

Acoustic Transfer of 25 nL Aqueous Droplets with the Echo[®] 525 Liquid Handler

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Abstract

The need to transfer fluids in the 1-10 μL volume range is an important concern when assembling biochemical assays. Liquid handlers capable of dispensing fluid volumes in the 1-10 μL volume range typically use pipette tips which are disadvantageous due to tip costs, tip cleaning requirements and the possibility of contamination. For researchers in the genomics and proteomics fields, the ability to accurately and precisely dispense all reagents in order to build a complete biochemical assay in the 1-10 μL volume range is highly beneficial.

This work highlights utilizing acoustic droplet ejection (ADE), a liquid transfer technology platform that eliminates the use of tips or nozzles and has been proven to accurately and precisely transfer fluids in the low-nanoliter volume range. Here we introduce the new Echo 525 liquid handling platform, which utilizes ADE technology to accurately and precisely dispense aqueous buffered solutions at 25 nL increments. The volumetric flow rate is approximately 5 $\mu\text{L}/\text{s}$. Both accuracy and precision of dispensing at this volume and flow rate is comparable to that of the Echo 555 liquid handling systems. We demonstrate the ability of a few general liquid classes to accurately and precisely transfer various aqueous reagents without requiring individual calibrations.

The Echo 525 Liquid Handler

The Labcyte[®] Echo 500 series revolutionizes liquid transfer by using acoustic energy to eject fluids. The Echo 525 liquid handler, the newest model of Echo liquid handlers, is designed for rapid transfer of biochemical and genomics reagents for assay assembly. The Echo 525 transfers 25 nL droplets of most biochemical reagents. These can be simple fluids (media for growing cells, buffer) or viscous solutions (lysis buffer, antibodies with glycerol, or transfection reagents). Microliter-scale volumes are transferred rapidly by repeating 25 nL transfers hundreds of times per second. The Echo 525 system enables contamination-free reagent transfer to precisely and accurately build biochemical assays. Miniaturization with the Echo 525 liquid handler retains high assay performance, allowing quantitative results at higher densities. The Echo liquid handler can be used to transfer virtually any volume to any well.

Accuracy and Precision of Droplet Transfers

To measure the accuracy and precision of the drop size in the Echo 525, single droplets and multi-droplet volumes were transferred from source wells containing various aqueous solutions with volumes ranging from 15 to 65 μL . In all cases tested in this experiment, the Echo system was able to detect the minimum fluid thickness in the center of all wells to support droplet ejection and maintain accuracy and precision. For fill volumes of 20 μL or below, the Echo system may determine the fluid thickness is it too low to support accurate and precise ejection when the fluid sits asymmetrically in the well (e.g., the meniscus is tilted at the outer wells due to centrifugation) or the solution has a particularly steep meniscus. If so, the instrument flags this well as not supporting transfer and reports this in a log file.

Materials

Phosphate Buffered Saline (Sigma-Aldrich, D1408)
Triton[®] X-100 (Sigma-Aldrich, T8787)
Fluorescein Sodium Salt (Sigma-Aldrich, F6377)
Sodium Hydroxide (J.T. Baker, 5674-02)
Echo[®] qualified 384-well polypropylene microplates (Labcyte Inc., P-05525)
384-well clear-bottom polystyrene microplates (Greiner Bio One, 781096)
Synergy H4 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Inc.)

Methods

To establish the drop size for the Echo 525 liquid handler, and to verify that the average drop size remained consistent through a multi-drop transfer, a series of solutions was prepared including 1X Phosphate Buffered Saline (PBS) and PBS containing 0.001%, 0.003%, and 0.042% (v/v) Triton X-100 surfactant. Those concentrations represent 5%, 14%, and 200% of the critical micelle concentration (CMC) of Triton X-100, respectively. Additionally, each solution contained 0.15 mM sodium fluorescein as a fluorescence tracer dye.

To show that the volume of a single drop was 25 nL, the Echo 525 liquid handler was used to transfer single-drop samples of 1X PBS, 5% CMC and 200% CMC solutions. The solutions were prepared in an Echo qualified 384-well microplate at a volume of 40 μL per well. One drop per well was then transferred from each source well to the corresponding well of a clear-bottom 384-well microplate.

To show that the average drop size remained consistent throughout transfers of multiple drops, the Echo 525 liquid handler was used to make 500 nL well-to-well transfers of the four aqueous solutions. All four solutions were prepared in an Echo qualified 384-well microplate in a quadrant pattern, with 96 wells filled to each of four volumes: 15, 20, 30, and 65 μL . Then 20 consecutive drops (500 nL total) were transferred from each source well to the corresponding well of a clear-bottom 384-well microplate. Following the transfers in both the single-drop and multi-drop cases, the clear-bottom microplates were back-filled with 50 μL per well of 10 mM sodium hydroxide using a conventional bulk filler. The microplate was then centrifuged for 1 minute at 1,000 rpm and then incubated for 30 minutes at room temperature. Next, the microplate was read on the Synergy fluorescence reader to determine the fluorescence level in each well. The fluorescence level was compared to a standard curve and reported as the volume of the Echo 525 transfer.¹

Results

Table 1. Accuracy and precision of 25 nL drop size on the Echo 525 liquid handler.

	1X PBS	0.001% Triton X-100	0.042% Triton X-100
N	384	384	384
Average	25.61	25.29	25.01
SD	0.45	0.38	0.39
CV	1.75%	1.52%	1.56%
Rel. Accuracy	2.45%	1.15%	0.02%

Measured volumes and CVs were obtained for transfers of buffer and of buffer with varying concentrations of surfactant. Table 1 shows the accuracy and precision of single-drop (25 nL) transfers for each of the four test solutions at four different source volumes. The average volume over 384 wells was within 3% of the nominal 25 nL value in all cases. The CV values were all less than 2%. Figures 1 and 2 show the accuracy and precision of 20-drop (500 nL) transfers of the same four solutions at four source volumes. Sample size for each combination of solution and source volume was N = 96. Accuracy and precision results in Figures 1 and 2 are shown for each source well volume. When results were pooled across all volumes for a given fluid, accuracy was typically within 3% of the nominal target volume, and less than 6% in all cases. CVs of pooled data were less than 4% for all fluids and volumes tested.

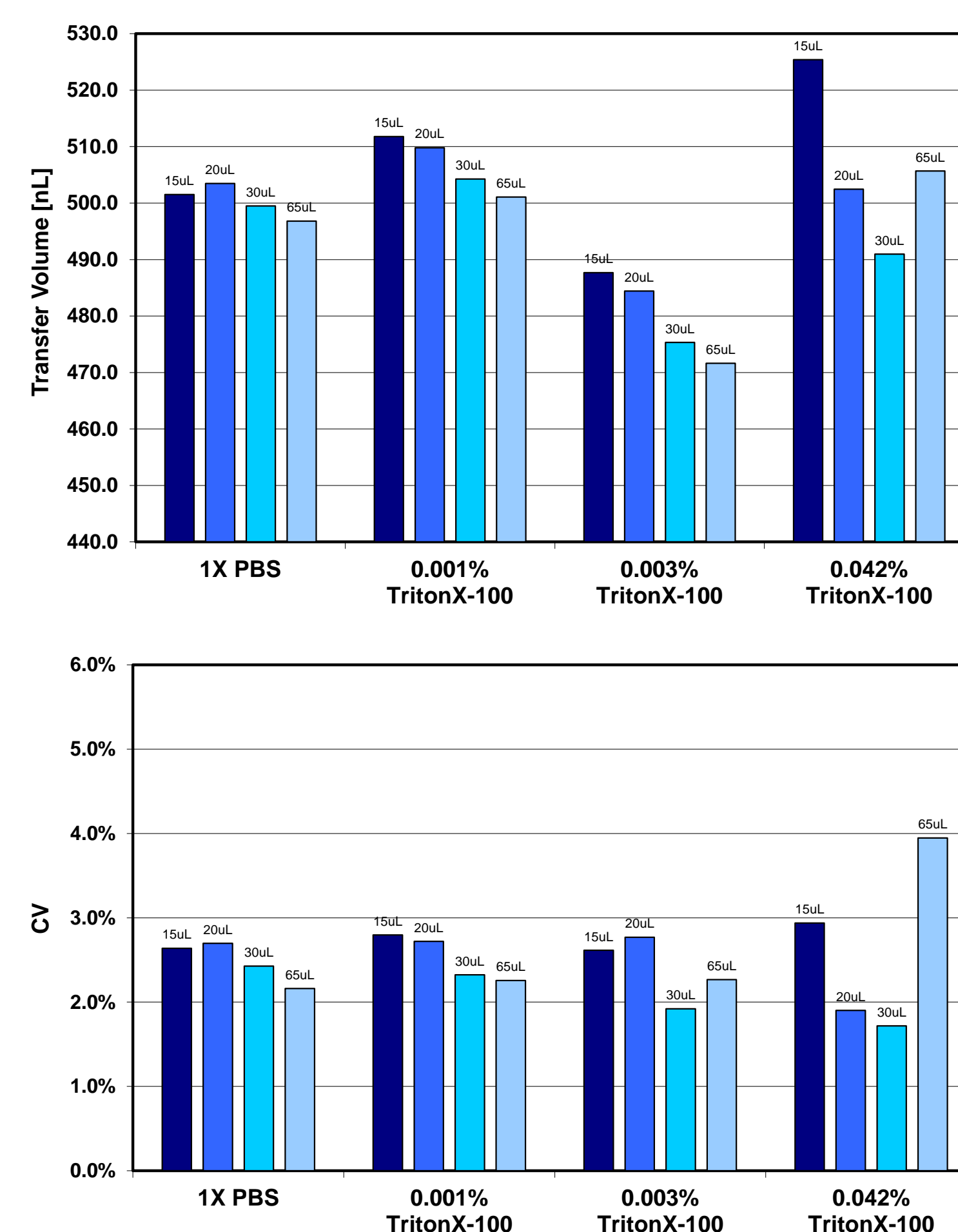


Figure 1 (top) and Figure 2 (bottom). Accuracy and precision of 500 nL transfers made with the Echo 525 liquid handler.

Suitability for Use in Genomics Applications as well as Biochemical Assays

To show that the Echo 525 liquid handler can dispense typical biochemical assay reagents with the required accuracy and precision, transfers of PCR master mix and ADME buffer containing a fluorescence marker were made and read on a fluorescence plate reader.

Materials

RealTime Ready DNA Probes Master Mix (Roche Applied Science, 05 502 381 001)
50 mM potassium phosphate buffer, pH 7.4 (Sigma-Aldrich, P8709)
Fluorescein Sodium Salt (Sigma-Aldrich, F6377)
Sodium Hydroxide (J.T. Baker, 5674-02)
Echo qualified 384-well polypropylene microplates (Labcyte Inc., P-05525)
384-well clear-bottom polystyrene microplates (Greiner Bio-One, 781096)
Synergy H4 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Inc.)

Methods

Samples of 1X RealTime Ready DNA Probes Master Mix and 50 mM potassium phosphate buffer, used as an ADME buffer, were prepared with 0.15 mM sodium fluorescein and transferred into an Echo qualified, 384-well PP source microplate. The Echo 525 liquid handler was used to perform one-to-many transfers from selected wells of the source plate to 384-well clear-bottom polystyrene destination microplates. The destination microplates were then back-filled with 50 μL per well of sodium hydroxide using a conventional bulk filler, centrifuged for 1 minute at 1,000 RPM, and incubated for 30 minutes at room temperature. Then the microplate was read on the Synergy fluorescence reader to determine the fluorescence level in each well. The fluorescence level was compared to a standard curve and reported as the volume of the Echo 525 transfer. For the master mix, two source wells were used with starting volume of 50 μL . Transfer volume was 100 nL, and transfers were made from each source well to all 384 wells of a destination microplate. Total transfer volume from each source well was 38.4 μL . For the ADME buffer, two setups were used. In the first, transfers as described above for master mix were made for four 50 μL source wells. In the second, 24 source wells with a 60 μL starting volume were used, and 16 transfers of 2,000 nL each were made to a single row of a 384-well destination plate. Thus for each source well a total of 32 μL was transferred, and all 24 wells were used to fill a single 384-well destination plate. The same Echo 525 calibration was used for transfers of master mix and ADME buffer. For all cases mentioned above, accuracy and precision were measured over the total number of transfers from a given solution.

Results

Table 2. Results of accuracy and precision measurements on representative biochemical assay components.

	RealTime Ready Master Mix	ADME Buffer	ADME Buffer
# Wells	2	4	24
Starting Volume	50 μL	50 μL	60 μL
Xfers per Well	384	384	16
Total Xfers	768	1536	384
Volume per Xfer	100 nL	100 nL	2,000 nL
Total Xfer Volume	76.8 μL	153.6 μL	768 μL
Relative Accuracy [% from nominal]	-1.70%	3.40%	-1.50%
CV [Across all Xfers]	2.74%	3.00%	2.23%

Table 2 shows the measured accuracy and CVs for master mix and ADME buffer. All average volumes were within 4% of the desired target, and all CVs were 3% or less. A single calibration was used for the master mix and ADME buffer transfers. The volumetric flow rate for the transfers reported in table 2 was 6.85 μL per second.

Summary

- The Echo 525 liquid handler can rapidly transfer a variety of aqueous reagents with high levels of precision and accuracy.
- The Echo 525 liquid handler transfers from individual wells or groups of wells to all wells of destination microplates.
- Conventional genomics and biochemical reagents and buffers, such as PCR master mix and ADME buffers, can be transferred with high precision and accuracy without the need for individual calibrations.

References

1. Harris and Mutz, JALA, 2006, 11(4), 233-239.



The Echo 525 liquid handler

- No tips or nozzles to clog
- No calibration required
- Rapid assay assembly
- Rapid assay optimization

