Automatic pipettes

According to the control method, we distinguish between manual and electronic automatic pipettes . In manual pipettes, the plunger is moved with the thumb using the control button. The correctness and accuracy of pipetting is significantly influenced by the experience and skill of the worker. In the case of electronic pipettes, the piston is moved by an electric motor. Compared to manual methods, it also offers programming of the pipetting method. Depending on the nature of the liquid, you can choose a different speed of piston movement when sucking in and expelling the liquid.

A disposable tip is attached to the body of the pipette (also *called pipetor*). The pipetted liquid comes into contact only with this tip.

According to the principle of their operation, automatic pipettes can be divided into two basic types

- **"Air displacement" pipette**

  This type of pipette uses the so-called air cushion principle. A certain volume of air always remains between the piston and the liquid. The volume of liquid drawn into the tip by the pipettor may differ slightly from the volume of air drawn in or pushed out by the piston, depending on the density and viscosity of the pipetted liquid, the wettability of the tip surface by the pipetted liquid, temperature and atmospheric pressure, and other influences. Therefore, the pipettor must be regularly calibrated and adjusted.
  Pipettes of this type are distinguished according to their design as single-channel (intended for pipetting one volume of a given liquid in time) or as multi-channel (most often eight or twelve-channel) intended for simultaneous pipetting of the same volume of a given liquid into several wells in a microtiter plate . Each channel in multi-channel pipettes has its own piston, therefore it is not necessary to use all channels at once (less than 8 or 12 tips can be connected).
  Automatic pipettes are designed either for one fixed volume or are adjustable for multiple volumes. Changing the volume setting is possible within a certain range (e.g. 10-100 µl) using the adjusting screw or knob

- **"Positive displacement" pipettors**

  This type of pipette sucks liquid into the tip directly without creating an air cushion, i.e. the piston is in direct contact with the measured liquid. *The liquid sucked into the tip (without air bubbles) is discharged all at once (syringe type) or in steps of the same volume (stepper* pipette). This type of pipettor is convenient to use for highly viscous or volatile liquids, or for repetitive pipetting

Direct pipetting

This is the most commonly used pipetting technique. During direct pipetting, a precisely set volume is sucked into the tip and in the next step it is completely pushed out of the tip into the selected container. Because a certain amount of liquid remains on the inner surface of the tip as a thin film, it is necessary to wet the tip with the measured liquid before pipetting. The direct pipetting technique is used for measuring most aqueous solutions, buffers , dilute acids and bases. Pipetting scheme-direct

Method

1. Place the tip on the dispenser. Press the controller button to the first position (a small resistance must be overcome when pressing the button).
2. Dip the tip of the dispenser about 2-3mm below the solution level. Slowly release the pressed button on the controller while sucking the sample into the tip.

   By slowly sucking the liquid into the tip, the possible formation of turbulence is limited, which can cause the formation of aerosol and gas bubbles coming out of the liquid. The optimal suction speed depends on the properties of the liquid (its density, vapor tension and viscosity).

   - Always check whether air bubbles have entered the tip (e.g. when the actuator piston is opened more sharply or the tip is incorrectly fitted).
   - For greater pipetting accuracy, remove your thumb completely from the controller button once it reaches the home position.
3. Slowly withdraw the tip from the liquid. Some of the contents of the tip may be lost when pulled out quickly. Wait, especially for larger 500-5000 µL pipettors, about 1-3 seconds before pulling the tip out of the liquid.
4. When expelling the liquid, hold the tip at a slight angle against the wall of the container (10-45º), just above the solution already in it, and smoothly press the control button with your thumb to the first position. Wait about 1 second and continue to quickly press the controller button to the second position (you will feel more resistance when pressed). Make sure that no droplets of liquid remain in the tip or splash on the walls of the container.
5. Hold down the controller button and pull the tip out along the wall of the container. Now enable the controller

button.

In direct pipetting, a certain error is created by the fact that a very thin film of the transferred liquid remains on the inner surface of the tip. With the mentioned procedure, we measure a slightly smaller volume than is set on the pipette, while the error depends mainly on the properties of the pipetted liquid and the material from which the tip is made. This error can be eliminated by wetting the inner surface of the tip with the measured liquid before pipetting. In practice, this means that we first suck the solution into the tip using the procedure described above, but instead of measuring it into the target container, we return it back to the storage container. At this moment, a film of pipetted liquid is formed on the inner wall of the tip, in the case of colorless solutions it is invisible to the eye with the correct technique. This is followed by measuring the liquid exactly according to the above procedure (only we do not insert a new tip). Since the amount of liquid,

If drops remain on the outer wall of the tip, it is possible to wipe them with a cotton wool with a light movement from top to bottom. Never touch the mouth of the tip with the pulp, otherwise you will suck out some of the liquid inside.

Reverse pipetting

During reverse pipetting, we draw a larger volume of liquid into the tip than we want to measure, and in the next step we push out the volume set on the pipette from the tip. This method of pipetting gives better results when working with viscous or highly volatile liquids, strongly wetting liquids and solutions that foam. It is also suitable for measuring very small volumes. After pipetting, there is always a residue of liquid in the tip, which can be squeezed back into the storage container or into the waste before removing the tip itself. Pipetting scheme-reverse

Method

1. Press the button to the second position (you will feel first a weak and then a greater resistance of the piston when pressing the button of the controller).
2. Dip the tip of the pipette about 2-5 mm below the surface of the solution. Slowly release the plunger while sucking the sample into the tip.
3. Slowly withdraw the tip from the liquid and remove any droplets adhering to the outer wall of the tip by touching the tip to the rim of the container.
4. When expelling a given volume of liquid, hold the tip at a slight angle against the wall of the container just above the solution already in it, and slowly and smoothly press the control button to the first position with your thumb.
5. Hold the controller button down in this position and pull the tip out of the container.
6. Push the part of the liquid that remains in the tip back into the original container or into the waste by pressing the controller button to the second position.
7. Hold the controller button down and pull the tip out of the liquid, then release the controller button.

Repeat pipetting

Schematic pipetting-repetitive This method of pipetting is intended for repeated pipetting of the same volume, e.g. for adding a reagent to a series of tubes or to wells in a microtitre plate. This is actually repetitive reverse pipetting. After sucking the liquid into the tip, repeat steps 2 to 4.

Pipetting heterogeneous samples

A technique suitable for pipetting heterogeneous samples such as blood, when it is not easy to rinse the tip before pipetting. It is similar to the direct technique, but the tip is not pre-wetted with the measured liquid. Instead, after the liquid has been transferred, it is repeatedly flushed with the solution with which the metered liquid is mixed. Scheme of pipetting-heterogeneous samples

Method

1. Press the button to the first position and dip the tip of the dispenser about 2-5 mm below the level of the solution.
2. Slowly release the plunger while sucking the sample into the tip.
3. Slowly withdraw the tip from the liquid and remove the drops of solution adhering to the outer wall of the tip by pulling the tip along the wall of the container.
4. Dip the tip of the dispenser into the target solution.
5. Press the control button to the first position and then slowly release it to the original position. This will draw the solution into the tip. Do not remove the tip from the solution and repeat this step until the inside of the tip is clean.
6. Along the wall, pull the tip above the solution level and empty it by pressing the controller button to the second position.
7. Hold down the controller button and pull the tip out of the container along the wall and then release the controller button.