Good Pipetting Technique - Simple Ways to Minimise Errors

As with many forms of instrumentation, a pipette will perform only as well as the operator's technique allows. Even with a high quality, well calibrated pipette using the finest precision tips, variations in user operation can alter delivery volumes and introduce inaccuracies. As many complex laboratory methods rely on basic pipetting in the initial set up stages it is vital to get this critical step right from the beginning.

Good technique increases accuracy by 0.1% to 5% Inaccuracy in results because of poor pipetting technique can be as high as 5%, enough to compromise your experimental results. However, by following some basic procedures that easily become routine practice, you can achieve consistent pipetting results all day every day.

> ***"...a small mistake in pipetting can cause a large error in the final result. It is, therefore, of great importance to evaluate and to reduce, wherever possible, both random and systematic errors in liquid sample handling." Sari Ylätupa, PhD, "Choosing a Pipetting Technique Affects the Results of Your Analysis", European Clinical Laboratory [1]***

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PIPETTE AND VOLUME SETTING

Start by choosing the optimal pipette for your dispensing volume. Work in the 35% to 100% volume range, for best accuracy and precision. Make sure you are using a well fitting tip designed for that pipette. When setting the micrometer turn it one third revolution above desired volume and then dial-down to required volume setting.

PRE-RINSING

When using a fresh pipette tip for the first time it is important to pre-rinse the tip at least twice before pipetting. This pre-rinsing provides identical contact surfaces for all aliquots. It also helps to neutralise capillary effects in micro-volume pipettes and – important for macro-volume pipettes – it equalises the air temperature inside the pipette to the sample temperature. This is especially important when pipetting hot or cold liquids. *Figure 1* shows how pre-rinsing twice achieves maximum accuracy, most critical for lowest volumes.

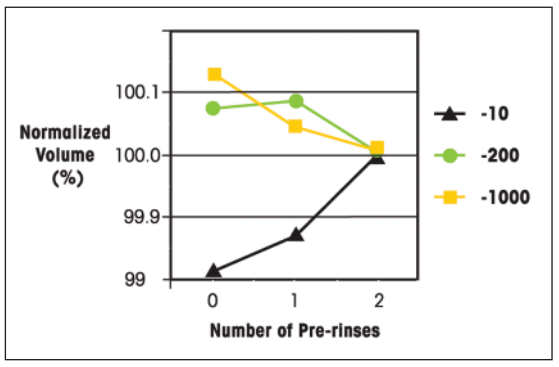

Figure 1. Prerinse graph

Figure 2. Correct Angle

TIP IMMERSION ANGLE

Keep the immersion angle as close as possible to the vertical position (*Figure 2*) otherwise the vertical liquid column is smaller and too much sample will be aspirated (*Figure 3*). When dispensing, the tip should be at a slight angle to the vessel wall in order to ensure good sample release. For micro-volume pipettes, the accuracy can be improved by up to 2.5% by using a close to vertical or within 20° of vertical position.

Figure 3. Incorrect Angle

DEPTH

Tip immersion depth can also have a significant effect on your results. Correct tip immersion depth is especially important for micro-volume pipettes. If the tip is immersed too far, more liquid will be aspirated due to increased pressure. Liquid retained on the tip surface can distort results.

If the tip is not immersed far enough, air can be drawn in, resulting in air bubbles and inaccurate volume. Correct tip immersion depth can improve accuracy by up to 5%. (*Figure 4*)

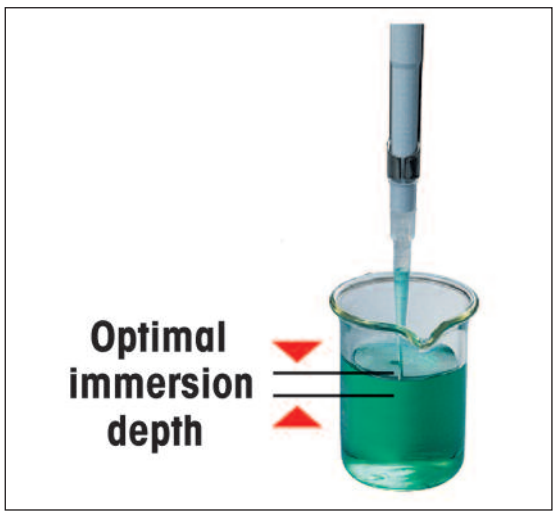

Figure 4. Optimal immersion depth

It is also advisable to hold the tip in the sample for one second (longer for large volumes) after aspiration and withdraw tip slowly and smoothly.

Table 1. Immersion Depth Guidelines

Pipette Volume	Immersion Depth
0.1-10µl	1-2mm
10-200µl	2-3mm
200-2000µl	3-6mm
>2000µl	6-10mm

CONSISTENCY

When depressing and releasing the plunger, maintaining a consistent pipetting rhythm, speed and technique is critical. Excessively fast and uncontrolled aspiration can lead to splashing, aerosols, contamination of shaft and piston, and even volume loss of the sample. Consistent pipetting speed can result in up to 5% better accuracy.

DISPENSING TECHNIQUE

For most applications, it's recommended to dispense with the end of the tip resting against the vessel wall (*Figure 5*). This reduces or eliminates sample remaining in the tip after dispensing. Remove the pipette by sliding the tip end up the side-wall in order to release any remaining droplet at the tip orifice. This technique can result in up to 1% better accuracy.
When dispensing your sample avoid aspirating too rapidly, use consistent pipetting speed, rhythm and plunger pressure.

Another technique is to dispense directly onto the liquid surface. The use of a thin-wall tip, for example, a RAININ FinePoint tip, is critical as these tips allow complete droplet release. If dispensing directly into the liquid, reverse mode pipetting is recommended in order to prevent sample pick-up after dispensing.

FREE P HAND-WARMING EFFECTS

When pipetting for extended periods, your hand can heat up the air inside the pipette (the air volume expands, leading to inaccurate results). Use pipettes made from high-grade PVDF polymers that provide better insulation against heat build up. Do not

Figure 5. Dispensing against vessel wall

continually hold the pipette in your hand between pipetting cycles. After pipetting, always put the pipette back on its stand.

ERGONOMIC FACTORS

To get the best results your hands must be free from fatigue. Many pipettes can become tiring to use for a prolonged time making good technique difficult to maintain. Using an ergonomic pipetting system such as the RAININ hand-friendly pipettes with LTS, eliminates these issues so that accuracy and precision is upheld.

Pipetting Don'ts

- DON'T immerse anything but the tip in your sample;
- Once filled, DON'T lay the pipette down or hold more than 20° from vertical;
- DON'T twist the volume higher than the maximum for the pipette;
- If the pipette is dropped DON'T assume it is operating correctly afterwards – check it; and
- DON'T turn the volume without unlocking.

Regular Maintenance will keep your Pipette Performing well

- Check and clean the shaft / seal and piston on a regular basis;
- Clean pipette if aerosol or splashing occurs without use of a filter tip; and
- Always fully dry the components before reassembly.

For further tips and advice on pipetting techniques, accuracy and precision please visit www.anachem.co.uk where you can also download our FREE Pipetting Techniques Poster – or contact response@anachem.co.uk to request your copy by mail.

REFERENCE

[1] Ylätupa Dr S. 'Choosing a Pipetting Technique Affects the Results of Your Analysis', European Clinical Laboratory 1996. 10:14.

Single System Provides Pure and Ultrapure Water Conveniently and Economically

Millipore Corporation has introduced the Milli-Q Direct system, the latest addition to the company's range of Milli-Q lab water purification systems.

The new system was designed to respond to the scientific community's need for an economical, single-source solution producing pure and ultrapure water from tap. The Milli-Q Direct system provides optimum water production, with water quality that exceeds the requirements of the most demanding norms.

Milli-Q Direct users are assured of having convenient and versatile water delivery: water can be dispensed either manually or automatically, at low or high flow rate. Additionally, a range of Application Pak final filters makes it possible to adapt the Milli-Q Direct system to the user's specific application(s) by adding a BioPak®,

VOC-Pak™, EDS-Pak®, LC-Pak™ or Millipak® polisher. The system's low footprint will also help lab managers save on space - the Milli-Q Direct can be bench- or wall-installed to ensure the best fit with their existing laboratory configurations.

Simplified procedures make maintenance for the system minimal, and an RFID tag ensures quick and easy traceability for consumables.

LAB PRODUCTS Circle no. 585

Autoclave Range Wins the Space Race

Astell have solved the eternal problem of how to double your laboratory's autoclave capacity without increasing the equipment footprint. Their new Duaclave range combines two identical chambers, one directly above the other within one frame.

The two chambers come equipped with all the features that Astell users have come to expect like delayed start, allowing sterilisation to take place overnight, Holdwarm capability to maintain the autoclave at a pre-set temperature after sterilisation until required and data archiving to ensure that all cycle details are automatically recorded and saved.

The two chambers are fully independent and each has its own Logi control system accessed via a full colour, icon-driven touch screen with password protected security. This means that, for example, while one chamber may be processing fluids, the other can either be sitting idle or perhaps completing a discard load.

The range includes both electrically heated and direct steam heated models, and options include load sensed process timing and assisted cooling.

As with all Astell autoclaves and sterilisers, the Duaclave range is CE marked and manufactured to comply with the Pressure Equipment Directive PD97/23/EC, the Medical Devices Directive MDD 93/42/EEC and all other applicable UK and international standards. Full IQ and OQ documentation is available for all models.

Astell Duaclave autoclaves are available in 33, 43, 63, 120 and 153 litre chamber sizes to suit most laboratory applications including sterilisation of reagents and media, glassware, laboratory instruments and discard.

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New Testing Products Guide Critical Patient Treatment

Oxoid announced that the Oxoid range of M.I.C. Evaluator™ (M.I.C.E.) Strips, for the accurate determination of minimum inhibitory concentration (MIC) values, has been expanded to include teicoplanin, meropenem, ceftriaxone and clindamycin. This range of antimicrobial susceptibility testing products has been designed for convenience and ease-of-use and provides important information to help guide the treatment of critically ill patients.

Oxoid M.I.C.E. Strips provide a gradient of stabilised antimicrobial, covering 15 doubling dilutions, on a convenient polymer strip format. Upon application, the antimicrobial is released from the strip, forming a defined concentration gradient in the surrounding agar. After an appropriate incubation, the MIC value is easily read off where the interface between the zone and growth of the organism touches the strip. M.I.C.E. Strips have been designed to make interpretation easy, through the emphasis on the reading scale of whole step dilutions, as stated in the standard methods.

Each strip is individually foil-wrapped with desiccant to maintain its integrity until use. For convenience, and to meet the needs of every laboratory, M.I.C.E. Strips are available in stackable boxes of 10 or 50. New antimicrobials are continually being added to the M.I.C.E. range.

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