The use and maintenance of liquid-handling devices

With the increased emphasis now placed on work-based learning, registration portfolios and the need for trainees and support workers to receive a solid grounding in basic laboratory techniques, *The Biomedical Scientist* once again goes back to basics. In this second in a series of articles, Peter Riddle considers the use and maintenance of liquid-handling devices.

Precise and accurate delivery of samples and reagents is fundamental to the sensitive techniques employed in today's laboratories, particularly with the use of such small volumes and the increase in molecular biology methods. The introduction of automatic pipetting devices has revolutionised liquid-handling procedures in the laboratory and offers many benefits (eg ease of use, speed of action, improved reproducibility and safety) over the more traditional methods using glass pipettes.

There is a considerable variety of pipettes on the market, ranging from the fixed-volume, single-channel, manually operated pipette to the very latest range of electronic multichannel instruments with fixed- or variable-volume settings. However, such pipettes fall into two basic groups (air displacement and positive displacement), each one utilising a different principle of operation.

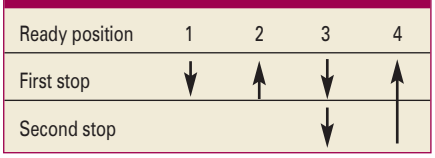
AIR-DISPLACEMENT PIPETTES

Air-displacement pipetting is used for standard applications. This type of pipette relies on a piston to create the suction necessary to draw the sample into a disposable tip. A wide range of volumes can be achieved and single or multistep options are available. When using the adjustable type, the volume required is achieved by turning the adjusting knob and the delivery volume is displayed on a digital readout on the handle.

Three techniques are in common use and there is also a method for whole blood. Following attachment of a disposable tip to the pipette and selection of the desired volume, the correct pipetting sequence consists of three actions: aspirate to draw up the sample, dispense to deliver the sample, and blow out to empty the tip.

There is a considerable variety of pipettes on the market, but each falls into one of two basic groups, air displacement or positive displacement.

FORWARD TECHNIQUE

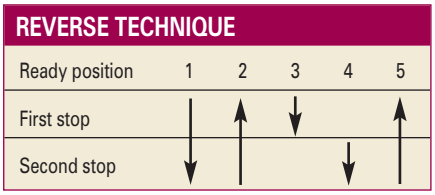


Forward technique

This technique is used for pipetting aqueous solutions:

1. Fill and empty the tip 2–3 times with the liquid and press the plunger to the first stop.
2. Immerse the tip in the liquid to about 5 mm, ensuring that the pipette is held vertically, and release the plunger slowly. This creates a partial vacuum and aspirates the specified volume.
3. Touch the tip against the side of the receiving vessel and dispense the liquid by pressing the plunger to the first stop. Then depress the plunger to the second stop to empty the tip.
4. Release the plunger to the 'ready' position.

REVERSE TECHNIQUE



Reverse technique

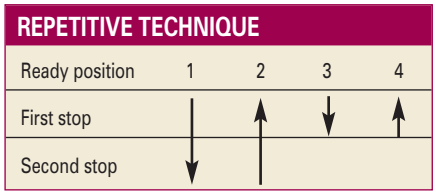
This technique is considered to be the best method for dispensing liquids with a high viscosity, those with a high vapour pressure,

Table 1. 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 20/40/60/8	Air displacement Positive displacement	Standard or wide orifice Positive displacement	Reverse	Pipette slowly to avoid bubble formation
Volatile compounds	Methanol, hexane	Air displacement Positive displacement	Filter Positive displacement	Reverse	Pipette rapidly to avoid evaporation. Carbon filter tips prevent vapour going into the pipette
Body fluids	Whole blood, serum	Air displacement	Standard or wide orifice tip	Pipetting of heterogeneous samples	Residual liquid can be found on the outer surface of the tip. Wipe the tip against the edge of the vessel to remove this liquid before dispensing
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	
Acids/alkalis	H_2SO_4, HCl, NaOH	Air displacement	Filter	Forward	
Toxic samples		Air displacement Positive displacement	Filter Positive displacement	Forward or reverse	

wetting agents, and for dispensing very small amounts:

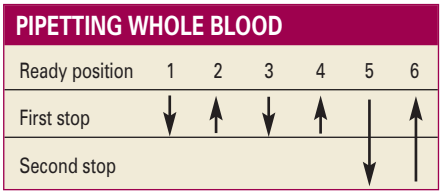
1 Fill and empty the tip 2–3 times with the liquid and press the plunger to the second stop.
2 Immerse the tip in the liquid to about 1 cm and release the plunger slowly. This action will fill the tip.
3 Touch the tip against the side of the receiving vessel and dispense the liquid by pressing the plunger to the first stop. Hold the plunger in this position. Some liquid will remain in the tip, and this should not be dispensed.
4/5 The liquid remaining in the tip should be thrown away with the tip.



Repetitive technique

This technique offers a rapid and simple procedure for repeated delivery of the same sample:

1 Fill and empty the tip 2–3 times with the liquid and press the plunger to the second stop.
2 Immerse the tip in the liquid to about 1 cm and release the plunger slowly. This action will fill the tip.
3 Touch the tip against the side of the receiving vessel and dispense the liquid by pressing the plunger to the first stop. Hold the plunger 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 steps 1 and 2 of the forward technique to fill the tip with blood and wipe carefully with a tissue.

1 Immerse the tip into the blood and press the plunger to the first stop. Make sure the tip is well below the surface.
2 Release the plunger slowly to the ready position. This action will fill the tip with blood. Do not remove the tip.
3/4 Press the plunger to the first stop and release slowly. Repeat this process until the interior wall of the tip is clear.
5 Press the plunger to the second stop and completely empty the tip.
6 Release the plunger to the 'ready' position.

Always hold the pipette in an upright position while aspirating. The accuracy of delivery can decrease three-fold if the pipette is held at an angle.

'There is now a greater need for full documentation of the procedures followed and the management of the data collected during measurement'

A pipette is now available in which all the functions of the pipetting cycle, including tip ejection, are triggered using a single button. With this system it is possible to discard the tip directly following blow out, without need for the piston to be reset, as is the case with the two-button pipette. This prevents the formation of aerosols, which could contaminate the pipette when performing tests on hazardous biological samples.

POSITIVE DISPLACEMENT PIPETTES

These pipettes are used for applications that require extreme accuracy. Liquid is delivered by means of a Teflon-tipped plunger that fits snugly inside the capillary, which can be glass or plastic. As the plunger tip and sample are in contact, such pipettes produce a high level of accuracy and reproducibility, and show practically no carry over of sample, allowing the capillary to be reused. Sample recovery is at least 90%, with reproducibility errors of 0.6% to 0.3% for volumes between 10 µL and 500 µL. Positive displacement pipettes are used in the same manner as forward mode air-displacement devices.

ENSURING OPTIMUM PERFORMANCE

The international standard ISO EN8655 defines terms for laboratory work involving dispensing devices, including pipettes. This standard unifies the wide range of standards and recommendations given for pipettes. The standard lists the methods for validating pipettes and these include: daily functional tests; volumetric tests; a monthly quick test to check the accuracy and precision of the pipette; and calibration tests that are performed every three months.

A pipette is accurate to the degree that the volume delivered is equal to the specified volume. Accuracy is expressed as the mean

and standard deviation (SD) for replicate measurements. Precision refers to the repeatability of the pipetting samples. It is expressed as the coefficient of variation (CV).

TESTING PROCEDURE

Reference should be made to BS EN ISO 8655-6:2002, which describes in detail a gravimetric procedure for testing piston pipettes.

The accuracy and precision of each pipette should be monitored throughout the year, and the frequency of this will depend on the amount of use or the requirements for accreditation. The manufacturer specifies tolerances for both parameters (technical specifications) and these can be used in evaluating the test results (NB: variable-volume pipettes should be tested at three or more points over their designated range; usually at the maximum [nominal] volume, at 50% of the maximum volume, and at the lower limit of their range).

The primary method for validating performance is a gravimetric technique:

- As a rule, the tolerances only apply to normal pipette operation (ie not to reverse pipetting) with deionised water as the test liquid.
- The minimum required balance sensitivity depends on the volume measured.
- The accuracy and the serviceability of the balance are decisive factors in the quality of the pipette calibration.
- The humidity in the sampling chamber must be kept at 60–90% during measurement to prevent the test liquid from evaporating; use of an evaporation trap is an economical way to ensure that the required humidity level is maintained.
- Most analytical balances have a rectangular draft shield, which is unsuited to pipetting, and it is best to have the balance equipped with a modified draft shield which will allow easy access to the sampling chamber, thus avoiding the need to open and close the draft shield; some balances are equipped with a motorised draft shield.

1 Make sure all items used (ie water, weighing vials, pipettes) are at room temperature.

Table 2. Water density.

°C	Density
20	0.9982
21	0.9980
22	0.9978
23	0.9976
24	0.9973
25	0.9971
26	0.9968
27	0.9965
28	0.9963
29	0.9960
30	0.9957

The introduction of multi-channel and automatic pipetting devices has revolutionised liquid-handling procedures in the laboratory.

2 Record the weight of the vial to the nearest 0.1 mg; record the temperature of the water.
3 Take up the distilled water with the pipette; wipe the outside of the tip.
4 Dispense the water into the weighed vial with the tip touching the side of the vial.
5 Record the weight of the vial plus the water to the nearest 0.1 mg.
6 Subtract the weight of the vial from the weight of the vial plus the water, and record the result; this is the weight of the first delivered sample.
7 Repeat steps 1 to 6 at least nine times, changing the disposable tip each time.
8 Obtain the average of the weights of the water.
9 Calculate the accuracy of the pipette using the formula:

$$\text{\% deviation from expected volume} = \frac{\text{expected volume} - \text{delivered volume}}{\text{expected volume}}$$

The delivered volume is calculated by dividing the mean (x) or average weight of water by its specific gravity at the measured temperature. Table 2 lists the specific gravity of water at probable laboratory temperatures.

The manufacturer usually provides acceptable limits for a particular pipette, but the value should not differ by more than 1.5% from the expected value. Precision can be indicated as the percentage coefficient of variation (%CV) or SD for a series of repetitive pipetting steps. The equations to calculate SD and %CV are:

$$SD = \sqrt{\frac{\Sigma(x-\bar{x})2}{n-1}}$$

$$\%CV = \frac{SD}{\bar{x}} \times 100$$

where x = sample weight, $\bar{x}$ = average or mean weight, Σ = sum, and n = number of weighings or pipettings. Required repeatability is usually ±1SD. The %CV will vary with the expected volume of the pipette, but the smaller the %CV value the greater the precision.

ISO 8655-7 recognises the use of single-dye photometry for liquid-handling device calibration. However, according to this standard, this analysis may include error contributions such as accuracy of the photometer and reagents, dye instability, and deviation from ideal Beer's Law behaviour.

EVALUATION SOFTWARE

There is now a greater need for full documentation of the procedures followed and the management of the data collected during measurement. These tasks are made easier by using software designed for testing and evaluating pipettes.

The system can be connected directly with the balance and the database can be programmed with all the relevant information on the pipettes used in the laboratory, including serial number, manufacturer, nominal volume, specified tolerances and the testing interval for each pipette.

On completion of the measurements, the program calculates the mean volume, accuracy and precision of the pipetted volume. The cost of setting up this system can be a limitation but it becomes more economical if shared between several departments.

Alternatively, a new online pipette validation tool is now available from a number of companies. All you need to do is enter the gravimetric readings into the online program, which will then perform a series of calculations and compare the data with the manufacturer's range expected for the pipette. A detailed report is generated to show the pipette's performance and whether or not it is within specification.

PIPETTE ADJUSTMENT

Adjustment allows any inaccuracy to be corrected if it is too high. Adjustments can also be carried out to adapt a pipette for regularly dispensing liquids of greater density. The manipulations for adjusting a pipette differ significantly between individual brands, and therefore no further details are given here. ■

Other topics covered by Peter Riddle (prriddle@hotmail.co.uk) in this series of updates include centrifugation (February, page 76), with articles on pH meters and balances scheduled to follow in subsequent issues.