

Selection and Development of Bulk Liquid Dispensing Technology

by Jason Greene

A vast majority of research is dependent on the transfer of a measured volume of liquid from one vessel to another, such as from a bulk container to a microplate. Because this technique is often employed up to several times per hour, the product used to perform the task must be user friendly and comfortably suited to the application. General-purpose and specialized pipets and peristaltic pumps dominate the liquid handling market for virtually every volume range, viscosity, and container size, although each may exhibit distinct disadvantages for niche applications such as assay development, where bulk dispensing is required in microplate format.

Pipet dispensing

Pipets, especially multichannel pipets, accurately and precisely deliver a predetermined fluid volume to a microplate well, or a single column/row of wells in a microplate. Pipets are especially useful for dispensing liquid in milliliter, microliter, or smaller volume ranges since most are highly precise and accurate. Although this method has been the overwhelmingly popular liquid handling choice for several decades, inconsistent and poor pipetting techniques are common issues that result in assay performance variability, even from a single operator. These problems can be compounded by repetitive strain injuries such as carpal tunnel syndrome.

Aside from operator error, dry pipet tips must be preconditioned by repeatedly drawing up and dispensing a chosen volume of liquid to reduce surface tension within the tip walls, which is of concern if using expensive reagents. Viscous liquids with high surface tension can dramatically affect assay results because the true volume of liquid dispensed through the small pipet tip opening can be distorted. Additionally, since pipets contain moving parts, they must be regularly cleaned and calibrated to ensure optimal dispense performance without damage from aerosols or deposits created from the liquids used.

Finally, with advances in microplate technology and miniaturization, laboratories have transitioned from 96-well microplates to higher-density microplate geometries such as 384- and 1536-well formats. These higher densities can be a daunting task for manual pipetting, thus requiring other methods of liquid dispensing.

Peristaltic pump dispensing

Peristaltic pumps are commonly used for continuous or semicontinuous liquid dispensing, and since the liquid is entirely contained within flexible tubing, they are ideal for sterile applications without risk of cross-contamination. They are also well suited for use when conservation or recycling of reagents is desired because the liquid can be pumped back to the source reservoir. Peristaltic pumps incorporate rollers fixed to a rotating rotor which, when encircled by flexible tubing, compress the tubing at regular intervals between two consecutive rollers, forcing a volume of liquid to pass through the tube. This volume is defined as a slug. Volume adjustments are determined by the distance between the rollers, rotor position, tubing tension, inner diameter of the tubing, and tip geometry. This dispensing style is suited for automated systems with a high degree of precision and accuracy. Since there are no moving parts in contact with the liquid, these pumps are relatively inexpensive to maintain, requiring only periodic replacement of the tubing.

The disadvantage of many peristaltic pumps is that, by design, their volume ranges are often not suitable for micro-



Figure 1 MicroFlo Select Dispenser.

Table 1 Effects on precision and accuracy at several dispense volumes when using three cassette sizes in the MicroFlo Select Dispenser

Volume (μL)	10- μL cassette precision			10- μL cassette accuracy		
	Mean	Standard deviation	% CV	Expected	Actual	(% Error)
10	1.876	0.043	2.30%	0.96	0.9628	0.29%
15	0.472	0.021	4.38%	1.44	1.4421	0.15%
20	0.612	0.011	1.78%	1.92	1.9165	-0.18%
25	0.760	0.019	2.45%	2.4	2.3994	-0.02%
50	1.483	0.012	0.83%	4.8	4.7841	-0.33%
75	2.214	0.020	0.91%	7.2	7.1803	-0.27%
100	1.311	0.009	0.66%	9.6	9.5474	-0.55%
	5- μL cassette precision			5- μL cassette accuracy		
1	0.194	0.025	12.88%	0.096	0.0956	-0.42%
2	0.398	0.029	7.32%	0.192	0.1955	1.82%
3	0.593	0.029	4.83%	0.288	0.2905	0.87%
4	0.787	0.035	4.41%	0.384	0.386	0.52%
5	0.956	0.007	0.72%	0.48	0.4729	-1.48%
10	1.902	0.015	0.78%	0.96	0.955	-0.52%
15	0.485	0.004	0.73%	1.44	1.4407	0.05%
	1- μL cassette precision			1- μL cassette accuracy		
1	0.194	0.004	2.03%	0.096	0.0982	2.29%
2	0.374	0.006	1.68%	0.192	0.1892	-1.46%
3	0.570	0.013	2.34%	0.288	0.2915	1.22%
4	0.751	0.008	1.08%	0.384	0.3918	2.03%
5	0.934	0.007	0.74%	0.48	0.4902	2.13%
10	1.825	0.011	0.58%	0.96	0.9735	1.41%
15	0.512	0.004	0.83%	1.44	1.4526	0.87%

dispensing since it is increasingly difficult to effectively overcome cohesive forces of the liquid for accurate dispensing at very low volume levels.

Accuracy is also compromised if the pump dispenses volumes of liquid (slugs) in fractions rather than full volumes, because the variation between the fractions is significant when compared to full volumes. This is especially problematic at microvolumes because the liquid is no longer forced out of the tubing by the force of the roller, but rather the speed of the rotor itself. Cohesive forces in the liquid cause uneven distribution of the liquid. In addition, cassettes, which contain the tubing, are rated for a limited lifetime because the tubing will stretch to a certain degree due to prolonged contact with the rollers. This stretching can adversely affect dispense performance, or worse, rupture the tubing.

An improved dispensing method

In order to provide accurate bulk dispensing that is both precise throughout a range of volumes and easy to use with low maintenance, **BioTek Instruments, Inc.** (Winooski, VT) designed the MicroFlo™ Select Dispenser (Figure 1). The flexible design of the device allows for dispensing into microplates from 6- to 1536-well formats with a variety of plate heights, and also dispenses into microtubes and other

tubes up to 4 inches in height. Dispense height is automatically adjusted by the instrument's software, and the dispenser can be automated for high-throughput applications. Stable-performing, long-lasting peroxide-cured tubing in each of eight channels eliminates the cumbersome process of preconditioning, and the low dead volume reduces reagent waste. The MicroFlo Select uses a specialized peristaltic design for a wide volume range from 1 μL to 9999 μL , with precision and accuracy comparable to, or better than, multi-channel pipets.

Three selectable cassette sizes (1 μL , 5 μL , and 10 μL) each incorporate the appropriate dimension tubing to produce full volumes of liquid instead of fractions, and also use optimized tip geometries, thus reinforcing the precision and accuracy of each volume dispensed. These cassettes can be autoclaved without the expense and time associated with recalibration, and both the cassettes and cassette tubing may be easily and quickly replaced as needed.

Experimental protocol

In order to determine precision and accuracy ranges of the MicroFlo Select using each of the three cassette sizes, a standard blue dye (FD&C #1) was dispensed per the following protocols: A 10- μL cassette with 0.022-in.-i.d. molded polypropylene tips was inserted into the

MicroFlo Select, the variable dispense rate was set to the highest setting, and the dispense height was set to 15.362 mm. The concentrated dye solution was undiluted for the 10- μ L dispenses, diluted by a ratio of 1:5 with distilled water for dispenses between 15 μ L and 75 μ L, and diluted again by a ratio of 1:2 before the 100- μ L dispenses so that the absorbance readings were in the range of 0.5 and 2.5 o.d. The prepared dye solutions were then dispensed into individual 96-well microplates (**Corning Life Sciences**, Lowell, MA) in quantities ranging from 10 μ L to 100 μ L, and distilled water was added to each microplate well with a calibrated μ Fill™ microplate dispenser (**BioTek Instruments, Inc.**) for a total volume of 250 μ L per microplate well.

A 5- μ L cassette with 0.014-in.-i.d. molded polypropylene tips was inserted into the MicroFlo Select, the variable dispense rate was set to the lowest setting for dispenses between 1 μ L and 4 μ L, and the medium setting was used for the remaining dispenses. The concentrated dye was undiluted for dispenses between 1 μ L and 10 μ L, then diluted per the above ratios for dispenses between 15 μ L and 50 μ L, and 75 μ L and 100 μ L, respectively. The prepared dye solutions were then dispensed into microplates in quantities ranging from 1 μ L to 100 μ L, and distilled water was added to each microplate well as described above for a total volume of 250 μ L per microplate well.

A 1- μ L cassette with 0.010-in.-i.d. molded polypropylene tips was inserted into the MicroFlo Select, and the variable dispense rate was set to the highest setting. The concentrated dye was undiluted for dispenses between 1 μ L and 10 μ L, and diluted per the above ratio of 1:5 for dispenses between 15 μ L and 50 μ L. The prepared dye solutions were then dispensed into microplates in quantities ranging from 1 μ L to 50 μ L, and distilled water was added to each microplate well as described above for a total volume of 250 μ L per microplate well.

All microplates were read using a PowerWave™ microplate spectrophotometer (**BioTek Instruments, Inc.**) at dual wavelengths (450 and 630 nm) to reduce background noise.

Results

The data in *Table 1* emphasize two important points. First, there is wide variability inherent to fractional slugs, especially in low volumes. For example, using the 10- μ L cassette to dispense 15 μ L, or one full slug and one-half slug of liquid results in a high degree of variability due to the effects of cohesive forces, while using a 5- μ L cassette or 1- μ L cassette to dispense 15 μ L with multiple full slugs of liquid (two and ten slugs, respectively) results in a high degree of precision. Second, all precision and accuracy data in the table, when used with the appropriate cassette, are comparable to, or better than, those expected with use of a multichannel pipet.

Summary

When dispensing liquids in microplate or even tube format, pipets and peristaltic pumps each offer advantages and disadvantages in ease of use, precision and accuracy, and flexibility. The MicroFlo Select Dispenser is the first instrument of its kind to combine the advantages of both technologies, with a wide range of choices in dynamic dispense volumes, microplate types and styles, tube sizes, and cassette selection, in one compact and user-friendly unit to accommodate bulk dispensing requirements and optimal assay integrity.

Mr. Greene is Product Manager, **BioTek Instruments, Inc.**, Highland Park, P.O. Box 998, Winooski, VT 05404-0998, U.S.A.; tel.: 888-451-5171; fax: 802-655-7941; e-mail: GreeneJ@BioTek.com.