye-pieces.

Do not interchange the objectives of two microscopes if the specified mechanical tube length is different.

NOSE-PIECE

The nose-piece is attached under the arm of the microscope tube. The nose-piece houses the objectives and rotates them. The objectives are arranged in sequential order of their magnifying power, from lower to higher. This helps to prevent the immersion oil from getting onto the intermediate objectives.

OBJECTIVES

The image of the specimen first passes through the objective. Objectives with magnifying powers 4x, 10x, 40x and 100x are commonly used. The magnifying power is marked on the lens and is usually colour-coded for easy identification.

THE 100X OBJECTIVE IS FOR OIL IMMERSION

The numerical aperture (NA) is the measure of light-gathering power of a lens. The NA corresponding to the various magnifying powers of the objective is:

Magnification	Numerical aperture
10x	0.25
40x	0.65
100x	1.25

A high NA indicates a high resolving power and thus useful magnification. To provide the best image at high magnification, immersion oil is placed between the slide and the oil immersion objective (100x). Unlike air, immersion oil has the same refractive index as glass. Therefore, it improves the quality of the image. If immersion oil is not used, the image appears blurred or hazy.

MECHANICAL STAGE

The mechanical stage holds the slide and allows it to be moved to the left, right, forward or backward by rotating the knobs.

It is fitted with fine vernier graduations as on a ruler. This helps in relocating a specific field of examination.

CONDENSER

The condenser illuminates the specimen and controls the amount of light and contrast. There are different types of condenser. Some condensers have a rack-and pinion mechanism for up-and –down adjustment.

The NA of a condenser should be equal to or greater than that of the objective with maximum NA.

An iris diaphragm is provided below the condenser. This adjusts the NA of the condenser when using objectives having low magnifying power.

A swing-out type filter holder may be fitted above or under the condenser. In some microscopes the filter holder may not be swing-out type. The filter holder holds detachable filters when required.

Condenser centering screws, when present, are used to align the condenser with the objective.

A condenser raising knob may be present (if centering screws are not present) or the distance may be fixed

TWO-SIDED MIRROR

A mirror is the simplest illuminator. The two-sided mirror provides necessary illumination through reflection of natural or artificial light. It has two surfaces, one plain for artificial light and other concave for natural light. It is supported on two sides by a fork fixed on a mount in a way that permits free rotation.

A mirror is usually fitted on a mount or at the base of the microscope.

BUILT-IN LIGHT SOURCES

An illuminator is built into the base of the microscope. A halogen bulb provides the best illumination. On top of the illuminator is an in-built filter holder to fit the filter of desired quality.

FILTERS

Blue filters are used to change the light from ordinary electric bulbs into a more natural white light.

Neutral density filters are used to reduce brightness without changing the colour of the background.

Green filters may be useful in some situations.

Blue/green filters are not recommended for Ziehl-Neelsen microscopy as the acid fast bacilli stained in red may not be clearly visible

IMMERSION OIL

Immersion oil must be used with objectives having NA more than 1.0. This increases the resolving power of the objective.

An immersion oil of medium viscosity and refractive index of 1.5 is adequate. Any synthetic non-drying oil with a refractive index of 1.5 and/ or as recommended by the manufacturer should be used.

Cedar wood oil should not be used as it leaves a sticky residue on the objective. If cedar wood oil is used, particular care then needs to be taken to ensure that the objective is thoroughly and promptly cleaned with xylene after each session of use. Petrol can be used in place of xylene for cleaning if xylene is not available.

Liquid paraffin should not be used as it has a low refractive index which produces an inferior image. It is also unsuitable for scanning specimens for long periods, as is required for accurate microscopy

COARSE AND FINE FOCUSING KNOBS

The coarse and fine focusing knobs are used to change the distance between the specimen slide and the objective. The coarse focusing knob alters this distance rapidly and is used to bring the specimen into the field of view using and objective having low magnification power. The fine focusing knob changes the distance very slowly and permits better viewing of the object. One revolution of the fine focusing knob should generally move the mechanical stage by 100 μm. The movement should be smooth and free from jerks

HALOGEN LAMP

Halogen lamps are low wattage, high intensity lamps and are the preferred light source. They emit white light, with higher luminosity. The halogen lamp has a compact filament and sustains a longer life. Though costlier, halogen lamps have the fore mentioned advantages over tungsten lamps

6.3 FUNCTIONING OF THE MICROSCOPE

There are three main optical pieces in the compound light microscope. All three are essential for a sharp and clear image. These are:

- Condenser
- Objectives
- Eye-pieces

The condenser illuminates the object by converging a parallel beam of light on it from a built-in or natural source. The objective forms a magnified inverted (upside down) image of the object. The eye-piece magnifies the image formed by the objective. This image is formed below the plane of the slide.

The total magnification of the microscope is the product of the magnifying powers of the objective and the eye-piece.

For example, if the magnifying power of the eye-piece is 10x and that of the objective is 100x, then the total magnification of the compound light microscope is: 10x x 100x=1000 fold magnification.

ROUTINE OPERATION OF THE MICROSCOPE

- Ensure that the voltage supply in the laboratory corresponds to that permitted for the microscope; use a voltage protection device, if necessary.

- Turn on the light source of the microscope

- With the light intensity knob, decrease the light while using the low magnification objective.

- Place a specimen slide on the stage. Make sure that the slide is not placed upside down. Secure the slide to the slide holder of the mechanical stage.

- Rotate the nose-piece to the 10x objective, and raise the stage to its maximum.

- Move the stage with the adjustment knobs to bring the desired section of the slide into the field of view

- Focus the specimen under 10x objective using the coarse focusing knob and lowering the stage. Always turn the stage up towards the objectives while looking from the side and not through the eye-pieces, so as to avoid turning it up too far and damaging the objective. Only thereafter do the actual focusing, looking through the eye-pieces, by lowering the stage away from the objectives.

- Make sure the condenser is almost at its top position. Centre the condenser using condenser centering screws if these are provided in the microscope. For this take out one eye-piece and while looking down the tube, close the iris diaphragm till only pin-hole remains. Check if this is located in the centre of the tube.

- Open the condenser iris diaphragm to 70%-80% to adjust the contrast so that the field is evenly lighted

Many modern microscopes have pre-centered and fixed condensers. In these no adjustments are required. To reduce glare adjust the opening of the iris diaphragm

- Adjust the inter - pupillary distance till the right and left images become one
- Focus the image with the right eye by looking into the right eye-piece and turning the focusing knob
- Focus the image with the left eye by looking into the left eye-piece by turning the diopter ring
- Put one drop of immersion oil on the specimen
- Change to 100x objective
- Increase the light by turning the intensity knob until a bright but comfortable illumination is achieved
- Focus the specimen by turning the fine focusing knob
- When the reading/observation has been recorded, rotate the objective away from the slide
- Release the tension of the slide holder, and remove the slide.
- Turn off the light
- If immersion oil was used, wipe it from the objective with lens paper or muslin cloth at the end of each session of use. In general, avoid wiping the objective except when it seems to be dirty. This is sufficient if good quality oil is used (use synthetic oil recommended by the manufacturer)
- Do not clean lenses frequently. This may cause scratching and chipping of lenses.
- Always keep the immersion oil bottle capped and free from dust and debris
- Use a dropper and not a glass rod to put immersion oil on the slides without touching it
- The cover slip should conform to the specifications for the objective of the microscope. Most oil immersion objectives are corrected for cover slip of 0.17 mm thickness.
- Do not increase the intensity of the light source beyond the maximum permitted value

- Do not use bad quality facial tissue or coarse cloth to clean the lens as the coarse fibres can scratch the surface of the lens

- Never touch electric bulbs with bare fingers. Natural oil from the skin may burn and darken its surface causing premature decrease in light intensity. Use lens paper to hold the bulb when inserting it

- Do not introduce bubbles into the immersion oil by stirring it, or sucking or expelling the oil violently. A bubble under the objective will cause glare and lower contrast, thus reducing the quality of the image.

- Do not use xylene (or petrol) excessively to clean the lens. Excess oil can be usually wiped off with lens paper or muslin cloth. If good quality immersion oil is used xylene is usually not needed. Avoid using cedar wood oil.

- Do not exchange objectives of two microscopes unless you are certain that their mechanical tube length specifications are identical

Eye strain should not develop if the microscope is used properly.

Never adjust the stage upward while looking through the eye-piece. It will cause the objective to push against the slide and may damage it.

Only the 100% objective can be used for viewing under immersion oil. All other lenses are to be used without immersion oil; keep them dry and avoid applying oil or any liquid to these lenses

6.4 MAINTENANCE OF THE MICROSCOPE

NOTE: In all cases, the manufacturer's manual should be consulted for specific instructions.

INSTALLATION AND STORAGE

- Install the microscope on a sturdy, level table. Equipment and instruments which generate vibrations, such as centrifuges and refrigerators, should not be placed on or near this table.

- The height of the table should be convenient for the use. As and alternative or in addition, and adjustable stool should be made available to make microscopy comfortable. The table should be away from water, sinks, and racks containing chemicals, to prevent damage to the microscope from splashes or spills.

- Always carry the microscope with one hand supporting the base and the other hand around the arm.

- Place the microscope in a location from which it need not be moved frequently

- Turn the nose-piece to the objective with lowest magnifying power before removing the slide and when the microscope is not in use

- If the microscope does not have a built-in light source then the table should be placed near a window away from direct sunlight and arrangements made for the provision of a lamp.

- In so far as is possible, the microscopy room should be free from dust and should not be damp.

- If the microscope is to be used every day, do not remove it from the site of installation, provided security is assured.

- When the microscope is not in use, keep it covered with a polythene or plastic cover and take necessary precautions against fungus.

Dust is the worst enemy of the microscope. Always keep the microscope properly covered. Fungus is also a major problem. Always keep the microscope in dry surroundings.

- In humid areas, store the microscope every night in a cabinet fitted with and electric bulb (5 W or 40 W). This is switched on at night to reduce humidity.

- If the microscope is used intermittently and requires storage for prolonged periods, keep it in an air-tight plastic bag with about 100 g of drying agent. Remember to regenerate/replace drying agents (silica gel or dry rice) fortnightly or as needed.
- If only a wooden box is available, keep the microscope in it with some dry silica gel.

MAINTENANCE OF LENSES

Avoid collection of dust and immersion oil on the objectives and eye-pieces by keeping the microscope covered. Do not allow immersion oil to touch any of the objectives other than the oil immersion objective. Always keep the eye-pieces in place to protect the inner surface of the objective. Close the holes of missing objectives in the nose-piece by using special caps that are provided, or by sealing with adhesive tape.

REMOVAL OF DUST FROM LENSES

Check for dust or dirt on the lenses (eye-pieces, objective, condenser and illuminator lenses) if the image appears hazy or with black dots.

- If the black dot moves when the eye-piece is rotated, this means that the dust is on the eye-piece.

- If the black dot moves when the slide moves then the dust is present on the slide.

- If these two are ruled out, presume that the dust is on the objective. Dust on objectives shows as dots if it is inside. If the dust is outside the objective it shows as a hazy image.

Do not remove the dust from the lenses by wiping these with a cloth as this can scratch the lens and damage it permanently. Use an airbrush or a camel-hair/artist's brush.

Dust can be removed with a camel-hair/artist's brush or by blowing air over the lens with an airbrush. Dust on the inner surface of the objective can be removed by using a soft camel-hair brush (artist's brush)

REMOVAL OF OIL FROM LENSES

The presence of oil on the lenses produces a hazy image. The localization of oil can be done by the same method as has been described above for localization of dust.

Oil should be removed with the help of lens paper using lens cleaning fluid as recommended by the manufacturer. This can be applied gently with lens paper. Do not use force to remove oil as this might result in scratches on the lens.

If the field of view is not clear despite cleaning, and the microscope works well with another lens, then the lens has been permanently damaged and must be repaired or replaced.

If the field of view is not clear even after changing the lenses(objective and eye-piece) there is probably dirt or fungus on the tube prisms. These can be checked by removing the eye-pieces, and examining the upper part of the microscope tube with the light fully opens. Fungus is seen as threads, dots or a woolly layer.

INSPECTION OF THE OBJECTIVE

- Carefully unscrew the objective from the nose-piece.
- Gently remove one eye-piece to use as a magnifier (or use a magnifying glass)
- Grasp the objective in one hand with the front lens face up.
- Hold the eye-piece in the other hand with the top lens facing down.
- Bring the eye-piece very close to your eye and focus on the objective. Change the angle of the objective so that light can reflect off its surface. The two lens surfaces will be about 2.5 cm apart. Try to avoid letting them touch each other.
- Inspect the objective for scratches, nicks, cracks, deterioration of seal around the lens, or oil seepage into the lens.

MAINTENANCE OF MECHANICAL MOVING PARTS

Mechanical moving parts of the microscope may become too stiff or too loose.

Stiffness is due to accumulation of dust or because the sliding channel has become rough. This problem can be solved by cleaning, polishing and lubricating the sliding channel and the rack and pinion. First remove the dust with a camel-hair/artist's brush or by blowing air; clean it with a solvent such as petrol, polish with metal polish and apply high quality silicone grease to lubricate the moving parts.

Stiff movements may also be due to mechanical bending of some part. Rectify the fault or call the service engineer.

With the prolonged use, the up and down movement of the mechanical stage becomes loose. The stage, therefore, slides down during examination resulting in loss of focus. Adjust the tension with the tension adjustment device as recommended by the manufacturer.

MAINTENANCE OF LIGHT SOURCE

The supply of voltage (110v or 220V) must always conform to that specified for the microscope. Adequate number of spare bulbs and fuses should be available. Do not touch the bulbs with bare hands. Provide adequate ventilation to take care of heat generated by light. Provide voltage protection, if necessary. Before switching the lamp on, adjust the variable voltage regulator to minimum. Switch on the lamp and slowly increase the voltage until the desired intensity is achieved.

DAILY MAINTENANCE

- Bring the variable voltage regulator setting to the minimum before turning off the lamp. Turn off the light source of the microscope.
- Gently wipe the immersion oil off the objective, condenser and mechanical stage with lens paper or muslin cloth.
- Replace the cover of the microscope and take necessary precautions against fungus.

MONTHLY MAINTENANCE

- Use an air brush to blow away dust. Clean the objectives, eye-pieces, and condenser with lens cleaning fluid. Do not put fluid directly on the lenses; instead, apply it to the lens paper and then clean.
- Remove the slide holder from the mechanical stage and clean.
- With a tissue moistened with water, wipe the dust off the body of the microscope and the window of the illuminator in the base of the unit.

MAINTENANCE EVERY SIX MONTHS

Thoroughly inspect, clean, and lubricate the microscope after consulting the manufacturer's manual. This should preferably be done by competent biomedical engineering staff

7. MANUAL PIPETTES

7.1 INTRODUCTION AND CLASSIFICATION

Manual pipettes are devices used for accurate volumetric measurements and transfers.
Two categories of manual pipettes are defined.

I. Volumetric or Transfer
II. Graduated or measuring pipettes

VOLUMETRIC PIPETTES
These pipettes hold and deliver only the specific volume indicated at the upper end of the pipette.
Either "to deliver" [TD] or "to contain" [TC]

VOLUMETRIC PIPETTES [TD]
Consist of a cylindrical bulb joined at both ends to narrower glass tubing. A calibration mark is etched around the upper suction tube and the lower delivery tube is drawn out to a graduated taper Specifications are indicated on the pipette. A volumetric transfer pipette is calibrated to deliver accurately a fixed volume of a dilute aqueous solution. (e.g. standard, calibrator or non viscous sample)The TD pipettes are filled and allowed to drain by gravity. The pipette must be held vertically and the tip placed against the wall of the receiving container, but not touching the liquid in it. The most commonly used sizes are 1, 2,3,4,5 and 10 ml.

VOLUMETRIC "TO DELIVER" [TD] / BLOW OUT PIPETTE
E.g. Ostwold - Foline
Similar to the volumetric pipettes but have their bulb closer to the delivery tip. Commonly used sizes are 0.5, 1.0, 2.0 and 3.0 ml. These pipettes are used for accurate measurement of viscous fluid (blood or serum).Using of a pipette bulb the liquid is drawn in to the pipette and the meniscus is read. The liquid is blown out of the pipette only after the liquid has drain to the last drop in the delivery tip.

MEASURING PIPETTES [TD] OR GRADUATED PIPETTES
These are glass tubes of uniform diameter with a tapered delivery tip. Graduations are marked at uniform intervals; these pipettes are used for measurement of regents only and not recommended for measurement of samples and calibrators.

MOHR TYPE PIPETTES
Mohr type pipette is calibrated between two marks on the stem. Solutions are delivered between the desired marks. Pipette should be selected, so that the greatest volume is used, therefore maximum accuracy will be achieved. Mohr pipette is never used as a blow out type of pipette but delivers only point to point.

SEROLOGICAL PIPETTES
'TO DELIVER' / BLOW OUT
The pipette is calibrated to the tip. Pipette is filled and allowed to drain and the remaining fluid is blown out.

PIPETTE 'TO CONTAIN' [TC]
These are calibrated for the total volume of liquid held in the pipette and must be washed out completely for delivery of the correct volume.

TECHNIQUE OF MANUAL PIPETTING
Before using a pipette, be sure that it is the correct size, clean, and inspect the tip for any damages.

- Place a rubber bulb on the stem of the pipette
- An automatic pipette filler may be used
- Lower the pipette in to the solution. Allow sufficient depth to fill the pipette above the calibration mark.
- Apply suction and fill the pipette above the calibration mark.
- Hold the pipette vertically. The index finger is placed over the upper opening of the pipette and the liquid is allowed to drain to the mark. Excess fluid outside the pipette is drain in to the solution by touching inner wall of the bottle.
- Transfer the pipette to the receiving container. Drain the pipette freely in a vertical position, and the tip against the side of the container.
- The pipette must be held in a vertical position when adjusting the liquid level to the calibration line and during delivery. The lowest part of the meniscus, when sighted at eye level should be in level with the calibration line on the pipette
- The pipette has been calibrated to deliver its specified volume in a vertical position with a constant rate of delivery
- Do not attempt to force the liquid from the pipette at a faster rate than free drainage permits
- When using volumetric pipettes the flow of the liquid should be unrestricted and the tip should be touching the inclined surface of the receiving container until 2 seconds after the liquid has ceased to flow
- Avoid air bubbles in the pipette

7.2 CALIBRATION OF PIPETTES

Gravimetric method by weighing the 20 pipettings of distilled water with same pipette on a sensitive balance. (Please refer the procedure given for micro pipette calibration)

7.3 SPECIFICATIONS

Volumetric glassware is grade A, B, and student grade. Accuracy of grade A glassware meet or exceed the requirements specified by the National Bureau of Standards. Volumetric glassware used in clinical chemistry laboratory is made of borosilicate glass. This has a high degree of thermal resistance. Commercial brands are known as Pyrex and Kimax [Kimble] Corex brand glassware is a special alumina-silica glass strengthened chemically

7.4 CLEANING

General laboratory use glassware should be rinsed with water and immediately place in a large plastic measuring cylinder full of water.
Then soaked in a weak detergent solution [Teepol] for 2-3 hrs
Rinse with tap water for several times and then with distilled water.
Dry in an oven at 60 ^{0}C, or in an incubator

DIRTY GLASSWARE

Put in a cylinder filled with dichromate solution and leave overnight .Pour the dichromate solution in to another cylinder. Rinse the pipette with water and dry in an oven at 60 ^{0}C

8. MICRO PIPETTES

Micropipettes contain or deliver small volumes of liquid ranging from 1 to 1000 µl. The most common type of semi automated device that uses either air displacement or positive displacement to dispense the contained fluid; some models with digital volume adjustments are also available

There are many brands of air displacement pipettes, but all are piston operated devices. A disposable and exchangeable polypropylene tip is attached to the barrel, and liquid is drawn into and dispensed from this disposable tip. Some instruments can automatically eject the used pipette tip and reload new one, minimizing analytical contamination.

There are also several brands of positive – displacement pipettes available. The capillary tips which may be made of siliconized glass, glass or plastic can be reused. These devices are particularly useful for handling reagents that will react with plastics. Positive displacement pipettors deliver liquids by means of a Teflon-tipped plunger that fits snugly inside the capillary. Carryover liquid is negligible in properly maintain instruments and in some instances a washing step is used in between samples.

Air displacement micropipette may be used in either of two modes, the forward mode or the reverse mode. The reverse mode is used with two- component stroke mechanism system only. The precession of these devices in the forward mode depends on the precise draining caused by the air pressure, and they are relatively sensitive to the physical characteristics of the liquid being pipetted. .Reverse mode operation on the other hand, is considerably less sensitive to the type of the liquid being dispensed. In the forward mode the piston is depressed to the first stop on a two –stroke device, the tip is placed in the liquid, and piston is slowly allow to rise back to the original position. This fills the tip with designated volume of liquid. The pipette tip is then drawn up the sidewall of the vessel so that any adhering liquid is removed. If there are any extraneous droplets the tip is wiped carefully with a lint free tissue, with care been taken not to "wick" out any sample from the pipette tip. The tip is then placed on the wall of the receiving vessel, and piston is depressed smoothly to the first stop on a two – stroke device allowing the liquid to drain. Then one should allow one second to elapse before depressing the piston to the second stop, blowing out the remaining liquid. When the reverse mode is used the liquid is aspirated after depressing to the second stop position. This overfills the pipette with sample. To dispense the liquid one then depresses the piston to the first stop and removes it after waiting one second.

Positive-displacement micropipettes are used in the same manner as forward mode air-displacement devices. Again careful wiping of the tip is crucial in order not to "wick" out a sample from the tip. The need for maintenance of the Teflon tip cannot be over emphasized.

8.1 QUALITY CONTROL OF MICROPIPETTES

GENERAL

The accuracy and precision of each micropipette should be calibrated on acquisition and monitored during the course of the year. The frequency of calibration will depend on the amount of use. Heavily used devices may need monthly calibration, whereas rarely use devices may need to be checked on with once or twice per year. Manufacturers of newer micropipettes are claiming two year calibration stability.
Routine Maintenance is critical. Air displacement pipettes have a fixed – length that must be maintained. In addition, there are seals to prevent air from leaking into the pipette when the piston is moved. These must be greased to maintain proper operation. The manufacturer will provide guidelines for performing this maintenance. Any worn part must be replaced and devices that do not meet specifications for precision or accuracy will generally require servicing by the manufacturer

Positively displacement pipettes in general require the similar maintenance with regard to spring checks and replacement of Teflon tips. Many of these devices also are supplied with a slide wire that is used to quickly check the plunger setting. This device cannot be used in place of routine performance checks. Follow the manufacturer guidelines.

QUALITY CONTROL VALIDATION

The primary method for calibration of micropipettes is gravimetric technique. A secondary method is spectrophotometric procedure with potassium dichromate. The later method is unacceptable for volumes of less than 10 μl.

The following protocol describes the gravimetric method for calibration.

DETERMINING PERFORMANCE OF VOLUMETRIC EQUIPMENT

PRINCIPLE:
The procedure is based on determining the mass of water samples delivered by the instrument. True mass and volume are then calculated, based on the density of water at specific temperature and corrections for air buoyancy.
Deliver a total of 20 distilled water samples into a covered weighing vessel and weigh each sample after delivery. Thereafter calculate the mean mass and the mean volume of the delivered sample. Determine the inaccuracy and the imprecision with which the mean volume of the volumetric equipment has been determined

When handling the vessel use a tweezer or another device so as not to contaminate the outside of the vessel. (The manual volumetric equipment should be rinsed before testing) (Air displacement semiautomatic pipettes should be calibrated for forward and reverse techniques separately.

Procedure

1. Place a small amount of distilled water in the weighing vessel to cover the bottom and place a cap on vessel.

2. Open door of balance chamber, place weighing vessel on balance pan and close the door of balance chamber.

3. Release the arrestment system. Take readout to zero (electronic balance) or record readout value (mechanical balance)

4. Arrest balance; open balance door, retrieve weighing vessel. Aspirate distilled water into the pipette; remove vessel cap, deliver the water sample into the vessel and replace vessel cap. To avoid evaporation the period with uncovered vessel should be as short as possible.

5. Place vessel on balance pan and close balance door. Release arrestment system and record balance readout.

6. Repeat steps 2, 3, 4 and 5 until 20 samples have been weighed.

7. Measure and record the water temperature and the barometric air pressure.

8. (The procedure can be adapted to minimize the handling of weighing vessel by opening and closing the top lid of the balance.)

CALCULATIONS

1. Calculate the individual weighing result (M_i) by subtracting the tare reading from the sample reading.

2. Calculate the mean mass (M) from the individual weighing results (M_i):
   $M = \frac{\Sigma M_i}{N}$
   Where N = Number of weighings

3. Calculate the mean volume (V_t) of the water samples from the mean mass (M) :
   $V_t = M \times Z$
   Where Z = conversion factor (ml/g), incorporating the density of water when buoyed in air, at the test temperature and air pressure. Values of Z for water at various temperatures can be found in the table below.

4. Calculate inaccuracy (E_t) of the volumetric equipment as the difference between the nominal volume (V_0) and the calculated mean volume (V_t) in percentage of nominal volume
   $E_t = \frac{V_t - V_0}{V_0} \times 100\ \%$

5. Calculate the within- run imprecision (Coefficient of variation, CV) from the distribution of individual mass (M_i) about their mean (M)

$$CV = \frac{SD}{M} \times 100\ \%$$

Where SD = standard deviation $= \sqrt{\{\frac{\Sigma (M_i - M)^2}{N - 1}\}}$

N= number of weighings

RECORDING SCHEME FOR DETERMINING PERFORMANCE OF VOLUMETRIC EQUIPMENT

Type of volumetric equipment :
Temperature of distilled water :
Barometric air pressure :

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Weight of weighing vessel and Sample																				
Wight of weighing vessel																				
Mass of sample (M_i)																				

Mean mass (M) $= M = \frac{\Sigma M_i}{N}$

Mean volume (V_t) $= V_t = M \times Z$

Inaccuracy (E_t) $= E_t = \frac{V_t - V_0}{V_0} \times 100\ \%$

Standard deviation (SD) $= \sqrt{\frac{\Sigma (M_i - M)^2}{N - 1}}$

Coefficient of variation (CV) $= \frac{SD}{M} \times 100\ \%$

Comments *(Compare with the factory performance given by the manufacturer)*

Value for Z (µl/mg) or (ml/g), as a function of temperature and pressure, for distilled water :i.e reciprocal of density

Temperature C	Air Pressure						
	600	**640**	**680**	**720**	**760**	**800**	**mmHg**
	800	**853**	**907**	**960**	**1013**	**1067**	**Mbar**
	80.0	**85.3**	**90.7**	**96.0**	**101.3**	**106.7**	**kPa**
15.0	1.0018	1.0018	1.0019	1.0019	1.0020	1.0020	
15.5	18	19	19	20	20	21	
16.0	19	20	20	21	21	22	
16.5	20	20	21	22	22	23	
17.0	21	21	22	22	23	23	
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024	
18.0	22	23	24	24	25	25	
18.5	23	24	25	25	26	26	
19.0	24	25	25	26	27	27	
19.5	25	26	26	27	28	28	
20.0	1.0026	1.0027	1.0027	1.0028	1.0029	1.0029	
20.5	27	28	28	29	30	30	
21.0	28	29	30	30	31	31	
21.5	30	30	31	31	32	32	
22.0	31	31	32	32	33	33	
22.5	1.0032	1.0032	1.0033	1.0033	1.0034	1.0035	
23.0	33	33	34	35	35	36	
23.5	34	35	35	36	36	37	
24.0	35	36	36	37	38	38	
24.5	37	37	38	38	39	39	
25.0	1.0038	1.0038	1.0039	1.0039	1.0040	1.0041	
25.5	39	40	40	41	41	42	
26.0	40	41	42	42	43	43	
26.5	42	42	43	43	44	45	
27.0	43	44	44	45	45	46	
27.5	1.0044	1.0045	1.0046	1.0046	1.0047	1.0047	
28.0	46	46	47	48	48	49	
28.5	47	48	48	49	50	50	
29.0	49	49	50	50	51	52	
29.5	50	51	51	52	52	53	
30.0	1.0052	1.0052	1.0053	1.0053	1.0054	1.0055	

If the expected volume of water is not dispensed, readjust the instrument and repeat the investigation. (The micropipettes are provided with tools for adjustment of volume. However it should be carried out only by competent technical/biomedical engineering staff.)

In hot climates and when controlling minute volumes, the evaporation of water during the calibration procedure must be taken into account. The evaporation of water during the calibration process is determined by weighing the vessel, already containing a few milliliters of water. Thereafter the pipetting steps are simulated exactly the same way but without delivering water. After this simulation the container is weighed again and the difference of the weight corresponds to the weight of water evaporated during the pipetting procedure. It must be added to the total weight of water delivered in the calibration procedure.

8.2 METHOD OF USING AUTOMATIC FILLERS

1. Press the bulb with pressing the valve marked A to expel the air first
2. Fix the filler to the pipette
3. Insert the pipette into the solution
4. By pressing the valve S fill the pipette with the solution , aspirate the solution to the level above the calibration mark
5. Adjust the solution up to the calibration mark by pressing the value E then deliver the solution using the value

9. ANALYTICAL BALANCE

Analytical balances are in principle divided into two groups

1. Mechanical balances on which the compensation of the load is done in a mechanical manner by transferring or removal of weights
   The mechanical balances can be further subdivided in
   a. Substitution balances (constant load one pan balances)
   b. Equal- arm two pan balances.

2. Electronic balances on which the compensation of the load is done by an electromagnetic force. No weights are used.

Prolonged use and improper operation can influence the performance of an analytical balance concerning accuracy and precision. A calibration is mandatory to achieve reliable measurements from an analytical balance. Information on how to perform the calibration may perhaps be found in the manual of the manufacturer. Some of the new electronic balances have internal calibration procedures (built-in control), which can be performed by pressing a push-button, but in most cases external weights are used, the mass of which are known. Ideally the external weights that are used in the calibration should be traceable to the national and international standard weights.
In principle the control of accuracy is performed by loading the balance with weights of known mass to cover the entire measuring range of the balance. The readings of the balance should be close to the mass of the weights used.

9.1 CALIBRATION OF THE ANALYTICAL BALANCE

APPARATUS AND MATERIALS

Weighing boats or appropriate containers
Weights (preferably traceable to national and international standard weights)
Electronic balance

PROCEDURE

ACCURACY

1. Place the balance in a level position
2. Close the door(s) of the balance chamber. Release the arrestment system and determine the zero point by turning the "zero knob"
3. Arrest balance, open the door of balance chamber, place a weight of known mass on the pan and close the door of balance chamber.
4. Compensate the load, release the arrestment system. Record the balance reading in a scheme
5. Repeat steps 2, 3 and 4 for all others of the weights in the weight set and record the balance reading in a scheme.
6. Calculate the deviations between the measured mass and the true mass of the weights. Determine the percentage of inaccuracy and decide on any adjustments to be made by competent biomedical staff.

RECORDING SCHEME FOR ACCURACY

Load(g)	Measured mass	True mass	Deviation

Calculation of percentage of inaccuracy (%) = $\frac{\text{True mass} - \text{Measured mass}}{\text{True mass}} \times 100$

Comments:

PRECISION

Perform a weighing 20 times of a selected weight. Between each weighing the balance must be arrested and the weight removed from the pan. Record the balance readings and calculate the mean and standard deviation.

RECORDING SCHEME FOR PRECISION

N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mass of weight 0.5 g Mi																				

Mean mass (m) $= \frac{\Sigma Mi}{N}$

Standard deviation $= \sqrt{\frac{\Sigma (Mi - m)^2}{N-1}}$

Coefficient of variation (CV) $= \frac{\text{Standard Deviation} \times 100}{\text{Mean Mass}}$

Comments:

9.2 USE OF BALANCES

- Choose a balance appropriate for the range of the object to be weighed and the precision of a particular analysis
- Prior to use, the balance should be checked for spilled substances and spilled solids should be brushed away..
- Ensure that the balance is leveled, on a stable surface.
- Place the balance away from air currents.
- The pan of balance should be checked to ensure that it is properly seated on the pan mechanism.
- Turn on the balance. If an error message is received in lieu of the tare value, notify your supervisor immediately.
- Press the tare button and wait until a stable zero point reading is obtained. Re-press the tare button if necessary.
- Select the range by pressing the control bar until the balance displays mg (mg=range)
- Releasing and then briefly pressing the control bar again, allow you to switch between the 30 g range and the 160 g weighing range.
- When the desired weighing range has been selected, wait: Display "----", then 0.0000. The balance is now in the weighing mode.
- Place the object to be weighed on the pan, in the centre area.

  Allow the reading to stabilize and record the weight

  If a container or weighing paper is to be used, press the tare button and wait until a stable zero point reading is obtained. Add the substance to be weighed, allow the reading to stabilize, and record the weight.
- Allow samples/ containers to reach room temperature before weighing. Hot samples will generate an upward convection of warm air that will cause an inaccurate reading.
- Samples that are extremely hygroscopic or lose water rapidly must be weighed in a closed container.

- Volatile liquids must be weighed in a closed container or with a trapping solvent.

- "Lumps" are often caused by moisture or electrostatic charge and may not constitute a representative sample.

- When weighing a sample or reagent, discard excess material that has been removed from the original container. Do not return material to its original container.

- Do not use regular paper for weighing. Use weighing boats or an appropriate container.

- Do not weigh containers containing a magnetic stir bar. The magnetic field generated may cause unpredictable effects on the electronic balance.

- Fingerprints may cause an inaccurate value. Make sure hands are clean and dry and do not contribute to the weighing.

- Be sure to use a clean and dry spatula or other transfer device to avoid contamination of the article to be weighed

- A brush designed for the purpose can be used to eliminate electrostatic charge on the balance pan. Do not weigh objects that can carry an electrostatic charge, such as some plastics. Changed materials can result in unpredictable balance behavior and weighing.

- Turn off balance. Wash and replace spatula in the rack. Clean up any rubbish or spillages near or on balance, close balance doors.

SPECIFICATIONS OF AN ANALYTICAL BALANCE

- Specify the type of balance required : Analytical balance
- State the purpose :To weigh chemicals and stains
- Range of weighing :
- Performance: State the required sensitivity (0.001g) will be adequate in most situations.
- Pan size
- Power source: State the available power source and request for a voltage stabilizer.
- Digital display
- Accessories; calibration weights and dust covers.
- Durability and robustness: Should be movable and be easily disassembled and reassembled.

10. CENTRIFUGE

10.1 INTRODUCTION

Centrifuges are used in the clinical laboratory to separate substances of significantly different masses or densities. The two substances to be separated can be a solid (particles) and a liquid or two liquids of different densities.

In a clinical chemistry laboratory the centrifuges are used primarily to separate clotted blood or cells from serum or plasma and body fluids. Although the choice of a specific relative centrifugal force (RCF) to carry out these separations is not critical, a force of 1000 to 1200 x g for 10 to 15 minutes is recommended. In some situations more time may be necessary.
Three general types of centrifuges are available

- Swinging bucket or horizontal head centrifuge
- Fixed angle or angle head centrifuge
- Ultracentrifuge

All centrifuges have a motor, drive shaft, and head or rotor, which may be in the form of a chamber with a cover. A power switch, timer, speed control, tachometer and brake are the components that control the centrifuge. When necessary, refrigeration units are included. Some centrifuges are equipped with an alarm that sounds when there is a malfunction such as a tube imbalance. Some centrifuges automatically shut down, preventing tube breakage and potential for exposure to bio hazardous agents. All modern centrifuges have a safety latch that prevents the operator from opening the instrument before the rotor has stopped.

The swinging bucket or horizontal head rotors hold the tubes in a vertical position when the centrifuge is at rest. This type use pairs of buckets or carriers that swing freely. The carriers are designed to accept a variety of cushioned inserts allowing centrifugation of small tubes or large bottles. The tubes move to and remain in horizontal position when the rotor is in motion. During centrifugation, particles constantly move along the tube while it is in the horizontal position, distributing the sediment uniformly against the bottom of the tube. After centrifugation is complete and the rotor has ceased turning, the surface of the sediment is flat with a column of liquid above it.

Fixed angle rotors keep the tubes at a specified angle, 25 to 52 degrees to the vertical axis of rotation. Different fixed angle rotors are required for different sized containers. During centrifugation, particles move along the side of the tube to form sediment that packs against the side and bottom

of the tube. The surface of the sediment in this case is parallel to the shaft of the centrifuge. As the rotor slows and then stops gravity may cause the sediment to slide down the tube forming a poorly packed pellet. Fixed angle rotors are used when rapid sedimentation of small particles are required. The design of these rotors is more aerodynamic, operation at speeds higher than those achievable with a swinging bucket rotor. RCF up to 14000 x g is achievable.

Ultracentrifuges are high speed centrifuges that use fixed angle or swinging bucket rotors. They are often refrigerated to counter the heat generated as a result of friction. This type is used to fractionate lipoproteins, perform drug binding assays and prepare tissue for hormone receptor assays. Analytical ultra centrifuges are used to determine sedimentation coefficients of proteins, allowing assessment of molecular weights.

The motor in a large centrifuge is usually a direct current, heavy duty electric motor. In smaller centrifuges the current is usually alternating. Power is transmitted to the rotor by the commutator and brushes. The rotor shaft is usually driven by a gyro system and the bearings are sealed, minimizing vibration and lubrication. Centrifuge speed is controlled by a potentiometer that modulates the voltage that is supplied to the motor. Speed is also determined by the mass of the load in the rotor. The tachometer measures rotor speed in rpm. The brake decelerates the rotor by reversing the polarity of the current to the motor. The timer permits the rotor to reach a pre programmed speed. The rotor then decelerates with out braking after a set time has elapsed.

Refrigerated centrifuges are used when the heat generated during centrifugation could cause evaporation or denaturation of protein or leakage of cellular components in the sample. The temperature can be controlled between – 15 and 25 ^{0} C, allowing centrifugation at higher speeds for prolonged periods.

The selection of centrifuge tubes and bottles is important. Tubes with tapered bottoms, which form more compact pellets, may be required under certain conditions such as preparing urine sediment for microscopic analysis. The tubes must fit snugly in the carriers the top of the tube must not protrude so far above the carrier that the rotor is impeded.

Balancing the tubes within the carriers is critical. All tubes should be filled with an equal amount of liquid by weighing the two sets of carriers in a two pan balance. In a situation where the tubes are unequal, dummy tubes should be included with distilled water or a comparative liquid. Each bucket should be balanced with respect to its pivotal axis. Improper balancing can cause the centrifuge to vibrate, disrupting the formed pellet. Bio hazardous material should be centrifuged with the caps or stoppers in place to minimise aerosols.

10.2 CARE, MAINTENANCE AND QUALITY ASSURANCE

- Always follow the manufacturer's instructions for installation, operation, cleaning, maintenance, lubrication, repair and replacement.

- The instrument must be positioned exactly horizontally to prevent the instrument moving away from its place during centrifugation.

- Check if the rubber buffers/cushions are in the buckets.

- Balance the tubes and buckets in the centrifuge.

- Turn the speed control slowly to the required speed. Do not use the centrifuge at a higher than necessary.
- Daily cleaning of the centrifuge buckets with a non corrosive disinfectant is recommended. The buckets should be inverted to drain dry.

- After any sample spillage wipe and disinfect immediately.

- When tube breakage occurs remove the broken pieces using forceps and thick gloves and dispose appropriately. The portions of the centrifuge in contact with the blood or potentially infectious agent must be immediately decontaminated. The centrifuge bowl should be cleaned with a disinfectant, and rotor heads and buckets should be autoclaved.

- Stop the centrifuge immediately if it develops an abnormal sound during operation.

- Preventive maintenance should be carried out by trained biomedical staff, on centrifuge speed using a tachometer every 6 months. The measured and rated speed should not differ by more than 5% under specified conditions. The accuracy of the centrifuge timer should be checked every 6 months. The temperature of the centrifuge should be checked at least monthly under standardized conditions. The agreement between the measured and expected temperature should be within 2^0C.Check brushes and bearings every 3 months. Replace if necessary. Check for corrosion and repaint if necessary.

- All maintenance function checks must be recorded, and all corrective actions documented.

10.3 SPECIFICATIONS

Facts to be considered when writing up specifications and during an evaluation of quotations for a centrifuge

Specifications should be written according your intended purpose of the equipment.

Decide on the type (swinging bucket/fixed angle/ultracentrifuge), capacity, table top/floor model, refrigerated or not (temperature range), maximum relative centrifugal force, variable speed and time control facility, bucket adaptor sizes, lid safety lock, availability of spare parts, power and voltage requirement, guarantee of replacement of the equipment due to mechanical failure in the first year, unit price with accessories and total price with accessories, In the specification include the phrase "complete functioning unit of a centrifuge" there by the supplier is required to suggest any other requirement to be fulfilled for the satisfactory functioning equipment. Include the purpose of the centrifuge especially when the specifications are written for an ultracentrifuge

When evaluating the quotations list your requirements and match with the features given in the quotations. Give reasons for acceptance and rejections. Valid technical reasons are required to justify the selection.

11. AUTOCLAVE

11.1 INTRODUCTION

An autoclave in a clinical chemistry laboratory is used mainly for sterilization of dirty glassware, instruments and material to be discarded. In a microbiology laboratory the main purpose is to sterilize the instruments, media and clean glassware. It is recommended to install two autoclaves in a microbiology laboratory to carryout the sterilization of "dirty glassware" and "clean glassware" separately

Autoclave that uses saturated steam under pressure is one of the most reliable methods available in the laboratory for the inactivation of all forms of microbial life. To ensure safety and quality control, all bio hazardous materials and items contaminated with potentially infectious agents should be decontaminated before use or disposal. Such items include laboratory equipment, glassware, and biomedical waste including sharps. Steam sterilization is not recommended for anhydrous substances, flammable materials, electrical equipment, radioactive material, or any item that may be damaged in the autoclaving process.

The most common steam sterilizer found in laboratories is the gravity displacement type.
Saturated steam enters the top of the chamber by a steam pressure control valve. As the steam enters, it pushes the air out through a trap in the drain line. Once all the air is evacuated, the trap closes. Steam continues to fill the chamber of the autoclave until a preset temperature and pressure is reached. The operating conditions are 121^0C and 15 pounds per square inch gauge pressure.

The sterilization procedure consists of three phases, namely heat-up time, the contact time, and the cool-down time. Once the temperature has come to equilibrium, a minimum of twenty minutes contact time for all surfaces that require sterilization is necessary to ensure complete biological inactivation. Usually the heat-up time section of the sterilization procedure is the time given for the chamber to heat up to the prescribed temperature; therefore, the run time used must be long enough for the entire package to equilibrate at 121^0 C and still give the load a twenty minute contact time.

11.2 OPERATING CYCLE CONDITIONS FOR STERILIZATION

Sterilizing temperature(^{0}C)	Approximate pressure (bar)	Maximum hold time (min)
115	0.75	30
122	1.15	15
128	1.50	10
136	2.25	03

11.3 OPERATIONAL AND SAFETY PRECAUTIONS

- All potentially infectious materials must be autoclaved before being washed, stored, or disposed as biomedical waste.

- Personnel who operate the instrument must be trained to understand proper packaging, loading, labelling, operation and emergency procedures. This training should be supervised and documented by the head of the unit.

- Autoclaves generate high heat and pressure; therefore, all users should understand the associated risks. Always manipulate hot items with a thick glove designed for this purpose. At the completion of the cycle, allow the instrument unit to cool down before opening. Then stand back and crack the door slowly so as to allow the excess steam to escape.
- Warning signs alerting users to these hot surfaces should be placed next to the equipment, to remind personnel of this hazard. Do not stack or store combustible materials or flammable liquids next to the instrument.

- Modern autoclaves have a safety interlocking system which prevents the instrument from working if the door is not properly closed. Extra precautions should be observed if the autoclave has no in built safety mechanisms.

- If steam is leaking around the door during the sterilization process, the door has not been sealed properly. In this event shut down the system as safely as possible. Let the unit cool, and reset the door. Give special attention to making sure that the door is sealed tight, and restart the run. If this problem persists, the unit needs to be serviced by a qualified technician.

- It is recommended that the biomedical waste be labelled as such using the universal biohazard symbol, and be sterilized by the end of each work day. Never leave non sterilized material inside the instrument, or sitting in the waste disposal room overnight. Materials that contain toxic agents, volatile chemicals, or radio nuclides should not be autoclaved.

- All biomedical waste that is to be autoclaved must be first placed in a labelled autoclave bag. Sharps such as needles and scalpels must be placed in a labelled and ridged sharps container before sterilizing. Do not place sharp pipettes or broken glass in bags. These waste items should go into boxes or pails to be autoclaved, or be sterilized using a liquid sterilizing agent such as 10% sodium hypochlorite.

- Biomedical waste that is to be sterilized in a chamber of an autoclave must have an indicator that demonstrates that the waste has been autoclaved before it can be disposed. It is recommended that potentially infectious waste must be labelled "BIOHAZARD", and has the biohazard label present on the container.

- Proper use of autoclave bags is necessary. Approximately 50 ml – 100 ml of water should be placed in the bag prior to the start to facilitate steam production. If this water is naturally occurring in the load, additional water will not need to be added. The bags should be properly wrapped to prevent spillage. If the tape is wrapped too tight, steam pressure can build up inside the bag causing it to rupture.

- Do not overfill bags or the unit, as this may interfere with the sterilization process due to poor steam circulation. The autoclave bags should be placed on stainless steel trays.

- Do not fill the container more than 75 percent capacity when autoclaving liquids. This will ensure that the fluid has room for expansion when heated. Since sterilizing liquids using steam can be problematic, it is best to sterilize liquid waste using a chemical agent if possible.

- If liquid is being autoclaved borosilicate glass (Pyrex) or polypropylene containers should be used. Loosen the caps on the vessels to allow for pressure build up during the process. Always place loose glassware and liquid containers inside a secondary container during the autoclaving process.

- Wear thick heat resistant gloves, splash goggles, and a rubber apron to remove liquids from the autoclave.

- The exhaust cycle must be set very slow for liquid loads. This allows time for the liquid to cool below 100 ^{0}C so the liquid does not boil as pressure is reduced. Upon the completion of the run, open the door slowly and stand back until the steam has cleared.

- The parameters for the sterilization cycle will depend upon the amount and type of material. . The exact operating procedure for each model and various loads will differ; therefore, it is recommended to write your own Standard Operating Procedure (SOP) for the steam sterilization This SOP should include the sterilization procedure for each type of load, amount, run and program. An autoclave log book

should be maintained including the date, time, type of load and operator's name

- Each autoclave unit should have preventive maintenance, including autoclave spore testing procedures and the responsibility should be given to a qualified repair technician.
- Operator preventive includes removal and cleaning of the drain strainer, if applicable once a week and monthly visually inspecting the autoclave gaskets, doors, shelves and walls for residue building and wear.

- Quality control monitoring program should be implemented and visible indicators should be included in all autoclave runs Biological indicators use heat resistant *Bacillus stearothermophilus* spores as a control to test for an effective kill during the process. Spore vials are placed in a challenging location in a medical waste bag during a run. A negative growth demonstrates that the equipment is working properly. If the tester obtains a positive growth, try the run again with a fresh vial. Consistent positive growth indicates a problem that must be repaired before the instrument can continue in service.

11.4 SPECIFICATIONS

IMPORTANT FEATURES TO BE INCLUDED IN SPECIFICATIONS OF AN AUTOCLAVE

- Capacity: The size and capacity of the autoclave will depend on the volume of work carried out. The chamber capacity should not be larger than required. Calculate the capacity using the following formula

  $$\text{Necessary capacity (litres)} = \frac{\text{Average daily work load (litres)} \times 1.5}{\text{Daily number of cycles}}$$

- Spare parts: A set of spare parts should be included in the specifications

- Performance and safety: interlocking safety device on the door or lid to prevent opening until the pressure has returned to atmospheric.

- Maintenance

- Heat source

- Power source

- Training

- Quality control method for testing performance of the autoclave should be included.

12. REFRIGERATOR

12.1 INTRODUCTION

Refrigerators provide storage facilities for reagents and specimens. Laboratory refrigerators must have a temperature monitoring system. For refrigeration, the heat flow, which is normally transmitted from a warm object to a cold object, needs to be reversed. Heat can be reversely transmitted by evaporation and condensation of a liquid. The evaporation of a liquid consumes energy to overcome the cohesion forces of the molecules in the liquid state. This energy is called "latent heat". The absorption of latent heat by surrounding environment causes a drop in temperature. Conversely, during condensation the latent heat of the gaseous phase is available to increase the temperature.

Refrigeration makes use of energy absorbed by evaporation and condensation of liquids that have a boiling point below room temperature at atmospheric pressure. CF_2CCl_2 (boiling point: -30°C) and ammonia (boiling point: -33°C) are commonly used as refrigerants. The liquid is circulated through a closed pipe system, where on one side it is vaporized (refrigeration chamber) and on the other side (outside the refrigeration chamber) it is condensed.

Refrigerators are constructed upon two principles for condensation, either absorption or compression.

The absorption principle is used in smaller refrigerators. The closed system consists of an evaporator and absorption vessel, a heating chamber and a condenser. Absorption refrigerators have a circuit containing a constant pressure.

The three materials used in this system are water, ammonia and hydrogen and cooling is achieved on the basis of

- The capacity of water to absorb large quantities of ammonia vapour
- The fact that ammonia will evaporate from this solution if heat is applied.
- The laws of partial pressure which show that in a space occupied by a mixture of a vapour and a gas, which do not react together chemically, each component exerts the pressure which it would produce if it alone occupied the said space. Therefore, the total pressure is the sum of these two pressures.

A water/ammonia solution flows by gravity from the absorption vessel to the heating chamber. The heating chamber is heated by an electrical heating system. The heated water/ammonia solution releases water and ammonia as a vapour. A water separator prevents drops of water from moving ahead and the water is returned to the absorbed vessel, while the light ammonia vapour enters the condenser. In the condenser, the ammonia vapour is cooled and the ammonia moves by gravity into the

evaporation chamber. The evaporation chamber is fed with hydrogen gas, which facilitates the evaporation of ammonia. During evaporation of the ammonia latent heat is absorbed so that the temperature drops in the compartment. The ammonia vapour is absorbed by the weak ammonia solution in the absorption vessel where heat is liberated and removed by air-cooled fins. A concentrated ammonia solution flows again by gravity to the boiler, where the cycle is repeated.

A compression system, which is used for cold rooms and also for small refrigerators, consists of an evaporator, an expansion valve or throttle pipe, a condenser and a compressor.

The refrigerant liquid evaporates in the evaporator, which is placed in the refrigeration chamber, and enters into the compression chamber, where it is compressed. The compression warms the vapour, and the higher pressure raises the boiling point if the refrigerant. When leaving the compressor at elevated pressure the vapour passes the condenser and condenses to liquid while cooling. The liquid flows through a capillary after which it is exposed to low pressure which reduces the boiling pressure, thus resulting in evaporation. This process requires energy and latent heat from the refrigerant, thus the temperature of the refrigerant drops below room temperature.

Some refrigerators have two condensers, one for cooling the refrigerant, the other for cooling the oil in the compressor.

12.2 MAINTENANCE OF REFRIGERATORS

GENERAL MAINTENANCE:

- Refrigerator must be placed so that sufficient air can pass the condenser (at the back of the refrigerator) for exchange of heat and also to facilitate cleaning of the condenser.
- The refrigerator door must seal perfectly to prevent warm outside air from entering the cool chamber.
- The refrigerator must have good insulating walls.

DAILY CHECKS

- Check temperature daily. It should not exceed 12^{o}C. Application of battery-driven mobile or stationery thermometers are recommended, preferably those including continuous printing or plotting of temperature measured when heat-sensitive reagents are stored for long periods.

MONTHLY CHECKS

- Clean cool chamber and defrost the evaporator monthly
- Clean refrigerator from the outside.
- Clean condenser for dust

TEMPERATURE CHART

REFRIGERATOR - LAB …….. SERIAL NO…….

MONTH …… YEAR……

Date	Time	Refrigerator Temperature	Freezer Temperature	Comments	M.L.T

12.3 SPECIFICATIONS

- Storage capacity: This is influenced by the availability of other refrigerators and the type and size of the laboratory.
  Refrigerator capacity between 75 and 120 litres with a freezer capacity of between 3 and 5 litres is recommended

- Performance : refrigerator temperature 0^0 C to 8 ^{0}C range
  Freezer temperature -15 ^{0}C to - 30 ^{0}C range

- Principle :absorption /compression
  Compression refrigerators are preferred as the thermostat ensures correct temperatures and requires less energy.

- 100% CFC (chlorofluorocarbon) free model

- Non frost preferred

- Power

- Upright refrigerator

- Refrigerator thermometer should be included

- Sample storage refrigerators are available with specially designed racks. Location of the sample is made easier with the assigned rack number/row number/column number.

13. FREEZERS

Freezes are required in clinical chemistry to store body fluids which are heat labile (hormones) and for long term storage of samples.

13.1 SPECIFICATIONS

- Capacity ; depends on the requirement of the laboratory
- Interior and exterior dimensions
- Temperature : - 15^0 C to -25^0 C
  Sufficient for storage of samples for one month
  Ultra low freezers - 80^0 C
  Required for storage of samples for more than one month
- Principle :compressor type
- Insulation : specify the insulation material
- Digital display of temperature
- Temperature control ;microprocessor
- Equipped with alarm systems : temperature, power and remote
- Racks – sizes
- Movable facility; castors
- Maintenance, operational and service manuals

14. HOT AIR OVEN

14.1 INTRODUCTION

Hot air ovens are used mainly for drying laboratory equipment in dry air. Some hot ovens are used for sterilization. Sterilization in dry air is only effective when the material is exposed for 60 minutes at 160^0 C or for 40 minutes at 180^0 C. It is important to remember that the timing of sterilization is sufficient when the holding period begins after the air in the oven has reached its expected temperature.

14.2 USE OF HOT OVENS

- Set the thermostat to the required temperature prior to sterilization.
- If there is a fan, check if it is working properly.
- Allow to continue heating for an additional 45 -60 minutes after the temperature reaches the pre set degree.
- Switch off the heat when the time is up.
- Wait until the temperature falls to 40^0 C before opening the door.
- Always follow the instructions of the manufacturer.

15. WATER BATH

15.1 INTRODUCTION

Water baths may be either circulating or non circulating in design. For clinical chemistry applications, non circulating baths are unacceptable because temperature control is inadequate, and circulating water baths, which have a tighter control, are necessary. Such baths are equipped with an external or internal circulating pump that maintains adequate thermal equilibrium. In some instances the pump may be coupled to a refrigeration unit to provide temperature control below room temperature. Good quality bath liquid is recommended to prevent salt deposition on the heat exchangers. Such deposits interfere with maintenance of adequate temperature control.(Type 1 or 2 reagent grade water/ distilled water is preferred to tap water). Some manufacturers recommend addition of diluted bactericidal to the bath liquid to minimise bacterial growth.

15.2 USE OF WATER BATHS

- The level of water in the water bath should be above the level of the solution in tubes to be incubated

- The water bath should have an immersion type thermometer calibrated against a calibration certified thermometer.

- Temperature should be maintained daily. Prior to incubating the tubes, check the temperature at the centre and four corners of the water bath.

- When incubating open containers, vials or tubes the water bath lid should be removed to prevent contamination and dilution of the incubated material by condensed water.

- The water bath must be refilled to prevent growth of algae and bacteria.

- Preventive maintenance: The bath should be filled with distilled water. The unit can be cleaned with commercial stainless steel cleaning agents. The heating element should always be kept clean. Objects liable to rust must not be placed in the bath.

15.3 SPECIFICATIONS

Specifications for a water bath

- Bath capacity: A bath capacity between 2-5 litres should be adequate for clinical chemistry laboratories.
- Construction Material: A tank made from stainless steel, transparent polycarbonate or with a polypropylene inner will be easier to clean.
- Temperature range : This may be fixed (37^0 C) or variable (30^0 C to 100^0 C)
- Temperature fluctuation: Only a narrow temperature range fluctuation (sensitivity of < 0.5^0 C) is allowable for clinical chemistry analysis. An electronic controller is recommended.
- Mixing unit: The water is circulated and maintained at a constant temperature by a unit equipped with a propeller. (immersion circulator)
- Heater : A heating unit may project down into the bath or be mounted at the base of the tank
- Spare parts: The local supplier should be able to provide a reliable supply of spare parts. Recommended spare parts include a thermometer, a heating element, a spare fuse and a thermostat.
- Power source: Consider the power requirements in relation to the availability of electricity to the laboratory. It should be used with a voltage stabiliser.

Specifications for a boiling water bath
(W.H.O recommended urea method – Diacetylmonoxamine method)

Should be electrically heated, constructed from 18/8 quality stainless steel, seamless (no welded joints), fitted with constant level device and three legs. Heater should be with built in safety cut-out. Unit should be complete with concentric rings and centre cover

16. PH METER

16.1 INTRODUCTION

A pH meter is an instrument widely used in a clinical chemistry laboratory. The concentration of hydrogen ions, which is conveniently expressed in terms of its negative decadic logarithm, pH, is measured in reagent solutions and buffer systems, as well as in clinical blood gas analysis

The pH measurement is based on the physical principle that an electric potential will be established on the surface of a metal (electrode) placed into a dilute salt solution. The electrodes are designed for specific measurement of a single type of ion in a mixture of other ions in solution. Glass electrodes are used for the measurement in the range of pH 0 to pH 14 and always in combination with "reference electrodes". The reference electrodes are electrodes which maintain a constant potential even when the ion concentration may vary in a test solution. The potential remains constant since the electrode metal is dipped in a chamber with a solution of constant salt concentration (salt bridge solution) Reference electrodes are made of mercury or precious metals. Salt bridge solutions are saturated solutions of KCl, K_2 SO_4, KNO_3 or LiCl. The widely used reference electrode for pH measurement is calomel electrode ($Hg/HgCl_2$). As the temperature affects the performance an automatic temperature compensation probe is included in modern pH meters.

16.2 ELECTRODE OPERATION AND CAUTIONS

Always follow the instructions provided in the operation/service manual. Only competent biomedical staff should carryout the maintenance of pH meters.

- When using the electrode under normal operating conditions, check that the KCL solution reaches the refilling hole level. Otherwise fill saturated KCL solution.

- When using a new electrode or a one that has been stored for a long time servicing and installation should be carried out by competent biomedical staff.

- After the measurement, fully wash the glass membrane and solution junction with pure water and then immerse the electrode in pure water with the refilling hole of the reference electrode opened for its storage

- However for more accurate measurement, it is recommended that the electrode be immersed in saturated KCL solution with the refilling hole closed.
- Errors may occur if solution other than the inner solution penetrate through the junction (contamination)

- Prior to storing the electrode for a long time, fully wash the glass membrane and junction with pure water, fill the inner solution up to the refilling hole level and then close the refilling hole with the plug. Finally, cover the electrode tip with a protection cap filled with saturated KCL solution

- Prior to performing accurate measurement, wash the electrode repeatedly 3 times or more, and then immerse the electrode in pure water or appropriate solution for more than 12 hours so that the membrane surface adapts well with water.

- If the glass membrane is dried, immerse the electrode in pure water for a few hours or in about 0.1 normal HCl solution for about 30 minutes and then wash the electrode with pure water. At this time, do not allow the penetration of pure water or HCl solution through the solution junction

- If the electrode is extremely dirty, wipe it off with gauze soaked with neutral detergent. (Do not use cleanser)

- High insulation is required at glass electrode terminals. Do not dip the terminals with any aqueous solution

- Solution containing hydrogen fluoride
  This solution dissolves glass so that no glass electrode is used in the solution

- Reference electrode
  A potential generated between the liquid junction of the reference electrode and measured solution is to extremely small to enable stable measurement.
  However, a complex potential is generated between the liquid junction and each of the following solutions to cause unstable indication and slow response. As a result an error may occur.

  - Strong acid and strong alkali solutions
  - Solutions having weak buffer action such as pure water, etc. In this case, the measuring method in addition to the reference electrode should be reviewed as described in item 1, 2
  - Non aqueous solutions
  - Solution which should not be reacted with chloride ion.(Solutions containing silver)

MEASUREMENT OF SPECIAL SOLUTIONS

- Glass electrode
  - High temperature and low temperature solutions
    The use of the electrode at any temperature beyond the range shown in the electrode table may shorten electrode life, or the use of the electrode at the temperature below the range shown in the same table may cause slow and unstable meter indication. Refer the operation manual for specific instructions.

  - Non-buffer solution
    If the measurement is inevitably required the effect of atmospheric carbon dioxide needs to be eliminated. Follow the procedure in the manual.

  - Non-aqueous solution
    Measurement is made in the same way as that of aqueous solution. However, no measured value can be compared with the value of aqueous solution, but it is only the relative value of a certain solvent.

  - Suspension
    Suspension may easily be clogged at the solution junction. Therefore in this case wash the solution junction or replace the reference electrode

16.3 CALIBRATION OF THE pH METER BY STANDARD SOLUTIONS

pH standard are available in tablet and powder forms. They should be dissolved in deionised water or glass distilled water. Accurately calibrated A grade glass ware (volumetric flask) should be used. Standard pH solution should be stored in polypropylene bottles

PROCEDURE

1. One point calibration
   The calibration is made at any one point of pH 6.86, pH 4.01 and pH 9.18 (at 25 ^{0}C)
2. Two point calibration
   This calibration is commonly made by using pH 6.86 (at 25 C) and any other standard solution
   First perform one point calibration using any 4.01 or 9.18 pH standard, then wash the electrode thoroughly and dry. Immerse the electrode in the standard solution of pH 6.86 and carry out the calibration.
3. Three point calibration
   This calibration is performed when the accurate measurement of acid or alkali solution is required
   The calibration is made at 3 pints of pH 6.86, pH 4.01 and pH 9.18; first perform the 2 point calibration. Then wash the electrode thoroughly and dry. Immerse the electrode in pH 9.18 standard solution and carry out the calibration.

CARE AND MAINTENANCE OF THE P H METER.

1. Do not flash water and/or solution on the meter surface
2. Fully insert the electrode plug into the electrode connector otherwise, measurement may become impossible due to imperfect contacts
3. If the meter does not operate normally (no display is shown, no display changes, no key operation is made, etc) turn off the power once and again turn on the power 2 to 3 seconds later.
4. Wipe of any spillage of stain on the meter surface with a soft cloth such as gauze slightly soaked with weak soapy water. Do not use thinner or toluene.
5. Carefully handle the electrode since it is fragile
6. When using the electrode remove a protection cap and a refilling cap from the electrode.
7. Do not return the solution used once back to a stock bottle
8. Fully wash the electrode with pure water. After washing, wipe off water drops remaining on the electrode surface with filter paper.
9. When the electrode is left in the air for a few days or more, junction potential may become unstable. Therefore in this case, prior to using the electrode, immerse it into saturated KCl solution for 24 hours more
10. Do not dilute the standard solutions.

When you are ordering a ph Meter select a model with a protective sheath to protect the glass electrode. The agent should have a good supply of electrodes, electrode leads, probes, anti-surge fuse, membranes, battery connectors, electrode buffer solutions and electrolyte maintenance solutions. Repair facilities should be available. The manufacturer should supply a user's manual (specify the language) which gives installation, operating and maintenance instructions with a table giving cleaning agents of glass electrode.

17 THERMOMETER

17.1 INTRODUCTION

Temperature sensitive instruments in the laboratory should be maintained at constant temperature. The types of thermometers include:

- Liquid - in – glass thermometer
- Thermistors
- Electronic digital thermometer

Liquid – in – glass thermometers are available for partial or total emersion. Partial emersion thermometers are used to measure the temperature of water baths, heating blocks and ovens. The emersion depth in engraved on the stem and is usually located about 76mm from the bulb. Total emersion thermometers are generally used to check refrigerator and freezer temperatures.

CALIBRATION OF LIQUID – IN – GLASS THERMOMETERS

Calibration of thermometers requires the use of a certified or traceable thermometer. It should be traceable to a national standard reference material programme.

17.2 THE PROCEDURE FOR VALIDATION OF NON-CERTIFIED THERMOMETERS

1. Check the mercury column for separation or gas bubbles
2. If gas bubbles are present, the device is not suitable to check temperatures. Contact the manufacturer for correction
3. Adjust the heating bath to the temperature required for analysis. It is important that the volume of the bath be at least 100 times greater than the volume of the fluid in which the thermometer being calibrated is placed. This will ensure maintenance of a uniform temperature throughout the bath.
4. Place the reference and non – certified thermometers in the test tubes filled with water to the appropriate depth. The thermometers should be placed close to one another but with sufficient space between to ensure adequate circulation in the bath.
5. After thermal equilibrium is reached, determine the temperature reading for both thermometers. Thermometers deferring from the reference thermometer by more than 1^{o}C should be discarded or returned to the supplier. Agreement within 0.1^{o}C is required for critical laboratory purposes such as enzyme analysis. If discrepancies are between 0.2^{o}C and 0.1^{o}C, the thermometer can be used for less critical functions such as monitoring ovens, refrigerators and freezers.
6. Assign each thermometer a log number and list it with the result of the calibration in a thermometer log book. Thermometers should be calibrated every 6 months.

18 PURIFICATION OF WATER

18.1 INTRODUCTION

Water is one of the most important and commonly used reagents in the clinical chemistry laboratory.

18.2 PURIFICATION PROCESSES

DISTILLATION

Water is purified by evaporation and steam condensation. The condensed steam is collected as distilled water. Two types of apparatus are available in laboratories.

1. Basic water still, which consists of a water flask with water feed, a heating element, a condenser column and a collection flask.
2. Automatic water stills with a capacity of producing four litres per hour is also recommended for use in the laboratory

The following aspects must be considered to ensure a continuous, safe production of distilled water.

- A suitable electrical supply or other energy source must be available and compatible with the equipment's requirements
- Constant supervision is required to ensure that here is sufficient supply of water
- In the case of basic water stills, the boiling flask and element must be checked for inorganic deposits and cleaned as appropriate. This must be done frequently in areas with hard water. All glassware must be inspected for fractures, especially the boiling flask, which represents a potential safety hazard
- In the case of automatic water stills, always follow the instructions of the manufacturer for installation, operation and maintenance. Special precautions must be taken to ensure continuous flow of water into the instrument. Like all laboratory glassware assemblies, after and extended period of use, water stills may suffer a reduction in wall thickness caused by the continuous process of solution attrition causing breakage of the glass.

DEIONISATION

In the deionisation process water is passed through a bed of mixed cation- and anion-exchange resins. Hydrogen and hydroxyl ions on the surface of the resins are displaced by cationic and anionic impurities. Deionisation is used with carbon adsorption which is very effective in removal of dissolved organic compounds.

REVERSE OSMOSIS

In reverse osmosis, water is forced under pressure through a semi permeable membrane, leaving behind remnants of the dissolved organic, ionic and suspended impurities.

ULTRA- FILTRATION

Water is passed through semi permeable membranes of pore size <0.22μm removing particulate matter, emulsified solids, most bacteria and pyrogens

ULTRAVIOLET OXIDATION AND STERILISATION

This is used after other purification processes to remove trace amounts of organic contaminants and bacteria

18.3 A COMPARISON OF WATER PURIFICATION PROCESSES

	MAJOR CLASSES OF CONTAMINANTS					
PURIFICATION PROCESS	Dissolved ionised solids	Dissolved ionised gases	Dissolved organics	Particulates	Bacteria	Pyrogens /endotoxins
Distillation	**E / G**	**P**	**G**	**E**	**E**	**E**
Deionization	**E**	**E**	**P**	**P**	**P**	**P**
Reverse Osmosis	**G**	**P**	**G**	**E**	**E**	**E**
Carbon Adsorption	**P**	**P**	**E / G**	**P**	**P**	**P**
Filtration	**P**	**P**	**P**	**E**	**E**	**P**
Ultra filtration	**P**	**P**	**G**	**E**	**E**	**E**
Ultraviolet Oxidation	**P**	**P**	**E / G**	**P**	**G**	**P**

E = Excellent (capable of complete or near total removal)
G = Good (capable of removing of large percentages)
P = Poor (little or no removal)

18.4 GRADES OF WATER PURITY

	TYPE I	TYPE II	TYPE III
Maximum bacterial content, colony forming units per millimetre (CFU/ml)	10 (but preferably bacteria free)	1000	Not specified
pH	Not specified	Not specified	5.0 – 8.0
Minimum resistivity, megaohm – centimetre (megaohm – cm 25°C)	10	1.0	0.1
Maximum silicate, mg/L of SiO_2	0.05	0.1	1.0
Particulate matter	0.22μm filter	Not specified	Not specified
Organic contaminants	Activated carbon	Not specified	Not specified

18.5 QUALITY CONTROL AND IMPURITY TESTING

Water must be monitored at regular intervals to evaluate the performance of the water purification system. As a minimum bacterial surveillance and resistivity determinations are necessary on frequent basis.

Microbial monitoring: The bacteria can inactivate reagents by metabolising certain reagent components. They contribute to the total organic contamination and can alter optical properties of test solutions. Adequate flushing should be carried out before the collection of the sample for microbial testing. (The system must also be flushed before one draws water for use in reagent preparation.) After collection the sample should be processed immediately.

Resistivity: Resistivity measurements are used to assess the ionic content of purified water. The higher the ion concentration the lower the resistivity. The resistivity must be at least 10 MΩ.cm (preferably 15 -18 MΩ.cm) to meet the type 1 specifications. In –line resistivity meters are recommended for systems that supply type 1 water. Frequency of testing is daily.

System documentation and record keeping: A procedure manual should be developed for the water purification system that includes
1. A quality assurance plan defining responsibilities of personnel
2. Procedures for preventive maintenance.
3. Quality control check lists
4. Worksheets for documenting daily, weekly, monthly and other testing
5. Documentation of all corrective actions taken.

19. AUTOMATION IN CLINICAL CHEMISTRY

Automated systems incorporate mechanized versions of basic manual laboratory techniques and procedures.

19.1 PROCESSING CONCEPTS

- Batch analysis
  A number of specimens are processed in the same analytical session or 'run'.
- Sequential analysis
  Each specimen in the batch enters the analytical process one after another, and each result or set of results emerges in the same order as specimens are entered
- Single channel analysis
  Each specimen is subjected to a single process so that results of a single analyte are produced.
- Multiple channel analysis
  Each specimen is subjected to multiple analytical processes so that a set of test results is obtained
- Random access analysis
  Any specimen by a command to the processing system can be analyzed by available process in or out of sequence with other specimens and with out regard to their initial order.

19.2 THE COMPONENT STEPS IN AUTOMATED SYSTEMS

- Specimen identification
  In the computerised systems entry of a test order for a uniquely identified patient generates a specimen label bearing a unique laboratory accession number.
  The unique label with a bar code is affixed to the specimen collection tube at the bed side of the patient. The specimen is sent to the laboratory with the request form. The patient's identification data with the clinical details are entered into the computer using a bar code reader. The primary tube enters the analyzer.

  In some analyzer systems separated serum cups are generated and placed on a tray. A work sheet generated with the patient's lab number, sample cup position and the requested tests. **Sample mix up is a possibility.**

- Specimen preparation
  Entry of the primary tube into the analyser avoids the possible mix up of specimens during the aliquoting of serum. The following methods of analysis minimize the errors.

Uses of whole blood for analysis – Inclusion of ion selective electrodes which measure ion activity rather than concentration are used.
Dry chemistry- whole blood is applied to the dry reagent films which are inserted into the analyzer

Automation of specimen preparation- Primary samples enters into the analyzer in a wheel, centrifuged and decants the supernatant in to secondary cups.

- Specimen handling and delivery
  Many analysers also sample from a cup or tube that is filled with serum transferred from the original tube. Each cup should be designed to minimise the dead volume (the excess serum that must be present in a cup to permit the aspiration of the full volume required for testing.)The cups should be made out of inert material that does not adsorb the analytes and their shape should be such that even without a cap little evaporation occurs. An analyser with a refrigerated loading zone and use of appropriate covers will reduce the degradation of samples. A mechanism for the removal of protein and other interferents should be in place.

- Sample and reagent delivery into the reaction cups
  A probe is a positive liquid displacement pipette used to deliver the sample/reagent in to the reaction cup/cell. In analysers that use one probe for both sample and reagent delivery a wash port with distilled water or separate pipette tips should be included to minimise the effects of carryover. The use of distilled water may dilute the samples. Separate probes for sample and reagent delivery are recommended. Inaccuracy and imprecision of the probes should not exceed 1%.

- Open versus closed reagent systems
  Some analysers that use liquid reagents are open systems in that most parameters related to an analysis may be modified by the operator and in that reagents from a variety of suppliers can be used. Closed system analysers require reagents in a unique container or format provided by the manufacturer.

- Reagent storage and delivery
  Most automated systems use liquid reagents stored in plastic or glass containers. They are stored in laboratory refrigerators and introduced into the instruments as required. The analyser should have a compartment maintained at 4^0 C - 8^0 C for reagent storage. Many analyser systems include facilities to identify the reagents, volume of the contents, expiration date and lot numbers.

- Storage of water (availability of a type 1 water),
  Many analysers need reagent grade type 1 water for the process of analysis. Pre treatment of water through the cartridges may be required.

- Method of analysis

  ABSORBANCE/ TRANSMITTANCE PHOTOMETRY

  Most analysers use absorbance/transmittance photometry method for clinical chemistry analysis. Reaction cups serve as the cuvettes which align with the light path to obtain the absorbance. The measurement of absorbance requires a radiant energy source, a means of spectral isolation, and a detector. The radiant energy sources used in automated systems include tungsten, quartz halogen, deuterium, xenon as well as lasers. Spectral isolation is commonly achieved with interference filters. The most popular detection component is the photomultiplier tube.

  Ion selective electrode for electrolyte measurement

  It is the measurement of the electrical potential difference between two electrodes (reference and measuring electrodes) A membrane is included to select the ion under measurement. Ion activity is measured and the relationship to the concentration established through calibrators. In the combined photometric/ion selective electrode analyser, samples should first be analysed in the ion selective electrode followed by chemical reactions

- Method of analysis in immunoassay systems

  ELISA –Antigen and antibody reaction identified through an enzyme system

  Chemiluminance – Antigen and antibody reaction identified through the emission of light from the chemical which reacts with the enzyme attached to the second antibody.Substrates are luminal, isoluminol, acridium esters or luciferin.Highly sensitive method

- Signal processing, data handling and microprocessors

  These functions achieved by the inclusion of digital computers into the system

19.3 FACTORS TO BE CONSIDERED IN THE SELECTION OF AN AUTOMATED SYSTEM

- Semi automated/fully automated system

- Availability of infrastructure facilities
  - Space
  - Air conditioning facility
  - Water supply
  - Electrical supply with back up generators
  - Ups (uninterrupted power supply)
  - Sample storage
  - Reagent storage capacity
  - Reagent supply (maintain a continuous supply)
  - Request the reagents in 2- 3 instalments

- Cost
  Initial capital expenditure
  Cost of the equipment, installation, training of personnel
  Estimate the yearly expenditure depending on the expected life span of the instrument with at least 10% increase every year
  Recurrent expenditure on consumables, spare parts
  Maintenance cost

- Manpower
  Technical competency of the operators

- Handling of sudden breakdown- back up system

- Long term shut down of instrument due to non availability of facilities (reagents)

- Analyzer specifications – method of measurement

20. MINOR EQUIPMENT AND CONSUMABLES

1. Magnetic stirrer
2. Pipette washer
3. Timer
4. Calculator
5. Vortex mixer
6. Desiccator
7. Test tube racks(stainless steel, polypropylene)
8. Test tube holders

20.1 GLASS WARE

1. Beakers
2. Volumetric flasks
3. Flat bottom flasks
4. Bijou bottles
5. Universal containers
6. Reagent bottles –glass(clear/amber)
7. Reagent bottles-polypropylene
8. Wash bottles (polypropylene)
9. Funnels
10. Cylinder- Measuring
11. Pipettes
12. Test tubes(12 x 50, 12 x 75 – for serum separation, 12 x 100, 16 x 100, 16 x 125, 16 x 150, 18 x 150 –for dilutions and analysis)
13. Centrifuge tubes 16 x 100 (round bottom, conical)
14. Distillation unit (condenser with distillation flask)

20.2 CONSUMABLES

1. Pipette tips
2. pipette tips boxes
3. Eppendorf vials
4. Test tube caps
5. Parafilm

20.3 REGISTERS

1. Inventory register
2. Consumable register for chemical and glassware
3. Health 500
4. Health 503
5. Maintenance log book for equipments
6. Cash and local purchase register
7. Specimen receiving
8. Specimen despatch register
9. Records of analysis, calculations ,technical and clinical validations
10. Statistics of testing

20.4 STATIONARIES

1. Files
2. Box files
3. CR books
4. Pens
5. Markers

21 CONDEMNING OF UNSERVICEABLE EQUIPMENT

(Extracted from the document prepared by Mr. M.M. Dassanayaka, Former Principal, School of Medical Laboratory Technology, Colombo, for the benefit of the inventory holders)

21.1 WRITING OFF FROM INVENTORY

All instruments and general items, which are unserviceable, should be removed from the inventory. This has to be done in accordance with the government regulations. Under financial regulation 877 the provisions are provided to the Heads of the Institutions or the regional heads to take necessary actions to delete such items from the inventory.

The officer handling the inventory should prepare the printed form general 47 in triplicate with all items need to be removed from this inventory. The folio number, the name of item as it appears in the inventory, and the number of years of use of equipment should be entered in this form. Certify the forms and forward them to the head of the institution through the immediate supervisory.

The board of survey
In a major institution the head of the institution/Director and in a smaller institution the provincial Head/Director has the power to appoint a three member board to inspect and decide on the action to be taken on the items listed in General 47.

The board has a chairman and they collectively decide on each item as to what action should be taken. They award a grading to each item by English litters D-destroy, R-Retain/Repairable S - sale, and T- Tender are the accepted letters to mark the grading of the items. When arriving at such decisions the board adheres to following guidelines.

1. Sale of items in auction should be done in the presence of at least one board member.
2. Destruction of items, burn, bury etc done in the presence of at least one board member
3. Items for sale should be advertised and displayed to the public
4. No item in working order should be forwarded for sale.
5. The state symbol should not appear on items for sale
6. A list of items sold or destroyed, the money collected on the sale should be certified by the board and the documents should be handed over to the head of the institution.
7. The heavy and costly equipments such as refrigerators, freezers, hot air ovens, air conditioners, colorimeters, balance, water baths etc. should be inspected by the staff of the Bio Medical engineering division. The equipment should carry a certificate issued by the head of the Bio Medical Engineering stating that the equipment is beyond economical repairs and recommended for condemning

The condemned items should be strike off from the inventory. For this purpose the head of the institution will issue a certificate on health 605 to the officer in charge of the inventory.

On receipt of this certificate the officer handling the inventory should make an appointment with the storekeeper to amend the sub inventory. He should then meet him with the health 605 certificate and the sub inventory.

The storekeeper is the officer responsible for maintaining correct records of items physically present in an institution. He should also have a record of their distribution in the institution.

Any adjustment made in the sub inventory should be carried on to all relevant registers so that the registers are balanced. The adjustments should be made in the presence of the SMLT and should record very clearly with the date and the number of the health 605 form that gave the authority for the change. Signatures of the storekeeper and SMLT are placed on the entries.

If the correct procedures are not adopted during a stock verification or and annual audit inspection the SMLT will be asked to make explanations. He may be then found fault for shortages in the inventory.

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4. The Microscope, a practical guide; WHO –South East Asia 1999
5. WHO Guidelines on standard operating procedures for clinical chemistry, Sep 2000
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7. Basics for quality assurance for intermediate and peripheral laboratories (WHO publication)
8. Selection of basic laboratory equipment for laboratories with limited resources.

WE SINCERELY THANK THE FOLLOWING COLLEAGUES / MEMBERS OF STAFF OF MRI AND WHO COUNTRY OFFICE

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