Refining Automated Liquid Dispensing

New Instrumentation Aims to Make Process More Accurate and Reproducible

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High-throughput screening (HTS) has allowed researchers to conduct large-scale biochemical assays for the rapid identification of active compounds, antibodies, or genes of interest. In order to ensure that potential drug candidates are accurately identified, with minimal off-target effects, high-throughput assays are incorporated as the starting point for drug design, as well as to provide a deeper understanding of various intra-cellular and cell-cell interactions.

With the ability to screen a wide range of static assays against a comprehensive library of candidate compounds, HTS provides a means of identifying and analyzing cellular events, including kinase activation/inhibition, up/down regulation of signal transduction pathways, and apoptosis. The assays that are incorporated into any one screening campaign are dependent on the therapeutic targets, which can include cancer, cardiovascular disease, and inflammation. However, for any assay, a precise and accurate method of liquid dispensing is essential to reduce the chances of incurring any potential error.

Compatible with 19 interchangeable pipetting heads, the Thermo Scientific Versette provides versatility, from single- to 384-channel automated pipetting.

The need for accurate dispensing of liquid volumes has ensured that multichannel liquid dispensers have become a mainstay in the majority of drug discovery laboratories. Such liquid transfer is often used in the dilution of concentrated stock solutions, which will subsequently be dispensed across the assay plate for analysis. However, there is the potential for error

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Selecting the Right Labels

Lab managers know all too well the wear and tear that instruments are subjected to in a laboratory environment. Exposure to chemicals, high and low temperatures, and other extreme conditions are routine in the lab, so it's crucial to not only select the appropriate instruments and labware, but also to consider a number of factors when choosing a labeling solution.

The use of permanent markers or laser-printer imaged paper labels is not ideal for many reasons, according to Eric Isberg, marketing manager for **Computype Laboratory** (www.computype.com).

"First of all, many solvents, or even water, can cause ink used on vials, tubes, plates, or slides to smear or bleed, leaving an unreadable mark," he explains. "Additionally, paper labels can be easily damaged, stained, or misshapen, and laser-printer imaged labels often

when creating any dilutions, as well as when dispensing compound solutions into the assay plate, which can propagate into false positive or negative results in the initial screen.

With additional uses in the pharmacological proofing of compounds, liquid-handling instrumentation has the capability to accurately perform repeated dispensing, mixing, and aspiration cycles in successive wells. The resulting drug titrations or dose-response curves can then be used to determine the potency of the drug candidates during the primary screen. Accuracy is, therefore, key in the creation of these dilution series to ensure that the most potent compounds are selected and moved forwards for further analysis, minimizing the time required for hit-to-lead and lead-to-market.

Due to the minute volumes typically handled during HTS, most commonly on the microliter scale, inaccuracies of just 1 µL can have an adverse effect on experimental integrity through the production of false positive or negative results. Although this is not detrimental to experimental data, false positives can significantly reduce laboratory efficiency.

Minimizing the number of false positives incurred will, therefore, aid in the reduction of unnecessary re-tests, while minimizing reagent loss. In addition, false negatives can lead to lost opportunities and the need for more regular and extensive re-testing to identify any potential lead compounds, which have been missed.

Versette Performance Data Using Multichannel Verification System

Volume (µL)	%CV	%Error
	1-channel	
2	5.3	5
3	1.1	3.8
30	0.7	1.5
	8-channel	
2	6.4	9.6
3	5	1.1
30	1.3	1.9
	12-channel	
2	2.6	5.3
3	1.8	4.1
30	0.6	1.2
	96-channel	
0.5	12.6	12.2
3	1.4	3.6
7	0.5	1.2
14	0.6	0.5
24	0.5	0.9
30	0.8	1

In order to ensure that dispensed volumes are highly accurate, we investigate the dispensing precision of microliter volumes using the Thermo Scientific Versette liquid-dispensing automation platform from **Thermo Fisher Scientific** (www.thermoscientific.com) (*Figure*). Performance was validated across a wide volume range using a single-, 8-, 12-, and 96-channel pipetting head to ensure accuracy and precision over the instrument's complete capability range.

Multichannel Verification

Liquid transfers were evaluated in-house using 10 previously determined optimization parameters, including air gap, blow out, aspiration and dispense speeds, overstroke, and tip height. These were subsequently evaluated using a multichannel verification technique—a dual-dye multichannel verification system that has been widely used in the automation field to calibrate liquid volumes from a variety of automation platforms. This system offers a systematic validation of the liquid-handling instrument and includes dye solutions (sample and diluent), a calibration plate, an orbital plate mixer, as well as spectrophotometric readout and data-analysis software.

The single-, 8-, and 12-channel heads were used to dispense three different volumes: 2, 3, and 30 µL, to evaluate accuracy over a wide range. In addition, the 96-channel head was used to dispense five volumes, from 0.5 to 30 µL, in order to determine that this new liquid handler can dispense liquids in a precise and accurate manner. The assessment of these parameters is critical to generate reliable data when using the instrument for automated applications.

The accuracy of the Versette was initially confirmed via the following process:

- Dye was dispensed at the selected volume into a 96-well plate.
- The plate was shaken at 1,200 rpm for one minute and centrifuged at 1,700 rpm for one minute.
- Spectrophotometric absorbance was measured.

The coefficient of variation (%CV) and margins of error (%error) were subsequently calculated using multichannel verification system software.

Results

The data obtained, including the %CV and %error were directly compared for a wide range of volumes using the 1-, 8-, 12-, and 96-channel pipetting heads (*Table*).

Consistent results were obtained from the Versette and the multichannel verification system, demonstrating that the volumes dispensed from this liquid-handling platform are accurate and precise. This is, in part, due to the pipetting mechanics, which are adjustable for improved accuracy. With five precalibrated liquid classes for adjustment between standard experimental solutions, performance can be optimized. Experimental error due to dispensing inaccuracies is, therefore, reduced.

Conclusion

Automated liquid handling is an integral part of any HTS protocol. In order to maintain experimental integrity and ensure that precise data is obtained, all potential sources of error must be significantly reduced. As such, ensuring the accuracy and precision of any liquid volumes dispensed is of extreme importance.

The modular Thermo Scientific Versette automation platform for liquid handling provides the functionality to dispense volumes from 0.1 to 1,250 µL in a precise and accurate manner. Compatible with 19 interchangeable pipetting heads, ranging from single to 384 channels, means a wide range of throughput requirements are easily met. As such, when used in the creation of dilution series, users can be confident that the occurrence of false positive or negative results is maintained at a minimum, while throughput can be maximized without any loss in precision. GEN

for Laboratory Applications

do not offer a high enough image resolution for barcode technology.

Isberg points out that these common issues can be avoided when you consider the following key criteria when selecting labeling solutions.

- What label material is best? "Lab managers most often find that polymer is the best label material because it is resistant to chemicals and performs well in hot, cold, and even wet environments," he says. "Also, polymer labels offer unique resistance to tearing and other physical damage."
- What are your imaging needs? For barcode and other high-density data storage images, Isberg maintains that thermal transfer printing is the optimal choice. Thermal transfer images use a heat-activated ribbon material in the label printer, which allows users to select the image durability and clarify which is best for their application. "These images are the most durable option for labs, as they will not smear after printing," he continues.
- What adhesive should be used? This depends on the surface finishes, material chemistries, and usage environments of labware. Adhesives used for plastics are often different than those used for glass, and there can even be different adhesives for different types of plastics. Also, adhesives used for cryogenic applications are unique.

"Be sure to share specifics about your lab equipment and conditions with your label provider to ensure you get the right adhesive for your needs," says Isberg. "By selecting engineered labels that are customized to your material, imaging, and adhesive needs, you can rest assured they will withstand your unique laboratory environment." ■