World Health Organization

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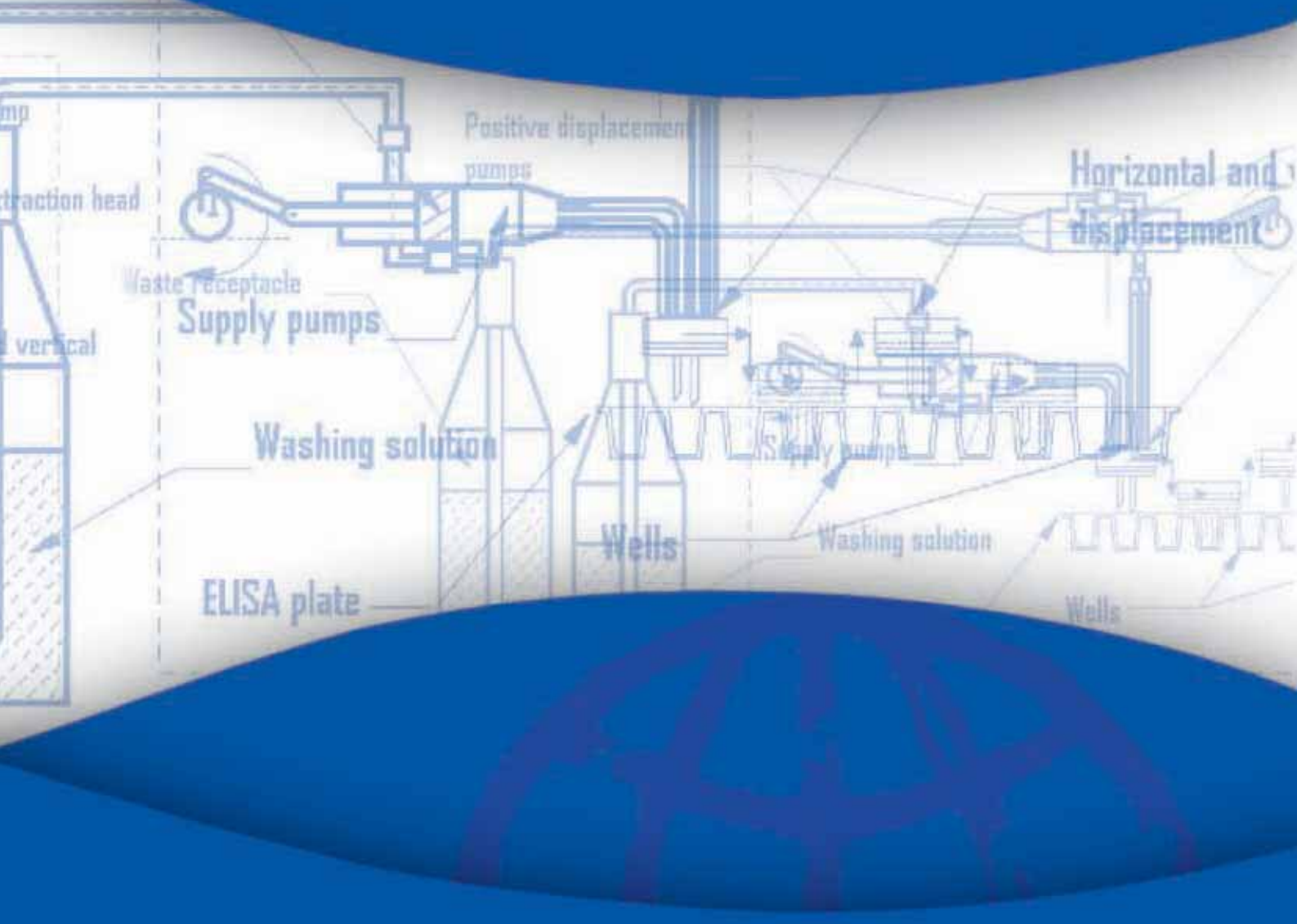
MAINTENANCE Manual *for* Laboratory Equipment

2nd Edition



MAINTENANCE Manual

for Laboratory Equipment

2nd Edition

Panamerican Health Organization
Regional Office of the World Health Organization

World Health Organization

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Acknowledgements

This manual is a revised edition of "Manual de mantenimiento para equipo de laboratorio" (PAHO, 2005) translated from Spanish into English. Revisions include additional chapters on laboratory equipment commonly used in some laboratories and updates allowing global use of the manual.

The revised version has been prepared under the direction of Dr Gaby Vercauteren, World Health Organization, Geneva, Switzerland and in coordination with Dr Jean-Marc Gabastou, Pan-American Health Organization/World Health Organization, Washington, DC, USA; translated by Ms Christine Philips; reviewed by Ms Mercedes Pérez González and adapted, revised and edited by Mrs Isabelle Prud'homme.

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WHO also thanks manufacturers who have granted permission to use their images in this publication.

Introduction

This manual has been developed to support personnel employed in health laboratories. Its purpose is to give a better understanding of the technical requirements regarding installation, use and maintenance of various types of equipment which play an important role in performing diagnostic testing. The manual also aims to provide support to personnel responsible for technical management, implementation of quality management and maintenance.

Due to the diversity of origins, brands and models, this manual offers general recommendations. Equipment-specific details are explained in depth in the maintenance and installation user manuals from manufacturers. These should be requested and ordered through the procurement processes of the individual agencies and professionals responsible for the acquisition of technology, or directly from the manufacturer.

This manual was originally developed by the Pan-American Health Organization (PAHO) to support improved quality programmes which PAHO promotes in regional laboratories. The English version was produced by WHO to further expand support for quality programmes in other regions. The revised edition now includes 20 equipment groups selected to cover those most commonly used in low to medium technical complexity laboratories across the world. Given the differences in technical complexity, brands and existing models, each chapter has been developed with basic equipment in mind, including new technology where relevant. The following information is included in each chapter:

- Groups of equipment, organized by their generic names. Alternative names have also been included.
- Photographs or diagrams, or a combination of both to identify the type of equipment under consideration.
- A brief explanation on the main uses or applications of the equipment in the laboratory.
- A basic description of the principles by which the equipment operates with explanations of principles or physical and/or chemical laws which the interested reader can – or should study in depth.
- Installation requirements with emphasis on the electrical aspects and the requirements for safe installation and operation, including worldwide electrical standards.
- Basic routine maintenance, classified according to the required frequency (daily, weekly, monthly, quarterly, annually or sporadically). The procedures are numbered and presented in the actual sequence in which these should take place (model-specific procedures can be found in the manuals published by the manufacturers).
- Troubleshooting tables with the most frequent problems affecting the equipment with possible causes and actions that may resolve these problems.
- A list of basic definitions of some of the specialized terms used.
- For some equipment, additional themes related to calibration, quality control and design (with operational controls).

This information, along with good use and care, helps to maintain laboratory equipment in optimal condition.

Chapter 1

Microplate Reader

GMDN Code	**37036**
ECRI Code	**16-979**
Denomination	**Photometric micro-plate reader**

The microplate reader also known as "Photometric micro-plate reader or ELISA reader" is a specialized spectrophotometer designed to read results of the ELISA test, a technique used to determine the presence of antibodies or specific antigens in samples. The technique is based on the detection of an antigen or antibodies captured on a solid surface using direct or secondary, labelled antibodies, producing a reaction whose product can be read by the spectrophotometer. The word ELISA is the acronym for "Enzyme-Linked Immunosorbent Assay". This chapter covers the use of microplate readers for ELISA testing. For additional information on the instrument principles of operation and maintenance, consult Chapter 11 discussing the spectrophotometer.

PHOTOGRAPH OF MICROPLATE READER

Photo courtesy of BioRad Laboratories

PURPOSE OF THE MICROPLATE READER

The microplate reader is used for reading the results of ELISA tests. This technique has a direct application in immunology and serology. Among other applications it confirms the presence of antibodies or antigens of an infectious agent in an organism, antibodies from a vaccine or auto-antibodies, for example in rheumatoid arthritis.

OPERATION PRINCIPLES

The microplate reader is a specialized spectrophotometer. Unlike the conventional spectrophotometer which facilitates readings on a wide range of wavelengths, the microplate reader has filters or diffraction gratings that limit the wavelength range to that used in ELISA, generally between 400 to 750 nm (nanometres). Some readers operate in the ultraviolet range and carry out analyses between 340 to 700 nm. The optical system exploited by many manufacturers uses optic fibres to supply light to the microplate wells containing the samples. The light beam, passing through the sample has a diameter ranging between 1 to 3 mm. A detection system detects the light coming from the sample, amplifies the signal and determines the sample's absorbance. A reading system converts it into data allowing the test result interpretation. Some microplate readers use double beam light systems.

Test samples are located in specially designed plates with a specific number of wells where the procedure or test is carried out. Plates of 8 columns by 12 rows with a total of 96 wells are common. There are also plates with a greater number of wells. For specialized applications, the current trend is to increase the number of wells (384-well plates) to reduce the amount of reagents and samples used and a greater throughput. The location of the optical sensors of the microplate reader varies depending on the manufacturers: these can be located above the sample plate, or directly underneath the plate's wells.

Nowadays microplate readers have controls regulated by microprocessors; connection interfaces to information systems; quality and process control programs, which by means of a computer, allow complete test automation.

Equipment required for ELISA testing

In order to perform the ELISA technique, the following equipment is required:

1. Microplate reader.
2. Microplate washer (Chapter 2).
3. Liquid dispensing system (multi-channel pipettes may be used).
4. Incubator to incubate the plates.

Figure 1 illustrates how this equipment is interrelated.

Mechanical phases of the ELISA technique

Using the equipment

When an ELISA test is conducted, it typically follows these steps:

1. A first washing of the plate may be done using the microplate washer.
2. Using a liquid dispenser or the multi-channel pipettes, wells are filled with the solution prepared to be used in the test.
3. The plate is placed in the incubator where at a controlled temperature, a series of reactions take place.

Stages 1, 2 and 3 can be repeated several times depending on the test, until the reagents added have completed their reactions.

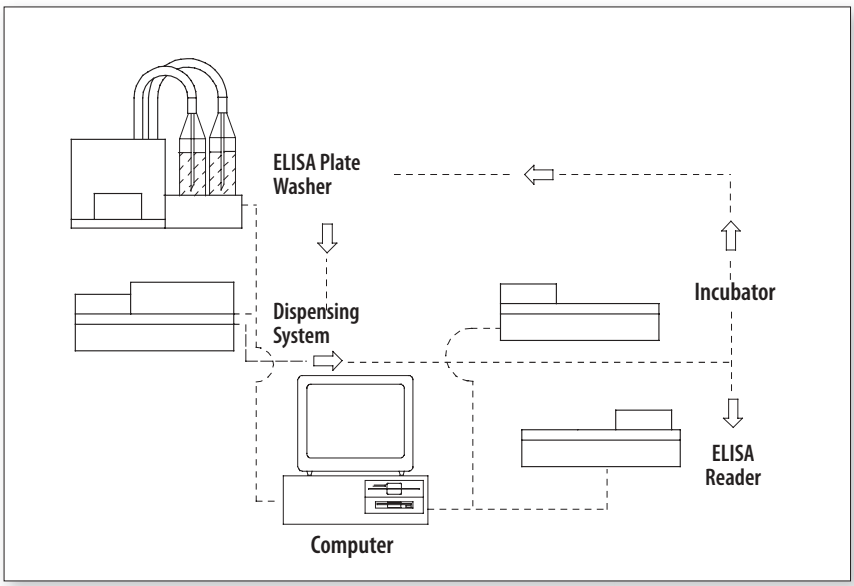
Finally, when all the incubation steps have been completed, the plate is transferred to the microplate reader. The reading of the plate is done and a diagnosis can be deduced.

Biochemical phases of the ELISA technique[1]

The ELISA technique from a biochemical point of view:

1. The plate wells are coated with antibodies or antigens.
2. Samples, controls and standards are added to the wells and incubated at temperatures ranging between room temperature and 37 °C for a determined period of time, according to the test's characteristics. During the incubation, the sample's antigen binds to the antibody coated to the plate; or the antibody in the sample binds to the antigen coated on the plate, according to their presence and quantity in the sample analyzed.
3. After incubation, the unbound antigen or antibodies are washed and removed from the plate by the microplate washer using an appropriate washing buffer.
4. Next, a secondary antibody, called the conjugate, is added. This harbours an enzyme which will react with a substrate to produce a change of colour at a later step.
5. Then begins a second period of incubation during which this conjugate will bind to the antigen-antibody complex in the wells.
6. After the incubation, a new washing cycle is done to remove unbound conjugate from the wells.
7. A substrate is added. The enzyme reacts with the substrate and causes the solution to change in colour. This will indicate how much antigen-antibody complex is present at the end of the test.
8. Once the incubation time is completed, a reagent is added to stop the enzyme-substrate reaction and to prevent further changes in colour. This reagent is generally a diluted acid.
9. Finally, the plate in is read by the microplate. The resulting values are used to determine the specific amounts or the presence of antigens or antibodies in the sample.

Figure 1. Equipment used in ELISA tests



Note: Some of the wells are used for standards and controls. Standards allow the cut-off points to be defined. The standards and controls are of known quantities and are used for measuring the success of the test, evaluating data against known concentrations for each control. The process described above is common, although there are many ELISA tests with test-specific variants.

[1] More detailed explanations must be consulted in specialized literature.

INSTALLATION REQUIREMENTS

In order for the microplate reader to operate correctly, the following points need to be respected:

1. A clean, dust free environment.
2. A stable work table away from equipment that vibrates (centrifuges, agitators). It should be of a suitable size so that there is working space at the side of the microplate reader. The required complementary equipment for conducting the technique described above is: washer, incubator, dispenser and computer with its peripheral attachments.
3. An electrical supply source, which complies with the country's norms and standards. In the countries of the Americas for example, 110 V and 60 Hertz frequencies are generally used, whereas other regions of the World use 220-240V, 50/60HZ.

Calibration of the microplate reader

The calibration of a microplate reader is a specialized process which must be executed by a technician or trained engineer following the instructions provided by each manufacturer. In order to do the calibration, it is necessary to have a set of grey filters mounted on a plate of equal geometric size to those used in the analyses. Manufacturers provide these calibration plates for any wavelength the equipment uses.

Calibration plates are equipped with at least three pre-established optic density values within the measurement ranges; low, medium, and high value. In order to perform the calibration, follow this process:

1. Place the calibration plate on the equipment.
2. Carry out a complete reading with the calibration plate. Verify if there are differences in the readings obtained from well to well. If this is the case, invert the plate (180°) and repeat the reading to rule out that differences are attributed to the plate itself. In general, it is accepted that the instrument does not need further calibration if the plate results are as expected at two wavelengths.
3. Verify if the reader requires calibration. If so, proceed with the calibration following the routine outlined by the manufacturer, verifying that the reading's linearity is maintained as rigorously as possible.
4. If the instrument does not have a calibration plate, verify it by placing a coloured solution in the wells of a plate and immediately carry out a complete reading. Then invert the plate 180° and read the plate again. If both readings display identical, average values in each row, the reader is calibrated.
5. Verify that the reader is calibrated, column by column. Place a clean, empty plate and carry out a reading. If there is no difference between each of the average reading of the first to the last column, it can be assumed that the reader is calibrated.

ROUTINE MAINTENANCE

Maintenance described next focuses exclusively on the microplate reader. The maintenance of the microplate washer is described in Chapter 2.

Basic maintenance

Frequency: Daily

1. Review that optical sensors of each channel are clean. If dirt is detected, clean the surface of the windows of the light emitters and the sensors with a small brush.
2. Confirm that the lighting system is clean.
3. Verify that the reader's calibration is adequate. When the daily operations begin, let the reader warm up for 30 minutes. Next, do a blank reading and then read a full plate of substrate. The readings must be identical. If not, invert the plate and repeat the reading in order to determine if the deviation originated in the plate or the reader.
4. Examine the automatic drawer sliding system. It must be smooth and constant.

Preventive maintenance

Frequency: Quarterly

1. Verify the stability of the lamp. Use the calibration plate, conducting readings with intervals of 30 minutes with the same plate. Compare readings. There must be no differences.
2. Clean the detectors' optical systems and the lighting systems.
3. Clean the plate drawer.
4. Verify the alignment of each well with the light emission and detection systems.

TROUBLESHOOTING TABLE		
PROBLEM	**PROBABLE CAUSE**	**SOLUTION**
The reader gives a reading that does not make sense.	The illumination lamp is out of service.	Replace the lamp with one with the same characteristics as the original.
The reader's readings vary from row to row.	Dirty optical sensors.	Clean the sensors.
	The illumination system's lenses or parts are dirty.	Clean the lighting system's lenses.
	Lack of calibration in one or more channels.	Verify the calibration of each one of the channels.
The reader displays high absorbance values.	Reagents expired and/or incorrectly prepared.	Check to see if the TMB is colourless and the preparation adequate.
	Contamination with other samples.	Repeat the test verifying the labelling, the washer and how the pipette was used.
	Incorrect wavelength filter.	Verify the recommended wavelength for the test. Adjust if it is incorrect.
	Insufficient or inefficient washing.	Verify the washing method used. Use an appropriate quality control test.
	Very long incubation time or very high temperature.	Check incubation times and temperatures.
	Incorrect sample dilution.	Check process for sample dilution.
	Some reagent was omitted.	Verify that the test has been carried out according to the established procedure.
The reader displays low absorbance values.	Very short incubation time and very low temperature.	Check temperatures and incubation times.
	The reagents were not at room temperature.	Check that the reagents are stable at room temperature.
	Excessive washing of the plate.	Adjust the washing process to what the test manufacturers indicate.
	Incorrect wavelength filter.	Verify the wavelength selected. Use wavelength recommended for the test.
	Expired or incorrectly prepared reagents.	Check the used reagents. Test the dilutions.
	A reagent was omitted.	Verify that the test was done according to the established procedure.
	The plate displays scratches at the bottom of the wells.	Prepare a new plate and repeat the test.
	Incorrectly selected or dirty plate.	Verify the type of plate used. Prepare a new plate and repeat the test.
	The plate wells have dried up.	Change the manner in which the plate is washed.
	The plate is incorrectly placed or is seated unevenly in the reader.	Check the placement of the plate. Repeat the reading.
	Humidity or fingerprints on the outer part of the bottom of the plate.	Verify that the plate under the bottom of the wells is clean.
	Residual quantities of washing buffer in the wells before adding the substrate.	Confirm that the washing buffer is completely removed.
	The substrate tablets do not dissolve completely.	Verify that the tablets dissolve correctly.
	The substrate tablet has been contaminated by humidity or metal clips or is not complete.	Test the integrity and handling of substrate tablets.
	The position of the blank well could have been changed and an incorrect quantity has been subtracted at each reading.	Verify that the plate set-up is correct.
The reader displays unexpected variation in the optical density readings.	The reader's lamp is unstable.	Replace the lamp with one that has similar characteristics as the original.
The reader displays a gradual increase or decrease from column to column.	Inappropriate calibration of the plate's advance motor.	Calibrate the advance so that at each step the wells remain exactly aligned with the lighting system.
The optical density readings are very low compared to the operator's optical evaluation criteria.	The reading is being carried out with a different wavelength than required for the test.	Verify the wavelength used when conducting the reading. If this is the problem, adjust the wavelength and repeat the reading. Verify that the recommended wavelength filter has been selected.

Low reproducibility.	Sample homogeneity.	Mix the reagents before use. Allow these to equilibrate to room temperature.
	Incorrect pipetting procedure.	Ensure pipette's tips are changed between samples and that excessive liquid inside is removed.
	Reader not calibrated.	Check the calibration. Use an appropriate quality control set.
	Reading without sufficient warming time of the instrument.	Wait until the reader has warmed up to its operating temperature.
	Expired reagents.	Verify the expiry dates of the reagents.
	Insufficient or inefficient washing.	Remove the buffer from the washer. Check that the wells are filled and aspirated in a uniform manner when washed.
The blank sample shows high absorbance.	Contaminated substrate.	Check that TMB is colourless and its preparation.
The data are not transferred from the reader to the microprocessor.	The reader and the microprocessor have differently defined codes.	Verify selected codes.
	Different (Baud) information transfer rates.	Confirm transfer rates selected.
	Incorrect configuration selected for the reception/ transmission plugs.	Review the configuration of the plugs. The configuration must follow parameters defined by the manufacturer.
Misaligned light beam.	The reader was transferred or moved without using the necessary precautions.	Call the specialized service technician.
	The light source – lamp – has been changed and the replacement has not been installed or aligned correctly.	Verify its assembly and alignment.
Incorrect identification of the sample.	The plate was incorrectly loaded.	Check the samples' identification process. Repeat the reading carrying out the adjustments.
	Incorrect identification of the sample registered in the reader.	Check the samples' identification process. Repeat the reading carrying out the adjustments.
Computer fails to indicate the error codes.	The programme which controls the activation of alarms and warning messages is defective or is not validated by the manufacturer.	Call the specialized service technician.
The reader demonstrates failure in detecting errors.	Various components of the system display failure, such as the liquid level detection system.	Call the specialized service technician.

BASIC DEFINITIONS

Chemiluminescence. Emission of light or luminescence resulting directly from a chemical reaction at environmental temperatures.

ELISA (Enzyme-Linked Immunosorbent Assay). Biochemical technique used mainly in Immunology to detect the presence of an antibody or an antigen in a sample.

ELISA plate. Consumable standardized to carry out the ELISA technique. Generally, plates have 96 wells in a typical configuration of 8 rows by 12 columns. There are also ELISA plates with 384 wells or up to 1536 wells for specialized high throughput testing in centres with high demand.

Microplate washer. Equipment used for washing plates during specific stages of an ELISA test with the aim of removing unbound components during reactions. Microplate washers use special buffers in the washing process.

Enzyme. Protein that accelerates (catalyses) chemical reactions.

Fluorophore. Molecules absorbing light at a determined wavelength and emitting it at a higher wavelength.

Microplate reader. The name given to spectrophotometers with the capacity to read microplates.

TMB. Tetramethylbenzidine, a substrate for the horseradish peroxidase (HRP) enzyme.

Chapter 2

Microplate Washer

GMDN Code	**17489**
ECRI Code	**17-489**
Denomination	**Micro-plate washer**

The microplate washer or "plate or ELISA washer" is designed to perform washing operations required in the ELISA technique. The microplate washer performs the washing of the ELISA plate's wells during the different stages of the technique.

PHOTOGRAPH OF MICROPLATE WASHER

Photo courtesy of BioRad Laboratories

PURPOSE OF THE MICROPLATE WASHER

The microplate washer has been designed to supply cleaning buffers required for the ELISA technique in a controlled manner. In the same fashion, the equipment removes from each well, substances in excess from the reaction. Depending on the test performed, the washer can intervene from one to four times, supplying the washing buffer, agitating and removing the unbound reagents[1] until the programmed times and cycles are completed. The washer has of two reservoirs; one for the washing buffer, the other for the waste generated during the washing process.

OPERATION PRINCIPLES

The microplate washer has been designed to perform washing operations in the ELISA technique. The equipment possesses at least, the following subsystems which vary depending on the manufacturer's design.

- ***Control subsystem.*** Generally, the washer is controlled by microprocessors allowing programming and controlling steps to be performed by the washer such as: number of washing cycles[2] (1–5); expected times; supplying and extracting pressures; plate format (96–384 wells); suction function adjustment according to the type of well[3] (flat bottom, V bottom or rounded bottom or strips used); volumes distributed or aspirated; the soaking and agitation cycles, etc.
- ***Supply subsystem.*** In general, this comprises a reservoir for the washing solution; one or several pumps; usually a positive displacement type syringe and a dispenser head that supplies the washing solution to the different wells by means of needles. The head usually comes with eight pairs of needles for washing and aspirating simultaneously the wells of the same row (the supply and extraction sub-systems converge on the head). There are models with twelve pairs of needles and others that conduct the washing process simultaneously in all the wells. Some washers offer the possibility of working with different types of washing solutions, performing the solution changes according to the program entered by the operator.

[1] See a brief explication of the ELISA technique in Chapter 1, *Microplate Reader*.

[2] The exact number of washing operations required depends on the assay used. This is explained in each manufacturer's test instruction manual.

[3] If the bottom is flat, the suction needle is located very close to one of well's faces; if it is rounded or V-shaped, the suction needle is centered.

- ***Extraction or suction system.*** This requires a vacuum mechanism and a storage system for gathering the fluids and waste removed from the wells. The vacuum may be supplied by external and internal pumps. Extraction is done by a set of needles mounted on the washer/dryer's head. The number of needles varies from one to three, according to the washer model used.

  If it uses only one needle, the washing and extraction operation is done with this single needle. If it uses two needles, one is used for supplying the washing solution and the other for extraction. If it uses three needles, the first is used for supplying the washing solution, the second for extraction and the third for controlling (extracting) any excess volume in the well. Generally, the extraction needle is longer than the supply needle, which enables it to advance (vertically) up to a height ranging between 0.3 and 0.5 mm from the bottom of the well.
- ***Advance sub-system.*** This is composed of a mechanism which moves the supply and extraction head horizontally to reach each well in the ELISA plate. When the horizontal movement to the following row occurs, there is a vertical movement towards the well to dispense or extract the washing solution. There are washers which carry out these operations in a simultaneous manner.

The sub-systems previously described are shown in Figure 2. Figure 3 shows the different types of wells most commonly found in microplates. Each kind of well is suitable for a particular type of test.

Washing process

The washing of the microplate is one of the stages of the ELISA technique. Special solutions are used in the washing steps. Among those most commonly used is phosphate buffer solution or PBS. The phosphate buffer solution has a stability of 2 months if kept at 4 °C. It is estimated that 1 to 3 litres of solution is required for washing one microplate and that 300 *µl* is used in each well per cycle. Washing can be done manually, but it is preferable to use an automated microplate washer for a better throughput and to minimize handling of potentially contaminated substances.

Among the washing processes used by microplate washers are featured:

- ***Aspiration from top to bottom.*** When the aspiration phase is initiated, the needles move vertically and the aspiration is initiated immediately as these enter into the liquid. The process continues until the needles reach their lowest position very close to the bottom of the wells. At this point they are stopped in order to avoid suctioning the air that should flow against the interior lateral walls of the wells. This type of aspiration prevents air currents from drying the bound protein on the surface of the wells.

Figure 2. Microplate washer

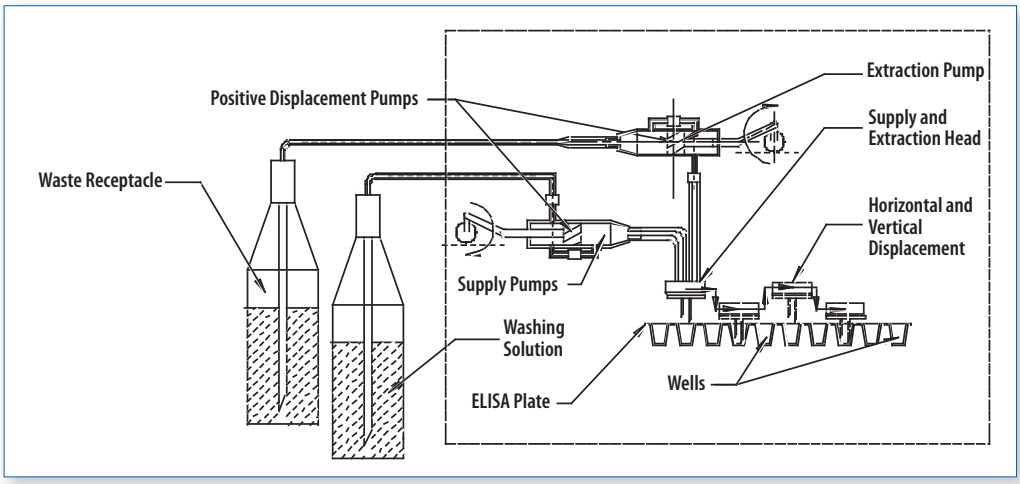
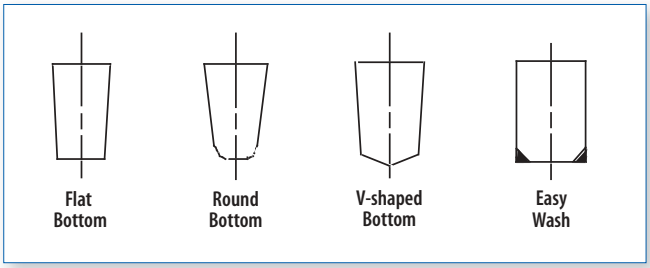


Figure 3. Well profiles



- ***Simultaneous distribution and aspiration.*** In certain types of washer, the washing and aspiration systems operate simultaneously, generating a controlled turbulence inside the well which removes the unbound substances during the incubations.
- ***Aspiration from the base of the wells.*** In this system, the aspiration of the fluid contained in the wells is performed initially with the aspiration needles in a position very close to the bottom, immediately beginning a suctioning cycle, usually time-controlled. This system may aspirate air if there are differences in the levels of the tanks.

Washer calibration

The microplate washer is critical for guaranteeing that the ELISA technique performs as expected. The alignment to be taken into account for the effective functioning of the equipment is presented next:

- ***Position of the needles (supply and aspiration head).*** The horizontal and vertical position adjustment with respect to the wells must be verified carefully. If the plate has flat bottom wells, the supply needle must be checked to see that it is situated very close to the well's wall. If the bottom is round or V-shaped, the suction needle should be located in the centre of the well: upon the vertical movement, a needle-base distance is maintained in the well, usually between 0.3 to 0.5 mm. The needles must never be allowed to touch the bottom of the wells to avoid mechanical interferences between the needle point and the well's base during the aspiration function.
- ***Aspiration time.*** Appropriately adjust the aspiration time so that a solution film adhered to the well's wall can flow towards the bottom. Avoid very long time lapses to prevent the coating on the wells from drying up. Check that the suction system's needles are clean (free of obstructions).
- ***Distributed Volume.*** Check that the volume distributed is as close as possible to the maximum capacity of the well; confirm that all the wells are filled uniformly (at the same level). Verify that the distributing needles are clean (free of obstructions).
- ***Vacuum.*** The suctioning system must be calibrated efficiently. If the vacuum is too strong, the test can be altered. In fact, it could dry out the wells and considerably weaken the enzyme activity in the wells and completely alter the test result. The majority of washers function with a vacuum ranging between 60 and 70% of atmospheric pressure. In some washers, the vacuum is made in an external pump which operates as an accessory of the washer. Its operation is controlled by the washer, which means that the vacuum pump operates only when required.

Washing process verification

To verify that the washing process is done according to the specifications of ELISA techniques, manufacturers of ELISA tests have developed procedures to be carried out regularly. One of the controls[1] is based on using the peroxidase reagent, which is dispensed using a pipette in the plate wells to be read at 405, 450 and 492 nm. At once the wells are washed and a colourless substrate is added (TMB/H_2O_2–Tetramethylbenzidine/Hydrogen Peroxide). Whatever conjugate remains will hydrolyze the enzyme and the chromogen will change to blue. After stopping the reaction with acid, the TMB will turn yellow again. The resulting colour intensity is directly related to the washing process efficiency.

INSTALLATION REQUIREMENTS

For the microplate washer to operate correctly, the following is necessary:

1. A clean, dust-free environment.
2. A stable work table located away from equipment that generates vibrations, (centrifuges, and agitators). It must be of a suitable size to locate the necessary complementary equipment: reader, incubator, distributor and computer with its peripheral attachments at the side of the microplate washer.
3. An electric outlet in good condition with a ground pole and, an electrical connection which complies with the country's or the laboratory's norms and standards. In the countries of the Americas, the 110 V and 60 Hz frequency is generally used. In other parts of the World, the 220-240 V and 50/60 Hz frequency is generally used.

ROUTINE MAINTENANCE

The routine maintenance described next focuses exclusively on the microplate washer. Maintenance of the microplate reader is dealt with in the Chapter 1.

Basic maintenance

Frequency: Daily

1. Verify the volume distributed.
2. Test the filling uniformity.
3. Verify the aspiration sub-system's efficiency.
4. Confirm the cleaning of the supply and extraction needles.
5. Clean the washer with distilled water after use, to remove every vestige of salt in the supply and extraction sub-systems' channels. The needles may be kept submerged in distilled water.
6. Verify that the body of the washer has been cleaned. If necessary, clean the exterior surfaces with a piece of cloth, moistened with a mild detergent.

[1] Procedure developed by PANBIO, *ELISA Check Plus*, Cat. Nº E-ECP01T.

Preventive maintenance

Frequency: Quarterly

1. Disassemble and clean the channels and connectors. Verify their integrity. If leaks or any vestiges of corrosion are detected, adjust and/or replace.
2. Verify the integrity of the mechanical components. Lubricate according to the manufacturer's instructions.
3. Test the adjustment of each one of the sub-systems. Calibrate according to the manufacturer's recommendations.
4. Confirm the integrity of the electrical connector and the inter-connection cable.
5. Clean the washer with distilled water after using it in order to remove every vestige of salt in the supply and extraction subsystems' channels.
6. Verify the integrity of the fuse, and that its contact points are clean.

Note: Trained technical personnel must carry out maintenance of the control system. If necessary, call the manufacturer or representative.

TROUBLESHOOTING TABLE

PROBLEM	PROBABLE CAUSE	SOLUTION
Upon completion of washing, residual solution remains in the wells.	The washer extraction system demonstrates failure.	Verify if the vacuum system is functioning at the appropriate pressure.
	The conducts/pipes of the vacuum system are of a different diameter than that recommended.	Check that the diameter of the channels corresponds to the recommendation by the manufacturer.
	The suction line shows obstructions.	Verify that the vacuum lines are clean.
	The container for storing the waste is full.	Confirm the waste recipient's level.
	The line filter is damp or blocked.	Verify the state and integrity of the suctioning system's filter.
	The needles' points are not placed correctly and do not reach the bottom of the wells.	Examine the placement of the needles' points.
	A different microplate is used in the test.	Verify the type of plate required for the test.
	The washer has not been purged sufficiently.	Check the purging process.
	The operator has not followed the manufacturer's instructions correctly.	Examine the process recommended by the manufacturer. Carry out the required adjustments.
	The plate placed in the washer is incorrectly aligned.	Check the placement of the plate in the washer.
The washing cycle is performing inadequately.	The washing solution reserve is exhausted.	Examine the cleaning solution storage receptacle. Replace the volume missing.
	The washer was not purged sufficiently at the beginning of the work cycle.	Clean adequately in order to homogenize the humidity in each one of its components and to eliminate air bubbles.
	The volume of washing solution distributed has been programmed erroneously.	Verify the required volume for each type of test and for each plate.
	The plate was placed incorrectly in the washer.	Check the correct installation of the plate in the washer.
	The cycle setting was incorrectly selected.	Review the cycle setting recommended for each type of plate.
	The plates used are different from those recommended by the manufacturer.	Verify that the plates used are completely compatible with the washer.
	The fluid level in the wells is inadequate.	
	The washing solution supply tube is not of the diameter or thickness specified by the manufacturer.	Check the manufacturer's specifications. If necessary, correct.
	The pressure is insufficient for delivering the adequate amount of washing solution.	Check the supply system and supply channels, there might be an obstruction in the filling line.
The washing container shows fungal and bacterial growths.	The system is not used frequently.	Check the procedures used for preventing fungal and bacterial growth.
	An adequate control procedure (disinfection) is not used.	Check the procedures used for preventing fungal and bacterial growth.
	The tubes and connectors are not changed with the required frequency.	Verify the change frequency suggested by the manufacturer and or the technical department.
	The washing solution has been contaminated.	Confirm the procedures used in the preparation and management of the washing solution with the aim of determining the cause of contamination and eliminate it.
	Maintenance has not been carried out according to its schedule.	Check the dates planned for carrying out maintenance. Inform those responsible.

BASIC DEFINITIONS

Buffer. A solution containing either a weak acid and its salt or, a weak base and its salt, which makes it resistant to changes in pH at a given temperature.

PBS. One of the solutions used to perform washing operations in ELISA tests. PBS is the acronym for Phosphate Buffer Solution. This is made of the following substances: *NaCl, KCl, $NaHPO_4 2H_2O$ and KH_2SO_4*. The manufacturers supply technical bulletins which indicate the proportions and instructions for preparing PBS. In general, one part of concentrated PBS is mixed with 19 parts of deionised water.

Plate (ELISA). Consumable with standard dimensions, designed to hold samples and reactions for the ELISA technique. In general, these have 96, 384 or 1536 wells and are made of plastics such as polystyrene and polypropylene. There are plates specially treated to facilitate the performance of the tests.

Positive displacement pump. A pump adjusted by a plunger moving along a cylinder. The mechanism is similar to that of a syringe. It is equipped with a set of valves for controlling the flow to and from the pump.

TMB/H_2O_2. (Tetramethylbenzidine/hydrogen peroxide). A set of reagents used for verifying the quality of washing done on the wells used in the ELISA technique.

Chapter 3

pH Meter

GMDN Code	**15164**
ECRI Code	**15-164**
Denomination	**pH Meter**

The pH meter is used for determining the concentration of hydrogen ions [H^+] in a solution. This equipment, provided it is carefully used and calibrated, measures the acidity of an aqueous solution. pH meters are sometimes called pH analysers, pH monitors or potentiometers.

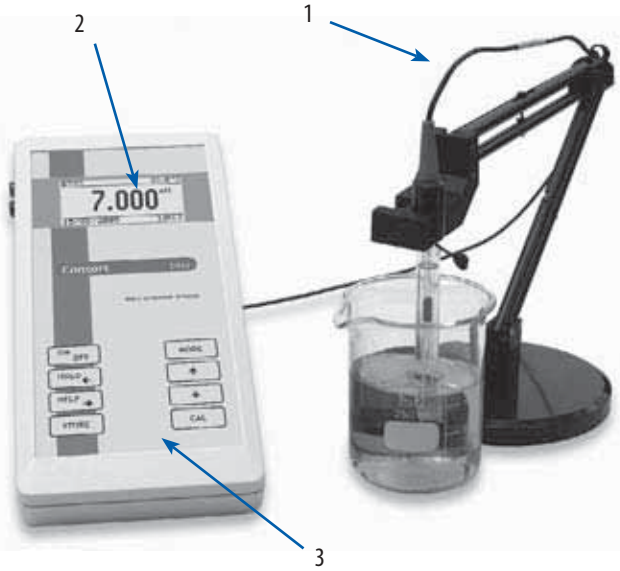
PURPOSE OF THE EQUIPMENT

The pH meter is commonly used in any field of science related to aqueous solutions. It is used in areas such as agriculture, water treatment and purification, in industrial processes such as petrochemicals, paper manufacture, foods, pharmaceuticals, research and development, metal mechanics, etc. In the health laboratory, its applications are related to the control of culture mediums and to the measurement of the alkalinity or acidity of broths and buffers. In specialized laboratories, diagnostic equipment microelectrodes are used to measure the pH of liquid blood components. The plasma pH allows the patient's health to be evaluated. It normally measures between 7.35 and 7.45. This value relates to the patient's metabolism in which a multitude of reactions occurs where acids and bases are normally kept in balance. Acids constantly liberate hydrogen ions [H^+] and the organism neutralizes or balances acidity by liberating bicarbonate ions [HCO_3^-]. The acid-base ratio in the organism is maintained by the kidneys, (organs in which any excesses present are eliminated). The plasma pH is one of the characteristics that vary with factors such as age or state of health of the patient. Table 1 shows typical pH values of some bodily fluids.

pH values of some bodily fluids

Fluid	pH Value
Bile	7.8 – 8.6
Saliva	6.4 – 6.8
Urine	5.5 – 7.0
Gastric Juice	1.5 – 1.8
Blood	7.35 – 7.45

PHOTOGRAPH AND COMPONENTS OF THE pH METER



1 Electrode carrying arm and electrode
2 Digital display
3 Control panel with temperature adjustment control, mode selection (Standby/mV/pH) and calibration controls

Photo courtesy of Consort

OPERATION PRINCIPLES

The pH meter measures the concentration of hydrogen ions [H^+] using an ion-sensitive electrode. Under ideal conditions, this electrode should respond in the presence of only one type of ion. In reality, there are always interactions or interferences with other types of ions present in the solution. A pH electrode is generally a combined electrode, in which a reference electrode and an internal glass electrode are integrated into a combined probe. The lower part of the probe ends in a round bulb of thin glass where the tip of the internal electrode is found. The body of the probe

contains saturated potassium chloride (KCl) and a solution 0.1 M of hydrogen chloride (HCl). The tip of the reference electrode's cathode is inside the body of the probe. On the outside and end of the inner tube is the anodized end. The reference electrode is usually made of the same type of material as the internal electrode. Both tubes, interior and exterior, contain a reference solution. Only the outer tube has contact with the measured solution through a porous cap which acts as a saline bridge.

This device acts like a galvanized cell. The reference electrode is the internal tube of the pH meter probe, which cannot lose ions through interactions with the surrounding environment. Therefore as a reference, it remains static (unchangeable) during the measuring process. The external tube of the probe contains the medium which is allowed to mix with the external environment. As a result, this tube must be filled periodically with a potassium chloride solution (KCl) for restoring the capacity of the electrode which would otherwise be inhibited by a loss of ions and evaporation.

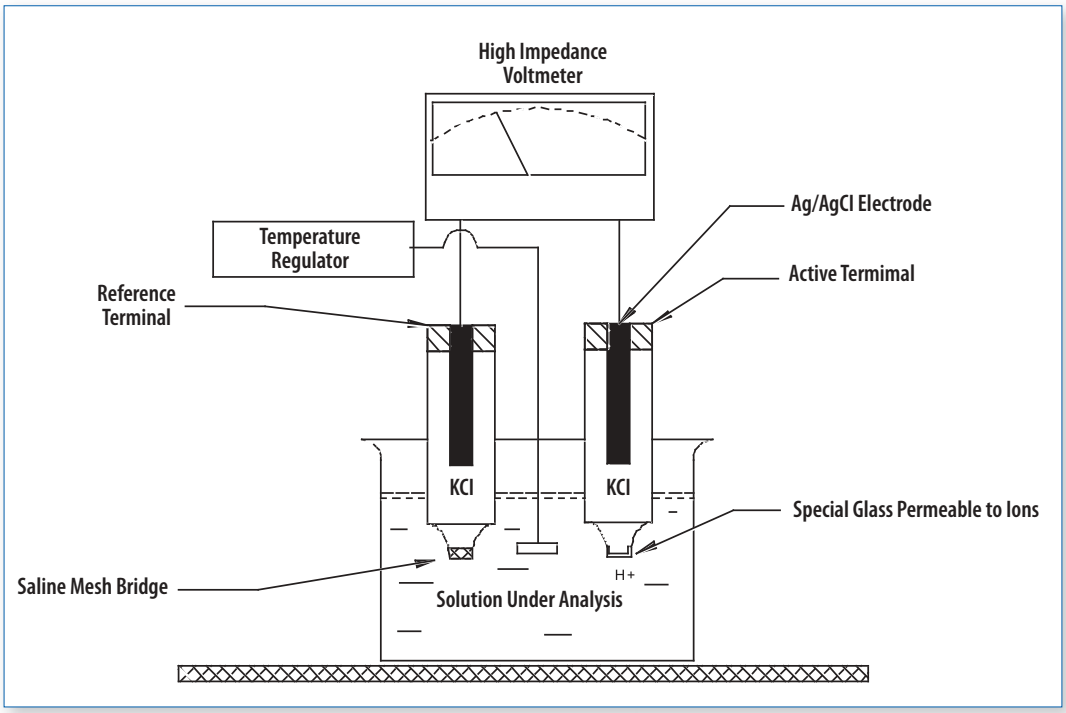
The glass bulb on the lower part of the pH electrode acts as a measuring element and is covered with a layer of hydrated gel on its exterior and interior. Metallic sodium cations [Na^+] are diffused in the hydrated gel outside of the glass and in the solution, while the hydrogen ions [H^+] are diffused in the gel. This gel makes the pH electrode ion-selective: Hydrogen ions [H^+] cannot pass through the glass membrane of the pH electrode. Sodium ions [Na^+] pass through and cause a change in free energy, which the pH meter measures. A brief explanation of the theory on how electrodes function is included in the appendix at the end of the chapter.

pH METER COMPONENTS

A pH meter generally has the following components:

1. ***The body of the instrument containing the circuits, controls, connectors, display screens and measuring scales.*** The following are among some of its most important components:
   a) ***An ON and OFF switch.*** Not all pH meters have an on and off switch. Some simply have a cord with a plug which allows it to be connected to a suitable electrical outlet.
   b) ***Temperature control.*** This control allows adjustments according to the temperature of the solution measured.
   c) ***Calibration controls.*** Depending on the design, pH meters possess one or two calibration buttons or dials. Normally these are identified by **Cal 1** and **Cal 2**. If the pH meter is calibrated using only one solution, the Cal 1 button is used; making sure that Cal 2 is set at a 100%. If the pH meter allows two point calibrations, two known pH solutions covering the range of pH to be measured are used. In this case, the two controls are used (Cal 1 and Cal 2). In special cases, a three-point calibration must be done (using three known pH solutions).
   d) ***Mode selector.*** The functions generally included in this control are:
      I. **Standby mode (0).** In this position the electrodes are protected from electrical currents. It is the position used for maintaining the equipment while stored.
      II. **pH mode.** In this position the equipment can take pH measurements after performing the required calibration procedures.

Figure 4. Diagram of a pH meter



III. **Millivolt mode (mV).** In this position the equipment is capable of performing millivoltage readings.

IV. **ATC mode.** The automatic temperature control mode is used when the pH is measured in solutions for which the temperature varies. This function requires the use of a special probe. Not all pH meters have this control.

2. ***A combined electrode or probe.*** This device must be stored in distilled water and stay connected to the measuring instrument. A combination electrode has a reference electrode (also known as *Calomel* electrode) and an internal electrode, integrated into the same body. Its design varies depending on the manufacturer.

TYPICAL CIRCUIT

Figure 6 features a typical circuit adapted to the control system of the pH meter. Each manufacturer has its own designs and variations.

Figure 5. Types of electrodes

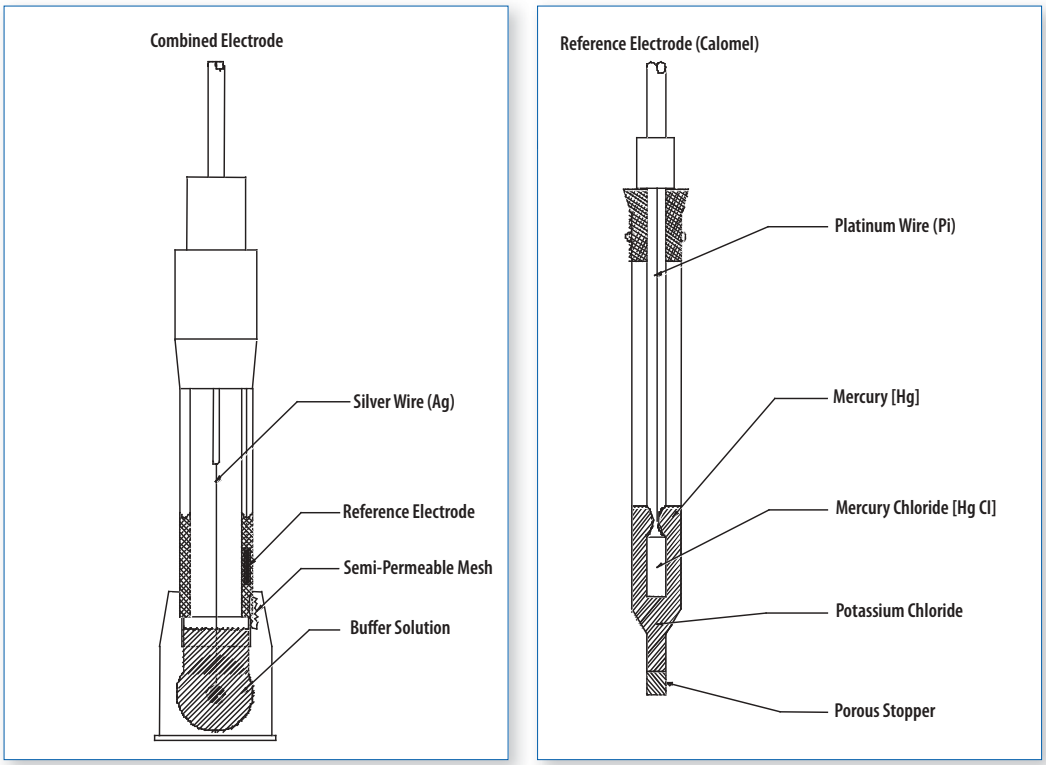
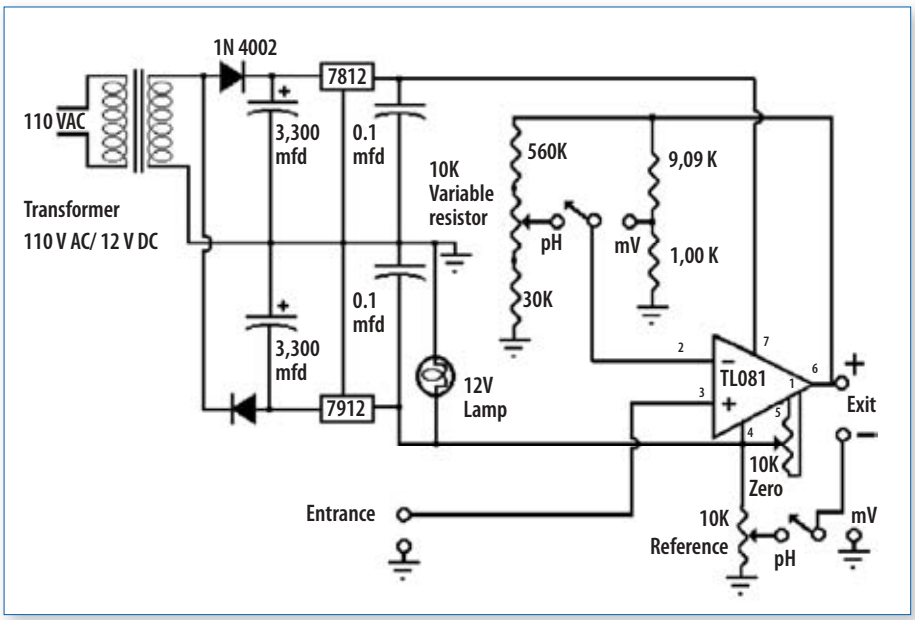


Figure 6. Example of a typical pH meter control circuit



Description of typical control circuit elements

System	Element	Description
Electric feeding and correction.	110 V/12 V AC transformer.*	A device converting the voltage of the 110 V to 12 V AC network.
	1N4002 rectifier diodes.	Diode controlling the type of wave and guaranteeing that is positive.
	Electrolyte condensers 3300 microfarads (µfd) (2).	Condensers absorbing the DC voltage to the diodes.
	Tri terminal regulators (7812, 7912).	A device regulating the voltage resulting from the interaction between diodes and condensers.
	0.1 microfarad (µfd) (2) electrolyte condensers.	Devices used to achieve stability at high frequency.
	12 V D C signal light.	Light indicating if the equipment is ON.
Measurement of pH and millivolts.	TL081 non-inverted type dual amplifier.	Millivolts circuits.
	(R1) 9.09 K Ω (ohm) resistors.	
	(R2) 1 K Ω (ohm) resistors.	
	(R3) 560 K Ω (ohm) resistors.	pH circuits.
	(R4) 10 K Ω(ohm) variable resistors.	
	(R5) 30 K Ω (ohm) resistors.	Ground resistance.
		The circuit gain is governed by means of the following equation: Gain = 1+ (R3+PxR4)/R5+ (1–P) xR4.
Outlet section.	Low cost DC voltmeter.	Permits readings in millivolts. The voltage read is 10 times that of the cell, allowing a resolution of 0.1 millivolts.
		The reading is done by using carbon/quinhydrone electrodes.

* Different voltage specifications are applicable in certain regions of the World.

INSTALLATION REQUIREMENTS

The pH meter works using electric current with the following characteristics.

Power: Single phase Voltage: 110 V or 220-230 V Frequencies; 50-60Hz depending on the World region.

There is also portable pH meters powered with batteries.

GENERAL CALIBRATION PROCEDURE

pH analyzers must be calibrated before use to guarantee the quality and accuracy of the readings following these procedures:

1. ***One point calibration.*** This is carried out for normal working conditions and for normal use. It uses one known pH reference solution.
2. ***Two point calibration.*** This is done prior to performing very precise measurements. It uses two known pH reference solutions. It is also done if the instrument is used sporadically and its maintenance is not carried out frequently.

Description of the process

Frequency: Daily

1. ***Calibrate the pH meter using one known pH solution (one point calibration).***
   - 1.1 Connect the equipment to an electrical outlet with suitable voltage.
   - 1.2 Adjust the temperature selector to the environmental temperature.
   - 1.3 Adjust the meter.
   - 1.4 Remove the electrodes from the storage container. The electrodes must always be stored in a suitable solution. Some can be maintained in distilled water, others must be kept in a different solution as their manufacturers recommend[1]. If for some reason, the electrode becomes dry, it is necessary to soak it for at least 24 hours before use.
   - 1.5 Rinse the electrode with distilled water in an empty beaker.
   - 1.6 Dry the electrode with material able to absorb residual liquid on its surface, without impregnating the electrode. To avoid possible contamination, the electrodes must be rinsed between different solutions.

[1] Verify the type of buffer solution recommended by the electrode manufacturer.

2. ***Place electrodes in the calibration solution.***
   2.1 Submerge the electrode in the standardization solution in such a manner that its lower extremity does not touch the bottom of the beaker. This decreases the risk of breaking the electrode. If the test requires that the solution be kept in motion using the magnetic agitator, special care must be taken so that the agitation rod does not hit the electrode as this could break it. Buffer solution is used as a calibration solution, because its pH is known and therefore will still be maintained even if a little contamination occurs. In general, a solution of pH = 7 is used for this purpose[1].
3. ***Turn the functions selector from Standby position to pH position.***
   3.1 This action connects the electrode to the pH measuring scale in the pH meter.
   3.2 Adjust the meter to read the pH of the calibration solution using the button marked Cal 1. This enables the meter to read the pH of the calibration solution.

   For example: For a solution at pH = 7, the needle can oscillate slightly in units of 0.1 pH; on average, the reading should be 7. The reading of the meter (reading scale) should be done perpendicularly, to avoid or eliminate parallel-type errors (reading errors produced by the shadow of the meter's needle, visible on the mirror of the reading scale). The pH meter is then ready (calibrated), to carry out the correct pH readings.
   3.3. Put the functions selector in the Standby position.
4. ***Measuring the pH of a solution.***
   4.1 Remove the electrode from the calibration solution.
   4.2 Rinse the electrode with distilled water and dry it.
   4.3 Place the electrode in the solution of unknown pH.
   4.4 Turn the functions selector from the Standby position to the pH position.
   4.5 Read the pH of the solution on the meter's scale or the screen. Register the reading obtained on the control sheet.
   4.6 Turn the functions selector again to the Standby position.

   If it is necessary to measure the pH of more than one solution, repeat the previously described procedures, rinsing the probe with distilled water and drying with clean, lint-free paper between readings. When the pH has to be measured in numerous solutions, the pH meter must be calibrated frequently, following the steps previously described.
5. ***Turn off the pH meter.***
   5.1 Remove the electrode from the last solution analyzed.
   5.2 Rinse the electrode in distilled water and dry it with a drying material that will not penetrate it.
   5.3 Place the electrode in its storage container.
   5.4 Verify that the functions selector is in the Standby position.
   5.5 Activate the off switch or disconnect the feed cable, if it lacks this control.
   5.6 Clean the work area.

GENERAL MAINTENANCE OF THE pH METER

pH meters have two general maintenance procedures: one concerning the analyzer's body, the other for the pH detection probe (electrodes).

General maintenance procedures for the pH meter's body

Frequency: Every six months

1. Examine the exterior of the equipment and evaluate its general physical condition. Verify the cleanliness of the covers and their adjustments.
2. Test the connection cable and its system of connections. Check that they are in good condition and clean.
3. Examine the equipment controls. Verify that these are in good condition and activated without difficulty.
4. Verify that the meter is in good condition. To do this, the instrument must be disconnected from the electric feed line. Adjust the indicator needle to zero (0) using the adjustment screw generally found below the pivot of the indicator needle. If the equipment has an indicator screen, check that it is functioning normally.
5. Confirm that the on indicator (bulb or diode) operates normally.
6. Verify the state of the electrode carrying arm. Examine the electrode attachment and assembly mechanism to prevent the electrode from becoming loose. Check that the height adjustment operates correctly.
7. Check the batteries (if applicable); change them if necessary.
8. Test its function by measuring the pH of a known solution.
9. Inspect the ground connection and check for escaping current.

[1] Verify the type of calibration solution recommended by the electrode manufacturer.

BASIC MAINTENANCE OF THE ELECTRODE

Frequency: Every four months

The measuring or detector electrode requires periodic maintenance of the conducting solution to obtain precise readings.

The recommended steps for replacing the electrolyte solution are the following:

1. Remove the detector electrode from the storage buffer solution.
2. Rinse the detector electrode abundantly with distilled water.
3. Remove the upper cover of the detector electrode.
4. Fill the conduit surrounding the internal electrode with a saturated potassium chloride (KCl) solution. Use the syringe or applicator supplied with the KCl solution. Verify that the tip of the syringe does not touch the inside of the electrode.
5. Close the electrode with its cover. Rinse the electrode in distilled water.
6. Keep the electrode in storage buffer solution while not in use.

Cleaning of the electrode

The type of cleaning required for electrodes depends of the type of contaminant affecting it. The most common procedures are summarized next:

1. ***General cleaning.*** Soak the pH electrode in a 0.1 M HCl solution or 0.1 M HNO_3, for 20 minutes. Rinse with water.
2. ***Removal of deposits and bacteria.*** Soak the pH electrode in a diluted domestic bleach solution (e.g. 1%), for 10 minutes. Rinse abundantly with water.
3. ***Cleaning oil and grease.*** Rinse the pH electrode with a mild detergent or with methyl alcohol. Rinse with water.
4. ***Cleaning of protein deposits.*** Soak the pH electrode in 1% pepsin and 0.1 M HCl for 5 minutes. Rinse with water.

After carrying out each cleaning operation, rinse with deionised water and refill the reference electrode before use.

Other precautionary measures

1. Do not strike the electrode. Given that the structure is generally made of glass and very fragile, it is necessary to manipulate it very carefully, preventing it from being knocked off.
2. Remember that the electrode has a limited lifespan.
3. While not in use, keep the electrode inside the storage buffer solution.

TROUBLESHOOTING TABLE

PROBLEM	PROBABLE CAUSE	SOLUTION
The pH meter shows unstable readings.	There are air bubbles in the electrode.	Soak the electrode to eliminate the bubbles.
	The electrode is dirty.	Clean the electrode and recalibrate.
	The electrode is not immersed.	Verify that the sample covers the tip of the electrode perfectly.
	The electrode is broken.	Replace the electrode.
The electrode's response is slow.	The electrode is dirty or greasy.	Clean the electrode and recalibrate.
The screen shows an error message.	Incorrect operating mode selected.	Verify the operation mode selected. Select a valid operation.
The screen shows a calibration or error message.	There is a calibration error.	Recalibrate the pH meter.
	The calibration of the buffer value is erroneous.	Verify the buffer values used.
	The electrode is dirty.	Clean and calibrate the electrode.
The pH meter is on, but there is no signal on the screen.*	The batteries are badly installed.	Verify the polarity of the batteries.
	The batteries are worn out.	Replace the batteries.
The battery indicator is flashing.*	The batteries are worn out.	Replace the batteries.

* Applicable to equipment equipped with batteries only.

BASIC DEFINITIONS

Buffer. A solution containing either a weak acid and its salt or, a weak base and its salt, which makes it resistant to changes in pH at a given temperature.

Calomel electrode. A reference electrode used with the active electrode for determining the pH of a solution. This electrode is constructed with a mercury base (Hg), a covering of dimercuric chloride (Hg_2Cl_2) and a potassium chloride solution of 0.1 M. It is represented as $Cl_2[Hg_2Cl_2, KCl]Hg$.

Dissociation. A phenomenon through which a break in the molecules occurs. As a result it produces electrically charged particles (ions).

Electrolyte. A solute which produces a conducting solution, e.g. NaCl (sodium chloride) and NH_4OH.

Gel. A semisolid substance (e.g. jelly) composed of a colloid (solid) dispersed in a liquid medium.

Ion. Neutral atom which gains or loses an electron. When the atom loses an electron, it becomes a positively charged ion, called a cation. If the atom gains or captures an electron, it becomes a negatively charged ion, called an anion.

Ion-sensitive electrode. A device which produces a difference in potential proportional to the concentration of an analyte.

Molarity. Number of Moles (M) in a substance in a litre of solution. (Number of moles of solute in a litre (L) of solution). The brackets around the ionic symbol indicate that it is treated as a molar concentration.

Mol. (abbreviation for molecule). A quantity of any substance whose mass expressed in grams is numerically equal to its atomic mass.

Mole (unit). The amount of a substance that contains as many atoms, molecules, ions, or other elementary units as the number of atoms in 0.012 kilogram of carbon 12. It corresponds to the number 6.0225×10^{23}, or Avogadro's number, also called gram molecule.
The mass in grams of this amount of a substance, numerically equal to the molecular weight of the substance, also called *gram-molecular weight*.

pH. Measurement of the concentration of the hydrogen ion (H^+) given in moles per litre (M) in a solution. The pH concept was proposed by Sørensen and Lindstrøm-Lang in 1909 to facilitate expressing very low ion concentrations. It is defined by the following equation:
$pH = -\log [H^+]$ or $[H^+] = 10^{-pH}$

It measures the acidity of a solution. Example, in water the concentration of $[H^+]$ is 1.0×10^{-7} M resulting in pH = 7. This allows the range of concentrations from 1 to 10^{-14} M, to be expressed from zero (0) to 14. There are diverse systems for measuring the acidity of a solution. An acidic substance dissolved in water is capable of producing H^+ ions. A basic substance dissolved in water is capable of producing $[OH^-]$ (hydroxides) ions.

An acid substance has a greater quantity of ions $[H^+]$ than pure water; a basic substance shows greater quantities of ions $[OH^-]$ than pure water. The concentrations of substances are expressed in moles per litre.

In pure water, the ion concentration $[H^+]$ and $[OH^-]$ is 1.0×10^{-7} M, it is thus considered a neutral substance. In reality, it is a weak electrolyte that is dissociated following the following equation:
$H_2O \; D \; [H^+][OH^-]$

In all aqueous solutions there is a balance expressed as:

$$\frac{[H^+][OH^-]}{H_2O} = K$$

If the solution is diluted, the concentration of the non-dissociated water can be considered constant:
$[H^+][OH^-] = [H_2O]K = K_a$

The new constant Ka is called a constant of dissociation or ionic product of water and its value is 1.0×10^{-14} at 25 °C.
$[H^+][OH^-] = 1.0 \times 10^{-14}$
$X \times X = 1.0 \times 10^{-14}$
$X^2 = 1.0 \times 10^{-14}$
$X = 1.0 \times 10^{-7}$

In pure water the concentrations of H^+ and OH^- are 1.0×10^{-7} M, a very low concentration, given that the molar concentration of water is 55.4 mol/litre.

Solution. Homogenous liquid mixture (with uniform properties) of two or more substances. It is characterized by the absence of chemical reactions among the components in the mixture. The component in greater proportion and generally in a liquid state is called solvent and that or those in a lesser quantity, the solutes.

Annex
The pH theory

pH electrodes ideally behave as an electrochemical cell and react to the concentration of ions [H^+]. This generates an electromotive force (EMF) which, according to the Nernst law is calculated using the following equation:

$$E = E^\circ + \frac{RT}{nF} \ln a_{H^+}$$

Given that:

$pH = -\ln a_{H^+}$ where a is the effective concentration of ions (Activity)

If n = 1, the equation is then rewritten as:

$$E = E^\circ - \frac{R'T}{F} pH$$

E° is a constant dependant on the temperature. If E° is substituted by E'T, the calibration will be more sensitive. Real electrodes do not always perform according to the Nernst equation. If the concept of sensibility (s) is introduced, the equation can be rewritten as:

$$E = E'T - s\frac{R'T}{F} pH$$

The values of E' and s are found when measuring the EMF in two solutions with known pH. S is the slope of E versus pH, while E' is found at the intersection with the axis y. When E' and s are known, the equation can be rewritten and the pH can be calculated as:

$$pH = \frac{E'T - E}{s\frac{R'T}{T}}$$

Chapter 4

Balances

GMDN Code	10261	10263	45513	46548
ECRI Code	10-261	10-263	18-449	18-451
Denomination	Balances	Electronic balances	Analytical electronic balances	Micro analytical, microelectronic balances

The balance is an instrument which measures the mass of a body or substance using the gravity force which acts on that body. The word comes from the Latin terms bis which means two and *lanx*, plate. The balance has other names such as scale and weight. It must be taken into account that the weight is the force which the gravitational field exercises on a body's mass, this force being the product of the mass by the local acceleration of gravity [F = m x g]. The term local is used to emphasize that this acceleration depends on factors such as the geographical latitude, altitude and the Earth's density where the measurement is taken. This force is measured in Newtons.

PHOTOGRAPHS OF BALANCES

Mechanical balance

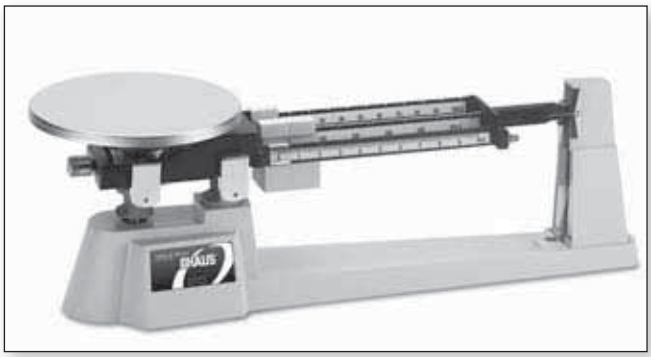


Photo courtesy of Ohaus Corporation

Electronic balance

Photo courtesy of Acculab Corporation

PURPOSE OF THE BALANCE

The balance is used for measuring the mass of a body or substance or its weight. In the laboratory, the balance is used for weighing as part of quality control activities (on devices like pipettes), in the preparation of mixtures of components in predefined proportions and in the determination of specific densities or weights.

OPERATION PRINCIPLES

There are differences in design, principles and criteria of metrology amongst balances. At present, there are two large groups of balances: mechanical and electronic balances.

Mechanical balances

The following are some of the more common ones:

1. ***Spring balance.*** Its function is based on a mechanical property of springs as the force exercised on a spring is proportional to the spring's elasticity constant [k], multiplied by its elongation [x] [F = -kx]. The greater the mass [m] placed on the balance's plate, the greater the elongation will be, given that the elongation is proportional to the mass and the spring's constant. The calibration of a spring balance depends on the force of gravity acting on the object weighed. This type of balance is used when great precision is not necessary.
2. ***Sliding weight balance.*** This type of balance is equipped with two known weights which can be moved on setting scales (one macro, the other micro). Upon placing a substance of unknown mass on the tray, its weight is determined by moving the weight on both setting scales until the equilibrium position is reached. At this point, the weight is obtained by adding both quantities indicated by the sliding masses' position on the scale.
3. ***Analytical balance.*** This balance functions by comparing known weight masses with that of a substance of unknown weight. It is composed of a base on a bar or symmetrical lever, maintained by a blade-like support on a central point called a fulcrum. At its ends, there are stirrups, also supported with blades which allow these to oscillate smoothly. From there, two plates are suspended. Certified weights are placed on one of the plates and unknown weights on the other. The balance has a securing system or lock, which allows the main lever to remain stable when not in use or when it is necessary to modify the counter-weights. The balance is inside an external box which protects it from interferences, such as air currents. Analytical balances can weigh ten thousandths of a gram (0.0001 g) or 100 thousandths of a gram (0.00001 g). This type of balance generally has a capacity of up to 200 grams.

Figure 7. Spring balance

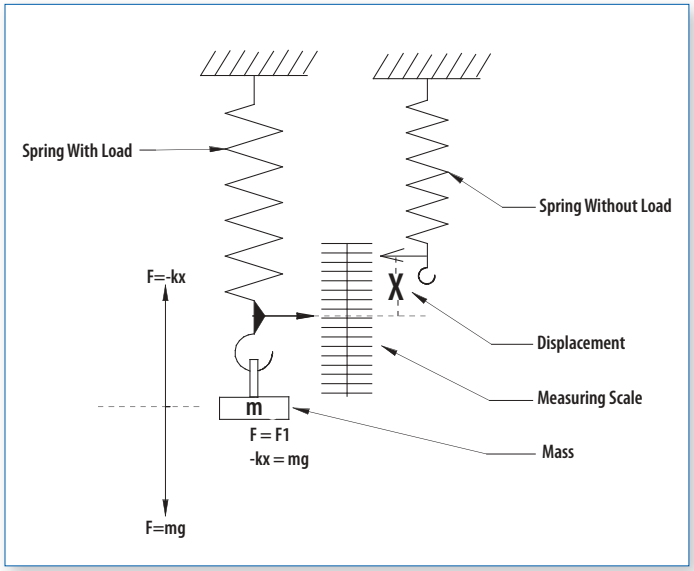


Figure 8. Sliding weight scale

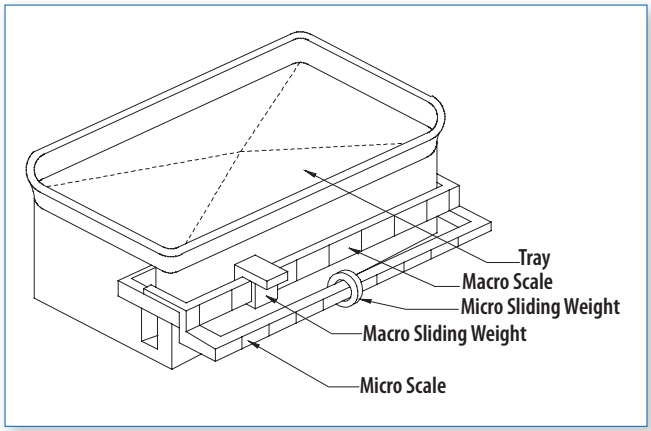
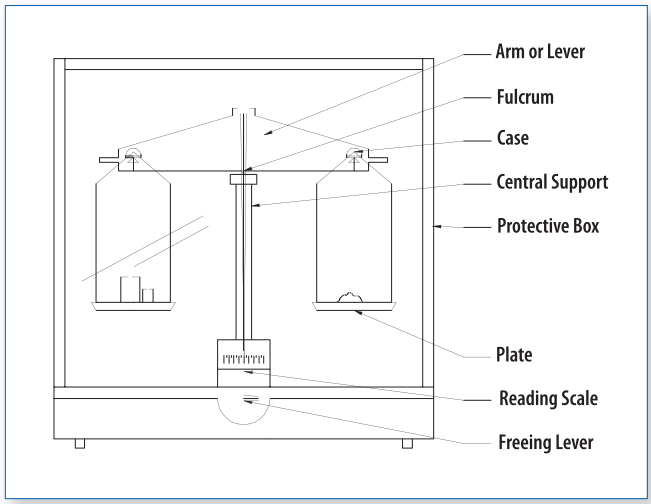


Figure 9. Analytical balance



It is necessary to have a set of certified masses. The set is generally composed of the following pieces:

Type of mass	Capacity
Simple pieces	1, 2, 5, 10, 20, and 50 g
	100, 200 and 500 g
Fractional pieces	2, 5, 10, 20 and 50 mg
	100, 200 and 500 mg

4. ***Upper plate balance (Top loading or parallel guidance balance).*** This type of balance has a loading plate located on its upper part, supported by a column maintained in a vertical position by two pairs of guides with flexible connections. The effect of the force produced by the mass is transmitted from a point on the vertical column directly or by some mechanical means to the loading cell. The requirement with this type of mechanism is that parallel guides must be maintained with exactitude of up to ± 1 *µm*. Deviations in parallelism cause an error known as lateral load (when the mass being weighed shows differences if the reading is taken at the centre of the plate or on one of its sides). The diagram shown below explains the operation principle some manufacturers have introduced in electronic balances.
5. ***Substitution Balance (Unequal-lever arm or two-knife balance).*** This is a balance with a single plate. An unknown mass is placed on the weighing plate. It is weighed by removing known masses from the counterweight side until it reaches a balanced position, using a mechanical system of cams. The fulcrum is generally off-centre in relation to the length of the load beam and located near the front of the balance. When a mass is placed on the weight plate and the balance's locking mechanism is released, the movement of the load beam is projected through an optical system to a screen located on the front part of the instrument.

Operation verification

The procedure used for verifying the functioning of a typical mechanical balance is described below. The described process is based on the substitution balance.

1. Verify that the balance is levelled. The levelling is achieved using a ring-shaped adjustment mechanism located on the base of the balance or by adjusting a bubble or knob on a scale located on the front of the balance's base.
2. Test the zero mechanism. Place the controls on zero and free the balance. If the reading does not stay at zero, adjust the zero mechanism (a grooved screw located in a horizontal position near the fulcrum). To do this, it is necessary to block the balance and slightly adjust the mechanism. The process is to be continued until the zero adjusts correctly on the reading scale.

Figure 10. Upper plate balance

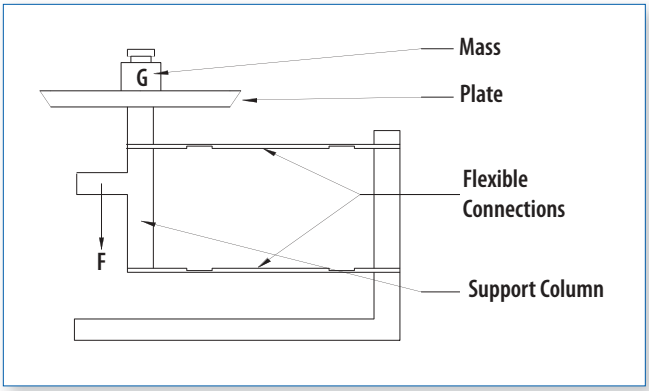
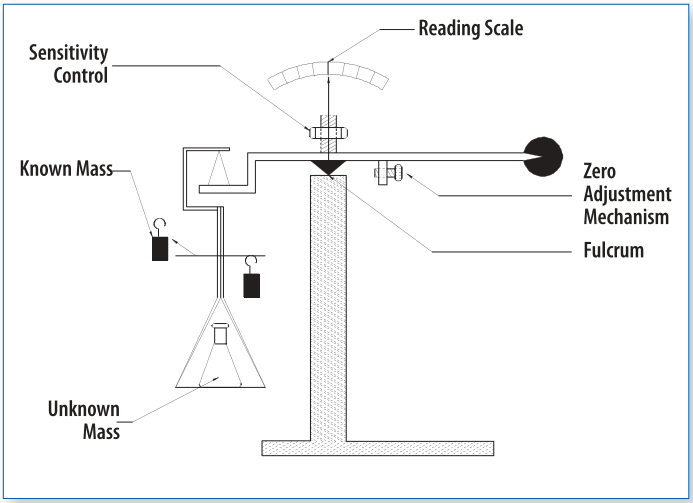


Figure 11. Substitution balance



3. Verify and adjust the sensitivity. This is always readjusted whenever some internal adjustment is done. It is performed with a known standard according to the following steps:
   a) Lock the balance.
   b) Place a standard weight (equivalent to the optical scale range) on the plate.
   c) Position the micro setting to one (1).
   d) Release the balance.
   e) Adjust to the zero position.
   f) Position the micro setting to zero (0). The balance should indicate 100. If the scale displays less or more than 100, the sensitivity control must be adjusted. This requires locking the balance, opening the upper cover and turning the sensitivity screw: If the scale registers more than 100; turn the screw in a clockwise position. If the scale registers less than 100, it is necessary to unwind the screw anticlockwise. Repeat the process until the balance is adjusted (adjusting the zero and the sensitivity).

4. Verify the plate's brake. It is mounted on a threaded axis which touches the plate in order to prevent it from oscillating when the balance is locked. In case of an imbalance, the axis must be rotated slightly until the distance between the break and the plate is zero when the balance is locked.

Maintenance of the mechanical balance

The maintenance of mechanical balances is limited to the following routines:

Frequency: Daily

1. Verify the level.
2. Verify the zero setting.
3. Verify the sensitivity adjustment.
4. Clean the weighing plate.

Frequency: Annually

1. Calibrate the balance and document the process.
2. Disassemble and clean the internal components. This must be done according to the process outlined by the manufacturer or a specialized firm must be contracted to do so.

Electronic balances

The electronic balances have three basic components:

1. A weighing plate. The object to be weighed placed on the weighing plate exercises a pressure distributed randomly over the surface of the plate. By means of a transfer mechanism (levers, supports, guides), the weight's load is concentrated on a simple force [F] which can be measured. [$F = \int P\partial a$]. The pressure's integral part on the area allows the force to be calculated.
2. A measuring device known as "load cell" produces an exit signal corresponding to the load's force in the form of changes in the voltage or frequency.
3. A digital analogous electronic circuit shows the final result of the weight digitally.

Laboratory balances operate according to the principle of compensation of the electromagnetic force applicable to displacements or torques. The combination of their mechanical components and automatic reading systems provides weight measurements at defined levels of accuracy depending on the model.

Principle. The mobile parts (weighing plate, support column [a], bobbin, position and load indicator [G] -the object in the process of being weighed-) are maintained in equilibrium by a compensation force [F] equal to the weight. The compensation force is generated by an electrical current through a bobbin in the air gap of a cylindrical electromagnet. The force F is calculated with the equation [F = I x L x B] where: I = electrical intensity, L = total length of the wire of the coil and B = magnetic flow intensity in the electromagnet's air gap.

With any change in the load (weight/mass), the mobile mechanical system responds by moving vertically a fraction of distance. Detected by a photosensor [e], an electrical signal is sent to the servo-amplifier [f]. This changes the flow of electrical current passing through the bobbin of the magnet [c] in such a manner that the mobile system returns to the balanced position upon adjusting of the magnetic flow in the electromagnet. Consequently, the weight of the mass [G] can be measured indirectly at the start of the electrical current flow, which passes through the circuit measuring the voltage [V] by means of a precision resistor [R], [V = I x R]. To date, many systems developed use the electronic system for carrying out very exact measurements of mass and weight. The following diagram explains how electronic balances function.

Figure 12. Components of electronic balances

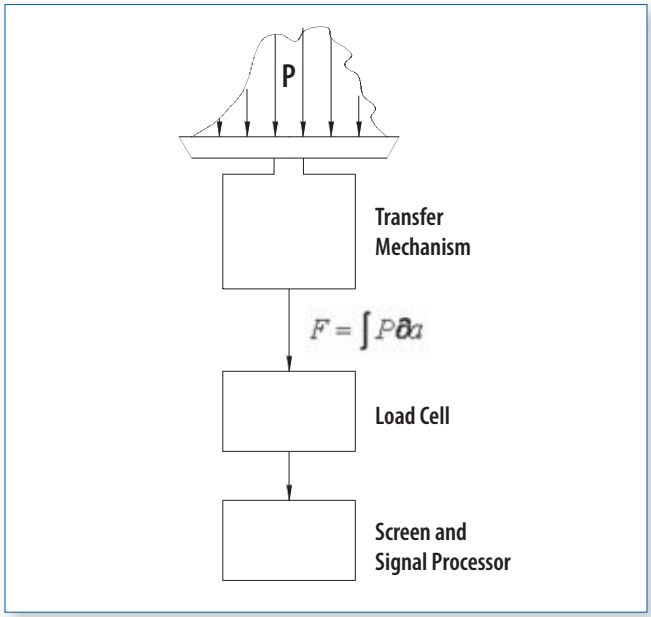
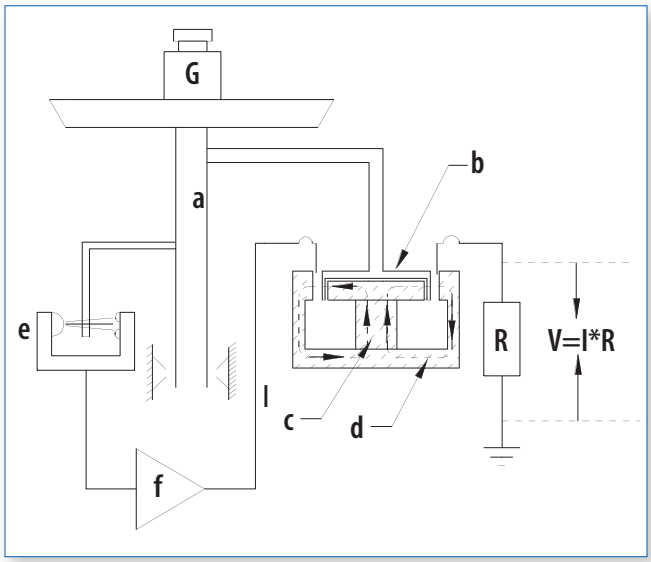


Figure 13. Compensation force principle



The signal processing system

The signal processing system is composed of the circuit which transforms the electrical signal emitted by the transducer into numerical data which can be read on a screen. The signal process comprises the following functions:

1. ***Tare setting.*** This setting is used to adjust the reading value at zero with any load within the balance's capacity range. It is controlled by a button generally located on the front part of the balance. It is commonly used for taring the weighing container.
2. ***Repeatability setting control.*** During a reading, weighed values are averaged within a predefined period of time. This function is very useful when weighing operations need to be carried out in unstable conditions, e.g. in the presence of air currents or vibrations. This control defines the time period allowed for a result to lie within preset limits for it to be considered stable. In addition, it can be adjusted to suit a particular application.
3. ***Rounding off.*** In general, electronic balances process data internally at a greater resolution than shown on the screen. The internal net value rounded off is displayed on the screen.
4. ***Stability detector.*** This light indicator fades when the weighing result becomes stable and is ready to be read. Alternatively in other balance models, this feature allows the display of the result on the screen when the measure of the weight becomes stable.
5. ***Electronic signalling process.*** It allows the processing and display of the weighing operation results. It may also allow other special functions such as piece counting, percentage weighing, dynamic weighing of unstable weight (e.g. animals), and formula weighing, among others. The calculations are done by the microprocessor following the instructions entered by the operator on the balance's keyboard.

Classification of balances

The International Organization of Legal Metrology (OIML) has classified the balances into four groups:

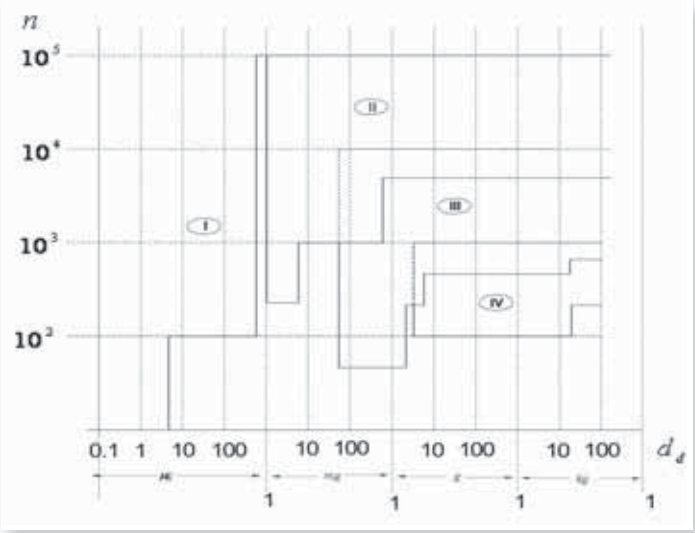
- Group I: special exactitude
- Group II: high exactitude
- Group III: medium exactitude
- Group IV: ordinary exactitude

The graph in Figure 14 shows the above-mentioned classification.

In the metrological classification of electronic balances, only two parameters are of importance:

1. The maximum load [Max.]
2. The value of the digital division [d][1]

Figure 14. Classification of balances by exactitude



The number of the scale's divisions is calculated by means of the following formula.

$$n = \frac{Max}{d_d}$$

The OIML accepts the following convention for laboratory balances.

1. Ultramicroanalytics $d_d = 0.1\ \mu g$
2. Microanalytics $d_d = 1\ \mu g$
3. Semi-microanalytics $d_d = 0.01\ mg$
4. Macroanalytics $d_d = 0.1\ mg$
5. Precision $d_d \geq 1\ mg$

[1] Kupper, W., Balances and Weighing, Mettler Instrument Corp., Princeton-Hightstown, NJ.

Electronic balance controls

A diagram of the typical controls on a modern electronic balance is shown in Figure 15. From this diagram it is necessary to point out the following:

1. Numerous functions are incorporated.
2. Various measuring units can be selected.
3. It is possible to know the day and hour when the measurements were taken.
4. The processes done can be documented and printed.
5. It is possible to select the language.

INSTALLATION REQUIREMENTS

For the satisfactory installation and use of a balance, the following is required:

1. An environment with no air currents or sudden changes in temperature and free from dust.
2. A perfectly levelled table/counter. A platform of high inertia, isolated from the structures located in its vicinity is ideal to reduce the effect of vibrations from certain equipment such as centrifuges and refrigerators. There must be a large enough area for installing the balance and any auxiliary equipment needed during the weighing processes. Likewise, the space required for cables such as the interconnection, electrical current cables and the information system connection to the printer must be anticipated.
3. Avoid installing equipment which produces elevated magnetic fields or vibrations like centrifuges, electrical motors, compressors and generators in its vicinity.
4. Avoid locating it directly under the air-conditioning system (air currents) and sunlight.
5. An electrical outlet which complies with the current electrical standards in the country or the laboratory. It must be in good condition and equipped with a ground pole and switches.

Electronic balance operation

The operation of a modern electronic balance is clearly detailed in its operator's manual from the manufacturer. In general, it must conform to the following procedure:

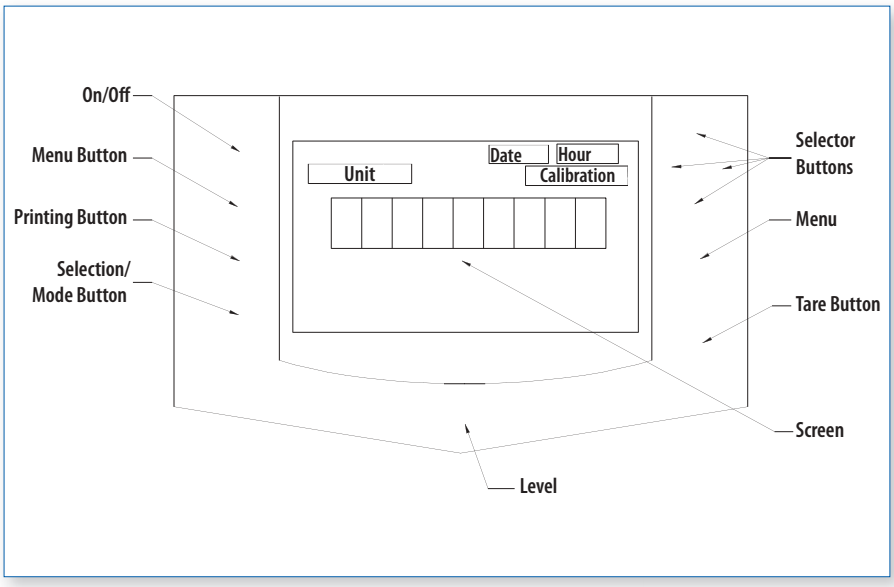
1. Allow the balance to equilibrate with the environment where it is installed.
2. Allow the balance to warm-up before initiating activities. Normally it is sufficient to have it connected to the electrical feed system. Some manufacturers suggest at least 20 minutes from the moment it is energized until use. Analytical balances Class 1 require at least 2 hours for warming before initiating use.

   Verify that the balance is calibrated. Electronic balances generally have a factory-made calibration stored in memory which can be used if it does not have calibration masses. If calibration is required, use calibrated masses as indicated by the manufacturer. The calibrated masses must conform or exceed the ASTM tolerances. For general information, the following table shows the accepted tolerance for the ASTM Class 1[1] masses.

Weight (grams)	Higher limit (g)	Lower limit (g)
100	100.0003	99.9998
200	200.0005	199.9995
300	300.0008	299.9993
500	500.0013	499.9988
1 000	1000.0025	999.9975
2 000	2000.0050	1999.9950
3 000	3000.0075	2999.9925
5 000	5000.0125	4999.9875

3. Follow the instructions indicated in the manufacturer's operations manual.

Figure 15. Analytical balance control panel



Calibration of balances

The calibration of balances must be done by personnel specially trained for this activity. It should be highlighted that it must be done based on the alignments of the OIML or an equivalent body such as the American Society for Testing and Materials (ASTM), institutions which have developed methodologies for classifying standard weights. The reference weights classification used by the OIML is covered in the table opposite.

[1] Field Services Handbook for High Precision Scales, IES Corporation, Portland, Oregon, 2004.

Table of OIML reference weights classification[1]

Class	Description	Tolerance	Uncertainty allowed	Frequency of recalibration
E1	Stainless steel weights without marks or adjusting cavity.	± 0.5 ppm per kg	± 1/3 of the tolerance	2 years
E2	Stainless steel weights without marks or adjusting cavity.	± 1.5 ppm per kg	± 1/3 of the tolerance	2 years
F1	Stainless steel weights with screw button for protecting the adjusting cavity.	±5 ppm per kg	± 1/5 of the tolerance	1 year
F2	Bronze plated weights.	± 15 ppm per kg	± 1/5 of the tolerance	1 year
M1	Bronze weights (that do not corrode or become stained) or of cast iron weights with a high quality paint finish.	± 50 ppm per kg	± 1/5 of the tolerance	1 year
M2	Bronze or cast iron weights (commercial weights).	±200 ppm per 1 kg	± 1/5 of the tolerance	1 year

Table of standard weights' use according to the balance's capacity

Capacity	Resolution							
	100 g	10 g	1 g	100 mg	10 mg	1 mg	0.1 mg	Ö0.01 mg
Up to 200 g	–	–	–	M1	M1	F2	F1	F2
200 g to 1 kg	–	–	M1	M1	F2	F1/E2	E2	E2
1 to 30 kg	M2	M2	M1	F2	E2	E2	E2	–
30 to 100 kg	M2	M1	F2	F1	E2	–	–	–
More than 100 kg	M2	M1/F2	F1	E2	–	–	–	–

Any calibration process must be done using standard weights. The results obtained must be analyzed to determine if these are within the acceptable tolerances. The standard weights must be selected based on the balance's capacity. The above table complements the previous. It provides guidance in determining the standard weights to use in the calibration of a balance according to its capacity.

ROUTINE MAINTENANCE

The balance is characterized as an instrument of high precision. For this reason, the operator is only responsible for minimal maintenance limited to the following:

Daily Activities

1. Clean the weighing plate so that it is kept free of dust. Cleaning is done by using a piece of clean cloth which may be dampened with distilled water. If there is a stain, a mild detergent can be applied. Also a paintbrush with soft bristles can be used to remove particles or dust deposited on the weight plate.
2. Clean the weighing chamber, externally and internally. Verify that the glass is free from dust.
3. Verify that the adjustment mechanisms on the front door of the weighing chamber works adequately.
4. Always use a clean, pre-weighed container for weighing (glass container or weighing paper if possible). Note that plastic can become electromagnetically charged and is not recommended for weighing powdered or granulated chemicals.
5. Any spill must be cleaned immediately to avoid corrosion or contamination. Use 70% ethanol to disinfect the pan of the balance.

Very important: Never lubricate a balance unless the manufacturer has expressly indicated it. Any substance interfering with the mechanism of the balance retards its response or definitely alters the measurement process.

Note: In general, the manufacturer or the specialized installation representative carries out the maintenance of the balances, according to procedures which vary depending on the type and model.

[1] Guidelines for calibration in laboratories, Drinking Water Inspectorate by LGC (Teddington) Ltd., December 2000.

TROUBLESHOOTING TABLE

Electronic balance

PROBLEM	PROBABLE CAUSE	SOLUTION
The balance does not turn on. T	he interconnection cable is disconnected or maladjusted on the balance.	Check the connection. Adjust the cable connector if this is the case.
	Electrical outlet has no power.	Check electrical feed.
The weight reading is incorrect.	The balance was not adjusted to zero before the reading.	Place the balance on zero; repeat the measurement.
	The balance is incorrectly calibrated. C	alibrate according to the procedure recommended by the manufacturer.
	The balance is not levelled.	Level the balance.
The balance does not show the desired units of measurement on the screen.	The units are incorrectly selected.	Check the procedure defined by the manufacturer to select the required measurement unit.
	The unit required not available or not activated.	Activate the measurement unit according to the procedure defined by the manufacturer.
The balance menu configuration cannot be changed.	The menu may be locked.	Check to see if the locking switch is activated. If this is the case, deactivate it.
The balance is incapable of keeping the selections or changes.	The End key has not been pressed to finish the process.	Verify that the changes and selections are done according to the manufacturer's instructions. Repeat the selection or change.
		Turn the balance off, wait a moment and switch on again.
The balance's reader is unstable.	There is vibration on the surface of the table/counter.	Place the balance on a stable surface.
	The front door of the balance is open.	Close the front door to measure.
The RS232 interface does not function.	The interconnection cable is maladjusted.	Check the connection of the interconnection cable.
The screen shows incomplete readings or is locked.	The microprocessor is locked.	Turn off the balance and a moment later put it on. If the situation persists, seek technical assistance from the service representative.
The screen displays an error code.	Various.	Verify the error codes in the balance's manual.

FUNCTIONAL ERROR	PROBABLE CAUSE
Readings not reproducible (hysteresis).	The measurement cell is dirty.
	The measurement cell is badly assembled.
Non-linear readings.	Defective electronic system.
	Mechanical system is in bad condition.
Digital reading continually goes up or down.	Defective electronic system.
	Change in room temperature.
The digital reading goes up and down continually.	Dirty measuring cell.
	Defective electronic system.
	Environmental problems like air currents, static electricity or vibrations.
The digital screen is blank or shows marks that make no sense.	Defective electronic system.
The screen indicates an overload or negative condition without a load being applied.	Measuring cell damaged by overload.
	Measuring cell is inadequately assembled.
The balance cannot be calibrated.	Defective calibration battery.
	Electronic system is defective.
	Measurement cell is inadequately assembled.

BASIC DEFINITIONS

ASTM. American Society of Testing and Materials.

Calibration. Determination of the correct value of an instrument's reading by measurement or comparison against a standard or norm. A balance is calibrated by using standard weights.

Certified masses. Masses conforming to the tolerance defined by the certification bodies. The ASTM classes 1 to 4 standards are those most widely used and must be used (a compulsory reference) for performing the calibration routines.

Exactitude. The sum of all the balance's errors. This is called *total error band*.

Hysteresis. The difference in the results when the load in the balance is increased or decreased.

Lateral load. A balance's ability to consistently read the value of masses, no matter where they are placed on the weighing scale. This is also called corner load.

Lateral load error. A deviation in the results when an object is weighed placing it in different parts of the weighing plate, i.e. in the centre of the plate and on one of its sides.

Linear error. A difference showed when the balance is loaded in a successive manner, increasing the quantity of weight in equal magnitude until it reaches its maximum capacity and unloaded in an analogous process. The differences shown between the readings obtained and the arithmetic values corresponding to the weights used are interpreted as non-linearity.

Linearity. Refers to the ability of a balance to perform accurate readings of weights throughout its weighing capacity . A graph showing weight compared to the weight indication on a perfectly linear balance should generate a straight line. In order to determine the linear error of a balance, certified masses must be used. The procedure allows the linear differences to be calculated by reading certified masses with and without preloading. The difference between the readings allows the linear error to be calculated.

Mass. A physical property of the bodies related to the quantity of matter, expressed in kilograms (kg), these contain. In physics, there are two quantities to which the name mass is given: **gravitational mass** which is a measure of the way a body interacts with the gravitational field (if the body's mass is small, the body experiences a weaker force than if its mass were greater) and the **inertial mass**, which is a quantitative or numerical measure of a body's inertia, that is, of its resistance to acceleration. The unit for expressing mass is the kilogram [kg].

OIML. International Office of Legal Metrology.

Sensitivity. The smallest mass detected by the balance or the smallest mass that the balance can measure correctly.

Sensitivity error. Constant deviation throughout the weighing range or capacity of a balance.

Traceability. The ability to relate the measurements of an instrument to a defined standard.

Chapter 5

Water Bath

GMDN Code	36754		16772
ECRI Code	15-108		16-772
Denomination	Water bath		Water bath, shaker

The water bath is an instrument used in the laboratory for carrying out serological, agglutination, inactivation, bio-medical, and pharmaceutical tests and even for industrial incubation procedures. In general they use water, but some baths use oil. The temperature range at which water baths are normally used range between room temperature and 60 °C. Temperatures of 100 °C can be selected, using a cover with special characteristics. Water baths are manufactured with chambers of a capacity ranging from 2 to 30 litres.

DIAGRAM OF A WATER BATH

Below is a basic diagram of a water bath. In the diagram, it is possible to observe the electronic control, the screen, the cover (an optional accessory) and the tank. Other components can be installed, e.g. a thermometer and an agitation unit to keep the temperature constant (not shown).

OPERATION PRINCIPLES

Water baths are made of steel and are generally covered with electrostatic paint with high adherence and resistance to environmental laboratory conditions. Water baths have an external panel on which the controls can be found. They also have a tank made of rustproof material with a collection of electrical resistors mounted on their lower part. By means of these, heat is transferred to the medium (water or oil) until reaching the temperature selected with a control device (thermostat or similar). The resistors may be of the following types:

- ***Immersion type.*** These resistors are installed inside a sealed tube and located on the lower part of the container in direct contact with heating medium.
- ***External.*** These resistors are located on the lower part but on the outside of the tank. These are protected by an isolating material which prevents heat loss. This type of resistor transfers the heat to the bottom of the tank through thermal conduction.

Figure 16. Water bath

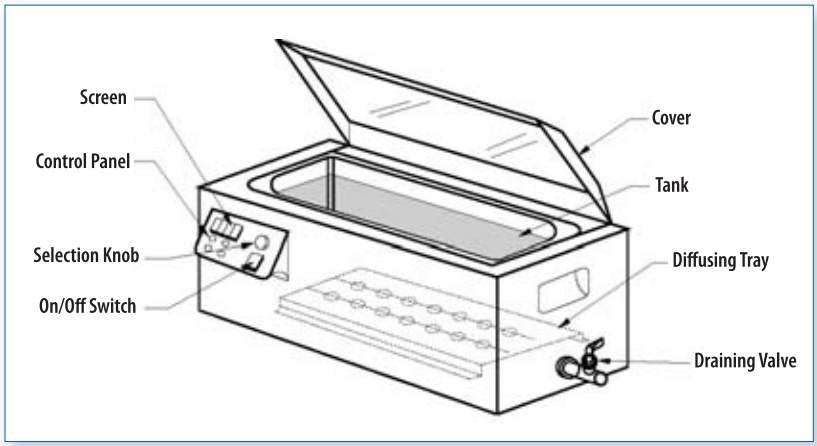
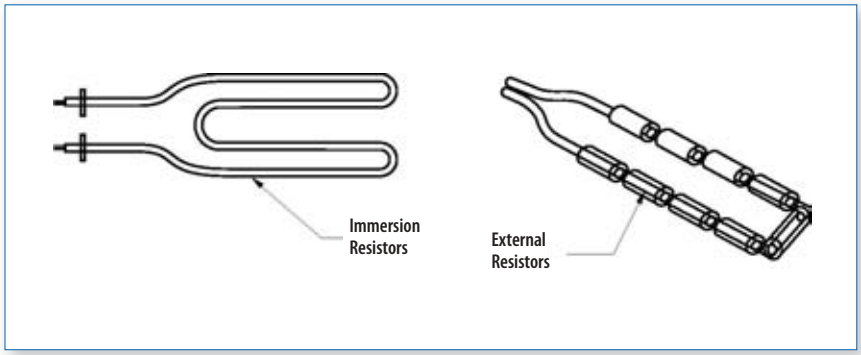


Figure 17. Immersion and external resistors



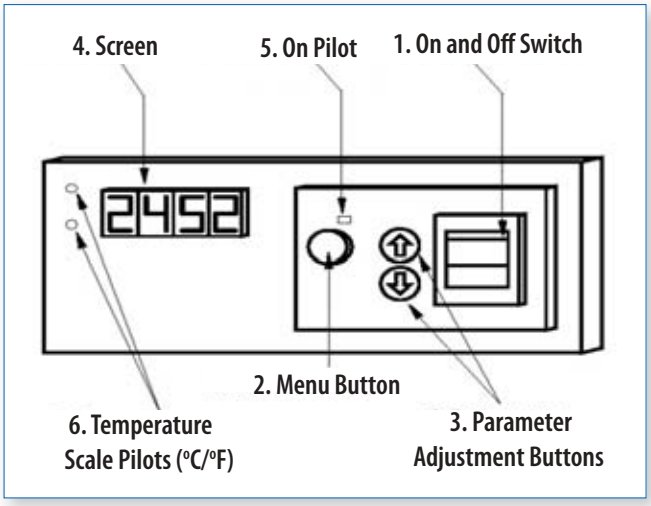
Certain types of water bath have a series of accessories such as agitation systems or circulators, generating carefully controlled movement of the heating medium to keep the temperature uniform. A table which describes the main types of water baths is shown below.

Class	Temperature range
Low temperature	Room temperature up to 60 °C
	Room temperature up to 100 °C
High temperature	Room temperature up to 275 °C. When it needs to reach temperatures above 100 °C, it is necessary to use fluids other than water as the boiling point of water is 100 °C under normal conditions
	This type of bath generally uses oils which have much higher boiling points.
Insulated	Room temperature up to 100 °C with accessories and/or agitation systems (with water).

WATER BATH CONTROLS

Water baths generally have very simple controls. Some manufacturers have incorporated controls with microprocessors. They vary depending on the type of bath. The diagram of a basic water bath's control panel is shown next.

Figure 18. Water bath controls



The control panel has these elements:

1. The on and off control switch
2. A Menu button for selecting the operation's parameters: operation temperature, alarm temperature, temperature scale (°C, °F)
3. Two buttons for parameter adjustment
4. A screen
5. A pilot light
6. Pilots (2) for identifying the temperature scale (°C, °F).

WATER BATH OPERATION

Installation

1. Install the water bath close to an electrical outlet. The outlet must have its respective ground pole in order to guarantee the protection and safety of the operator and the equipment. Water baths generally operate at 120 V/60 Hz or 230 V/60Hz. Its installation and use is facilitated by a sink close by for supplying and draining of water.
2. Verify that the location selected is levelled and has the necessary resistance to safely support the weight of the water bath when it is full of liquid.
3. Ensure that the location has a suitable amount of space for putting the samples and the accessories required for the normal operation of the water bath.
4. Avoid placing the water bath where there are strong air currents which can interfere with its normal operation. For example: in front of an air-conditioning unit or window.

Safety

1. Avoid the use of the water bath in environments where there are flammable and combustible materials. The equipment has components (resistors generating very high temperatures) which could start an accidental fire or explosion.
2. Always connect the equipment to an electrical outlet with a ground pole to protect the user and the equipment from electrical discharges. The electrical connection must comply with the required norms of the country and the laboratory.
3. Use the water bath exclusively with non-corrosive or non-flammable liquids.
4. Use personal protective elements when working with the water bath. The bath has resistors which can cause burns if inadvertently touched, even a considerable time after turning off the equipment.
5. When working with substances that generate vapours, place the water bath under a chemical hood or in a well ventilated area.
6. Remember that liquids incubated in the water bath tank can produce burns if hands are inadvertently placed inside it.
7. Take into account that the water bath is designed for use with a liquid inside the tank. If the inside is dry, the temperature of the tank can become very high. Use the diffusing tray for placing the container inside of the filled tank of the water bath. This has been designed for distributing the temperature in a uniform way.
8. Avoid using the water bath if any of its controls is not working, e.g. the temperature or limit controls.

Using the water bath

Before using the water bath, verify that it is clean and that accessories needed are installed. The steps normally followed are:

1. Fill the water bath with fluid to keep the temperature constant (water or oil). Verify that once the containers to be heated are placed, the fluid level is between 4 and 5 cm from the top of the tank.
2. Install the control instruments needed, such as thermometers and circulators. Use additional mounts provided for this purpose. Verify the position of the thermometer's bulb or thermal probe to ensure that the readings are correct.
3. If water is used as the warming fluid, verify that it is clean. Some manufacturers recommend adding products which prevent the formation of fungus or algae.
4. Put the main switch Nº 1 in the ON position (the numbers identifying the controls herein correspond to those shown in the diagram). Some manufacturers have incorporated controls with microprocessors which initiate auto-verification routines once the ON switch is activated.
5. Select the operation temperature using the Menu Nº 2 button and the buttons for adjusting the parameters.
6. Select the cut-off temperature (in water baths with this control). This is a safety control which cuts off the supply of electricity if it exceeds the selected temperature. This is selected also by using the menu button and is controlled by the parameter adjustment buttons.
7. Avoid using the water bath with the substances indicated below:
   a) Bleach.
   b) Liquids with high chlorine content.
   c) Weak saline solutions such as sodium chloride, calcium chloride or chromium compounds.
   d) Strong concentrations of any acid.
   e) Strong concentrations of any salt.
   f) Weak concentrations of hydrochloric, hydrobromic, hydroiodic, sulphuric or chromic acids.
   g) Deionised water, as it causes corrosion and perforation in the stainless steel.

Maintenance

Warning: Before carrying out any maintenance activity, disconnect the equipment from the electrical feed outlet.

Water baths are equipment whose maintenance is simple. The recommended routines mainly focus on the cleaning of external components. The most common routines are featured next.

Cleaning

Frequency: Monthly

1. Turn off and disconnect the equipment. Wait until it cools to avoid the risk of burns and accidents.
2. Remove the fluid used for heating. If it is water, it can be poured through a siphon. If it is oil; collect into a container with an adequate capacity.
3. Remove the thermal diffusion grid located at the bottom of the tank.
4. Disassemble the circulator and clean to remove scale and potential algae present.
5. Clean the interior of the tank with a mild detergent. If there is any indication of corrosion, use substances for cleaning stainless steel. Rub lightly with synthetic sponges or equivalent. Avoid using steel wool to remove rust stains as these leave particles of steel which could accelerate corrosion.
6. Avoid bending or striking the temperature control capillary tube generally located at the bottom of the tank.
7. Clean the exterior and interior of the water bath with clean water.

Lubrication

Frequency: Daily

For water baths with an agitation unit or circulator system:

Lubricate the axis of the circulator's electric motor. Put a drop of mineral oil on the axis so that a good lubricating condition is maintained between the motor's bearings and its axis.

Periodic inspection

Frequency: Quarterly

Check the thermometer or temperature controls every three months using known standards. If no reference standard is available, use an ice/water mixture and/or boiling water. Note that the thermometer or the water bath temperature controls should also be checked when the equipment is first installed after purchase.

TROUBLESHOOTING TABLE

PROBLEM	PROBABLE CAUSE	SOLUTION
There is no power to the instrument.	The water bath is disconnected.	Connect the water bath.
	The switch is defective.	Change the switch.
	The fuse is defective.	Substitute the fuse.
The water bath is not getting hot.	The temperature control not set.	Set the temperature control.
	The resistor(s) is/are defective.	Change resistor(s).
	The limit control is not set	Set the limit control.
The temperature is higher than that selected.	The temperature control is defective.	Change the temperature control if required.
	Verify the selection of the parameters.	
The samples are warmed slowly.	The tank is empty or contains very little fluid.	Fill the tank up to the recommended level.
The temperature is increasing very slowly.	The resistor(s) is/are defective.	Change the resistor(s).
	The temperature control is defective.	Substitute temperature control.

BASIC DEFINITIONS

Circulator. An apparatus that shakes or stirs fluids to keep their properties (temperature, color, density) homogenous. These are also called agitators.

Diffusing tray. Device located at the bottom of the water bath to support the containers located inside the tank. It also allows thermal convection currents generated in the fluid contained in the tank to circulate from top to bottom and back to the top, maintaining the temperature homogeneous at the level selected by the operator. In general the diffusing tray is made of stainless steel.

Electrostatic painting. A painting process that uses the particle-attracting property of electrostatic charges. A potential difference of 80-150kV is applied to a grid of wires through which the paint is sprayed to charge each particle. The metal objects to be sprayed are connected to the opposite terminal of the high-voltage circuit, so that they attract the particles of paint. The piece covered with paint particles is then placed in an electrical oven to melt the particles, making them adhere strongly to the piece.

Fuse. A safety device which protects the electrical circuits from excessive current. Fuses are made of materials whose dimensions and properties equip them to work well within some predefined conditions. If for some reason the design parameters are exceeded, the material burns out and interrupts the passage of the electrical current.

Immersion resistor. An electrical resistor (see definition below) inside of a sealed tube. These are generally used for heating fluids as water or oil.

Resistance. Opposition that a material or electrical circuit imposes to the flow of electric current. It is the property of a circuit that transforms electrical energy into heat as it opposes the flow of current. The resistance [R], of a body of uniform section such as a wire, is directly proportional to the length [l] and inversely proportional to the sectional area [a]. The resistance is calculated by the following equation:

$$R = k \times \frac{l}{a}$$

Where:

k = constant that depends on the units employed
l = Length of the conductor
a = sectional area of the conductor

The ohm (Ω) is the common unit of electrical resistance; one ohm is equal to one volt per ampere.

Chapter 6

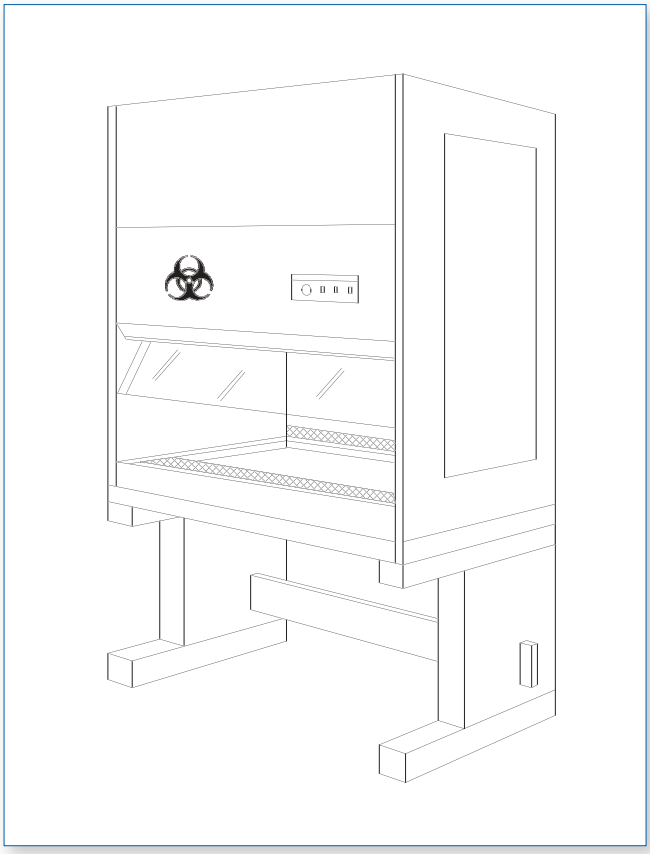
Biological Safety Cabinet

GMDN Code	15698	20652	20653	20654
ECRI Code	15-698	20-652	20-653	20-654
Denomination	Cabinets, biological safety	Cabinets, biological safety, class I	Cabinets, biological safety, class II	Cabinets, biological safety, class III

This equipment is designed for controlling aerosols and microparticles associated with managing potentially toxic or infectious biological material in laboratories in activities such as agitation, centrifugation, pipetting, and opening of pressurized containers. Safety cabinets have been designed to protect the user, the environment and the sample manipulated using appropriate ventilation conditions. They are also known as *laminar flow cabinets* and/or *biosafety cabinets*.

ILLUSTRATION OF A BIOLOGICAL SAFETY CABINET

Figure 19. Biological safety cabinet



PURPOSES OF THE EQUIPMENT

The biological safety cabinet is used for the following:

1. To protect the worker from risks associated with the management of potentially infectious biological material.
2. To protect the sample being analyzed from becoming contaminated.
3. To protect the environment.

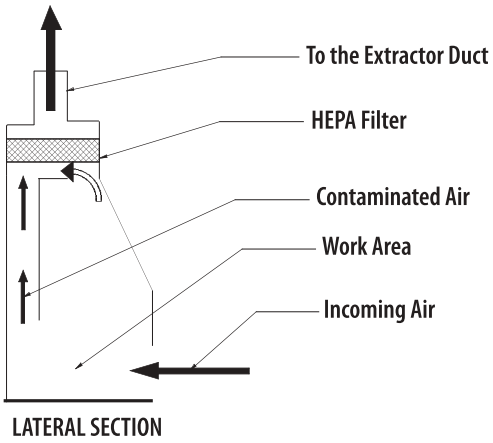
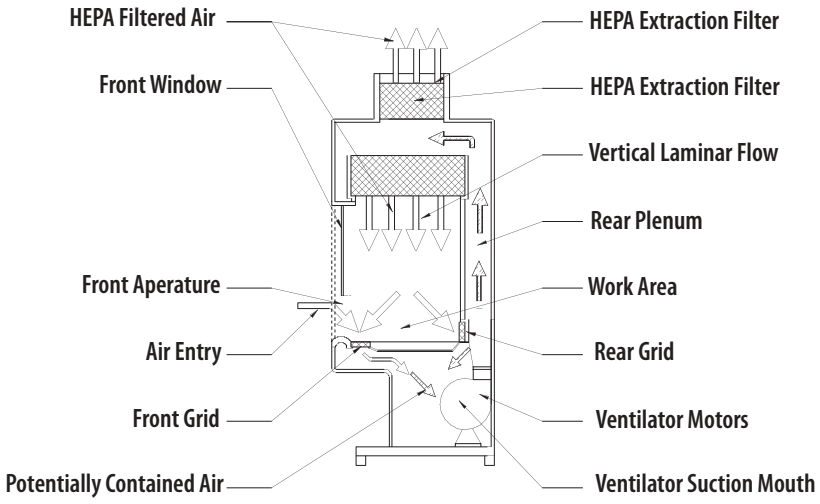
The cabinets are used for routine work related to pathogens (parasites, bacteria, virus, fungus), cell culture and under very precise conditions, the management of toxic agents.

OPERATION PRINCIPLES

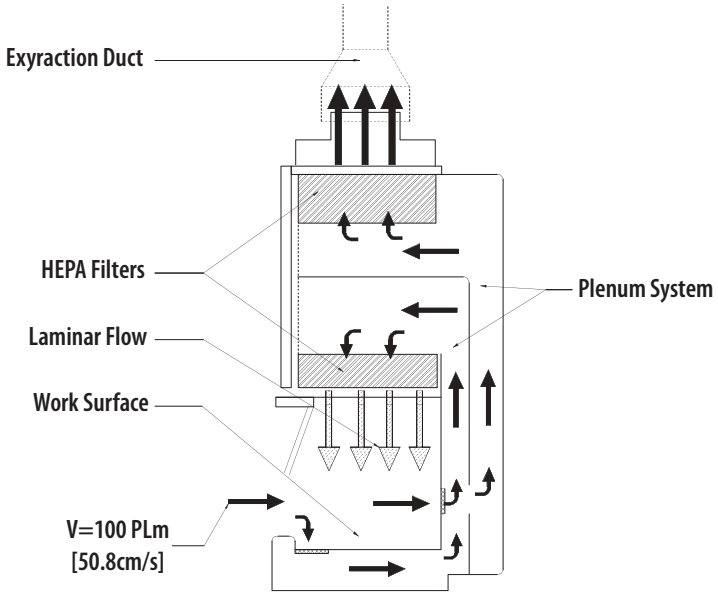
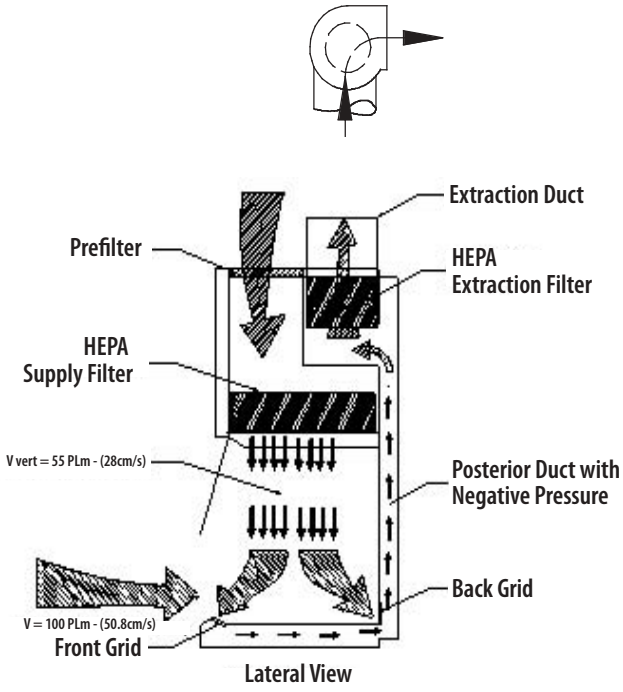
The biological safety cabinet is a chamber generally constructed of steel. It has a front glass window of adjustable height, a ventilation system with an electrical motor, a ventilator and a set of ducts which while functioning, generate a negative pressure condition inside the cabinet. This forces the air to flow from inside the cabinet through the front opening to generate a curtain of air protecting the operator. Internally, the air is conducted through a series of grids and ducts to be finally treated in HEPA[1] filters. Depending on the design of the cabinet, the air is recycled inside the laboratory or extracted and renewed in diverse proportions. The air flow, which in Class II cabinets moves from the filter towards the work surface, is laminar. A summary of the existing type of cabinets and their principal characteristics is presented next.

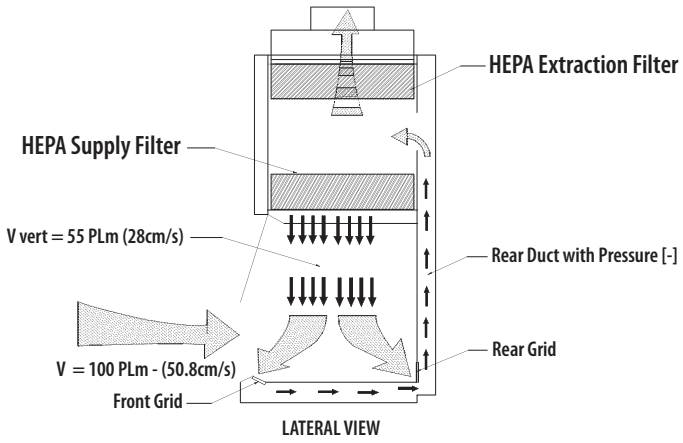
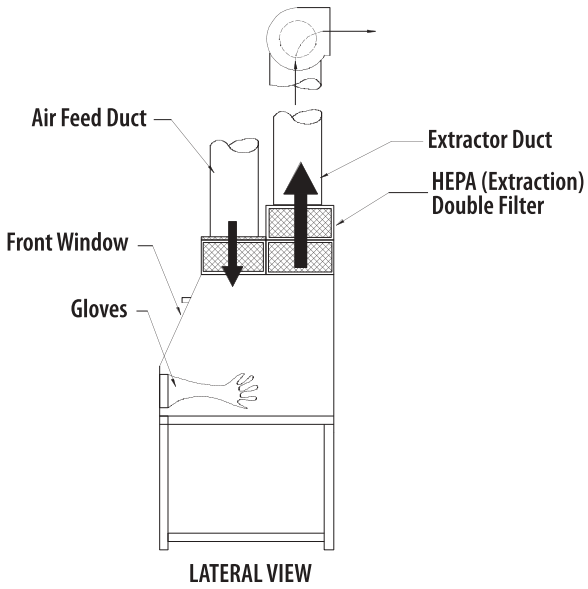
[1] HEPA: High Efficiency Particulate Air.

Summary of biological safety cabinet types

Type of cabinet, with illustration	Characteristics
CLASS I — TYPE A	
	1. Protection provided: to the operator and the environment. 2. Air velocity on entering the cabinet: 38 cm/s. 3. Suitable for working with bio-safety level[1] 1, 2 or 3 agents. 4. Filtration HEPA, located in extraction system which may or may not be connected to the exterior. 5. Disadvantage: Does not protect the sample manipulated in the cabinet.
CLASS II — TYPE A	
	1. Protection offered: To the operator, the product and environment. 2. Air velocity on entering the cabinet: 38 cm/s. 3. Suitable for working with agents with biosafety level 1, 2 or 3. 4. Filtration system: two HEPA filters, one located on the work surface; the second on the extraction system which may or may not be connected to the exterior. If they are connected to the exterior, it utilizes a bell type connection. 5. They recycle approximately 70 % of the air volume and renew 30 % of it.

[1] See biosafety classifications levels of agents in the following section *"Biological safety"*.

Type of cabinet, with illustration	Characteristics
CLASS II — TYPE B1	
	1. Protection provided: to the operator, the product and the environment. 2. Air velocity entering the cabinet: 50.8 cm/s. 3. Suitable for working with agents with biosafety level 1, 2 or 3. 4. Filtration system: Two HEPA filters. It extracts potentially contaminated air (70 %) through a duct and recycles inside of the cabinet, after filtering, air taken from the exterior, through the front grid (30 %). 5. All biologically contaminated ducts have a negative pressure. 6. Allows work with small quantities of toxic and radioactive chemicals.
CLASS II — TYPE B2	
	1. Protection provided: to the operator, the product and the environment. 2. Air velocity on entering the cabinet 50.8 cm/s. 3. Suitable for working with agents of biosafety level 1, 2 or 3. 4. Filtration system: Two HEPA filters. It is known as the total extraction cabinet. It does not have any type of recirculation. 5. All biologically contaminated ducts have a negative pressure. 6. It has an extraction duct which allows work with toxic and radioactive chemicals.

Type of cabinet, with illustration	Characteristics
CLASS II — TYPE B3 OR A/B3	
	1. Protection provided: to the operator, the product and the environment. 2. Air velocity on entering the cabinet: 50.8 cm/s. 3. Suitable for working with agents of biosafety level 1, 2 or 3. 4. Filtration system: Two HEPA filters. 5. All biologically contaminated ducts have a negative pressure. 6. It is known as a combined cabin. It can be connected by means of a duct. It is denominated as Type B3. If the duct is missing, it is a Type A. It recycles 70 % of the air volume inside the cabinet.
CLASS III	
	1. Protection provided: to the operator, the product and the environment. 2. Filtration system: two HEPA filters in series in the extraction; a HEPA filter in the admission. 3. Suitable for working with agents classified biosafety level 4. 4. Totally sealed cabinet. The intake and extraction elements are conducted through a double -door pass-through box. The manipulation of materials is done by using sealed gloves at the front of the cabinet.

BIOLOGICAL SAFETY[1]

Microorganisms have been classified into four categories based on factors such as pathogenicity, infectious doses, transmission modes, and host range, availability of preventive measures and effectiveness of treatment for the disease caused.

1. ***Risk level 1 group*** is composed of biological agents very unlikely to cause sickness in healthy humans or animals. (No individual and community risk).
2. ***Risk level 2 group*** is composed of pathogens which cause sickness in humans or animals but unlikely to be dangerous to laboratory workers, the community, domestic animals or the environment under normal circumstances. Those exposed in the laboratory rarely become seriously ill. There are preventive measures and effective treatment available and the risk of dissemination is limited. (Moderate individual risk, limited community risk).
3. ***Risk level 3 group*** is composed of pathogens which usually cause serious sicknesses to human beings and animals and produce a serious economic impact.

   However, infection by casual contact by one individual to another is not common. The sicknesses these produce are treatable by antimicrobial or anti-parasitic agents. (High individual risk, low community risk).
4. ***Risk level 4 group*** is composed of pathogens which usually produce very serious sicknesses in human beings or animals, frequently without treatments available. These agents are easily spread from one individual to another or from animal to human being or vice versa, directly or indirectly or by casual contact. (High individual risk, high community risk).

INSTALLATION REQUIREMENTS

The following are requirements for a cabinet to function adequately:

1. A laboratory area protected from air currents from windows or air-conditioning systems. The cabinet must also be located far from the laboratory circulation zones in order to avoid air currents that could affect the curtain of air inside the cabinet. It must also be verified that the cabinet is not installed alongside other types of cabinets such as chemical hoods.
2. An electrical connection equipped with the respective control and safety elements; the electrical outlet with a ground pole.
3. A levelled and firm table designed for supporting the weight of the cabinet and allowing the operator to work comfortably. There must be free space for placing the feet and its height must be adequate.
4. The floor on which it is located must be flat and levelled.
5. The free space around the cabinet recommended by the manufacturer must be respected. Likewise, the height of the room must be verified (the ceiling must be of recommended height so that it can function without hindrance).
6. Type B cabinets must have an extraction duct equipped with the following required control devices: regulating valves that allow the flow of air to be isolated and regulated.
7. Gas connections must be in the immediate vicinity of the cabinet in order to facilitate the connection to these service valves.
8. The cabinet must be certified annually to verify that it complies with the established requirements in the NSF 49 Regulation.

USE OF THE SAFETY CABINET

Correct utilization of the biological safety cabinet is achieved by complying with the following instructions:

1. Plan the work to be done in the biological safety cabinet in advance. Determine what procedure and equipment will be used. Coordinate the time of the cabinet's use with the other laboratory professionals in order to avoid interruption or undesired traffic while it is in use.
2. Turn on the cabinet. Turn off the UV lamp if lit. Turn on the fluorescent light lamp and the cabinet's ventilator. Verify that the grids in front and behind are free of obstructions. Prepare the work area. Allow the cabinet to function for at least 15 minutes.
3. Wash hands and forearms with germicidal soap. Put on the personal protective apparel: coat/overall with long sleeves and adjustable cuffs, protective eyeglasses and mask if the work requires it. Prepare the interior surfaces of the cabinet applying 70% ethanol or a suitable disinfectant. After this, let the air flow through.
4. Only load and install the materials and equipment required for the test or manipulation. Distinguish between the clean areas and dirty areas. Place the material in such a way that the clean materials do not mix or cross used or dirty materials or impede the circulation of the internal air through the front and back grids. Place a biosafety bag for disposing waste materials, a container with disinfectant for the pipettes and a container for storing sharps. Avoid locating very large objects near one another. Upon finalizing the placing of the materials, the flow of air must be allowed to sweep through the cabinet for approximately 3 to 5 minutes in order to eliminate any particle produced or freed during the loading of materials and equipment.
5. Initiate activities. Slowly introduce hands into the work area. Carry on the processes and tasks in a methodical and careful manner (from the clean areas to the

[1] *The Laboratory Biosafety Guidelines*, 3rd. Edition-Draft, Health Canada, 2001.

potentially contaminated areas). Keep the materials at least 10 cm behind the front grid. Try to perform the most risky and contaminating activities towards the back of the cabinet's work area. Avoid the use of open flames of lighters since this breaks the laminar flow pattern and may burn the filter. Avoid removing hands from the work area until all procedures are accomplished and the potentially dangerous materials are disposed of in the biosafety bag or in the pipette and sharp containers.

6. Clean the cabinet, allowing the air to flow freely for 3 to 5 minutes upon ending all the procedures.
7. Decontaminate the surfaces of all the materials and equipment in contact with the biologically contaminated material. Apply 70% ethanol or a suitable disinfectant and allow drying. Lift the equipment and materials and disinfect the area underneath. Cover the open containers before removal from the work area. Transfer materials to their appropriate place (incubator, autoclave, etc.).
8. Discard the gloves and remove personal protective elements. Dispose of these following the laboratory's established procedure. Wash hands with a lot of water and soap.
9. Turn off the ventilator, the fluorescent lamp, close the front opening and turn on the ultraviolet light.

Note: In case of a leak or spill inside the cabinet while in use, it must be kept in operation and all the objects or equipment involved must undergo a process of surface decontamination. This will prevent the cabinet from releasing contaminants.

Decontamination of the cabinet

The decontamination of the biological safety cabinet is an activity which must be done before any maintenance work involving opening its surfaces or internal components. Whenever any of the processes indicated next are needed, decontamination of the cabinet must be done previously.

1. Changing of filters.
2. Conducting tests requiring access to the interior surfaces or exposure of the cabinet.
3. Before conducting certification tests when the cabinet has been used with classified agents such as level 2 or 3 biological risk agents.
4. Before moving the cabinet to a different location.
5. After a spill of a material containing high risk agents.

The most suitable decontamination procedure must be defined by the professional responsible for industrial safety and professional risks. In annex G of the NSF 49 Standard, the procedure for decontaminating the cabinet using depolymerised paraformaldehyde is described. Only professionals who have received the relevant training must conduct such procedures.

ROUTINE MAINTENANCE

Warning: The maintenance of internal components must only be done by trained and qualified personnel. In order to carry out maintenance on the internal components, decontamination must be done previously. Personal protection must be worn to perform the routines.

General maintenance required for the biological safety cabinet is for the most part simple to perform. The routines and frequencies are shown below:

Frequency: Weekly

1. Decontaminate the work surface and the interior surfaces of the cabinet with 70% ethanol.
2. Clean the front glass door and the surface of the ultraviolet lamp, using a domestic cleaning solution.
3. Verify the precision of the manometer's reading, indicating any fall in pressure flowing through the HEPA filter. Register the date and the reading in the cabinet's log book.

Frequency: Monthly

1. Clean the exterior surfaces, especially the front and the upper part using a piece of damp cloth in order to remove the dust.
2. Disinfect the surface of the lower compartment with 70% Ethanol or a suitable disinfecting solution.
3. Verify the state of the service valves.
4. Do the tasks due on a weekly basis.

Frequency: Annually

1. Carry out the certification process according to established outlines in the NSF 49 regulation.
2. Check the intensity of the UV lamp[1] with a radiometer. Substitute it if necessary.
3. Test the state of the fluorescent lamp. Substitute it if necessary.
4. Perform the tasks due on a monthly basis.

Removal of the work surface

For the removal of the work surface the following procedure is required:

1. Decontaminate the surface before removing it.
2. Loosen and remove the attachment screws located on the front part of the work surface.
3. Loosen, but do not remove the attachment screws located on the back part.
4. Raise the front end and remove it, pulling it towards the front part of the cabinet.
5. Decontaminate the interior part of the work surface.
6. To assemble it, perform the activities described in steps 2, 3 and 4 in reverse order.

[1] UV lamps have irradiation capacity lasting approximately 7,500 hours. Some manufacturers suggest annual substitution.

Changing of the ultraviolet lamp

In order to change the ultraviolet lamp, the manufacturers' instructions must be followed. In general, the following procedures are done:

1. Turn on the cabinet and leave it working for 5 minutes.
2. Raise the front window to its maximum position.
3. Decontaminate the interior surfaces and the UV lamp.
4. Disconnect the electrical feed to the cabinet.
5. Disconnect the UV tube from its connectors turning it 90 degrees. Next, install a spare part with the same characteristics as the original. Some manufacturers have installed the lamps on a plate located in the front of the cabinet, which is necessary to unscrew and lift so that the assembly of the lamp is kept visible. Once this is done, the lamp can be substituted as indicated above.

Specialized maintenance

Eventually, the cabinet will require specialized maintenance. The following are some procedures to be done according to the manufacturer's technical service manuals by a specialized contractor.

1. Annual certification in accordance with Regulation NSF 49 outlines.
2. Motor change. Generally, it uses maintenance-free sealed rollers and function by induction through frequency control. This motor does not have brushes. (*)[1].
3. Replacing ventilators. (*)
4. Replacing the HEPA filter (*). The replacement frequency depends on the use of the cabinet and the system of environmental control installed in the laboratory. If there is a good control of dust, the filter could last many years.
5. Repair of the electronic control system: flow control alarms, position of the window, velocity controls.
6. Repair/cleaning of the flow regulator valves, bell type adjustment fittings.

Cabinet certification

The certification process of the biological safety cabinets is regulated by Standard NSF 49, which applies to all Class II cabinets. This defines materials, design criteria and construction, operation parameters and tests which allow the cabinet to be guaranteed as safe and suitable for the work performed. The following is a list of tests, in which standards mentioned are included. The standards must be consulted for details. The certification process comprises the following tests:

1. ***Air tightness test.*** This is done on the exterior surfaces. Determine if joints, seals, penetration and solderings are free from leaks.
2. ***HEPA filter leak tests.*** Determines the integrity of the supply and extraction of HEPA filters, their location and mounted frames.
3. ***Temperature increase test.*** Determines the maximum temperature increase in the cabinet when the ventilator and lights are operating.
4. ***Noise test.*** Determines the level of noise produced by the cabinet.
5. ***Luminous intensity test.*** Determines the luminous intensity on the cabinet's work surface.
6. ***Vibrations test.*** Determine how much vibration there is in the cabinet when it is functioning.
7. ***Protection test*** to personnel, to the product and cross contamination biological tests. The test determines if aerosols are contained in the cabinet, if external contaminants reach the work table area and if aerosols are reduced by the cabinet.
8. ***Stability test.*** Determines if the cabinet has structural stability. Analyzes the resistance to shaking, to distortion by means of applied force, to deflection of the work surface subjected to load and resistance to the tilting of the work surface due to heavy loading conditions.
9. ***Vertical flow velocity test.*** Determines the velocity of the air moved vertically towards the work surface.
10. ***Entry flow velocity test.*** Determines the velocity at which the flow enters the cabinet through the front opening and the cabinet's extraction volume.
11. ***Smoke test.*** Determines if the flow of air along the entire perimeter of the front opening advances towards the cabinet, and if the vertical flow moving towards the bottom does not show dead points or flow backs on the work surface.
12. ***Drainage escape test.*** Defines the contention capacity for spills below the work surface.
13. ***Motor/ventilator system functioning test.*** Determines if the system provides the necessary static pressure.
14. ***Electric system test.*** Determines if there are potential risks of electrical discharges. Measures the escaping currents, the polarity, the functioning of the ground defect protection system and the ground circuit resistance.

FUNCTIONAL EVALUATION (ALTERNATIVE)

In case there are biological safety cabinets in the laboratory, but no authorized certification services available, the personnel responsible for maintenance has the option of conducting annual revision procedures based on Standard NSF 49. Duly documented, it should identify with low levels of uncertainty if the cabinet is in good condition and its operation normal[2]. The following are outlines of how these activities must be done.

1. ***Installation evaluation.*** Verify that the cabinet installation conditions are in accordance with the recommendations from the manufacturer.

[1] (*) These require specialized decontamination beforehand.
[2] The functional evaluation is essentially based on the availability (institutional or zonal) of properly trained and experienced technicians and engineers.

2. ***Operational evaluation.*** Test to see if the cabinet is working in accordance with its manufacturing and design characteristics.
3. ***Performance evaluation.*** Verify the cabinet's capacity to provide an adequate work space in normal and critical working conditions.

In the following table are featured the parameters to be taken into account in the functional evaluation. These are generally included in inspection forms[1] designed for this purpose.

[1] Each institution designs its own formats for record keeping of technical maintenance.

Table of functional evaluation of biological safety cabinets

Parameters	Observation
Institutional identification of cabinets	**Brand, model, type, series, location, inventory code, date.**
ELECTRICAL	
Voltage	Voltage measurement. Requires a voltmeter.
Amperage	Amperage measurement. Requires a voltmeter or amperemeter clip.
Motor/ventilator	Verification of operation temperature. Verify noise level and vibration.
Illumination – Fluorescent	Confirmation that the lamp is functional.
– Ultraviolet	Confirmation of the operational hours of the lamps and their light intensity. Requires a radiometer.
Electrical outlet	Integrity revision, quality of the contact and available voltages.
Switches	Control of state and integrity.
Integrity cables and connectors	Visual verification.
Alarms	Testing of state and calibration.
PHYSICAL	
Internal/external finishes	Visual verification.
State of filters and pre-filters	Visual verification. There must be no leaks, neither in the filtering material nor in the seals.
Seals/gaskets	Visual verification. There must be no leaks.
Sliding window	Visual verification. Must be able to be moved smoothly and maintain the selected positions.
OPERATIONAL	
Flow velocity	Control of velocity according to the class and type of cabinet. Requires an anemometer (wind gauge).
Noise level	Requires audiometer.
Pressure differential in the HEPA filter.	Take a manometer reading of the cabinet.
PERFORMANCE	
Counting of particles	Method defined in the Federal Standard 209D, E. Requires DOP generator, photometer and particle counter.
CONDITIONS OF THE INSTALLATION AREA	
Temperature	Requires thermometer: approximately 20–22 °C.
Humidity	Requires hygrometer: approximately 45–55 %.
Cleanliness	Must be adequate.
Air currents	There must be no air currents to affect the working of the cabinet.

TROUBLESHOOTING TABLE[1]

PROBLEM	PROBABLE CAUSE	SOLUTION
Neither the light nor the ventilation system in the cabinet works.	The cabinet is disconnected from the electrical outlet.	Verify that the cabinet is connected to an electrical outlet and that the cable is well connected to the cabinet's electrical box.
	There is no electrical feed in the connection.	Confirm that the electrical outlet is energized and that the circuit breaker is not deactivated (thermo magnetic protection). Restart switches.
The cabinet's ventilator is functioning but the light does not.	The lamp is defective.	Replace the lamp. Use one with the same characteristics of the original
	The lamp is badly connected.	Check the lamps connection. Adjust to the correct position.
	The thermo magnetic protection of the service breaker is activated.	Reconnect the circuit breaker.
	The lamp's wire is disconnected.	Check the lamp's wire.
	The lamp's ballast is defective.	Replace the ballast.
The ventilator is not blowing but the light is coming on.	The front window is closed.	Open the window until it reaches the work position.
	The ventilator's motor is defective.	Replace the motor ventilator set.
	The ventilator's motor is disconnected.	Check the motor's connections.
The manometer indicates an increase in the fall of pressure through the filter.	Retention of particles in the HEPA filter has increased.	Normal process during the useful life of the filter.
	There is blockage in the grids or return slots.	Verify that the grids are not obstructed by equipment or material.
	The extraction pipe is obstructed.	Test that there are no existing blockages or restrictions in the extraction pipe.
	There is a blockage or restriction under the work surface.	Verify that the pipe below the work surface is free of obstructions.
There is contamination in the samples manipulated in the cabinet.	Work procedures are inadequate.	Check that the cabinet is being used according to procedures and good practices.
	Restrictions in the return slots or blockage of the extraction duct.	Test the return and extraction system to see if they are free from obstructions.
	The cabinet's external factors affect its flow patterns on the inside and cause contamination.	Verify the installation of the cabinet and the procedures that are being carried out.
	The HEPA filter is defective.	Replace the HEPA filter and certify the cabinet.

[1] Purifier® Delta® Series, *Biological Safety Cabinets, User's Manual*, Kansas City, Labconco Corporation, Part Nº 36960-20, Rev. A ECO B296.

BASIC DEFINITIONS

Aerosol. A suspension of fine solid or liquid particles in the air. Their average diameter ranges between 10^{-4} and 10^{-7} cm.

Air supply. Air which enters the cabinet through the front or work opening and replaces the air extracted from the cabinet.

Biological Safety cabinet. Equipment with appropriate ventilation conditions protecting the user, the environment and the sample from aerosols and microparticles, associated with the management of potentially infectious biological material in laboratories as a result of activities such as agitation, centrifugation, use of pipettes and opening of pressurized containers.

Certification. Procedure establishing that the biological safety cabinet's functioning complies with criteria and minimum requirements to operate safely. Standard NSF 49 applies to the Class II cabins, Type A, B1, B2 and B3.

Decontamination. Removal or destruction of infectious agents; removal or neutralization of toxic agents.

HEPA filter. A filter with the ability to remove particles with average diameters of 0.3 *µm* with 99.97 % efficiency. These filters are constructed of Boron silicate micro fibres bonded together with a water resistant adhesive. The filtering material is folded inside of a frame with the aim of increasing the filtration area.

Laminar flow. Non-turbulent flow of a viscous fluid (e.g. air) in layers near a boundary. It occurs when Reynolds number [Re] is less than 3000.

NSF. An acronym of the *National Sanitation Foundation*, a non-profit organization dedicated to research, education and service, which seeks to resolve problems related to human beings, promote health and enrichment of the quality of life through conservation and improvement of the environment. NSF standards supply the basic criteria for promoting salubrious conditions and public health protection.

Toxic. A substance with a physiologically adverse effect on the biological systems.

Ultraviolet light (UV). This is electromagnetic radiation, the wavelength of which is between 200 and 390 nm. It is used in biological safety cabinets for its germicidal properties.

Work surface. A surface used when performing work, operation or activity inside the biological safety cabinet in this case.

Chapter 7

Centrifuge

GMDN Code	**15115**	**10778**	**10778**
ECRI Code	**15-115**	**15-117**	**15-116**
Denomination	**Centrifuges, standing, low velocity, non-refrigerated, for blood bank**	**Centrifuge, standing, refrigerated**	**Standing centrifuge**

The word *centrifuge* comes from the Latin word *centrum* which means *centre* and *fugere* which means to escape. The centrifuge is designed to use the centrifugal force generated in rotational movements to separate the constitutive elements of a mixture. There is a wide range of centrifuges capable of serving specific industry and research needs. This chapter focuses on standing centrifuges normally used in public health and clinical laboratories.

PHOTOGRAPH OF CENTRIFUGE

Photo courtesy of Beckman Coulter

PURPOSE OF THE CENTRIFUGE

The centrifuge uses centrifugal force (the force generated when an object rotates around a single point), for separating solids suspended in a liquid by sedimentation, or liquids of diverse density. The rotational movements allow forces much greater than gravity to be generated in controlled periods of time. In the laboratory, centrifuges are generally used in processes such as the separation of solid components from biological liquids through sedimentation and in particular of blood components: red cells, white cells, platelets among others and for conducting multiple tests and treatments.
There are several kinds of centrifuges. The most widely used in public health, surveillance and clinical laboratories are the table-top centrifuge, the ultracentrifuge, the haeamatocrit centrifuge and the standing centrifuge.

OPERATION PRINCIPLES

Centrifuges represent a practical application of Newton's law of motion. When a body of mass [m] turns around a central point [O], it is subjected to a *centripetal* force [N] directed towards the rotation axis with a magnitude $N = m\omega^2R$, where [m] is the mass of the body, [R] is the radius and ω is the angular speed. Centrifuges possess a rotating axis on which is mounted a rotor with sample receiving compartments. Tangential speed is defined by the following equation: $VT=\omega R$.

When the system spins at a speed of ω radians per second, the samples are subjected to the *centrifugal* force **Fp** of the same magnitude as **N**, but in an opposite direction. The figure shown below[1] features a diagram of the concept, of its actual application and of the obtained result. This **Fp** force acts on particles in the substance centrifuged, causing them to separate as a result of differences in density. Denser particles will settle at the bottom of the tube in shorter periods of time, while lighter ones require longer periods of time, settling onto those of greater density. The relationship between the centrifugal acceleration [$\omega^2 r$] to a given radius [r] and the force of gravity [g] is known as the *relative centrifugal field or [RCF]*[2].

$$RCF = \frac{r\omega^2}{g}$$

The RCF is the tool which allows rotors of different specifications to be compared when equivalent centrifugal effects are required.

COMPONENTS OF THE CENTRIFUGE

The most important components of a centrifuge are the following[3]:

The electric/electronic control which generally has the following elements:

1. On and off control, operation time control (timer), rotation speed control (in some centrifuges), temperature control (in refrigerated centrifuges), vibration control (safety mechanism) and brake system.
2. Refrigeration system (in refrigerated centrifuges).
3. Vacuum system (in ultracentrifuges, not shown in the figure).
4. Base
5. Lid/cover
6. Casing
7. Electric motor
8. Rotor. There are different types of rotors. The most common are the fixed angle, the swinging buckets, the vertical tube and the almost vertical tube types, which are explained next.

Sectional diagram of a centrifuge (numbers correspond to descriptions in the text above)

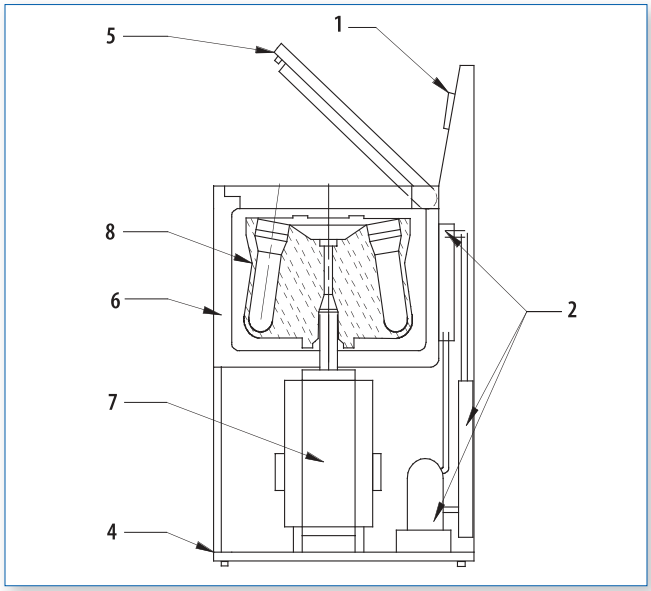
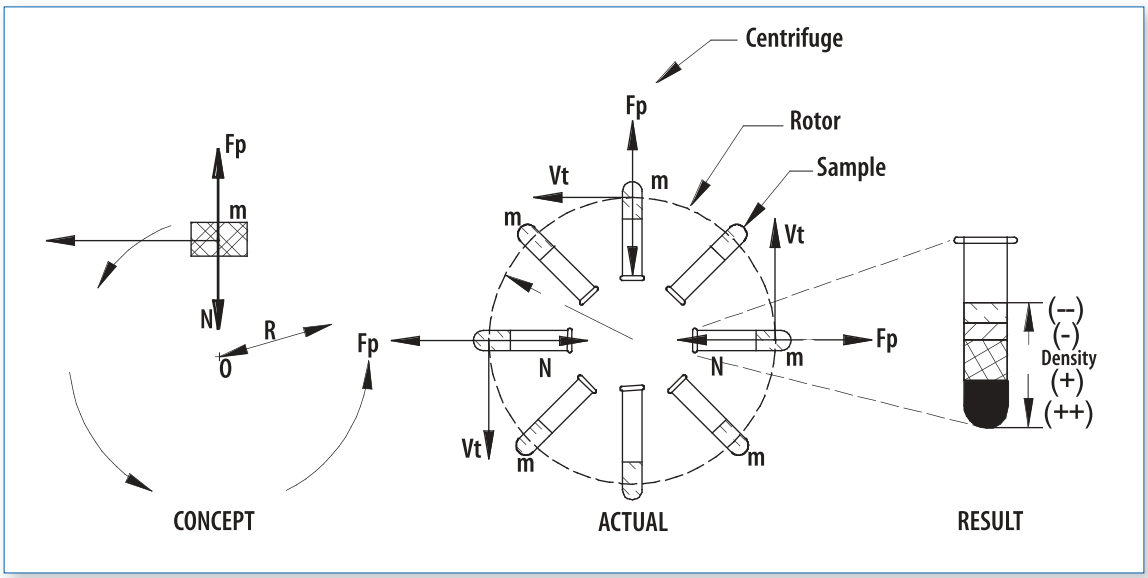


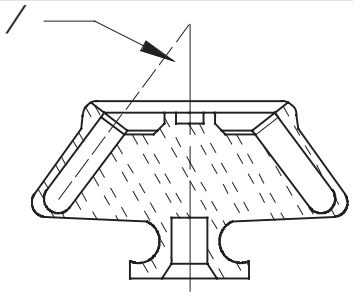
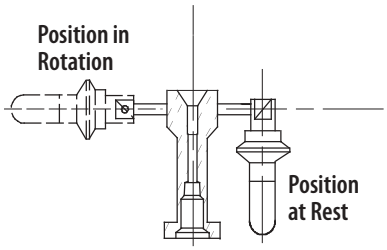
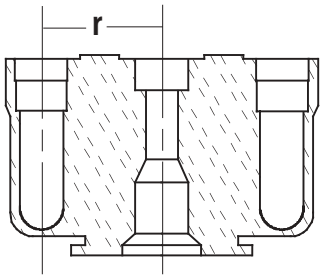
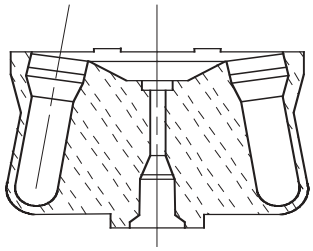
Figure 20. Centrifugal force concept



[1] Newton's law of movement, together with the explanation of the inertia marks of reference can be consulted in books on physics, chapters on uniform circular movement.
[2] RCF. Relative Centrifugal Field.
[3] The numbers identifying each component correspond to those in the sectional diagram of the centrifuge.

Types of rotors

Centrifuges use many different types of rotors. Among the most commonly used are the following:

Type of rotor	Characteristics	Transversal cross-section
Fixed angle rotors.	These are general purpose rotors. They keep tubes at a fixed angle [α] which by design, is specified between 20 and 45 degrees. They are used for sediment sub-cellular particles. The angle shortens the trajectory of the particles and the centrifugation time compared to the swinging buckets rotors.	
Swinging buckets rotors.	These are used for carrying out isopycnic studies (separation by density) and rate-zonal studies (separation by sedimentation coefficient), where maximum resolution of the zones is required for the sample.	
Vertical tube rotors.	This type of rotor keeps tubes parallel to the rotational axis. Thus, separate bands are formed across the tube's diameter, not its length. These rotors are used for carrying out isopycnic studies and in some cases, zonal limit separations where a short centrifugation time is important. These rotors use specially designed tubes.	
Almost vertical tube rotors.	This type of rotor is designed for gradient centrifugation when some sample components do not participate in the gradient. The small angle of these rotors reduces the centrifugation time in comparison to fixed angle rotors.	

Normally, manufacturers specify rotors to be used in centrifuges by providing specialized publications of tables with the following information:

1. ***Type of rotor.*** Specifies the type of rotor for which the technical information is being provided.
2. ***Nominal capacity of the rotor.*** Defines the capacity in litres or litre submultiples. For example: 6 litres; 250 ml, etc.
3. ***Maximum speed.*** This indicates the maximum speed at which this particular rotor should be operated in revolutions per minutes (RPM).
4. ***Maximum Relative Centrifugal field*** (RCF) obtained by that type of rotor.
5. ***k Factor,*** the sedimentation coefficient, defined by the following equation:

$$k = \frac{\ln(r_{max}/r_{min})}{\omega^2} \times \frac{10^{13}}{3600}$$

Where:

ω= angular speed in radians per second

r_{max} = maximum radius in mm, measured in the centrifugation tube

r_{min} = minimum radius in mm, measured in the centrifugation tube

The time required for sedimentation can be calculated in hours using this factor.

6. Information on the compatibility of the rotor with other models of centrifuges from the same manufacturer.

Recently manufactured centrifuges have incorporated numerous improvements into their design to provide greater safety and longer operational life. Among advances mentioned are controls based on microprocessors. By means of *software* controlled by a keyboard, these have several different operational programs in memory. According to the type of rotor being used and procedure conducted, these programs control the centrifugation time, the required temperature, the rotor's revolutions, the acceleration and deceleration, alarms warning the operator about any anomaly during operation.

Manufacturers have also incorporated induction motors (without brushes) in centrifuges. These have the advantage of electronically controlling currents and magnetic fields regulating the rotor's speed which reduces the frequency of maintenance. Operation and maintenance of such equipment must be carried out according to the manufacturer's recommendations.

INSTALLATION REQUIREMENTS

Centrifuges require the following for normal operation:

1. An electrical connection with a capacity suitable for the equipment providing stable single phase or triphase type voltage (depending on the model and specification given by the manufacturer). In general, centrifuges use 110V or 220 V/60 Hz.
2. A clean, dust free environment with a firm levelled floor.
3. If the centrifuge is refrigerated, it needs a free space on the side of the condenser for adequate heat transfer.
4. A cabinet in which the centrifuge accessories such as the alternate rotors can be kept.

ROUTINE MAINTENANCE

The routine maintenance required by a centrifuge depends on multiple factors such as the incorporated technology, usage intensity, training of users, quality of the electrical feed and environmental conditions. The following are general recommendations regarding adequate use and most common maintenance for guaranteeing correct operation. The routines or specialized repairs will depend on manufacturers' recommendations for each brand and model. Always disinfect the rotor bowl, centrifuge head, buckets and trunnion rings as applicable before any servicing of centrifuges used to prepare clinical or infectious samples.

Priority recommendation. Verify that only qualified personnel trained and familiar with the use, care, risks and handling of the centrifuge operates it. It is the laboratory directors' responsibility to supervise and take necessary precautions so that personnel operating centrifuges understand the implications of working with such equipment.

APPROPRIATE MANAGEMENT AND STORAGE RECOMMENDATIONS[1]

Rotors

1. Register the date of purchase of each one of the rotors, including information related to the serial and model number.
2. Read and understand the rotor manuals, equipment and tubes before use. Comply with indications for use and care specified by the manufacturer.
3. Use rotors only in centrifuges for which these have been manufactured. Do not interchange rotors without verifying the compatibility with the centrifuge.
4. Register operation parameters for each rotor in a log book in order to determine its remaining operational life and to acquire its replacements when needed.
5. Use the recommendations regarding maximum speed and sample density from the manufacturer. Each rotor is designed for supporting a maximum level of effort; these specifications must be followed rigorously.

[1] http://www.sunysb.edu/facilities/ehs/lab/cs.shtml

6. Obey the recommendation related to reducing the operation speed when working with high density solutions in stainless steel tubes or plastic adaptors. Manufacturers provide the related information.
7. Use titanium rotors if working with saline solutions frequently.
8. Protect the rotors' coating in order to avoid the metal base from deteriorating. Do not use alkaline detergents or cleaning solutions which can remove the protective film. The rotors generally made of aluminium [Al] are covered by a film of anodized aluminium which protects their metal structure.
9. Use plastic brushes when cleaning the rotor. Metal brushes scratch the protective coating and generate sources for future corrosion. Corrosion is accelerated in operation conditions and shortens the rotor's operational life.
10. If there are spills of corrosive substances, wash the rotor immediately.
11. Air dry the rotor once cleaned and washed with water.
12. Store vertical tube rotors and almost vertical tube rotors with the larger side facing downwards and without their covers.
13. Store rotors in a dry area. Avoid leaving them in the centrifuge.
14. Store swinging buckets rotors without the compartments' covers.
15. Lubricate spiral and O-rings, according to the manufacturer's recommendation.
16. Observe recommendations related to guaranteed times and operational life of each type of rotor.
17. Avoid using rotors whose operational lives have ended.
18. Use a shield if working with radioactive material.
19. Load or unload rotors inside a biological safety cabinet if working with materials classified as Biosafety level II or higher.
20. Never try to open the cover of a centrifuge while it is functioning and never try to stop the rotor by hand.

Tubes

Tube care includes aspects such as filling of the tubes, adequate temperature selection, centrifugation speed limitations, washing and sterilization. The principle recommendations are the following:

1. Wash tubes, adaptors and other accessories by hand using a 1:10 mild detergent solution in water and a soft textured brush (not metallic). Avoid using automatic dishwashers.
2. Avoid using alcohol and acetone since such liquids affect the structure of the tubes. Manufacturers recommend the solvent to be used with each type of centrifugation tube material.
3. Avoid drying tubes in a drying oven. Dry always with a stream of hot air.
4. Verify if the tubes are reusable or not. If they are disposable, use them only once.
5. For sterilizing, it is necessary to verify the material from which the tube is made, as not all can stand sterilization by heat. Glass tubes are normally sterilized with vapour at 121 °C for 30 minutes.
6. Store tubes and bottles in a dark, fresh, dry place, far from chemical vapours or ultraviolet radiation sources.
7. Verify maximum filling levels and the sealing of thin wall tubes in order to avoid collapse inside the rotor by the action of the centrifugal force. Comply with manufacturers recommendations.

Preventive maintenance

Warning: Never carry out a technical intervention in a centrifuge if it has not been previously decontaminated.

The most important maintenance routines performed on a centrifuge are the following:

Frequency: Monthly

1. Verify that the centrifuge external components are free of dust and stains. Avoid affecting the rotor with spills. Clean the rotor compartment using a mild detergent.
2. Test that the rotors' connecting and adjustment mechanisms are in good condition. Keep the points lubricated as the manufacturer recommends.
3. Verify the locking /safety mechanism of the centrifuge's cover. This is fundamental in guaranteeing operators' safety as this mechanism keeps the cover of the centrifuge closed while the rotor is turning.
4. Check the lubrication state of elements such as for O-rings as the manufacturer recommends. Always use lubricants according to the manufacturer's instructions (frequency and type of lubricants). In recently manufactured centrifuges, there are sealed ball bearings which do not require lubrication.
5. Verify the state of gaskets and watertight joints.

Frequency: Annually

1. Verify that electronic cards are clean and well connected.
2. Test operation controls needed for selection of the different parameters of the centrifuge: speed, time, temperature, alarms selectors and analogous or digital instruments.
3. Verify compliance with electrical standards. Use an electric safety analyzer: earth resistance test, escaping current test.
4. If the centrifuge is refrigerated, test the temperature by using an electronic thermometer. The temperature must not vary by more than ± 3 °C.
5. Examine the exactitude of the time controls. Use a timer. The time measured must not vary by more than ± 10 % of the programmed time.

6. Verify the actual rotation speed against the selected one using a normal load. The testing is done with a tachometer or a photo tachometer. If the hatch is not transparent, the procedure indicated by the manufacturer must be followed.
7. Confirm the functioning of the brake system.
8. Verify the functioning of the refrigeration system in refrigerated centrifuges. The following are the most important activities:
   a) Check the selected temperatures. These should not vary by more than 3 °C from the temperatures measured on the digital thermometer.
   b) Verify the state of the air intake filter. If the filter is obstructed, clean or substitute with an equivalent.
   c) Conduct a detailed cleaning of the diffusing wing of the condenser to eliminate the filth deposited. This maintains the heat transference rate according to the design specifications. If abnormal functioning is detected, seek assistance from a specialized service technician.

Note: Avoid spilling liquids on control keys. The keys must be operated with the fingertips: The operator should avoid using fingernails, as this can result in the perforation of their protective membrane.

Every six months:

Verify the state of the motor's brushes, if the centrifuge has a motor with brushes. Substitute with new ones (with the same specifications as the original) if necessary. Perform this routine every six months.

Tools and required instrumentation

In order to carry out the maintenance inspections normally required for a centrifuge, the following tools or instruments are necessary:

1. A key for tightening and slackening the rotor's nuts.
2. An electrical safety analyzer or an instrument for measuring escaping current.
3. A timer.
4. An electronic thermometer with exactitude of 0.5°C for refrigerated centrifuges.
5. A tachometer or photo tachometer.

TROUBLESHOOTING TABLE

Rotors[1]

PROBLEM	PROBABLE CAUSE	SOLUTION
Severe vibration.	The rotor is unbalanced.	Balance the rotor's load. Fill all the opposite tubes with the same level of liquid of same density.
		Distribute the weight of the opposite tubes symmetrically.
		Load fixed angle or vertical tube rotors symmetrically.
	The speed selected is near the rotor's critical speed range.	Select a rotation outside of the critical speed range.
	The rotor is incorrectly mounted.	Verify the rotor's assembly. Test that it is well adjusted.
	There is a lack of lubrication in the rotor's supports.	Lubricate the pivoting axis according to the manufacturer's recommendation. For e.g. each 250 centrifugation procedures.
Rotor covers, canister or cubes difficult to loosen after centrifugation.	A vacuum is being produced during centrifugation.	Open the ventilation line in the upper part of the rotor or bucket to eliminate the vacuum.
	The rings are contaminated with filth, dried lubricants or metallic particles.	Perform routine cleaning of the rings and lubricate. Use recommended products recommended by the manufacturers.

[1] *Rotors and Tubes for Beckman Coulter J2, J6 and Avanti® J series centrifuges, User's Manual,* Palo Alto, California, The Spinco Business Center of Beckman Coulter, 2001.

Tubes

PROBLEM	PROBABLE CAUSE	SOLUTION
The tubes leak.	The covers are badly secured.	Adjust the covers.
	The tubes are too full.	The meniscus must be lower in order to prevent leaks.
	The maximum recommended level has been exceeded in the open tubes.	Verify the volume and speed recommendations for the centrifugation.
	A deficient seal is presumed in the rapid seal tubes.	Press lightly, after heat sealing (only if the contents are not affected). If leaks are visible, seal again.
The tubes are cracked or broken.	The tubes can be broken or become fragile if they are used below the recommended temperature.	If the sample is frozen, warm to 2 °C before centrifuging. Evaluate how the tubes behave at low temperatures before centrifuging.
	The tubes become fragile with age and use.	Discard expired tubes, use new ones.

Various systems

PROBLEM	PROBABLE CAUSE	SOLUTION
The main switch is in the on position but the centrifuge is not functioning.	There is no power to the instrument.	Verify the power supply.
The centrifugue cover cannot be opened.	The centrifuge is off.	Turn the centrifuge ON. Press the handle and open the cover.
The balance indicator is activated.	The load to be centrifuged is unbalanced.	Balance the load to centrifuge.
	The centrifuge is not levelled.	Level the centrifuge.
There is a vibration at low speed.	The rotor adjustment mechanism is slack.	Correctly adjust the fastening system.
	The load is unbalanced.	Verify the balance of the load to be centrifuged.
	The selected speed is close to the rotor's resonance point.	Select a more elevated rotation speed or use a different type of rotor.
There are fluctuations in the rotation speed.	The transmission belts are in a bad condition (*).	Turn off the centrifuge. Verify the condition and state of the belts. The belts must be tempered.
The rotation speed does not reach the selected speed.	The brushes are defective.	Turn off the centrifuge. Verify the condition of the brushes. If this is the problem, put new brushes with the same specifications as the originals.
	The speed control calibration is maladjusted.	Adjust the speed control calibration.
The chamber is cold but the rotor is warm.	The temperature is incorrectly selected.	Verify the temperature selection.
The display which signals the state of the brushes is on.	The brushes are in a bad condition.	Turn off the centrifuge. Verify the condition of the brushes. Substitute the brushes by others with the same specification.

(*) Valid procedure in centrifuges with potential belt transmission system.

BASIC DEFINITIONS

Anodized coating. A hard, thin layer of aluminium oxide, which is deposited on the surface of a rotor by means of electrochemical processes with the aim of preventing corrosion. The coating is often finished in various colours.

Angular speed. The turning rate of a body measured in radians per second. It is calculated using the following formula:

$$\omega = \frac{2\pi \times \text{rpm}}{60}$$

Where:
rpm = revolutions per minute
π = constant with a value of 3.1416

Brush. A device that transmits electrical energy between the external electrical connection (cables in a static state) and the internal components (in rotation) of a motor. In general, brushes are manufactured in very soft textured graphite and, in motors, must be changed regularly (every six months).

Centrifugal force. Apparent force equal and opposite to the centripetal force, driving a rotating body away from the centre of rotation and caused by the inertia of the body. It is one of the components of the inertia vector, which equals the set of forces acting on a body. Its magnitude is always [m x a_n] and its direction radial, moving away from the centre.

Density. A body's mass by volume unit, generally expressed in gram per cm^3.

$$D = \frac{m}{V}$$

Isopycnic separation. A method for separating particles based on the density of the particle's flotation. It is known as sedimentation in balance. The speed of a particle due to differences in density is given in the formula:

$$v = \left(d^2 (\rho_p - \rho_c) \Big/ 18\mu \right) \times g$$

Where:
v = speed of sedimentation $\left(\frac{dr}{dt}\right)$

d = diameter of the particle
ρ_p = density of the particle
ρ_c = density of the solution
μ = viscosity of the liquid medium
g = gravitational force

Radian. A unit of angular measure equal to the angle subtended at the centre of a circle by an arc equal in length to the radius of the circle. It is expressed as the ratio between the arc formed by the angle with its vertex in the centre of the circle, and the radius of that circle.

RCF (Relative centrifugal field or force). A relationship between the centrifugal acceleration and a specific speed and radius, [$r\omega^2$] given with the normal gravity acceleration. It is calculated by means of the following equation:

$$\text{RCF} = \frac{r\omega^2}{g}$$

Where:
R = radius in mm
ω= angular speed in radians per second $\omega = \frac{2\pi \times \text{rpm}}{60}$

g = Standard gravity acceleration = 9 807 mm/s^2

Resonance. A situation in which a mechanical system vibrates as a response to a force applied at the system's natural frequency.

Sedimentation. Particles from a suspension settling at the bottom of the liquid as a result of the action of the gravitational force. During centrifugation, this process is accelerated and particles move away from the rotational axis.

Chapter 8

Water Distiller

GMDN Code	**40478**
ECRI Code	**15-136**
Denomination	**Distillation units**

The word *distiller* comes from the Latin word *distillare* which means to vaporize liquids through heat. The water distiller, also called distillation unit or water still, used in the laboratory, purifies running water by means of controlled vaporization and cooling processes. Upon applying thermal energy to water in a liquid phase by a warming process, it is changed into vapour. This allows the water molecules to separate from the molecules of other substances mixed or diluted. The water vapour is collected and passed through a condenser, where it is cooled and returned to the liquid phase. Then, the condensed water is collected into a different storage tank. Distilled water shows pure characteristics compared to running water; it is practically free of contaminating substances.

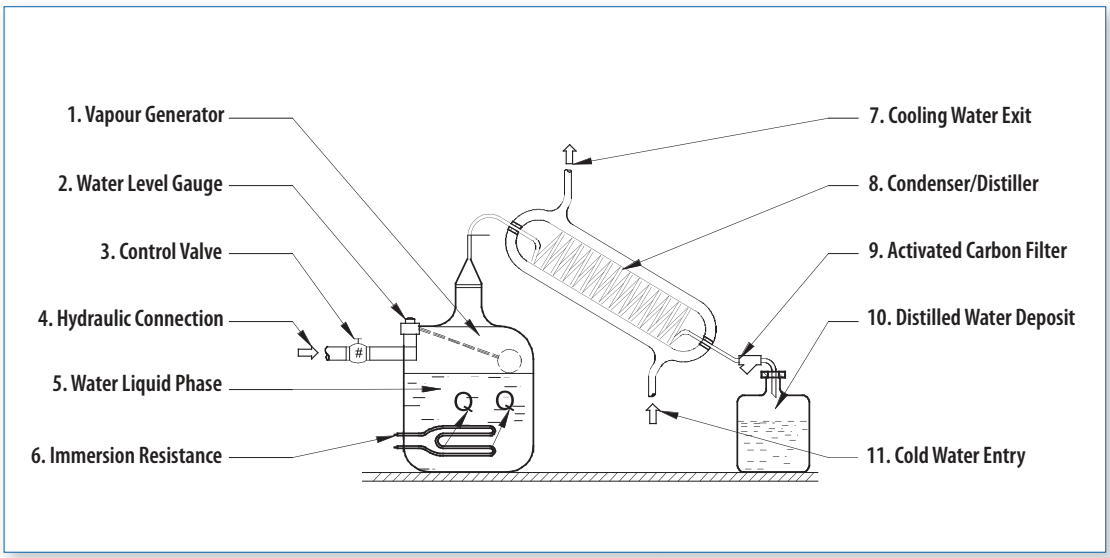
PURPOSE OF THE WATER DISTILLER

The water distiller facilitates obtaining very pure water from potable water normally provided by the aqueduct services in urban centres. Distilled water is characterized by a lack of solids in suspension. It is used in multiple applications in centres which provide health services, especially in laboratory units, in washing, sterilization and dietetics. The more specialized the procedures are in the laboratory, the greater will be the level of purity required. For example: the preparation of reagents or biological material requires water of the highest quality. Distillation is one of the fundamental processes to achieve this (although it may not be the only one required). Water used in laboratories must be free of pyrogens, with a concentration of total solids no greater than 1 ppm, a pH value between 5.4 and 7.2 and an electrical resistance of at least 3 x 10^5 ohm/cm at 25 °C[1].

[1] *Warming cabinets, sterilizers, and associated equipment*, Division 11– Equipment, USACE/NAVFAC/ AFCESA, UFGS-11710, July 2003.

DIAGRAM OF A WATER DISTILLER

Figure 21. Water distiller



OPERATION PRINCIPLES

The function of a distiller is based on a phenomenon demonstrated in nature known as the water cycle. The energy coming from the sun heats the water from the seas and transforms part of it into water vapour. This vapour is concentrated in clouds. When atmospheric conditions are suitable, these cool and condense the water which returns to the surface of the Earth in the form of rain.

Functioning of the water distiller

The water distiller reproduces the natural phenomenon described above. The configuration and design vary depending on the volume of water required. The following is a general explanation of the components of a distiller and a description of how these function.

1. ***Vapour generator.*** Also known as the boiling tank, this component is the container where the water to be distilled is stored. In general, it has a hydraulic connection which allows the water evaporated and distilled to be replenished. It is generally made of glass in small distillers or of stainless steel with copper, tin or titanium coverings in large capacity machines. It can have level, flow and water quality feed controls, which protect the distiller in case some irregularity in the water supply occurs. As a source of energy, it uses the water vapour coming from a boiler or vapour generator, or the thermal energy from electrical immersion resistors through direct conduction. These cause the water temperature to rise until, in normal conditions (atmospheric pressure equal to an atmosphere and gravity acceleration equal to 9.80665 m/s^2) water in the liquid phase is transformed into vapour at 100 °C.
2. ***Water level.*** Device which allows the quantity of water to be regulated inside the vapour generator. It is joined directly to the connection which supplies the water used by the distiller. When the quantity of water in liquid phase contained in the boiling tank decreases, the device allows the quantity of liquid evaporated to be recovered.
3. ***Control valve.*** Mechanical or electromechanical device which allows the flow of water towards the vapour generator tank to be regulated.
4. ***Hydraulic connection.*** Network which supplies water in liquid phase to the vapour generator tank.
5. ***Water in liquid phase.*** Water inside the vapour generator tank. It receives thermal energy from the immersion resistors and it is converted to vapour when the required temperature and pressure conditions are met.
6. ***Immersion resistors.*** Devices generating heat when an electrical current circulates through them. These are isolated by a ceramic cap and protected from the external environment by a metal shield.
7. ***Refrigeration water outlet.*** Line carrying the water used for condensing the water vapour thus removing the thermal energy from it (cooling).
8. ***Condenser.*** Device in which the vapour loses thermal energy, cools and returns to its liquid phase. In order to accelerate the process, forced convection by low temperature fluid circulation (air or water) around the line through which the vapour flows is used.
9. ***Filter.*** Distillers have activated carbon filters located at the exit of the condenser or collector. These eliminate flavours or particles which may be present in the vapour being condensed.
10. ***Distilled water container.*** Device in which the fluid completing the distillation process is collected. Distilled water must be stored in special plastic containers to avoid ionic contamination. Polyethylene, polypropylene or polytetrafluoroethylene containers are generally used.

INSTALLATION REQUIREMENTS

Depending on the design, capacity and type of distiller, the required installation may vary. The most common requirements are the following:

1. A well ventilated environment in which the equipment can be installed. This is necessary because the distiller transfers heat to a fluid and increases the temperature of the area where it is installed. It is necessary to leave free space around the distiller so that the flow of air is facilitated. Some distillers are assembled inside a metal box and need to be installed on a support to facilitate the circulation of air under them.
2. A potable water connection. Typically the required hydraulic connection has a diameter of 1/2". To ensure a smooth operation, the quality of the water feeding the distiller must be evaluated to determine if it is necessary to install a treatment system[1] to prevent the presence of incrustations or sediments in the vapour generating tank and on immersion resistors. Potable water is used for feeding the vapour generator and for refrigerating the condenser[2].
3. A distilled water connection. The distilled water produced is initially collected into a storage tank. In large capacity equipment, it is distributed to consumption points from the tank by means of a network. In small or medium equipment, it is transferred to containers from which it is used at the feed points.
4. Cleaning connection. This is used to drain impurities which may accumulate in the vapour generator tank using a siphon located near the distiller.

[1] Water treatment has been designed for removing substances normally present in water due to the great solvent capacity of water. The substances in general are inorganic ions (anions and cations) such as bicarbonate, sulphite, chloride, calcium, magnesium, sodium, potassium, magnesium, iron, nitrates and traces of many others.

[2] Some manufacturers cool the condenser through the use of ventilators which make air circulate on the condenser's fins, generating heat transference processes by forced convection from the diffusion surface to the environment.

5. An electrical connection equipped with control and safety devices complying with the national and international electrical standards used in the laboratory, adapted to the capacity of the resistive elements of the distiller. In general, the voltage is 220-240 V, 50/60 Hz.

Note: Always verify manufacturer's recommendations on installation to ensure the distiller is operating according to the specifications.

ROUTINE MAINTENANCE

The maintenance depends on the design and capacity of the distiller. The maintenance described in this manual focuses on a distiller equipped with a stainless steel vapour generator tank with immersion resistors and a condenser refrigerated through a ventilator impelling air (on or through the condenser's diffusing fins).

Warning: Before carrying out an inspection or routine maintenance, verify that the distiller is turned off and disconnected from the electrical source.

Inspection and cleaning of the vapour generator tank

Frequency: Monthly

1. Remove the protective panel or open the door allowing access to the boiling tank or vapour generator.
2. Remove the cover of the boiling tank.
3. Visually verify if the interior walls or the immersion resistors show solid deposits or sediments. The quantity of deposits present depends on the quality of water fed to the distiller. If there is an accumulation of sediments, it must be cleaned to avoid damaging the resistors[1].
4. Clean accumulated deposits. In general, the cleaning process requires a chemical product especially designed for removing them. The product must be selected according to the characteristics of the water used. This is determined by a chemical analysis.
5. Drain water from the generator tank until its level is approximately 10 cm above the location of the water level probe or the immersion resistance (verify that the water level is higher than the base of the tank to ensure that all of the elements stay submerged in water).
6. Add the chemical product recommended for the type of water used.
7. Mix well.
8. Allow the chemical to act overnight or as recommended by the manufacturer.
9. Drain the contents of the tank on the following morning.
10. Add clean water, wash and drain until the chemical has been completely removed along with the mineral residues from the affected surfaces.
11. Reinstall the cover.
12. Place the front panels or adjust the door.
13. Operate the equipment normally.

Warning: Under no circumstances, should the solution used for removing sediments be distilled.

Change of the activated carbon filter

Frequency: Every three months

Normally, the activated carbon filter is submerged in water below the dispenser system which comes from the distilled water storage tank. It is assembled on a casing installed on the distilled water distribution line. In general, it is a device which can be easily substituted. The following process is generally done:

1. Unscrew the top of the filter.
2. Remove the used filtering element.
3. Install a new element with the same characteristics as the original.
4. Reinstall the top of the filter.

Warning: The filter is adjusted inside its casing by means of O-rings or gaskets that must be installed carefully within their grooves in order to avoid leaks of distilled water.

Cleaning of the condenser

Frequency: Annually

1. In order to clean the condenser, it is necessary to remove the protective panels or open the door, giving access to the condenser.
2. Verify that the distiller is disconnected from the electrical outlet.
3. Remove the condenser. Disconnect the linkage system for the entry of vapour and the connection which links the condenser to the distilled product storage tank.
4. Remove screws joining the ventilator with the condenser. Disconnect the ventilator terminals from its connection points.
5. Remove the ventilator and clean the dirt accumulated on the blades. Lubricate the rotation axis with mineral oil (two drops).
6. Remove the condenser. Aspirate dirt, dust and fluff accumulated on the surface of the diffusing fins. Compressed air or a brush dampened with soap and water can also be used.
7. Rinse the parts.
8. Dry.
9. Assemble again in the reverse order to that described.

Sterilization of the distilled water storage tank

[1] The minerals deposited on the cover of the immersion resistors are particularly poor heat conductors in that they impede an efficient transfer of heat between the immersion resistance and the water in the distillation process. This makes the temperature of the resistance rise above that it would reach in normal operating conditions, deteriorating its condition and integrity..

Frequency: Occasionally

Before operating a new water distiller, it is recommended to insure that the distilled water storage tank is sterile and clean. To carry out the sterilization, use a chemical process with domestic bleach (chlorine based), for example. The procedure is as follows:

1. Verify that the main switch is off.
2. Open the front panel in order to access the storage tank for the distilled product.
3. Remove the activated carbon filter from its housing.
4. Prepare a chlorine bleach solution with a concentration of 200 ppm and add it to the storage tank.
5. Allow the solution to interact with the tank for at least three hours.
6. Empty the storage tank using the drainage line.
7. Turn on the distiller and allow the storage tank to be filled with distilled water.
8. Drain the storage tank again.
9. Install the activated carbon filter in its place.
10. Allow the distiller to fill the storage tank with distilled water. The activated carbon filter will remove any remnant of chlorine bleach used.

TROUBLESHOOTING TABLE

PROBLEM	PROBABLE CAUSE	SOLUTION
The distiller does not produce distilled water.	There is no energy supply.	Verify that the electric connector is well adjusted in the electrical outlet.
		Confirm that there is power in the circuit feeding the distiller.
		Verify that the main switch is in the on position.
		Test to ensure that there is water in the vapour generator or boiling chamber.
	The immersion resistance is burnt out.	Verify the integrity of the immersion resistance. Measure electrical continuity or resistance in ohms. Substitute with another that has the same characteristics as the original.
There is water around the distiller.	The distiller or some of its components are incorrectly adjusted.	Test the filter to ensure that the activated carbon is well installed and that water flows through it.
		Verify that the collector tank of condensed liquid is properly placed.
		Confirm that the drainage installation does not have leaks.
There is vapour around the distiller.	The distiller's ventilation is inadequate.	Verify that the distiller has free space around it and at the back.
		Test that there are no objects interfering with the flow of air towards the distiller.
		Remove any object affecting the flow of air
	The refrigeration ventilation does not function.	Verify the condition of the ventilator. If it is turned ON and not functioning, substitute the ventilator with another with the same characteristics as the original.
The distilled water has a flavour.	The carbon filter is worn out.	Replace the activated carbon filter.

BASIC DEFINITIONS

Distillation. A process through which a fluid in liquid phase is heated until converted into vapour and then cooled and condensed back into liquid phase. The distillation process is used for separating mixed substances, taking advantage of their difference in volatility. To obtain very pure substances, consecutive distillation cycles are performed with the aim of progressively eliminating other substances present in the mix.

Hardness (of water). A chemical characteristic of water determined by the carbonate, bicarbonate, chlorine, sulphate and occasionally calcium nitrate and magnesium content. The resulting resistance is undesirable in some processes. There are two types of **resistors in water.**

- **Temporary hardness.** This is determined by the magnesium and calcium carbonate and bicarbonate content. It may be eliminated by boiling the water and subsequently filtering out the precipitate. It is also known as *carbonate resistance.*
- **Permanent hardness.** This is determined by all the calcium and magnesium salts, except the carbonates and bicarbonates. It cannot be eliminated by the boiling of water and it is also known as *non-bicarbonate resistance.*

  Interpretation of resistance:
  Resistance as $CaCO_3$ interpretation
  0–75 soft water
  75–150 water with little resistance
  150–300 resistant water
  > 300 water with great resistance
  In potable water, the maximum limit allowed is 300 mg /l.
  In water for heaters, the limit is 0 mg / l.
- **Calcium resistance or hardness (RCa^{++}).** Quantity of calcium present in water.
- **Magnesium resistance or hardness (RMg^{++}).** Quantity of magnesium present in water.
- **Total resistance or general hardness [TH].** Quantity in calcium [Ca] solution and magnesium [Mg] as cations, without taking into account the nature of the anions present in the water. It is expressed as ppm (parts per million) of calcium carbonate *($CaCo_3$)*.

Incrustation (scale). A name given to solids in suspension deposited in layers on the surface of water storage containers.

Solution. A homogenous mix of two or more substances characterized by the absence of chemical reactions between the components of the liquid mixture. The liquid component which generally appears in greater proportion is called the *solvent* and that found in a lesser quantity in solution, the *solute*.

Chapter 9

Dilutor

GMDN Code	**15133**
ECRI Code	**15-133**
Denomination	**Dilutors**

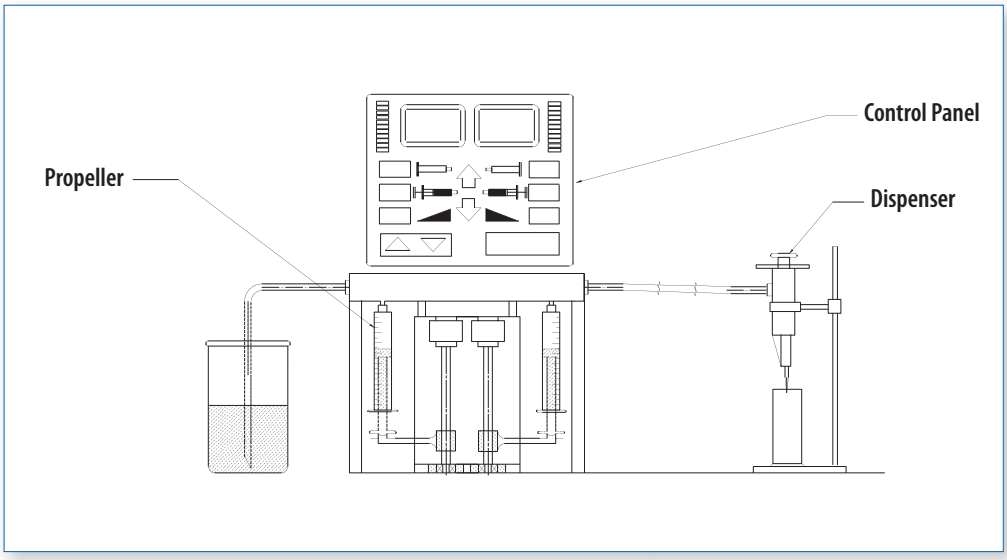
The dilutor is used for diluting substances. Dilute comes from the Latin word *diluere* and means to add liquid to a solution. Solutions are defined as homogeneous mixtures of two or more components which may be gaseous, liquid or solid. To dilute is to reduce the strength of a fluid in a solvent, generally water. The dilutor facilitates the preparation of liquid mixtures, until these achieve a proportion (concentration) suitable for use in different diagnostic processes. The identification of this type of equipment is generalized using the word *dilutor*.

PURPOSE OF THE DILUTOR

The purpose of the dilutor is to prepare mixtures of substances to achieve determined concentrations and volumes as done with a pipette, but with the advantage of an automated or programmed process. Dilutors vary in size and complexity. Their capacity depends on the models and manufacturers. They can control known volumes between 25 µl (microlitres) and 25 ml (millilitres).

DIAGRAM OF A DILUTOR

Figure 22. Dilutor diagram



OPERATION PRINCIPLES

The dilutor has various components which interact in a coordinated manner to handle liquids and mix volumes with great precision, which allows known solutions of between 1 µl and 25 ml to be prepared. The dilutor has in general, the following components:

1. A propulsion system
2. A control system
3. A dispensing system

Propulsion system

This is generally constituted of positive displacement systems as found in syringes. One or more selectable syringes (with a varying capacity) is/are used in the dilutor to control the volume to be mixed or diluted. The syringes' pistons are moved by a mechanism which controls their position. Aspirated volumes or deliveries are calculated by means of the following equation:

$$\partial V = A\partial l$$

Where:

$\partial V =$ fraction of the volume delivered by the syringe when the piston has a displacement ∂l.

A = piston area.

The total volume aspirated or delivered is the corresponding integral:

$$V = A\int_{l_o}^{l_1} \partial l$$

where l_o and l_1 correspond to the positions that define the piston's displacement.

Controlling how the pistons move facilitates good control over the volumes handled. The displacement system is activated by an electric motor which moves a very precise nuts and screws system and changes the position of the piston. A set of valves controlling the aspiration and supply processes complements the syringes and their displacement systems. The configuration of the dilutor depends on the model and manufacturers.

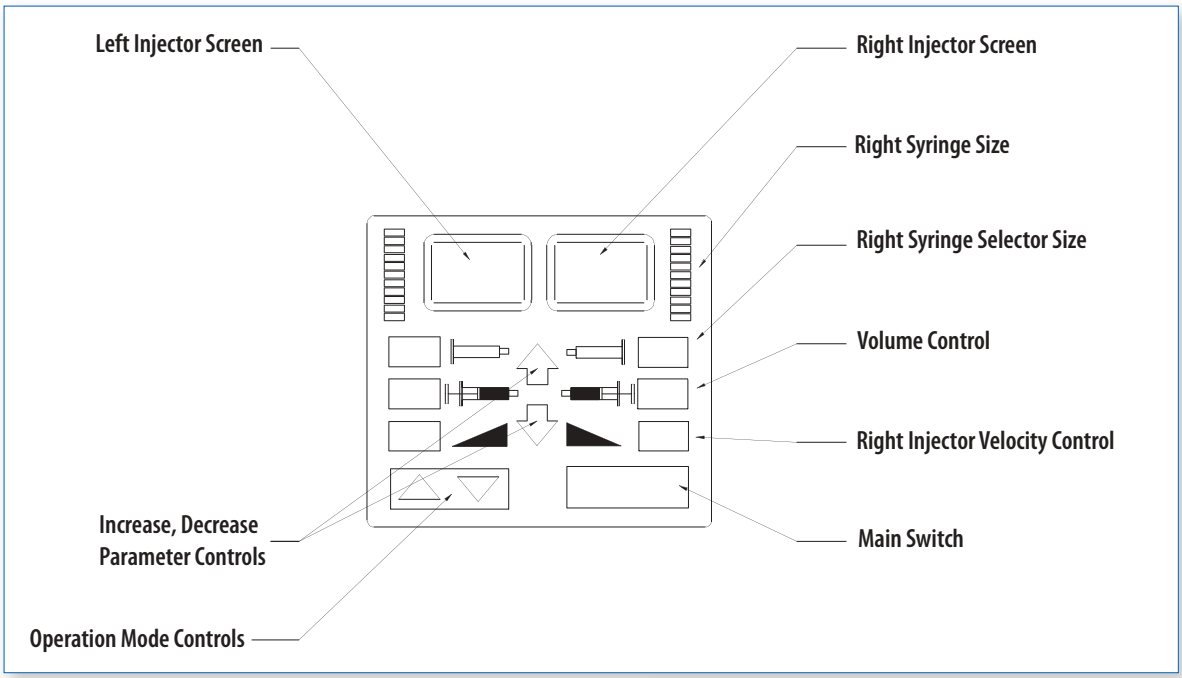
Control system

Modern dilutors have a control system which is automatic or controlled by microprocessors. The latter allow the following to be selected and controlled:

1. Mixing processes and/or dissolution of substances (programmable)
2. Predefined volume supply
3. Supply or suction velocities
4. Number of required cycles
5. Size or volume of selected syringes
6. Time
7. Priming and cleaning cycles
8. Quality control procedures

In order to give a clearer idea of the technical complexity achieved, a diagram of the control system based on a microprocessor displaying some of the dilutor functions is shown next. The controls for this type of device are generally symmetrical if they control two injectors.

Figure 23. Dilutor controls

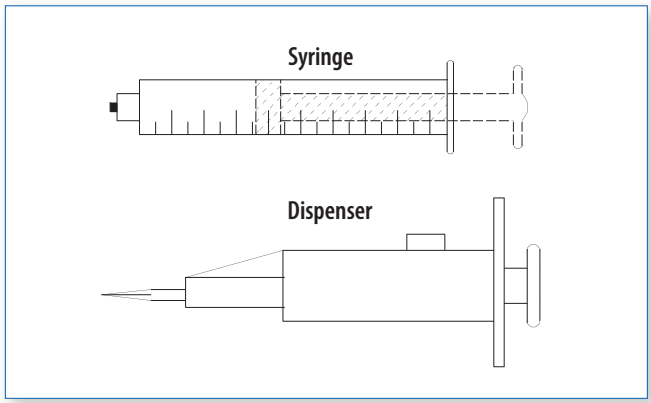


Dispenser system

The dispenser system is composed of a set of high precision syringes and devices called dispensers, through which fluids are supplied according to their volumes and selected velocities. These syringes are selected and installed in the dilutor depending on the densities, viscosities, and volumes of fluids to be manipulated. The fluids are transported through flexible tubes, whose diameters, lengths and chemical compatibility are taken into account in the design and manufacturing process for suitability with the selected activity. These tubes are linked using connections manually adjustable. Normally, the syringes are classified according to their use (e.g. syringes for reagents, diluents, samples), and the volume these manipulate. The following table shows an example of how they are classified according to their size and managed volumes.

Opposite, the components of the dispensing system (syringe and dispenser) are shown.

Figure 24. Syringe and dispenser



INSTALLATION REQUIREMENTS

The dilutor must be installed on a clean, dry and extremely levelled counter or work surface, far from areas where there may be vapours which can affect its functioning.

There must be free space around the equipment for facilitating ventilation and the passage of cables and interconnection lines and cables with the solvent containers, computers or supply systems. The space around the dilutor should be approximately 10 cm.

There must be a 115 V, 60 Hz electrical outlet in good condition with a ground pole or alternatively one of 220–240 V, 50/60 Hz, depending on the manufacturer's specifications and/or the electrical norms in the country of use.

ROUTINE MAINTENANCE

The routine maintenance focuses mainly on eliminating contaminants which may accumulate inside the fluid mechanisms and/or lines. The most common routines are the following:

Cleaning of exterior surfaces

Frequency: Daily

Warning: Disconnect the dilutor from the electrical feed outlet before beginning the external cleaning process.

1. Clean the exterior surfaces using a clean piece of cloth dampened with a mild detergent mixed with water.
2. Lightly rub the surfaces of the dilutor and the accessories.
3. Dry the treated surfaces.

Warning: Avoid humidity from entering the compartment of the electrical and electronic components.

Table of syringe size/volumes managed

Part No. (Depending on the manufacturer)	Model (Depending on the manufacturer)	Syringe size	Range (Processed volume)	Duct size[1]	
				Aqueous solution	Viscous liquids
DM	DM	25 µl	2.5–25 µl	18	18
DM	DM	50 µl	5–50 µl	18	18
DM	DM	100 µl	10–100 µl	18	18
DM	DM	250 µl	25–250 µl	18	18
DM	DM	500 µl	50–500 µl	18	18
DM	DM	1 ml	100–1 000 µl	18	18
DM	DM	2.5 ml	250–2 500 µl	18	12
DM	DM	5 ml	500–5 000 µl	12	12
DM	DM	10 ml	1 000–10 000 µl	12	12
DM	DM	25 ml	2 500–25 000 µl	12	12

[1] Table 2.4, Microlab 501A, 503A, 504A, *User's Manual*, Hamilton Company.

Cleaning of syringes, hoses or lines

Warning: If the dilutor has been in contact with dangerous substances, the safety and prevention procedures implemented in the laboratory must be respected.

Frequency: Daily

1. Feed the system with a cleaning solution. Consult the manufacturer to enquire about the solution to use. Verify that each system's elements come into contact with the solution and that air bubbles have been eliminated. This process is known as *priming*. In order to feed the system, the dilutor is connected to a container in which the used solution is present. Once the priming is complete; the waste solution goes into another container for final disposal.
2. Clean the system. In order to carry out cleaning, a fluid which complements the cleaning solution is circulated (consult the manufacturer's recommendations). It is common to use deionised water as a cleaning fluid. Depending on the substances processed in the dilutor, other cleaning agents can be used such as ethanol, urea, or a 10% bleach solution in deionised water.

Cleaning of the fluid conduction system

Frequency: Before putting into service for the first time

1. Prepare a container with cleaning solution and place the filling tube inside (manufacturers recommend using cleaning agents compatible with the dilutor).
2. Place the waste line inside the waste container.
3. Run a feed or priming cycle until the fluid's lines becomes clean.
4. Remove the filling tube from the cleaning solution and place it inside a container with deionised water. Start a feed or priming cycle again until the fluid trajectory is free of cleaning solution. Discard the fluid and rinse the waste container.
5. Suspend the feed cycle.
6. Place the fluid propulsion system in the rest position.
7. Use the system as it is clean and ready.

Procedure for storing the dilutor

Frequency: Whenever stored for a prolonged period of time

1. Purge and prime the system using methanol (facilitates drying).
2. Remove the tubes and syringes.
3. Store the syringes in their original protective covers.
4. Cover the body of the dilutor in order to protect it from dust.
5. Store.

Quality control

The quality control of dilutors is similar to that of pipettes. In order to resolve uncertainties, please see the explanation regarding how calibration is conducted in Chapter 16 on pipettes.

TROUBLESHOOTING TABLE

PROBLEM	PROBABLE CAUSE	SOLUTION
The dilutor does not turn on.	There is a fault in the electrical feed.	Check the electrical connection.
	The electrical feed is disconnected.	Connect electrical feed cable.
	The protection fuse is open.	Check the protection fuse. Substitute with an equivalent one if it is burnt.
The dilutor operates well, but there are no messages or indications on the screen.	There is possible damage to the LCD screen or in the emission diodes of the LED light.	Verify that the control is well connected to the propulsion system.
		Call the manufacturer's service technician.
The control keys do not function.	The dilutor is on the Pause mode.	Press the start/end button to complete the path of the piston.
The dilute is obstructed.	There is an internal error.	Press the start/end button to complete the path of the piston and to restart the cycle.
		Call the manufacturer's service technician, if the fault persists.
The dilutor does not aspirate nor dispense.	The hydraulic systems' tubes are defective or blocked.	Verify that tubes, syringes and connectors are free from blockages. Clean or substitute.
	Incorrect connection of tubes and syringes	Test that the tubes, joints, connections and syringes used are well adjusted.
	The propulsion system is defective.	Call the manufacturer's service technician.
	The valves are defective.	Remove the valves. Verify that their seals are clean and reinstall. Substitute for an equivalent valve if necessary.
The dilutor does not produce precise results.	There is air in the fluid circuit.	Verify that the feeding tubes are completely submerged inside the containers which contain the reagents.
		Confirm that the different connectors are adjusted.
		Verify that the syringes are correctly installed and there are no leaks.
		Test to ensure that the tubes or valves have no leaks.
		Reduce the operational speed of the syringe to eliminate cavitation problems.
	The delivery tube is incorrectly selected for the syringe's capacity.	Verify the recommended size of the tube used and its connections. For small volumes, use the dimensions recommended by the manufacturer.
A small air gap appears on the tip of the probe after the final aspiration.	The aspiration tube is dirty.	Change or clean the aspiration tube.
	The aspiration mode is incorrect.	Reduce the aspiration speed.
Air is persistently present or there are constant leaks in the fluid trajectory.	Cavitations are present in the system. The aspiration speed is very high.	Reduce the propulsion system's speed. Remember that the more viscous the fluids, the lower the speed must be used to manipulate them.
	The connections are loose, worn out or defective.	Adjust the connections by hand. Substitute to tubes with dimensions corresponding with the fluids processed.
	The piston is defective or the syringe is damaged.	Replace the piston or the syringe.
	There is a defective valve.	Replace the valve.
The dilutor is heating.	There is inadequate ventilation.	Check the ventilation.
	The room temperature is too high.	Check the air conditioning system in the area.
	The work cycle is very intense.	Use the dilutor with less intensity.

BASIC DEFINITIONS

Cavitations. A phenomenon in fluids when a vacuum is created upon emptying a vessel. The pressure decreases until it reaches the vapour pressure of the fluid. This produces diverse phenomena such as vaporization of gases dissolved in the liquid or, in the case of water, the formation of vapour bubbles collapsing after an infinitesimal time lapse, perforating the surfaces of conducts in the immediate vicinity. This occurs in dilutors when using large capacity syringes with elevated propulsion speed.

Concentration. A quantity measurement of a chemical substance present in a solution. The concept is expressed as the quantity of a substance dissolved into a solvent. Concentration is expressed in diverse forms; the most common are: molarity [M], molality [m], normality [N], percentage rate of solute.

Dilution. To reduce the concentration of a solution by adding other fluids. The fluid added is known as the *diluent*. Adding the molecules of a liquid substance with the molecules of another liquid substance. In order to determine the volume V1 of liquid needed to obtain V2 volume at a concentration C2 from a stock solution of concentration C1, the following equation is used:

$$V_1 = \frac{V_2 C_2}{C_1}$$

Dispenser. A device used for distributing liquids.

Dispensing. Distributing a fluid at a constant volume or in a progressive form.

Dissolution. Process by which a chemical in solid form is dissolved in a solvent (e.g. water or other liquid). The chemical now in solution is called the *solute*.

Equivalent – gram [Eq]. Mass in grams of solute divided by its equivalent weight [EW]:

$$Eq = \frac{mass(g)}{EW\ (g)}$$

Equivalent weight [EW] (of one substance). Results from dividing the molecular weight [MW] by its valency.

$$EW = \frac{MW\ (g)}{valency}$$

Molality [m]. Number of moles of a given substance, for every 1000 g of solvent. Thus an m molal solution is obtained by adding m moles of the substance to 1000 g of water.

Molarity [M] (of a solution component). Number of moles of solute for each litre of final solution. A solution n Molar of a salt is obtained by adding n moles from that salt to water until obtaining one (1) litre of solution. Normally, the formula employed is the following:

$$M = \frac{moles}{Vol(L)}$$

Mole. Molecular weight (MW) of the solute expressed in grams:

$$moles = \frac{mass(g)}{EW}$$

Normality [N] (of a solute). Number of moles of solute per litre of final solution.

$$N = \frac{Eq}{Vol(L)}$$

Solution. A homogeneous liquid mixture of two or more substances. The dissolved chemical(s) called the solute(s) usually name the solution. The substance in which the solute(s) are now dissolved is called the solvent. There is a usually greater quantity of solvent than solute(s) in a solution.

Weight/Volume. Relationship in clinical biochemistry expressing the mass of the solution in grams or its submultiples per volume unit in litres or submultiples of a litre. For example: g/l, mg/ml.

Note: Another type of notation known as "part per unit" is used for measuring extremely low concentrations. For example: parts per million (ppm) means that there is a particle of a given substance for each 999 999 particles of other substances.