

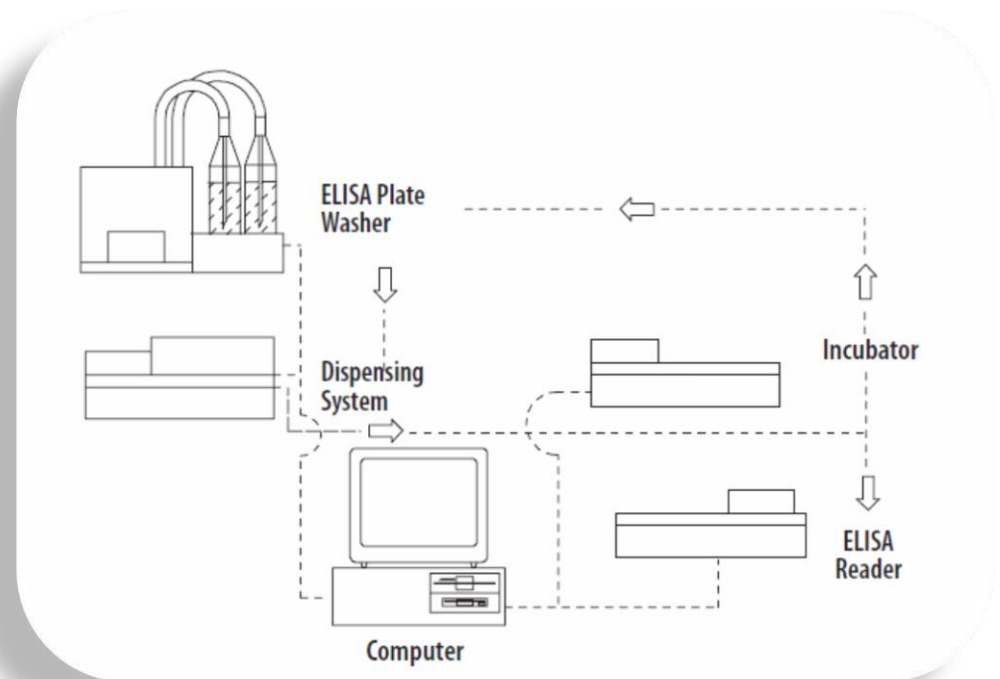
ELISA Instrument

Quality assurance

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Equipment required for ELISA testing

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



Microplate Reader

wavelength range to that used in ELISA,
generally between
400 to 750 nm (nanometres)

Some readers (ultraviolet range between
340 to 700 nm.

INSTALLATION REQUIREMENTS

1. A clean, dust free environment.
2. A stable work table away from equipment that vibrates (centrifuges, agitators).
3. An electrical supply source, which complies with the country's norms and standards.



Calibration of the microplate reader

- Executed by a technician or trained engineer
- Following the instructions provided by each manufacturer.
- It is necessary to have a set of **grey filters** mounted on a plate of equal geometric size
- Calibration plates are equipped with at least three pre-established Optic density values within the measurement ranges; low, medium, and high value.



Calibration of the microplate reader

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.

ELISA Check



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.

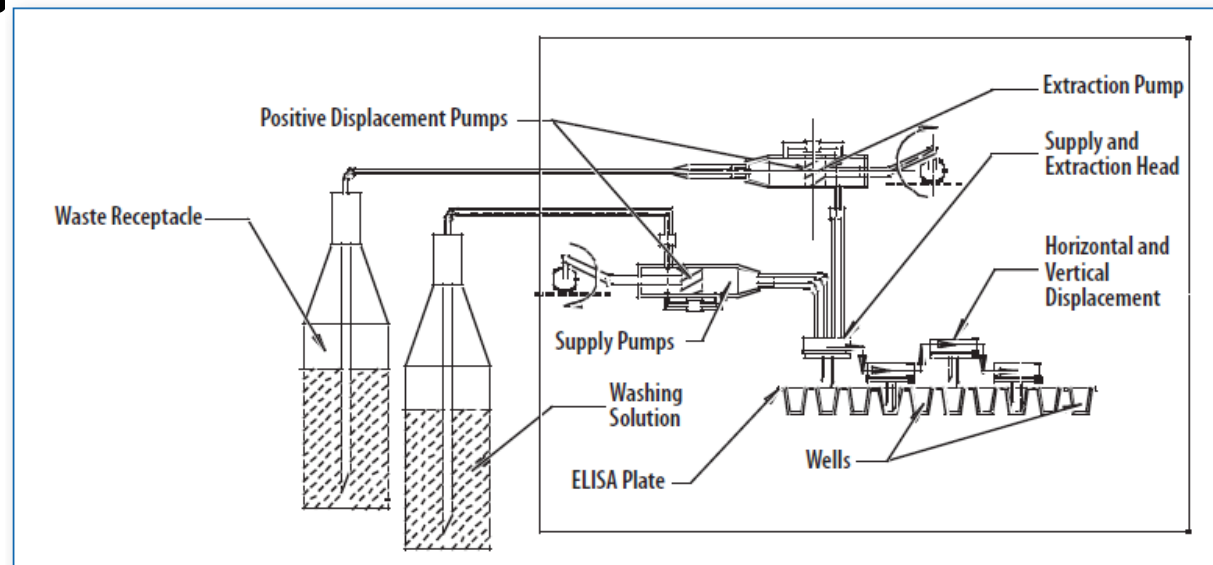


Microplate Washer

- The microplate washer has been designed to perform washing operations in the ELISA technique

Comprise:

- ***Control subsystem.***
- ***Supply subsystem***
- ***Extraction or suction system***
- ***Advance sub-system***



Microplate Washer

- **INSTALLATION REQUIREMENTS**

- 1. A clean, dust-free environment.**

- 2. A stable work table located away from equipment**
that generates vibrations, (centrifuges, and agitators).

- 3. An electric outlet in good condition with a ground pole.**



Microplate Washer Calibration

- **Washer calibration** The microplate washer is critical for guaranteeing that the ELISA technique performs as expected.

- **Position of the needles (supply and aspiration head).**

Situated very close to the well's wall.

The suction needle should be located in the centre of the well:

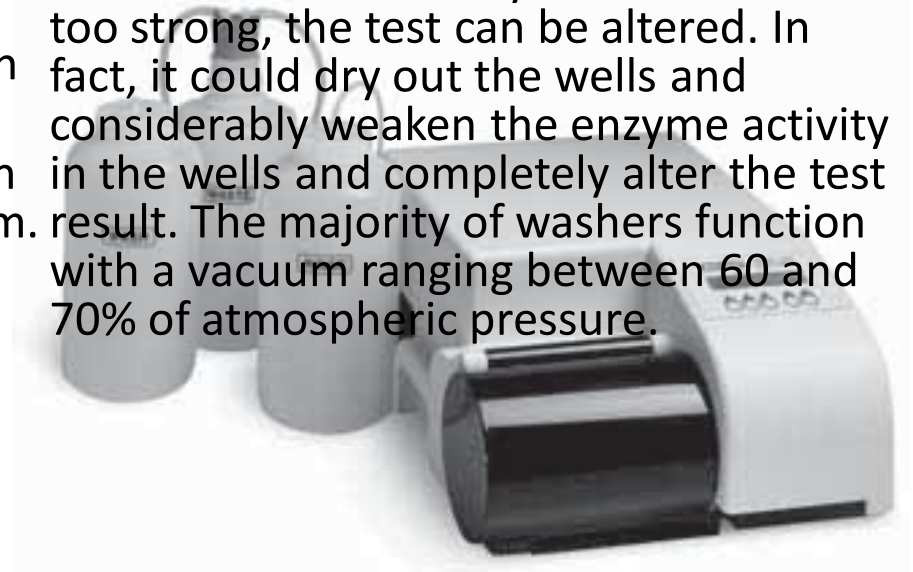
A needle-base distance is maintained in the well, usually between 0.3 to 0.5 mm.

The needles must never be allowed to touch the bottom of the wells to avoid mechanical interferences

- **Aspiration time.**
- **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.



Microplate Washer Performance Verification

- **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**
- 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/H₂O₂ – 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.



ELISA Check

Microplate Washer Maintenance

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 subsystems' 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.



Microplate Washer Preventive Maintenance

- **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.

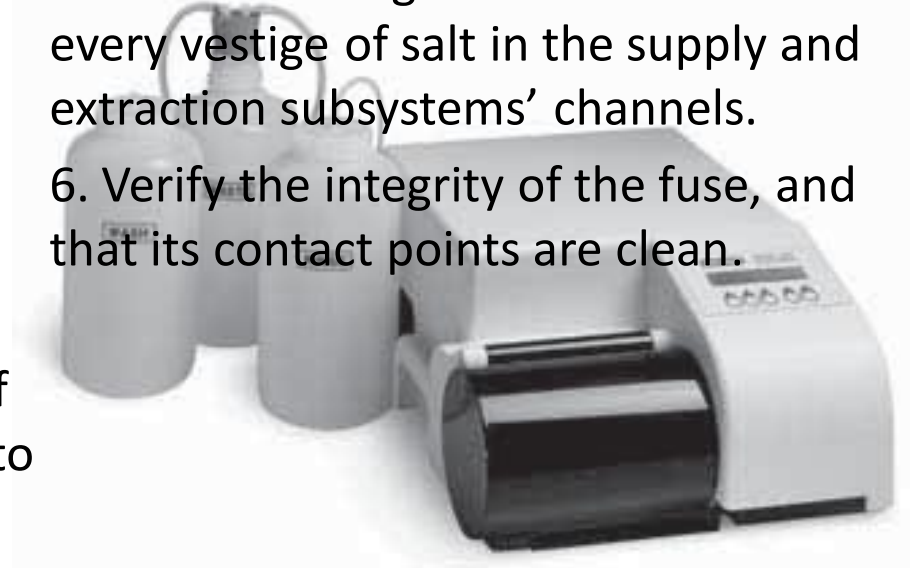
3. Test the adjustment of each one of the subsystems. Calibrate according to

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.



Pipettes and Best pipetting practice



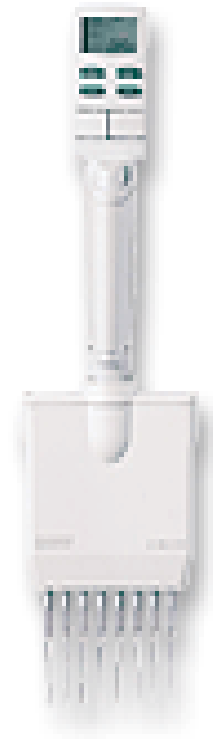
Manual
single
channel



Manual
multi-
channel



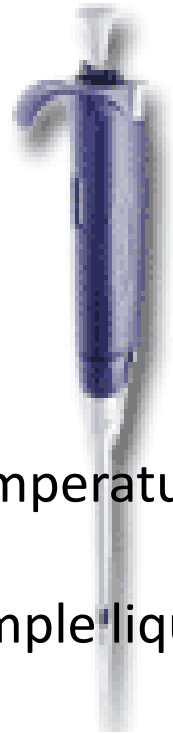
Electronic
single
channel



Electronic
multi-
channel

Ways to optimize pipette performance

- Choose the right pipette for the job.
- Check for leaks or any other pipette malfunctions
- Choose the correct pipette tip
 - Correct size
 - Correct style
- Have pipettes calibrated and serviced regularly.
- Allow all liquids and equipment to equilibrate to ambient temperature before beginning work.
- Pre-rinse the pipette tip by aspirating and dispensing the sample liquid at least 3 times before aspirating a sample for delivery.
- Immerse the tip vertically into the sample liquid well clear of the container walls and bottom and at a depth of approximately 2 – 5mm below the meniscus.



Tips to minimize pipetting errors

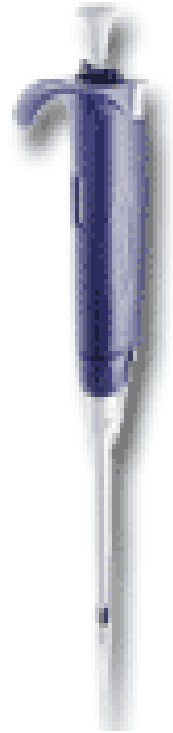
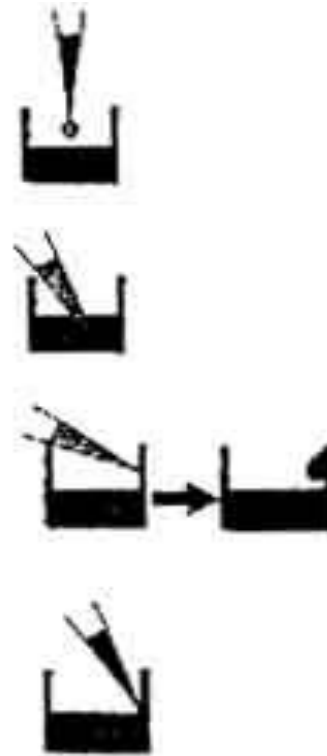
- Aspirate using a consistent speed, rhythm, and plunger pressure.
- Hold the tip in the sample for 1 second after aspiration and withdraw the tip slowly and smoothly without touching the sides of the container.
- To dispense touch the pipette tip to the sidewall of the container where sample is to be delivered just above the liquid the sample is being dispensed into. Use consistent speed, rhythm, and plunger pressure to dispense.
- Put the pipette in its stand between pipetting cycles to avoid warming the pipette in your hand – this can affect the volume of liquid dispensed.



PIPETTING

GUIDE TO PIPETTING

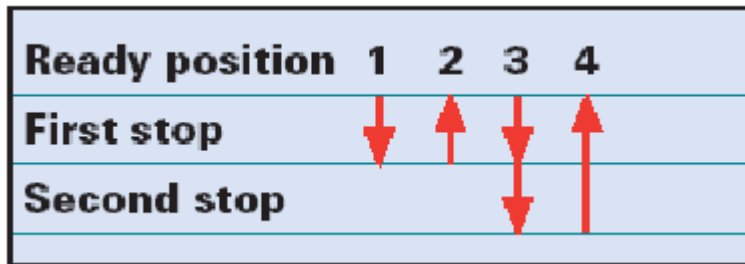
- **ONLY USE FIRST STOP !**
- **DO NOT DRIP**
- **DO NOT PRESS HARD INTO WELL**
- **DO NOT USE TOO ACUTE AN ANGLE**
- **MAKE SURE TIP TOUCHES SIDE OF WELL AND LIQUID**



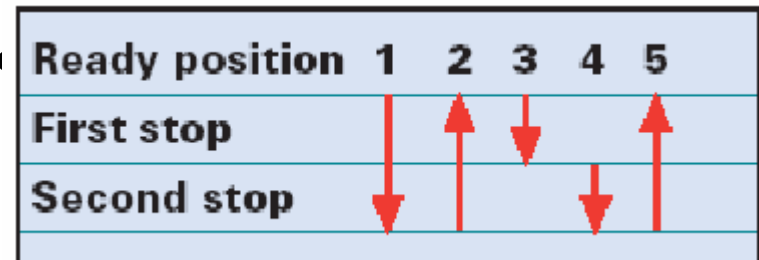
Pipetting tips



- **Forward Pipetting technique**



- **Reverse Pipetting technique**

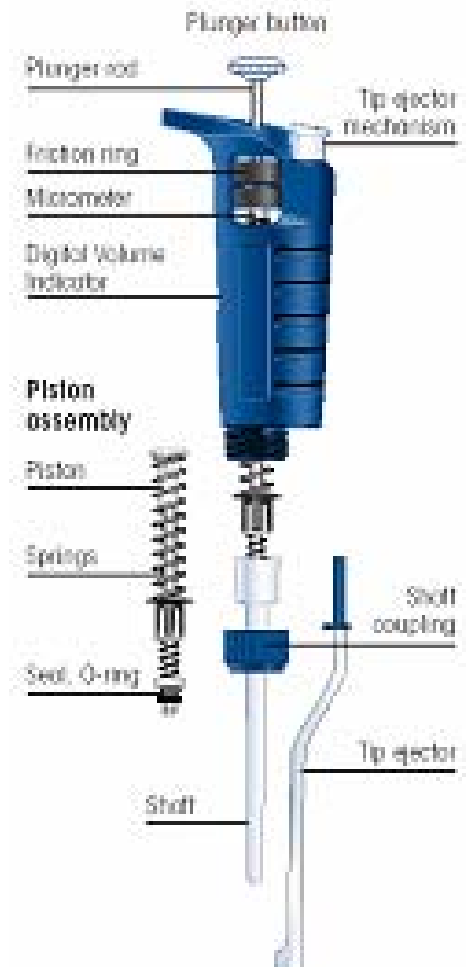


- **Highly viscous fluid**
- **Avoid foaming**

Pipette preventive maintenance

- As part of Preventive Maintenance, each Pipette disassembled according to manufacturer's diagrams and cleaned thoroughly with the appropriate chemical solutions.
- All parts are carefully inspected, piston is polished in most cases, piston seal and o-ring are replaced as well as all defective and malfunctioning parts. [see 12Point Inspection diagram]Thank You

12-Point Inspection



Pipette calibration

- Gravimetric
- Colourimetric



Thanks for your attention