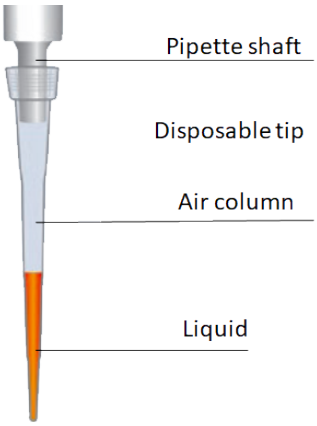
Liquid Handling of Micro to Low Milliliter Volumes

Introduction

Many applications in the lab require handling liquids in the micro- to low milliliter volume range. The validity of lab data and repeatability of results depends upon the accuracy and precision of this liquid handling. There are a variety of tools available that are capable of handling liquids in these volume ranges. How does one choose the correct instrument or tool for a specific application? To choose the correct tool, one must understand how the tool works and which application(s) it is best suited for. When performing low to medium throughput liquid handling work, a hand-held instrument is most often used. Two broad categories of these instruments are air displacement pipettes and positive displacement pipettes.

Air Displacement Pipettes

Air displacement pipettes are used in most labs and for a wide variety of applications. They are the 'workhorse' of the lab. As the name implies, a column of air does the actual work of moving the liquid with these instruments. The piston in the pipette moves an air column which allows liquid to be drawn into a disposable tip and then dispensed out. With an air displacement pipette, there should never be any direct contact between the liquid and the pipette or piston. If liquid is drawn into the pipette at any time, the pipette must be cleaned before further use to maintain proper performance and accuracy.



Because air displacement pipettes use a column of air to move the liquid, they are best-suited for liquids that have physical characteristics similar to water. When used with proper technique and a properly fitting tip, they provide very good accuracy. However, the further the physical characteristics of the pipetted liquid differ from water, such as its density, viscosity or vapor pressure, the less accurate the pipetted volume will be.

Air displacement pipettes can be either manually or electronically operated. Manual pipettes, such as the BRAND® Transferpette® S, are available as either single or multichannel pipettes. Single channel pipettes can be fixed volume or variably adjustable, and volumes can range from 0.1 µl up to 10 mL. Multichannel manual pipettes are most commonly available as 8- or 12-channel pipettes, although other sizes are available for specialized applications, and generally range in volumes from 0.5 µl to 300 µl.

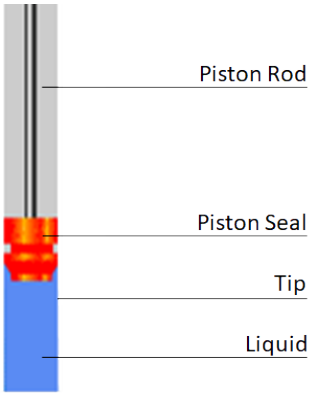
With manual air displacement pipettes, the piston movements are controlled by the user. The pipetting stroke has two stops. In standard or forward pipetting, the first stop is for aspirating and dispensing, and the second is for blow-out. Variability in the volumes dispensed with an air displacement pipette depends on several factors which include, but are not limited to, pre-wetting the tips, the depth and angle that the tip is immersed in the liquid, the aspiration and dispensing speed, as well as the wait time before blow-out.

Motorized, or electronic, air displacement pipettes are also available in both single and multichannel models, and in a wide range of volumes. The single channel Transferpette electronic, for example, offers volumes from 0.5 µl to 5 mL, while the multichannel, either 8- or 12-channel, ranges from 0.5 µl to 300 µl. Some manufacturers offer electronic multichannel pipettes in volumes up to 1250 µl.

Air Displacement Pipettes continued

An advantage of electronic air displacement pipettes is that user-to-user variability is minimized because piston movements are controlled electronically. An electronic pipette also reduces the amount of operating forces required during pipetting and significantly reduces the amount of repetitive motion required. It should be noted that while it is possible to dispense partial volumes, or multi-dispense, with an electronic air displacement pipette, a feature which most electronic models offer, multi-dispensing cannot be performed accurately with an air displacement pipette. For accurate multi-dispensing, a positive displacement pipette should be used. However, for labs that want to minimize user-to-user variability or have medium throughput pipetting needs, an electronic air displacement pipette is a very helpful tool.

Positive Displacement Pipettes



Positive displacement pipettes, in contrast to air displacement pipettes, have the liquid in direct contact with the piston of the instrument. This difference allows for greater accuracy with liquids that have a wider range of densities, viscosities and vapor pressures than can be pipetted accurately with an air displacement pipette. Liquids do not have to be similar to water in their physical characteristics to be pipetted accurately with a positive displacement pipette. However, because the liquid comes into contact with the piston, in order to minimize carryover or cross-contamination, the tips must be rinsed or changed between samples. The tips for a positive displacement pipette take more time and effort to change, but they can be reused. They are also more costly than the disposable tips used with air displacement pipettes and, with some positive displacement pipettes, the piston seal will wear over time and must also be replaced. Positive displacement pipettes also provide accurate dispensing of partial volumes. If accuracy is required when multi-dispensing partial volumes, a positive displacement pipette is the correct tool for the application.

The BRAND® Transferpettor™ is an example of a manual positive displacement pipette, and is available in volumes from 100 µl to 10 mL. It can be used for dispensing viscous or volatile liquids that cannot be accurately dispensed with an air displacement pipette. The tips and piston seals for the Transferpettor are reusable, and the piston seal is designed to push the liquid entirely out of the tip, minimizing carryover between samples. However, as noted previously, changing the tips and piston seals is more labor intensive than with air displacement pipettes. Thus, positive displacement tools are generally chosen only when needed for handling more challenging liquids.

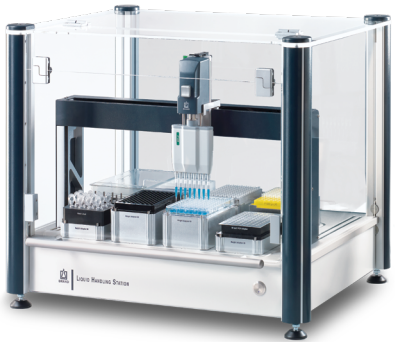
A specialized version of positive displacement pipette is the repeating or ‘stepper’ pipette. This is a category of positive displacement pipettes that is specifically designed to handle multi-dispensing of partial volumes accurately. Since they are a type of positive displacement pipette, the liquid comes into contact with the piston of the tip used with the pipette, providing accurate dispensing of a wide range of liquid types.

Manual repeating pipettes typically have a fixed number of ‘intervals’ that they can dispense – traditionally 50 - each representing 2% of the nominal volume of the tip. The user then would select between one to five of these intervals for each dispensing, or ‘step’, giving some flexibility in the dispensed volume. More advanced versions allow for the addition of ‘half intervals.’ For example, the HandyStep S has nine step settings (1 to 5 with ½ settings) which allows for dispensing of 59 different volumes ranging from 2 µl to 5 mL - depending on the size of the tip. Manual repeating pipettes are very simple, reliable tools that offer accuracy and convenience at an economical price point.

BRANDTECH®

Electronic repeating pipettes, such as the HandyStep® touch and touch S, offer much more versatility than a simple manual repeating pipette. They offer more flexibility in volume setting and more options in functionality. With an electronic repeating pipette, it is possible to perform auto-dispensing, sequential dispensing of different volumes, multi-aspirating of different volumes, save favorites, and even titration. Also, because the piston movements are electronically controlled, there is a wide range of speeds for both aspiration and dispensing, which is helpful for liquids that have a tendency to foam or bubble. And, of course, an electronic instrument reduces strain due to repetitive motion, which can be an important consideration when performing repeat dispensing for long periods of time.

Automated Liquid Handling



When a labs' throughput begins to increase, or for other considerations, an automated pipetting system may be the appropriate tool. Liquid handling automation differs from hand-held pipetting in that it uses a robotic system to move liquid from a source to a destination. Automated liquid handling systems include a wide range of instrumentation from robots that take up very little space to those that can take up a whole room. Some systems, like the BRAND Liquid Handling Station, use air displacement liquid ends which are suitable for many types of liquids. They have the ability to reproducibly compensate for liquids that would be a challenge with manually operated air displacement pipettes. However, it is necessary to avoid chemicals that may be damaging to the materials and components of the instrument. The volume range for pipetting robots can range from nanoliter to milliliter volumes; and sources and destinations can range from something as small as a PCR tube or 1536-well microplate all the way up to reservoirs holding a 240 mL capacity. Additionally, you will find a wide range of system structures. Some systems are enclosed, while others are completely open. Having an open system can be useful for certain applications, but offers no sample and user protection.

Traditionally, robotics in the lab have been reserved for highly repetitive and extremely high throughput, complex procedures. However, automated systems are now available in a wide range of size and capacity, making robotics accessible and sensible for even low to medium throughput procedures.

What are the benefits of using automation for liquid handling versus using manual or electronic hand-held pipettes, and when does it make sense to switch to automated liquid handling in your lab? There are several benefits to switching to automated liquid handling: Time, Repeatability, Safety, Optimization and Tracking.

Time: Installing liquid handling automation in your lab can offer tremendous benefits in time. A liquid handling robot will not necessarily perform a given task or assay quicker than a person, but it will free laboratory staff from having to do menial and complex liquid handling tasks, allowing them to focus their efforts on more productive lab work.

Repeatability: Using automation for liquid handling removes user variation. This benefits the lab by reducing repetition of experiments due to different technique among laboratory staff. Also, by having a predefined process, experimental failure due to procedural error will be limited. A liquid handling robot does not forget where it last pipetted in a plate.

Automated Liquid Handling continued

Safety: An automated liquid handling system can be beneficial for the safety of samples as well as staff. An enclosed pipetting robot offers samples protection from the environment. Additionally, the robot performs the physical work of repetitive pipetting reducing the risk from repetitive motion injury keeping staff at full productivity.

Optimization: With a liquid handling robot that allows for easy method set up, protocols can be optimized for time, reagent and consumable usage. Combined with the benefit of improving repeatability, this adds up to savings of time and money!

Tracking: Because a liquid handling robot is operated by some type of computer system, processes are easily tracked. Methods should be easy to create, edit and share. Samples can be tracked and the details of a method 'run' recorded.

As noted previously, automated liquid handling systems can range from those that take up very little space to ones that can take up a whole room. When first transitioning to automation, a smaller more affordable system is the appropriate choice. The LHS and the LHS Flow with integrated HEPA filtration, are benchtop systems offered by BRAND. Both units are extremely compact (~2 ft x 2 ft, L x W) and have eight positions (seven working) on the work table. They can be used with a variety of consumables such as PCR tubes or plates, 96 and 384-well microplates and reagent reservoirs. Three single channel and two multichannel liquid ends offer a volume range of 1 µL to 1000 µL. A variety of accessories and consumables are available to customize each system for the user's application: racks for the different labware, height adapters to bring the consumables to an even height for efficient pipetting, reagent reservoirs, robotic tips, and more. Heating can be controlled with an optional heater/shaker unit, and cooling of PCR tubes or plates is possible using a PCR cooler. With software that is especially user-friendly, method creation is accomplished with a graphical interface - no programming knowledge is needed. The LHS systems can be used for a wide variety of applications including PCR setup, serial dilutions, and more. They are a good example of benchtop liquid handling systems which can be used as a transition to automated liquid handling in the laboratory.

Summary

When selecting the correct tool or instrument for a specific application, there are key questions that can be asked:

- What is the liquid to be transferred?
- What is the volume to be transferred?
- What is the frequency of transfer?
- What other special needs or requirements does the lab have?

In summary, the manual air displacement pipette is the workhorse of the lab. This is the 'go-to' instrument for most people. With volume ranges from 0.1 µl to 10 mL it can handle a lot of tasks, and a lot of different liquid types. If user-to-user variability or repetitive strain are a concern, then an electronic air displacement pipette should be considered. When pipetting viscous or volatile liquids, or performing accurate repeat pipetting, a positive displacement pipette is the instrument of choice. Finally, if the laboratory has increasing throughput liquid handling needs, needs to improve repeatability or simply wants to optimize processes, it may be time to consider a switch to automation.