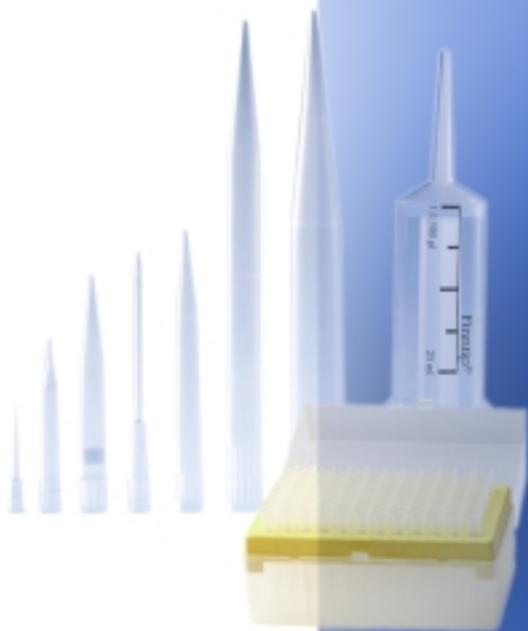


Liquid Handling Application Notebook

Tips on how to **pipette**



Thermo Labsystems





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Pipetting terms

Aspirate - to draw up the sample

Dispense - to deliver the sample

Blow-out - to empty the tip completely

Calibration check - checking the difference between the dispensed volume and the selected volume

Adjustment - altering the pipette so that the dispensed volume is within the specifications

Types of pipettes

Although Thermo Labsystems supplies pipettes for all application needs, most laboratories are equipped with two types of pipettes.

Air displacement pipettes are meant for general use with aqueous solutions.

Positive displacement pipettes are used for high viscosity and volatile liquids.

Both types of pipettes have a piston that moves in a cylinder or in a capillary. In air displacement pipettes, a certain volume of air remains between the piston and the liquid. In positive displacement pipetting, the piston is in direct contact with the liquid.

Air displacement pipetting

Air displacement pipetting, used for standard pipetting applications, is highly accurate. However, conditions such as atmospheric pressure as well as the specific gravity and viscosity of the solution may have an effect on the performance of air displacement pipettes.

- **Finnpipettes for air displacement pipetting:**
Finnpipette Digital, Finnpipette Colour, Finnpipette Fixed Volume, Finnpipette BioControl, Finnpipette Multistepper, Finnpipette Varichannel
- **Finntips for air displacement pipetting:**
Finntip (standard), Finntip Filter, Finntip BioCon, Finntip Multisteppper, Finntip Band 4, Finntip Wide



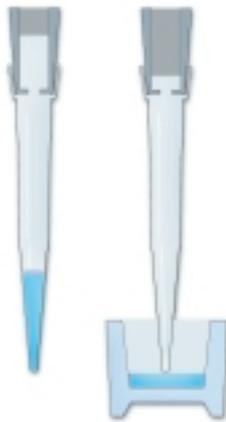
1.



2.



3.



4.

How does an air displacement pipette work?

1. The piston moves to the appropriate position when the volume is set.
2. When the operating button is pressed to the first stop, the piston expels the same volume of air as indicated on the volume setting.
3. After immersing the tip into the liquid, the operating button is released. This creates a partial vacuum and the specified volume of liquid is aspirated into the tip.
4. When the operating button is pressed to the first stop again, the air dispenses the liquid. To empty the tip completely the operating button is pressed to the second stop (blow out).

Positive displacement pipetting

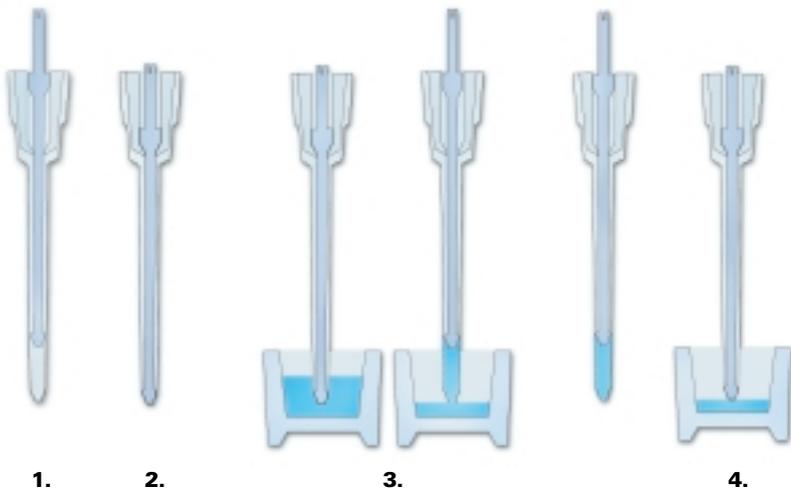
Positive displacement pipetting is used for applications like PCR and other DNA amplification techniques. The microsyringe tips used in positive displacement pipettes are disposable. This helps to avoid sample-to-sample cross-contamination (also known as sample carry-over), and contamination due to the aerosol effect.

- **Finnpipettes for positive displacement pipetting:**

Finnpipette Stepper, Finnpipette PDP

- **Finntips for positive displacement pipetting:**

Finntip Stepper, Finntip PDP



How does a positive displacement pipette work?

1. The piston moves to the appropriate position when the volume is set.
2. When the operating button is pressed to the first stop, the piston descends to the tip opening.
3. After the tip is immersed into the liquid, the operating button is released. The plunger is then raised and a partial vacuum is created. This causes the liquid to enter the tip.
4. When the operating button is pressed again, the piston descends, expelling the liquid from the tip.

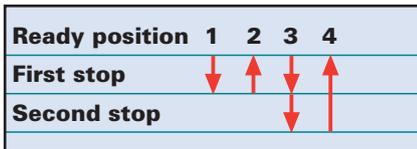
General guidelines and pipetting techniques

General guidelines

- Check your pipette at the beginning of your working day for dust and dirt on the outside. If needed, wipe with 70% ethanol.
- Set the volume within the range specified for the pipette.
- Hold the pipette so the 'grippy finger rest' rests on your index finger.
- To maximise accuracy, the pipette, tip and liquid should be at the same temperature.
- Check that you are using tips recommended by the manufacturer. To ensure accuracy, use only high-quality tips made from contamination-free polypropylene.
- Tips are designed for single use. They should not be cleaned for reuse as their metrological characteristics will no longer be reliable.
- Pre-rinsing (three - five times) the tip with the liquid to be pipetted improves accuracy, especially when using positive displacement tips.
- Avoid turning the pipette on its side when there is liquid in the tip. Liquid might go to the interior of the pipette and contaminate the pipette.
- Avoid contamination to or from fingers by using the tip ejector.
- Always store pipettes in an upright position when not in use. Finnpipette stands are ideal for this purpose.
- Check calibration regularly, depending on the frequency of use and on the application, but at least once a year. If used daily, a three-month interval is recommended. Follow the instructions for recalibration in the instruction manual (see also page 17).

Forward pipetting

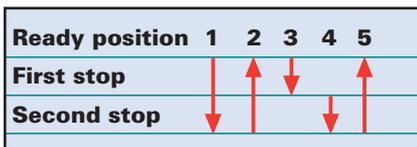
1. Press the operating button to the first stop.
2. Dip the tip into the solution to a depth of 1 cm, and slowly release the operating button. Wait 1-2 seconds and withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid.
3. Dispense the liquid into the receiving vessel by gently pressing the operating button to the first stop. After one second, press the operating button to the second stop. This action will empty the tip. Remove the tip from the vessel, sliding it up the wall of the vessel.
4. Release the operating button to the ready position.



Reverse pipetting

The reverse technique is used for pipetting solutions with a high viscosity or a tendency to foam. This method is also recommended for dispensing small volumes. Reverse pipetting is only possible with air displacement pipettes.

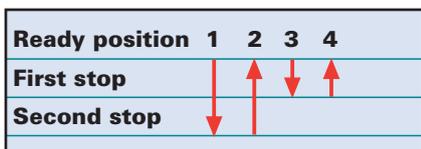
1. Press the operating button to the second stop.
2. Dip the tip into the solution to a depth of 1 cm, and slowly release the operating button. This action will fill the tip with a volume that is larger than the set volume. Wait 1-2 seconds and withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid.
3. Dispense the liquid into the receiving vessel by pressing the operating button gently and steadily to the first stop. This volume is equal to the set volume. Hold the button in this position. Some liquid will remain in the tip, and this should not be dispensed.
4. The liquid remaining in the tip can be pipetted back into the original solution or disposed together with the tip.
5. Release the operating button to the ready position.



Repetitive pipetting

This technique is intended for repeated pipetting of the same volume.

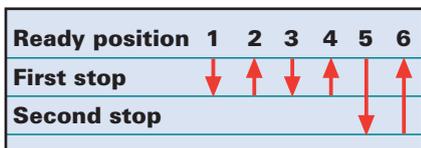
1. Press the operating button to the second stop.
2. Dip the tip into the solution to a depth of 1 cm, and slowly release the operating button. Withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid.
3. Dispense the liquid into the receiving vessel by gently pressing the operating button to the first stop. Hold the button in this position. Some liquid will remain in the tip, and this should not be dispensed.
4. Continue pipetting by repeating steps 2 and 3.



Pipetting whole blood

Use forward technique steps 1 and 2 to fill the tip with blood (do not prerinse the tip). Wipe the tip carefully with a dry clean cloth.

1. Dip the tip into the blood and press the operating button to the first stop. Make sure the tip is sufficiently below the surface.
2. Release the operating button slowly to the ready position. This action will fill the tip with blood. Do not remove the tip from the solution.
3. Press the operating button to the first stop and release slowly. Repeat this process until the interior wall of the tip is clear.
4. Press the operating button to the second stop and completely empty the tip. Remove the tip by sliding it along the wall of the vessel.
5. Release the operating button to the ready position.



Recommendations for pipetting different compounds

Solution/compound	Examples	Pipette	Tip	Pipetting technique	Comments
Aqueous solution	Buffers, diluted salt solutions	Air displacement	Standard	Forward	
Viscous solution	Protein and nucleic acid solutions, glycerol, Tween 20/40/60/80	Air displacement Positive displacement	Standard or wide orifice Positive displacement	Reverse	Pipette slowly to avoid bubble formation.
Volatile compounds	Methanol, hexane	Positive displacement Air displacement	Positive displacement Filter	Forward	Pipette rapidly to avoid evaporation. Carbon filter tips prevent vapor going into the pipette very effectively
Nucleotide solutions	Genomic DNA, PCR products	Air displacement Positive displacement	Filter or wide orifice Positive displacement	Forward	For genomic DNA wide orifice tips can be used to eliminate mechanical shearing.
Radioactive compounds	¹⁴ Carbonate, ³ H-thymidine	Air displacement Positive displacement	Filter Positive displacement	Forward	
Acid/alkalis	H ₂ SO ₄ , HCl, NaOH	Air displacement	Filter	Forward	
Toxic samples		Positive displacement	Positive displacement		

Pipetting guidelines for selected compounds

Body Fluids

Whole Blood

Pipette + tip combination:

Choose an air displacement pipette and a standard or wide orifice tip.

Technique:

Use the whole blood pipetting technique. Reverse pipetting should be used if high accuracy is needed.

Notice:

Some blood can remain in the tip and on the outer surface. Wipe the tip against the edge of the vessel to remove excess liquid outside the tip before dispensing.

Serum

Pipette + tip combination:

Choose an air displacement pipette and a standard or wide orifice tip.

Technique:

Use the whole blood pipetting technique. Reverse pipetting should be used if high accuracy is needed.

Notice:

Residual serum can sometimes be found on the outer surface of the tip. Wipe the tip against the edge of the vessel to remove excess liquid outside the tip before dispensing.

Oily fluids

Glycerol

Pipette + tip combination:

Choose an air displacement pipette and a standard or wide orifice tip.

Technique:

For high accuracy of performance, use the reverse pipetting technique.

Notice:

Oily fluids are difficult to pipette because of formation of air bubbles. Filling must be done very slowly to prevent air bubbles. Wipe the tip against the edge of the

vessel to remove excess liquid outside the tip before dispensing. The use of a positive displacement pipette and tip is also useful for pipetting glycerol.

Tween 20, 10% solution

Pipette + tip combination:

Choose an air displacement pipette and a standard or wide orifice tip.

Technique:

Use the reverse pipetting technique.

Notice:

Tween has a very high viscosity; to make pipetting easier, it should be diluted to a 10% solution. In any case pipetting will not be accurate; some liquid will stay inside the tip. Aspiration and dispensing should be done slowly. The use of a positive displacement pipette and tip is also advisable for pipetting Tween 20.

Bronidox L, 10% (preservative)

Pipette + tip combination:

Choose an air displacement pipette and a standard or wide orifice tip

Technique:

Use the reverse pipetting technique.

Notice:

Bronidox L is very viscous; the aspiration and dispensing should be done slowly or a positive displacement pipette and tip should be used.

Salt solutions

10 x PBS, 0.1M

NaCl, 3M

Pipette + tip combination:

Choose an air displacement pipette and a standard tip.

Technique:

Use the forward pipetting technique. Prewetting of the tip before aspiration increases accuracy.

Concentrated acids and bases

H_2SO_4

Pipette + tip combination:

Choose an air displacement pipette and a filter tip.

Technique:

Use the forward pipetting technique.

NaOH

Pipette + tip combination:

Choose an air displacement pipette and a filter tip.

Technique:

Use the forward pipetting technique.

Notice:

Some acids or bases vaporise easily (e.g. trifluoroacetic acid). Do the pipetting quite rapidly to minimise vapour formation.

Nucleic acids

DNA & RNA solutions

Pipette + tip combination:

Choose an air displacement pipette and a filter tip or a positive displacement pipette and tip.

Technique:

Use the forward pipetting technique

Notice:

For genomic DNA wide orifice tips can be used to eliminate mechanical shearing.

Volatile compounds

Pipette + tip combination:

Choose an air displacement pipette and filter tip or positive displacement pipette and tip.

Technique:

Use the forward pipetting technique.

Notice:

1. To get accurate results, calibrate the pipette with the volatile compound you want to pipette. If you use air displacement pipettes, aspirate and dispense the liquid a few times keeping the tip in the liquid. By doing so, the air inside the pipette will be saturated with vapour of the volatile compound.
2. Pipette rapidly to avoid evaporation when using air displacement pipettes.
3. It is recommended to use positive displacement pipettes for highly volatile compounds, since the built-in piston tip is in direct contact with the liquid.

Preventing cross-contamination

Pipette-to-sample

A contaminated pipette or contaminated tips can cause contamination of samples.

Prevention:

- Use sterilised tips or sterilised filter tips and if possible autoclave the pipette.
- Change the tip after pipetting of each sample.

Sample-to-pipette

Samples or aerosols from samples can enter the cone of the pipette.

Prevention:

- Keep the pipette vertical when pipetting in order to prevent liquid from running into the pipette body.
- Release the push-button slowly.
- To avoid aerosol contamination, use filter tips or use a positive displacement pipette and tips.
- Store the pipette vertically.

Sample-to-sample (carry-over)

The remains of sample A can mix with next sample B inside the tip and may cause a false test result.

Prevention:

- Change the tip after each sample.
- If you suspect that your pipette is contaminated, autoclave or clean your pipette (see "Maintenance of your Finnpipette" and "Autoclaving", page 27).

Finntip Filter tests

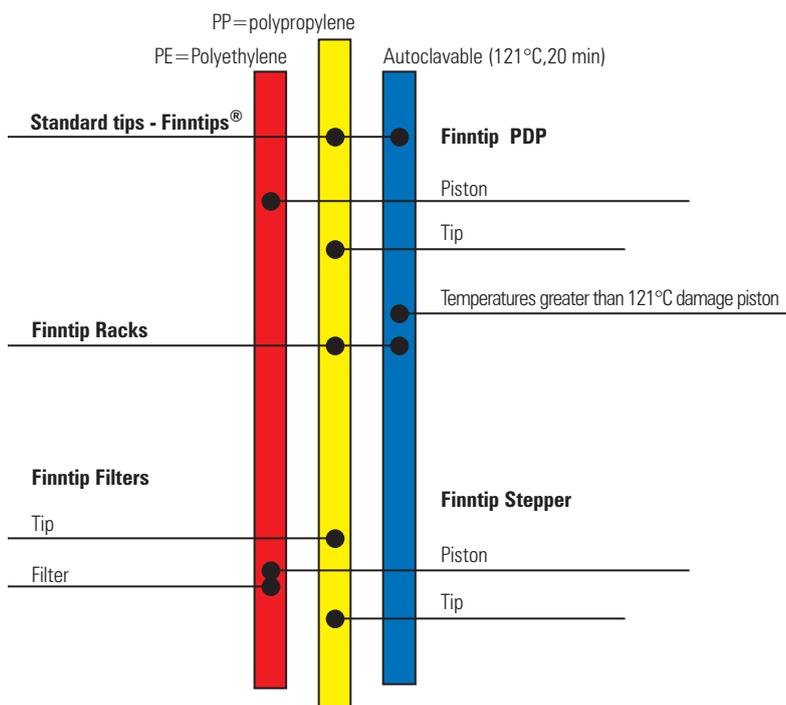
Acid test

- 35% and 5% trifluoroacetic acid (TFA) was used as test liquid.
- Finntip Filters prevented acids vapours of a 35% TFA solution of coming through the filter.
- When pipetting a 5% solution of TFA using a standard tip, no vapour came into the interior of the pipette.

DNA test

- DNA aerosols with sample concentrations of 20, 50 and 100 $\mu\text{g } \mu\text{l}^{-1}$ were blown onto the filters.
- Finntip Filters prevented DNA aerosol of these concentrations coming through filter into the interior of the pipette.

The plastics of various Finntips



Troubleshooting

Finnpipette® Digital		
Problem	Possible cause	Solution
Leakage	<i>Tip incorrectly attached.</i>	<i>Attach tips firmly, keeping pipette vertical and pressing pipette evenly to rack. Do not bang.</i>
	<i>Wrong tip size or shape.</i>	<i>Check that the size and shape are correct.</i>
	<i>Pipette incorrectly assembled after taking apart.</i>	<i>Check the assembly according to the instructions.</i>
	<i>Foreign bodies between the piston, O-ring and tip cone.</i>	<i>Clean tip cone module; attach new tips.</i>
	<i>Insufficient amount of grease on cylinder and O-ring.</i>	<i>Clean and grease O-ring and tip cone; apply grease.</i>
Inaccurate dispensing	<i>Damaged O-ring.</i>	<i>Change the O-ring.</i>
	<i>Incorrect operation.</i>	<i>Follow instructions carefully.</i>
	<i>Pipette incorrectly assembled after disassembling.</i>	<i>Reassemble according to the instructions.</i>
	<i>Unwanted substances inside the pipette. Clean the interior of the pipette.</i>	
	<i>Calibration altered; caused by misuse, for example:</i> <ul style="list-style-type: none"> • <i>Faulty calibration - High viscosity fluids may require recalibration</i> • <i>Uneven dispensing</i> 	<i>Recalibrate according to the instructions.</i> <i>Recalibrate with the liquids in question. Make sure that temperatures of tip and liquids used are the same.</i>
Aseptic working	<i>Contaminated tips.</i>	<i>Autoclave at 121°C for 20 minutes, working with gloves.</i>
Finnpipette® BioControl		
Problem	Possible cause	Solution
Leakage	<i>Tip incorrectly attached.</i>	<i>Attach tips firmly.</i>
	<i>Foreign bodies between the piston, O-ring and tip cone.</i>	<i>Clean and grease O-ring and tip cone. Use silicon grease.</i>
	<i>Insufficient amount of grease on cylinder and O-ring.</i>	<i>Clean and grease O-ring and tip cone. Use silicon grease.</i>
	<i>Damaged O-ring.</i>	<i>Change the O-ring.</i>
Inaccurate dispensing	<i>Incorrect operation.</i>	<i>Follow instructions carefully.</i>
	<i>Tips attached incorrectly.</i>	<i>Attach tips firmly.</i>
	<i>Calibration altered possibly by misuse.</i>	<i>Recalibrate.</i>
Display is blank	<i>Inappropriate calibration. High viscosity liquids may require recalibration.</i>	<i>Recalibrate.</i>
	<i>Battery is discharged.</i>	<i>Mount the pipette in the recharge stand, and make sure that the light beside the display is on.</i>
Display is blank	<i>Power is OFF.</i>	<i>Move the pipette slightly.</i>
	CALIBRATE text on the pipette won't operate	<i>Pipette is in reset mode.</i>
Pipette won't operate	<i>Tip cone module is improperly attached.</i>	<i>Release the latch, attach module firmly to the pipette and lock the latch; press trigger to the second stop.</i>
Module does not engage	<i>New module.</i>	<i>Close the latch without the module. Accept the volume range. Drive the coupler out of the pipette and keep pressing the trigger. Open the latch and push the module in the pipette a few times. Release the trigger and try again.</i>

Calibrating your pipettes

Calibration of Pipettes

Calibration of pipettes officially means determining the difference between the dispensed volume and the selected volume. Adjustment means altering the pipette so that the dispensed volume is within certain specifications.

All Finnpiettes are factory calibrated and adjusted to give the volumes as specified with distilled or deionised water. During factory calibration, performance is checked with five different weighings at both the maximum volumes of the range and at the minimum or 10% of the maximum volume, whichever is higher. Finnpiettes are designed to permit recalibration and adjustment for different temperatures and various viscous liquids.

Pipette calibration standards

The most common standards concerning pipette calibration are in a state of change. The German DIN 12650 is most commonly used in Europe, but a new version of the international standard ISO 8655 is coming in the near future, which will be a further development of DIN 12650. The latest drafts of both standards specify the gravimetric test method for accuracy, precision and permitted errors. The specifications of all Finnpiettes conform to both DIN 12650 and ISO 8655 standards.

Calibration of Pipettes in a Quality System

The main objective of pipette calibration in a quality system is to ensure that measurements are made with the intended accuracy. Very often error limits are taken from the manufacturer's specifications, while far less accuracy is required to perform the work. If these limits are not easily obtained, or vary, another option is to set the limits according to accepted standards (DIN 12650 or ISO 8655). However if the laboratory work requires the highest accuracy, the manufacturer's limits should be used. Basically every user should define their own limits, according to the application used and the ambient conditions.

Finnpipette Calibration Software is a Windows-based program designed for calibrating Finnpiettes and pipettes of any other brand.

To use the calibration software, simply install it according to the instructions. Set the environmental conditions and start the calibration. Follow the on-screen menus to complete the procedure. Once the settings are entered, the program calculates the mean volume, accuracy and precision of the pipetted volume based on up to 15 separate weighings. When using Sartorius or Precisa balances, the program can be linked directly to the balance. The advanced Finnpipette Calibration Software automatically generates complete documents with passed/failed marks depending on the limits in use for the quality system.

Device requirements and test conditions

Balance:

The scale graduation value of the balance should be chosen according to the selected pipette volume.

Volume range	Readable graduation	Sartorius model (example)
Under 10 µl	0.001 mg	Sartorius MC5
10 - 100 µl	0.01 mg	Sartorius MC210
Above 100 µl	0.1 mg	Sartorius MC210

Note:

Check the calibration of your balance regularly using known weights.

Test liquid:

Water, distilled or deionised, "Grade 3", conforming to ISO 3696. The test water is held in the calibration room for at least 2 hours before calibration to reach equilibrium with the test room conditions.

Test room:

Tests are performed in a draught-free room at a constant ($\pm 0.5^\circ\text{C}$) temperature of 20°C to 25°C . Relative humidity must be above 55%. Especially with volumes under 50 µl, the air humidity should be as high as possible to reduce the evaporation loss effect.

- The pipette, the water and the air in the test room should be at the same temperature.
- A new tip should be pre-wetted 3 to 5 times to improve the accuracy.
- Always pipette water from a reservoir, do not take it back from the balance.
- Check the calibration regularly, depending on the frequency of use and on the application, but at least once a year. If used daily, a three-month interval is recommended.

Procedures to check calibration:

Perform the calibration check using the pipetting technique you use in your applications.

Manual single channel pipettes

- The pipette is held in the calibration room for at least 2 hours before calibration to reach equilibrium with the test room conditions.
- The pipette is checked at the maximum volume (nominal volume) and at the minimum volume or 10% of the maximum volume, whichever is higher. For example, Finnpiquette 0.5 - 10 µl is tested at 10 µl and 1 µl.
- A series of ten pipettings is performed with both volumes.
- Calculate the accuracy and precision using the formulas below. If the calculated results are within the limits given in the Instructions-for-use booklet, the pipette calibration is correct. If not, the pipette has to be adjusted (see page 19) with the lower volume and checked again.

Manual multichannel pipettes

- The pipette is held in the calibration room for at least 2 hours before calibration to reach equilibrium with the test room conditions.
- The pipette is checked at the maximum volume (nominal volume) and at the minimum volume or 10% of the maximum volume, whichever is higher. For example, Finnpiquette 0.5 - 10 μl is tested at 10 μl and 1 μl .
- Both volumes are tested with the two end channels.
- A series of ten pipettings is performed with both volumes.
- Calculate the accuracy and precision using the formulas below. If the calculated results are within the limits given in the Instructions-for-use booklet, the pipette calibration is correct. If not, the pipette has to be adjusted with the lower volume (see below) and both end channels have to be checked again.

Finnpipette BioControl single channel module

- The pipette is held in the calibration room for at least 2 hours before calibration to reach equilibrium with the test room conditions.
- Use the Finnpiquette BioControl calibration software that comes with the pipette.
- The pipette is checked at the maximum volume (nominal volume) and at the lower calibration volume, which can be found from the Instructions-for-use booklet or from the Finnpiquette BioControl calibration software.
- A series of ten pipettings is performed with both volumes.
- Calculate the accuracy and precision of both series using the formulas below and compare to the limits given in the "Checking the calibration" chapter in the Instructions-for-use booklet. If the calculated results are within the selected limits, the pipette calibration is correct. If not, adjust the pipette as described below.

Finnpipette BioControl multichannel module

- The pipette is held in the calibration room for at least 2 hours before calibration to reach equilibrium with the test room conditions.
- The pipette is checked at the maximum volume (nominal volume) and at the lower calibration volume, which can be found from the Instructions-for-use booklet or from the Finnpiquette BioControl calibration software.
- Both volumes are tested with the two end channels.
- A series of ten pipettings is performed with both volumes.
- Calculate the accuracy and precision of both series using the formulas below and compare to the limits given in the "Checking the calibration" chapter in the Instructions-for-use booklet. If the calculated results are within the selected limits, the pipette calibration is correct. If not, adjust the pipette as described below.

Procedures to adjust the pipette:

Manual single channel pipettes

- The adjustment is done at the lower volume.
- Place the service tool that comes with the pipette into the openings of the

- calibration nut at the top of the handle.
- Turn the service tool clockwise to increase the volume or counter clockwise to decrease the volume.
- After the adjustment, check the calibration as described above.

Manual multichannel pipettes

- The adjustment is done at the lower volume with one of the middle channels.
- Place the service tool that comes with the pipette into the openings of the calibration nut at the top of the handle.
- Turn the service tool clockwise to increase the volume or counter clockwise to decrease the volume.
- After the adjustment, check the calibration as described above.

Finnpipette BioControl single channel module

- Adjustment is done in the calibration mode. To change the current calibration, do as follows:
 1. Start the calibration software that comes with the pipette.
 2. Choose the volume range.
 3. Enter the mean volume of the ten pipettings performed with both volumes (done in the calibration check) to the fields.
 4. Take the pipette and determine the values of the HK- and PK-factors: push down the MODE-button and keep it down and then push the + and - buttons as well. CALIBRATE text is now blinking, push SET to accept. The current HK-factor is now blinking followed by the PK-factor. Note: These factors have different values depending on the module, so please check that you choose the right module.
 5. Enter these factors to the OLD FACTORS fields of the calibration software.
 6. To get the new factors, click the CALCULATE button. The new factors will be displayed.
- Alternatively the Finnpipette Calibration Software may be used to determine the new HK- and PK-factors.
- Take the pipette again and change the HK- and PK-factors with + and - buttons, then accept the new values by pushing the SET button.
- After the adjustment, check the calibration as described above. When using the Finnpipette Calibration Software, continue according to the on-screen menus after entering the new values for HK- and PK-factors to the handle unit.

Finnpipette BioControl multichannel module

- Adjustment is done in the calibration mode. To change the current calibration, do as follows:
 1. Start the calibration software that comes with the pipette.
 2. Choose the volume range.
 3. Enter the mean volume of the ten pipettings performed with both volumes (done in the calibration check) to the fields.
 4. Take the pipette and determine the values of the HK- and PK-factors: push down the MODE-button and keep it down and then push the + and - buttons as well. CALIBRATE text is now blinking, push SET to accept. The current HK-factor is now blinking followed by the PK-factor. Note: These

factors have different values depending on the module, so please check that you choose the right module.

5. Enter these factors to the OLD FACTORS fields of the calibration software.
 6. To get the new factors, click the CALCULATE button. The new factors will be displayed.
- Alternatively the Finnpiquette Calibration Software may be used to determine the new HK- and PK-factors.
 - Take the pipette again and change the HK- and PK-factors with + and - buttons, then accept the new values by pushing the SET button.
 - After the adjustment, check the calibration as described above. When using the Finnpiquette Calibration Software, continue according to the on-screen menus after entering the new values for HK- and PK-factors to the handle unit.

Formulas for calculating results

Conversion of mass to volume

$$V = (w + e) \times Z$$

V = volume (µl)

w = weight (mg)

e = evaporation loss (mg)

Z = conversion factor for mg/µl

Note:

Evaporation loss can be significant with low volumes. To determine mass loss, dispense water into the weighing vessel, note the reading and begin timing with a stopwatch. Check how much the reading decreases during the 30 seconds. Compare this to the pipetting time. Typically, the pipetting time might be 10 seconds and the mass loss 2 mg. If an evaporation trap or lid on the vessel is used, an evaporation correction is unnecessary.

The conversion factor Z is for calculating the density of water suspended in air at the test temperature and pressure. See the conversion Table 1 on page 23.

Accuracy (systematic error)

Accuracy is the difference between the dispensed volume and the selected volume of a pipette.

$$A = \bar{V} - V_s$$

A = accuracy

\bar{V} = mean volume

V_s = selected volume

Accuracy is expressed on the calibration certificate as a relative value:

$$ACC\% = 100\% \times \frac{A}{V_s}$$

Precision (random error)

Precision refers to the repeatability of the pipettings. It is expressed as standard deviation (s) or coefficient of variation (cv). In addition to the features of the pipette, laboratory practice and user experience are the main factors affecting precision.

$$s = \sqrt{\frac{\sum_{i=1}^n (V_i - \bar{V})^2}{n - 1}}$$

s = standard deviation

\bar{V} = mean volume

n = number of measurements

V_i = single measurement result (i = 1...n)

CV (or CV%) is the relative value of standard deviation.

$$CV = 100\% \times \frac{s}{\bar{V}}$$

Error according to DIN 12650 (F-value)

The DIN standard does not give individual limits for accuracy and precision, but uses a combined error limit: the F-value.

$$F = |A| + 2 \times s$$

The relative F-value is calculated:

$$F\% = |A\%| + 2 \times cv$$

Table 2 shows the error limit according to the DIN 12650 standard for single-channel air displacement pipettes. For multichannel pipettes, these limits are doubled. With variable volume pipettes, the nominal volume is the maximum volume. The absolute μl limit of the nominal volume applies to every selected volume throughout the volume range.

For example, for a 20 - 200 μl pipette, the error limit is $\pm 2.0 \mu\text{l}$ for every selected volume. If the nominal volume of the pipette is between those in the table, the relative error limit F% of the nearest volume is used. If the nominal volume is exactly between the two volumes in Table 2, the relative error limit F% of the lower volume is used.

Conversion tables

Table 1:
Values of the conversion factor Z ($\mu\text{l mg}^{-1}$) as a function of temperature and air pressure, for distilled water.

Temperature °C	Air pressure hPA (mbar)					
	800	853	907	960	1013	1067
15	1.0018	1.0018	1.0019	1.0019	1.0020	1.0020
15.5	1.0018	1.0018	1.0019	1.0020	1.0020	1.0021
16	1.0019	1.0020	1.0020	1.0021	1.0021	1.0022
16.5	1.0020	1.0020	1.0021	1.0022	1.0022	1.0023
17	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024
18	1.0022	1.0023	1.0024	1.0024	1.0025	1.0025
18.5	1.0023	1.0024	1.0025	1.0025	1.0026	1.0026
19	1.0024	1.0025	1.0025	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0028	1.0028
20	1.0026	1.0027	1.0027	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0030	1.0030
21	1.0028	1.0029	1.0030	1.0030	1.0031	1.0031
21.5	1.0030	1.0030	1.0031	1.0031	1.0032	1.0032
22	1.0031	1.0031	1.0032	1.0032	1.0033	1.0033
22.5	1.0032	1.0032	1.0033	1.0033	1.0034	1.0035
23	1.0033	1.0033	1.0034	1.0035	1.0035	1.0036
23.5	1.0034	1.0035	1.0035	1.0036	1.0036	1.0037
24	1.0035	1.0036	1.0036	1.0037	1.0038	1.0038
24.5	1.0037	1.0037	1.0038	1.0038	1.0039	1.0039
25	1.0038	1.0038	1.0039	1.0039	1.0040	1.0041
25.5	1.0039	1.0040	1.0040	1.0041	1.0041	1.0042
26	1.0040	1.0041	1.0042	1.0042	1.0043	1.0043
26.5	1.0042	1.0042	1.0043	1.0043	1.0044	1.0045
27	1.0043	1.0044	1.0044	1.0045	1.0045	1.0046
27.5	1.0044	1.0045	1.0046	1.0046	1.0047	1.0047
28	1.0046	1.0046	1.0047	1.0048	1.0048	1.0049
28.5	1.0047	1.0048	1.0048	1.0049	1.0050	1.0050
29	1.0049	1.0049	1.0050	1.0050	1.0051	1.0052
29.5	1.0050	1.0051	1.0051	1.0052	1.0052	1.0053
30	1.0052	1.0052	1.0053	1.0053	1.0054	1.0055

Table 2:
DIN 12650 error limits for single channel air displacement pipettes

Nominal volume	Maximum error F	Relative error F%
1 μl	$\pm 0.15 \mu\text{l}$	$\pm 15.0\%$
2 μl	$\pm 0.20 \mu\text{l}$	$\pm 10.0\%$
5 μl	$\pm 0.30 \mu\text{l}$	$\pm 6.0\%$
10 μl	$\pm 0.30 \mu\text{l}$	$\pm 3.0\%$
20 μl	$\pm 0.40 \mu\text{l}$	$\pm 2.0\%$
50 μl	$\pm 0.80 \mu\text{l}$	$\pm 1.6\%$
100 μl	$\pm 1.50 \mu\text{l}$	$\pm 1.5\%$
200 μl	$\pm 2.00 \mu\text{l}$	$\pm 1.0\%$
500 μl	$\pm 5.00 \mu\text{l}$	$\pm 1.0\%$
1000 μl	$\pm 10.00 \mu\text{l}$	$\pm 1.0\%$
2000 μl	$\pm 20.00 \mu\text{l}$	$\pm 1.0\%$
5000 μl	$\pm 50.00 \mu\text{l}$	$\pm 1.0\%$
10000 μl	$\pm 100.00 \mu\text{l}$	$\pm 1.0\%$

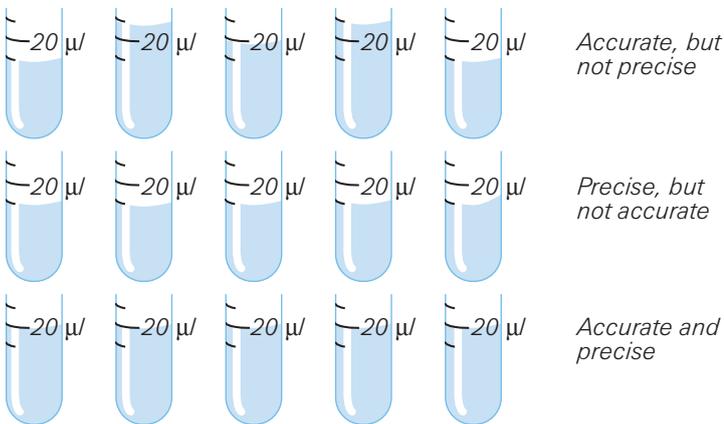
These limits apply to manufacturers with a controlled environment. If the tests are performed by a user in a normal laboratory environment, the limits in the table may be doubled.

Ensuring optimum performance

Error-free pipetting requires both precision and accuracy. A number of factors can affect these specifications, which are the main quantitative parameters for evaluating pipette performance.

What are accuracy and precision?

For example when the set volume is 20 μl :

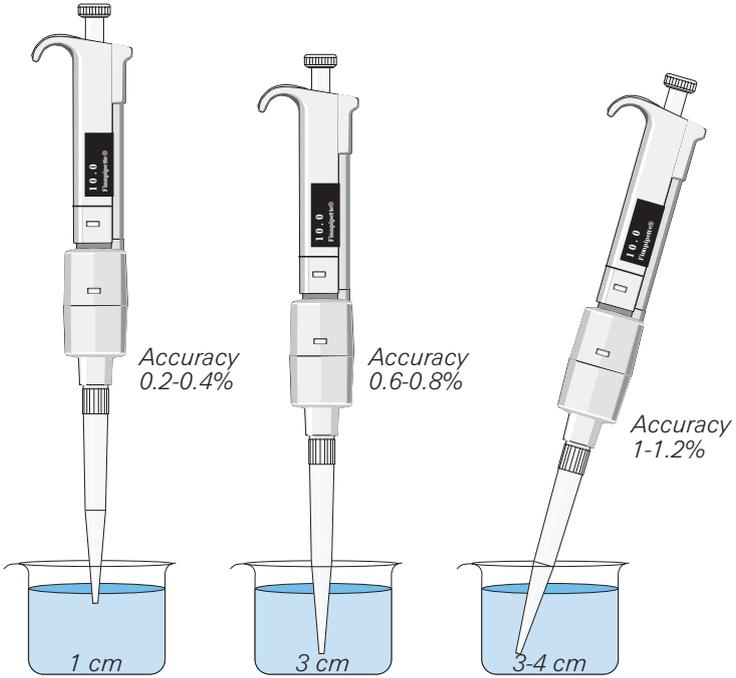


Accurate, but not precise: The mean volume is the correct (set) volume, but separate pipettings differ from the set volume.

Precise, but not accurate: There is no variation between the separate pipettings, but the mean volume differs from the set volume.

Accurate and precise: The mean volume is the set volume and there is no variation between different pipettings.

The effect of the pipetting position (e.g. using a 2-10 ml pipette)



1. Pipette vertical, tip immersed about 1 cm into the liquid.
2. Pipette vertical, tip immersed about 3 cm into the liquid.
3. Pipette at a 30 - 40° angle; tip immersed about 3-4 cm into the liquid.

Factors affecting the accuracy of air displacement pipettes

Temperature

The most important factor in pipetting accuracy is the liquid temperature. The figure below shows the change in volume when the liquid has a different temperature than the pipette and air. If the temperature of the liquid, pipette and air is the same, the accuracy is not significantly affected.

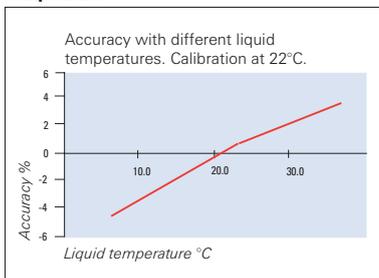
Density

Density is the mass/volume ratio of the liquid. The density varies according to the temperature and air pressure. Typically, the density of water is 0.996 kg/dm³, for ethanol 0.79 kg/dm³ and for sulfuric acid (H₂SO₄) 1.85 kg/dm³.

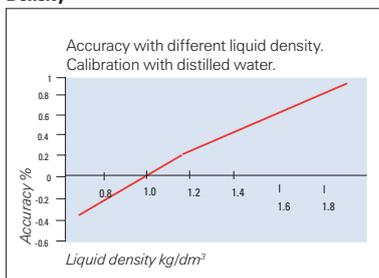
Altitude

The geographic altitude affects the accuracy through the air pressure. The air pressure decreases at higher altitudes and the conversion factor Z decreases as well. The boiling point of some liquids can also change to quite close to room temperature, increasing the evaporation loss dramatically.

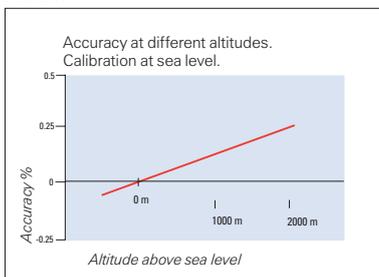
Temperature



Density



Altitude



Maintenance of your Finnpiquette

Finnpipettes are easy to service in the laboratory using the tools provided with the pipette. All Finnpipettes come with detailed instructions on how to disassemble the pipette. Instructions for routine in-lab maintenance are also included.

Short-term checking:

At the beginning of each workday, the pipette should be checked for dust and dirt on the outside surfaces. Particular attention should be paid to the tip cone. No other solvents except 70% ethyl alcohol should be used to clean the pipette.

Long-term maintenance:

If the pipette is used daily, it is recommended to check the calibration every three months.

General guidelines for cleaning the pipette:

1. Open the pipette with the maintenance tool.
2. Check for dust and dirt, and wipe clean.
3. Use only 70% ethyl alcohol to sterilise. The tip cone and tip ejector can be left in an alcohol bath overnight.
4. Grease the piston, O-ring and other cleaned parts with the silicon grease that comes with the pipette.
5. Assemble the pipette.

Note:

The calibration has to be checked after cleaning the pipette (see page 17 for the calibration check procedure).

Autoclaving

Please, follow these instructions carefully in order to avoid damage to tips and pipettes.

1. Autoclave tips at 121°C (248°F) for 20 minutes. Immediately after autoclaving, the tips are moist. Allow moisture to evaporate before using the tips, preferably overnight.
2. All Finnpiquette Digital models can be autoclaved in one piece at 121°C (248°F) for 20 minutes. The tip cone modules of the BioControl and the tip cone of single-channel Finnpiquette Colour models can also be autoclaved.
3. After autoclaving, the pipette must be cooled at room temperature for at least two hours before use.
4. Check the calibration (see page 17) of the pipette after every autoclave treatment.

UV resistance

Finnpiquette Digital models are UV resistant. The handle however might change colour from grey to light yellow. If the inner parts of the pipette are exposed to UV light, please check there is sufficient grease on the piston and O-rings.

General guidelines for decontaminating Pipettes when Working with Different Liquids

Liquid	Handling, Special features	Decontamination
Aqueous solutions and buffers	Pipettes are calibrated with distilled water. Results are extremely accurate.	Open pipette, rinse contaminated parts well with distilled water, and allow to dry at maximum 60°C in dryer compartment. Lubricate piston if necessary using the grease that comes with the pipette.
Inorganic acids	It is advisable to occasionally rinse the lower part of the pipette with distilled water if high-concentration acids are pipetted frequently. Using Filtertips is also recommended.	The plastics used in Thermo Labsystems pipettes are acid resistant. However, aerosols from the acids can enter the lower part of the pipette and affect the performance of the pipette. Clean as described above in "Aqueous solutions and buffers".
Alkalis	It is advisable to occasionally rinse the lower part of the pipette with distilled water if high-concentration alkalis are pipetted frequently. Using Filtertips is also recommended.	The plastics used in Thermo Labsystems pipettes are alkali-resistant. However, aerosols from the alkalis can enter the lower part of the pipette and affect the performance of the pipette. Clean as described above in "Aqueous solutions and buffers".
Potentially infectious liquids	To avoid contamination, Filtertips should be used. Alternatively, positive displacement systems can be used.	Use 70% ethyl alcohol to disinfect. The tip cone and tip ejector can be left in an ethyl alcohol bath overnight. Autoclave the Finnpiptette Digital in one piece at 121°C for 20 min. Viruses and spores can be inactivated by wiping the the pipette with a tissue moistened with 5% sodium hypochlorite. Moisten a clean tissue with distilled water and wipe the surface well.
Cell cultures	To guarantee sterility, Thermo Labsystems Filtertips should be used.	Proceed as described above with "potentially infectious liquids".
Organic solvents	1. Density is different than that of water. Therefore, it is necessary to adjust the pipette. 2. Pipetting should be carried out rapidly, due to the high vapor pressure and changes in the wetting pressure. 3. After pipetting is finished, open the pipette and allow the liquid to evaporate.	This evaporation process is normally sufficient for liquids with a high vapour pressure. Alternatively, immerse the contaminated parts in detergent. Rinse well with distilled water and dry as described above.
Radioactive solutions	To avoid contamination, Filtertips should be used. An alternative would be to use positive displacement systems.	Open pipette and place contaminated parts in complex solutions or special cleaning solutions. Rinse well with distilled water and dry as described above.
Proteins	To avoid contamination, Filtertips should be used. An alternative would be to use positive displacement systems.	Open pipette, rinse pipette with detergent. Rinse well with distilled water and dry as described above. Lightly lubricate piston.
Nucleic acids	To avoid contamination, Filtertips or positive displacement systems should be used.	If you have an autoclavable pipette, autoclave it according to the manufacturer's instructions. Otherwise open pipette, wipe with 90% ethanol, followed by 2M sodium acetate and again 90% ethanol.

Chemical compatibility of plastics

These are general guidelines, not performance guarantees. Factors such as concentration, temperature and length of exposure can affect performance.

	Finntip BioMate (nose cone)	Plungers (Stepper, Finntip PDP)	Tip cones (BioControl, Finnpipette, Digital MCP SCP)	Tip cones (Finnpipette MCP Classic, MCP Colour)	BioMate (adaptor)
Chemical Class	Polypropylene (PP)	Polyethylene (HD-PE)	Polyvinylidene fluoride (PVDF)	Polycarbonate (PC)	Silicone
Acid, mineral					
Boric acid	Good	Good	Good	Good	Good
Chlorosulphuric acid	Poor	Fair	Fair	-	-
Hydrogen chloride 20%	Good	Good	Good	Good	Fair
Hydrogen chloride 25%	Good	Good	Good	Poor	Fair
Hydrogen fluoride 25%	Good	Good	Good	Good	Poor
Nitric acid 70%	Fair	Good	Good	Poor	Poor
Perchloric acid	Fair	Good	Good	Poor	Poor
Phosphoric acid 1%	Good	Good	Good	Good	-
Phosphoric acid 10%	Good	Good	Good	Poor	-
Sulphuric acid 50%	Good	Good	Good	Good	Poor
Sulphuric acid 98%	Poor	Good	Good	Poor	Poor
Acid, organic					
Acetic anhydride	Fair	Good	Poor	Poor	Poor
Formic acid concentrate	Good	Good	Good	Poor	Fair
Lactic acid	Good	Good	Good	-	Good
Maleic acid	Good	Good	Good	Good	-
Palmitic acid	Good	Good	Good	Good	-
Salicylic acid	Good	Good	Good	Good	-
Tannic acid	Good	Good	Good	Good	Fair
Alcohol					
Allyl alcohol	Good	Good	Good	Poor	-
Amyl alcohol	Good	Good	Good	Poor	Poor
Ethanol	Good	Good	Good	Fair	Fair
Ethylene glycol 60%	Good	Good	Good	Good	Good
Ethylene glycol 100%	Good	Good	Good	Poor	Good
Furfuryl alcohol	Good	Good	Good	Poor	Good
Glycerol	Good	Good	Good	Good	-
Isobutanol	Good	Good	Good	Poor	Good
Methanol	Good	Good	Good	Good	-
Aldehyde					
Acetaldehyde	Good	Good	Good	Poor	Fair
Formaldehyde 37%	Good	Good	Good	Good	Fair
Aliphatic hydrocarbon					
Heptane	Fair	-	Good	-	-
Hexane	Fair	Poor	Good	-	-
Amide					
Dimethylformamide	Good	Good	Poor	Poor	-
Amine					
Aniline	Good	Good	Good	Poor	Fair
Pyridine	Good	Good	Good	Poor	Poor
Triethanolamine	Good	Good	Good	Good	-
Aromatic hydrocarbon					
Benzene	Poor	Fair	Good	Good	-
Toluene	Poor	Fair	Good	Good	Poor
Base					
Aluminium hydroxide	Good	Good	Good	Good	-
Ammonia concentrate	Good	Good	Poor	Poor	-
Calcium hydroxide	Good	Good	Fair	Poor	-
Potassium hydroxide 10%	Good	Good	Poor	Poor	Fair
Sodium hydroxide 10%	Good	Good	Poor	Poor	Fair

These are general guidelines, not performance guarantees. Factors such as concentration, temperature and length of exposure can affect performance.

	Finntip BioMate (nose cone)	Plungers (Stepper, Finntip PDP)	Tip cones (BioControl, Finnpipette, Digital MCP, SCP)	Tip cones (Finnpipette MCP Classic, MCP Colour)	BioMate (adaptor)
Chemical Class	Polypropylene (PP)	Polyethylene (HD-PE)	Polyvinylidene fluoride (PVDF)	Polycarbonate (PC)	Silicone
Ester					
Dibutyl phthalate	Fair	Good	Good	Poor	-
Ether					
Diethyl ether	Fair	Good	Poor	Poor	-
Polyalkylene glycol	Good	Good	Good	Poor	-
Polyethylene glycol	Good	Good	Good	Good	-
Polyethylene sulfide	Good	Good	Good	Poor	-
Propylene oxide	Good	Good	Good	Poor	-
Halogenated hydrocarbon					
Bromochloromethane	Poor	Poor	Poor	Poor	Poor
Carbon tetrachloride	Fair	Poor	Good	Poor	Poor
2-Chloroethanol	Good	Good	Good	Poor	-
Chlorobenzene	Poor	Fair	Good	Poor	Poor
Chloroform	Fair	Fair	Good	Poor	Poor
Dichloroethane	Fair	Fair	Good	Poor	-
Heterocyclic compounds					
Tetrahydrofuran	Fair	-	Fair	-	-
Ketone					
Acetone	Fair	Good	Poor	Poor	Poor
2-Butanone	-	-	Poor	-	-
Methyl ethyl ketone	Good	Good	Poor	Poor	Poor
Phenol					
Phenol	Fair	Good	Good	Poor	Poor
Salt, inorganic					
Aluminium chloride	Good	Good	Good	Poor	Fair
Aluminium fluoride	Good	Good	Good	Poor	-
Ammonium carbonate	Good	Good	Good	Good	Fair
Barium chloride	Good	Good	Good	Good	Good
Calcium chloride	Good	Good	Good	Good	-
Calcium sulphate	Good	Good	Good	Good	-
Copper (II) chloride 5%	Good	Good	Good	Good	Good
Iron (II) chloride	Good	Good	Good	Poor	Fair
Iron (III) nitrate	Good	Good	Good	Poor	-
Iron (III) sulphate	Good	Good	Good	Good	Fair
Lithium bromide	Good	Good	Good	Good	-
Magnesium chloride	Good	Good	Good	Good	Good
Magnesium (I) nitrate	Good	Good	Good	Good	-
Mercury (II) chloride	Good	Good	Good	Poor	-
Nickel nitrate	Good	Good	Good	Good	Good
Potassium carbonate	Good	Good	Poor	Poor	-
Potassium chlorate	Good	Good	Poor	Good	Fair
Silver nitrate	Good	Good	Good	Good	Good
Sodium carbonate	Good	Good	Good	Good	Good
Sodium fluoride	Good	Good	Good	Good	-
Sodium hypochlorite 5%	Fair	Good	Fair	Good	Fair
Tin (II) chloride	Good	Good	Good	Good	Fair
Tin (IV) chloride	Good	Good	Good	Good	Fair
Zinc chloride	Good	Good	Good	Poor	-
Zinc sulphate	Good	Good	Good	Good	-
Miscellaneous					
Dimethyl sulfoxide	Good	-	Poor	-	-
Urea	Good	Good	Good	-	Fair
Good: resistant, no affect			Poor: not resistant, will result in severe degradation		
Fair: limited resistance, only for short exposure			-: no data available		

Frequently asked questions

Finnpipette Digital

Question:

How can I sterilize the pipette?

Answer:

Finnpipette Digital is fully autoclavable (121°C, 20 min). The pipette can also be exposed to UV radiation. The colour of the handle may turn yellowish after prolonged exposure.

Question:

Which chemicals can I pipette without damaging the pipette?

Answer:

You can find the information on the chemical resistance of different plastics on page 29 and 30 of this Application Notebook. However, factors such as concentration of the chemical, temperature and length of exposure can affect performance. Materials should be tested under actual conditions to determine suitability for specific applications.

Finnpipette BioControl

Question:

How many times I can pipette on a full battery without recharging it?

Answer:

It depends on the module you are using. With the 8-channel module 50 - 300 µl, you can pipette at least 500 times.

Question:

How can I sterilise the Finnpipette BioControl?

Answer:

The tip cone module can be sterilised by autoclaving at 121°C for 20 min. Frequent autoclaving can cause some discoloration. This will not affect the accuracy and precision of the pipette. Never autoclave the handle!

Question:

Does Finnpipette Biocontrol have a mixing option?

Answer:

There is no special mixing mode, but you can mix in the pipette mode simply by aspirating and dispensing several times without taking the pipette out of the liquid.

Question:

What are the HK- and PK-factors? And where can I find them?

Answer:

The HK- and PK-factors are values needed to adjust the Finnpipette BioControl. You can find the current HK- and PK-factors by pushing down the MODE-button

and then push the + and - buttons as well. CALIBRATE text is now blinking, push SET to accept. The current HK-factor is now blinking followed by the PK-factor. Note: These factors have different values depending on the module, so please check that you choose the right module.

Finntips

Question:

What kind of material Finntips are made off?

Answer:

They are made of contaminant free virgin polypropylene.

Question:

How can you sterilise tips?

Answer:

By autoclaving at 121°C for 20 min. Finntips are also available in presterilised racks. The Finntip Filter is not autoclavable, but is available in irradiated racks.

Question:

From where can I get a Certificate of Conformity for the Finntips I purchase?

Answer:

You can contact Thermo Labsystems at <http://www.labsystems.fi/contact.htm>.

Question:

What is the filter pore size of Finntip Filter tips?

Answer:

The average pore size is 18 - 35 µm. The pores however don't have a straight shape. All pores together form the filter matrix with an efficiency that is about 1/3 of the average pore size.

General liquid handling questions

Question:

What is the difference between air displacement and positive displacement pipettes?

Answer:

Both types of pipettes have a piston that moves in a cylinder, or capillary. In air displacement pipettes, a specified volume of air remains between the piston and the liquid. In positive displacement pipettes, the piston is in direct contact with the liquid. This keeps air from entering the tip, preventing contamination effectively. Air displacement pipettes are meant for general use with aqueous solutions. Positive displacement pipettes are used for high viscosity and volatile liquids.

Question:

How can I pipette viscous liquids?

Answer:

You can do so using an air displacement pipette with standard or wide orifice tip (reverse pipetting, slowly). An alternative to this is to use a positive displacement system.

Question:

How can I prevent liquid dropping out of the tip when pipetting volatile compounds?

Answer:

If you use air displacement pipettes, aspirate and dispense the liquid a few times keeping the tip in the liquid. By doing so, the air inside the pipette will be saturated with vapour of the volatile compound. It is recommend using positive displacement pipettes for highly volatile compounds, since the built-in piston tip is in direct contact with the liquid.

Question:

How accurately can I pipette warm or cold liquids.

Answer:

The pipettes are calibrated by weighing distilled or deionised water of 20 - 25°C. With warm liquids, you will get a smaller mass with a certain volume, with cold liquids, you will get a higher mass.

Question:

What is the difference between the old and the new calibration procedure used to calibrate Finnpiettes?

Answer:

Since 1.7.2000 Finnpiettes are checked at two volumes (maximum and minimum or 10% of the maximum, whichever is higher) instead of one. This new procedure is more accurate than the old one.

Making your lab work lighter

High quality lab work needs good ergonomics. Work goes smoother and does not cause too much stress. Nowadays a lot of attention is paid to the ergonomics of lab equipment and furniture, but it is also important to do things in the right way.

Here you find some examples of how to improve your way of working (with the kind permission of the Centre for Occupational Safety, Finland).

- **Pipetting while standing:**

Lower the bench level when using graduated pipettes or bulb pipettes.



- **Pipetting while sitting:**

Put the elbow on the bench (or on a separate support) Keep the wrist in its natural position.



- **Stirring:**

Put the mixing device close to you and put the elbow or the arm on the bench.



- **Working at the microscope:**

Put your elbows on a special support and keep your wrist in its natural position.



- **Working at the laminar flow cabinet or chemical hood:**

There should be free space for the legs. Sit as close as possible to the hood.



- **Using the computer:**

Put the arms on the table or on the chair's armrests. Put the screen 15 - 20 cm below eye level. Use the mouse with both hands.

Make repetitive work lighter:

- * Use both hands: use your left hand (if you are right handed) when using a vortex mixer, an electronic pipetting aid or the computer mouse.
- * Avoid unnecessary squeezing of lab tools (e.g. pipettes).
- * Choose lighter or electronic pipettes and dispensers.
- * Use tubes that can be opened and closed easily.

Finnpipette Warranty Policy

Warranty policy

- 3-year warranty for all manual pipettes
- 1-year warranty for the electronic pipette
- the warranty only covers defects in material and workmanship

Warranty service policy

Thermo Labsystems provides the spare parts free of charge

- Thermo Labsystems does not cover any warranty labour costs

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