Poster Presentation

NanoQuot™, A Cost-Effective, Flexible Nanoliter Dispenser for High Throughput Screening

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Abstract

BioTek has developed the NanoQuot™, a dispenser capable of running stand-alone or computer controlled as part of a robotics system. Positive gas pressure system, in conjunction with eight precision valves, provides accurate and precise dispensing of volumes ranging from 100 nanoliters to 40 microliters in 96-, 384-, and 1536-well plates. The dispense head is adjustable for different plate heights, and removable, allowing the NanoQuot to be shared by multiple groups without the worry of contamination. The NanoQuot's low input gas pressure requirement allows house air, purified pressured gas, or a dedicated small compressor to be used. The instrument comes pre-calibrated with three liquid types and the end user can create new profiles with their liquids. Besides aqueous solutions, the NanoQuot can also handle viscous solutions and organic solvents. The user interface provides complete programming capabilities from the keypad. Information regarding design and performance, including accuracy and precision, will be provided.

Figure 1. NanoQuot™ Microplate Dispenser

Introduction

Scientists in drug discovery and life science research appreciate the benefits of assay miniaturization. Miniaturization offers many advantages including increased sample capacity, speed, faster time to market, and drastically reduced costs of expensive reagents or compounds. Towards this end, BioTek has developed the NanoQuot™ Microplate Dispenser capable of running stand-alone in a pilot assay laboratory or computer controlled as part of an HTS laboratory's robotics system (Figure 1). A positive gas pressure system, in conjunction with eight precision solenoid valves, provides accurate and precise dispensing of volumes ranging from 100 nl to 40 µl in 96-, 384-, and 1536-well plates (Figure 2). The dispense head and tubing set is easily removed and replaced allowing the NanoQuot to be shared by multiple lab groups without the worry of contamination. The Z-axis height adjustment allows for the accommodation of half-height plates. As the NanoQuot does not require a high input pressure (30 PSI), house air, purified pressured gas, or a dedicated small compressor can be used. Prior to shipment, BioTek performs a 13-point calibration procedure on three different liquid types and pre-loads the liquid profiles prior to shipment. The end user can easily create new profiles with their specific liquids. The NanoQuot handles viscous solutions such as 40% glycerol and 24% PEG-400 or organic solvents such as DMSO and acetonitrile. The uncomplicated user interface provides complete programming capabilities from the keypad. For more complete automation, robotic interfaces can be developed using a programmer's package. The NanoQuot's diminutive size, with a 12 x 12 inch footprint and a height of 8 inches, allows it to be used almost anywhere.

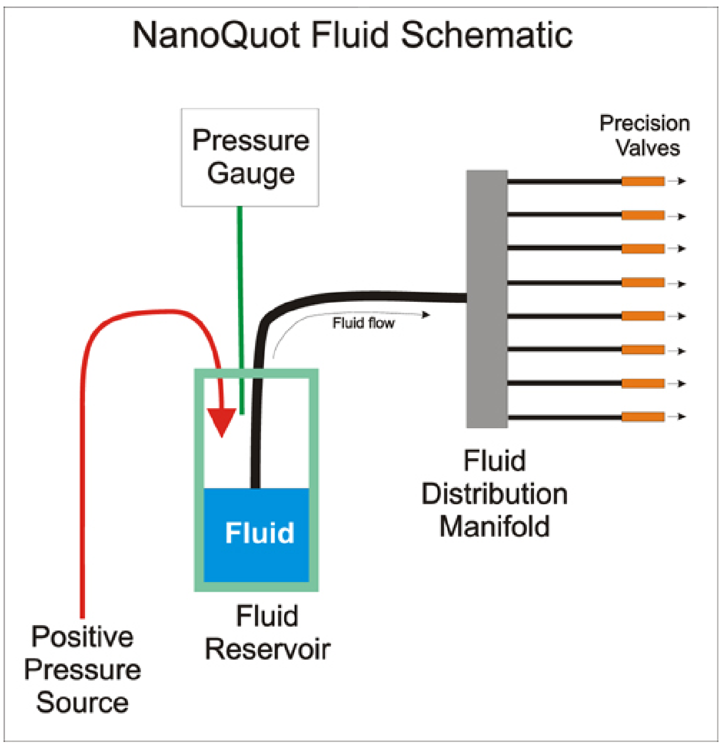

Figure 2. Fluid Path Design of the NanoQuot. Positive pressure drives fluids from the reservoir through a distribution manifold, which divides the fluid into 8 separate tubes. Eight precision valves control the fluid dispense into wells of the microplate. Pressure of the reservoir is monitored by a pressure gauge linked by tubing.

Materials and Methods

Dispense accuracy and precision were determined using a gravimetric method and the absorbance of dye solutions respectively. Determinations using the gravimetric method were performed by entire plates using a Sartorius A 120S analytical balance. After dispensing fluid to the plate using the NanoQuot, the plate was quickly re-weighed. The resultant weight change, when divided by the number of wells, returns an average per-well dispense volume when corrected for solvent specific gravity. When calculating the precision of dispense using the dye method, an aqueous solution containing FD&C blue number 1 dye was dispensed into plates with the NanoQuot™ Microplate Dispenser. Diluent was then dispensed using a Precision™ XS Microplate Sample Processor and the absorbance at 630 nm (450 reference) was measured using a Synergy™ 2 or Synergy™ HT Multi-Detection Microplate Reader (BioTek, Winooski, VT). The resultant absorbance values were then used to calculate dispense precision.

Results

Several different volumes of deionized water were dispensed into 384-well microplates. As demonstrated in Figure 3 the precision of dispensing, as measured by %CV, was very good across the entire volume range tested. The coefficient of variation (%CV) was always below 5% and for volumes of 0.5 µl or greater the %CV were found to be less than 3% for volumes up to 40 µl. When various volumes of an aqueous dye solution from 100 nl to 2 µl was dispensed and the mean absorbance of each plate was plotted a linear relationship is observed (Figure 4). These data indicate that the NanoQuot can reliably dispense across much of its volume range using a single calibration.

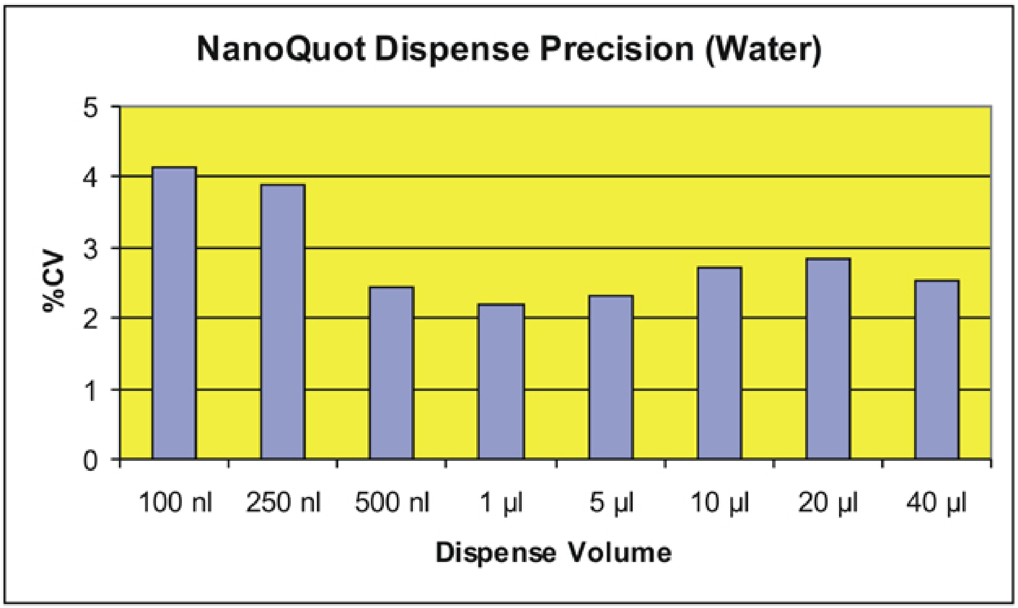

Figure 3. NanoQuot Dispense Precision. Various volumes of deionized water containing blue tracer dye was dispensed into 384-well microplates using a NanoQuot™ Microplate Dispenser. After adding deionized water diluent to a total volume of 100 µl, the absorbance of the wells of the plate was determined using a Synergy™ HT Multi-Detection Microplate Reader. The absorbance data was exported to Microsoft Excel and the mean, standard deviation and %CV calculated. The %CV for each plate was then plotted.

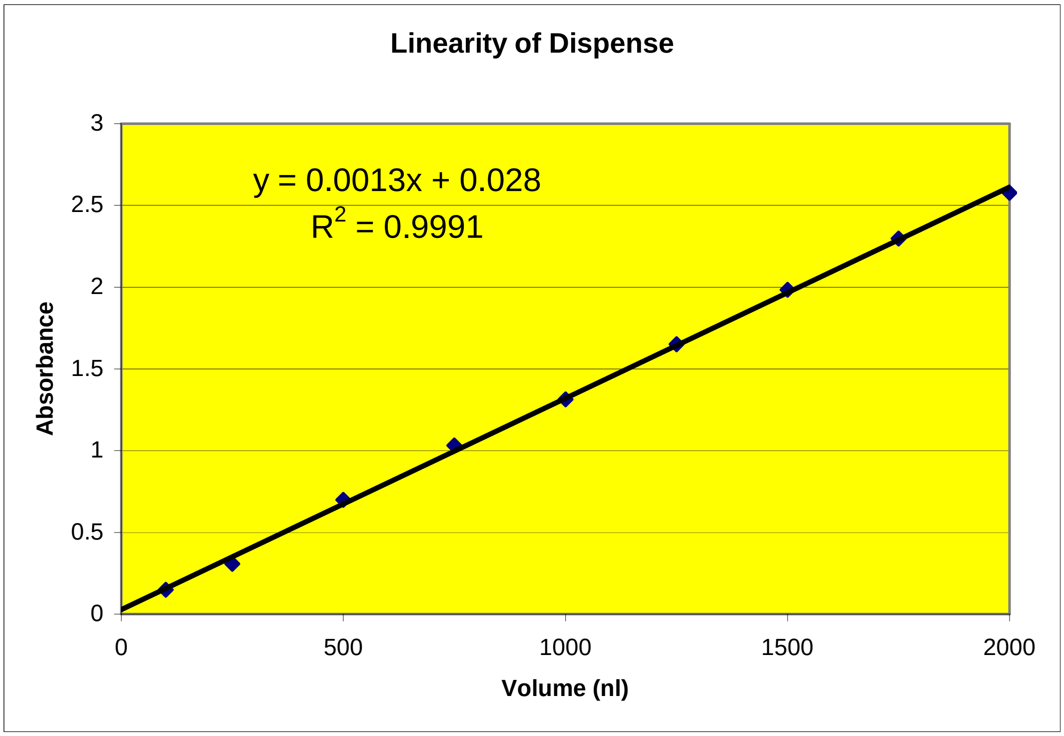

Figure 4. Linearity of Dispense. Different volumes of aqueous dye solutions were dispensed into wells of a 384-well microplate and the absorbance of each well was measured. The data for each plate was then exported to Microsoft Excel and plotted. Each data point represents the mean of 384 determinations.

Dispensing the lowest allowable volume range is often the most difficult for liquid handlers. As demonstrated in Figure 5, the NanoQuot can consistently dispense 100 nl to all the wells of a 384-well microplate. There is little variation between different valves and little variation between dispenses by the same valve. This is corroborated by the data presented in Figure 6, which depicts a 500 nl dispense of DMSO. These data are presented as the output of each individual valve. The uniformity between valves is indicated by the similarity of all the data lines. Sharp spikes would indicate deviations within any one valve either upward or downward at a particular dispense number.

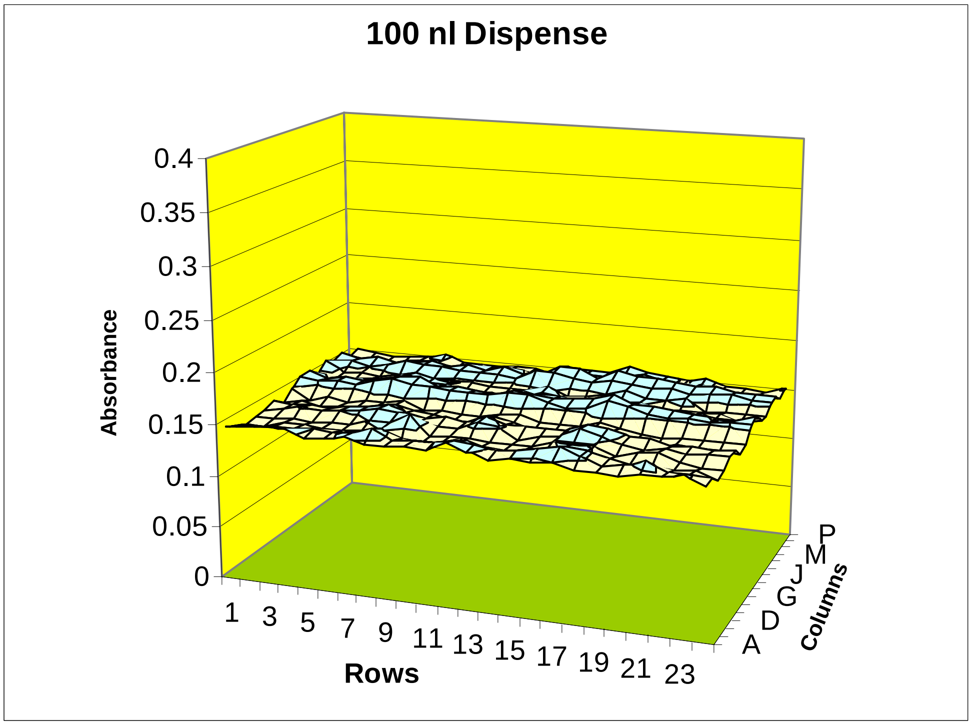

Figure 5. Uniformity of Dispense. The NanoQuot™ Microplate Dispenser was used to dispense 100 nl of aqueous dye solution into 384-well microplates. After the addition of dye solution, 100 µl of diluent was added and the absorbance for each well determined. Data for the entire plate was plotted using Microsoft Excel.

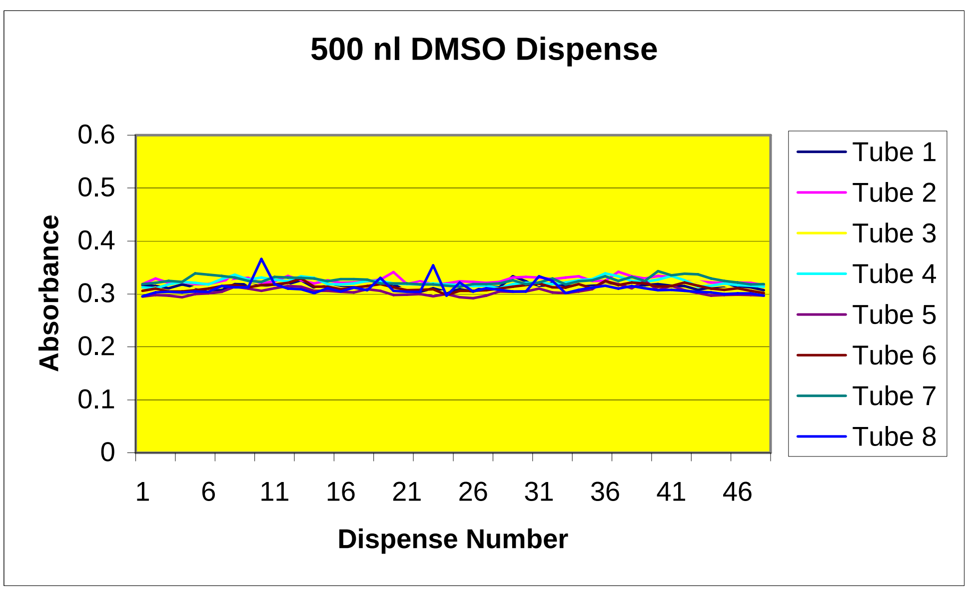

Figure 6. Valve-to-Valve Uniformity. DMSO containing a dye tracer (500 nl) was dispensed into a 384-well plate. After dispensing, 100 µl of water was added and the absorbance at 630 nm of each well was measured using a Synergy™ HT Multi-Detection Reader. The data was then exported to Microsoft Excel and each valve tube's dispense was plotted separately.

The effect of lowering the positive pressure was also examined. There are several different plate types and styles whose geometry can preclude the use of the maximal dispense pressure. The well depth of low profile plates is approximately half that of a normal 384-well microplate. Use of maximal pressure can lead to splashing after dispensing into the well. In addition, round bottom 384-well plates are prone to splashing as well. Table 1 demonstrates the precision performance of the NanoQuot. Despite using a positive pressure of 10 PSI in lieu of the recommended 15 PSI, the NanoQuot provides very acceptable precision for the solutions tested.

Table 1. Precision of Dispense for Various Solutions at 10-PSI Positive Pressure

Solution	Dispense Volume (μl)		
	0.5	**1.0**	**2.0**
100% Ethanol	4.2*	6.7	5.8
70% Ethanol	5.1	4.3	4.1
50% Ethanol	7.4	7.1	3.7
PBS	6.7	5.6	3.8
Acetonitrile	4.8	4.1	5.1
10% DMSO	4.8	4.3	3.4
98% DMSO	8.7	6.9	5.0
1% BSA	8.9	7.8	3.7
10% Glycerol	8.1	8.7	3.9

**Data represent the %CV across a 384-well plate.*

Several different solutions containing a tracer dye were dispensed into 384-well microplates with the NanoQuot™ Microplate Dispenser using positive pressure of 10 PSI. After the addition of water diluent, the absorbance was measured using a Synergy™ 2 Multi-Detection Microplate Reader and the %CV calculated from the subsequent absorbance values. These data represent the mean and standard deviation calculated from 384 determinations.

Under some circumstances, multiple dispenses of reagents, such as multiple reagents or repetitive dispenses of the same reagent, are made into the same well. These types of dispenses are prone to splashing, due to the shortened distance from the dispense-valve tip and the fluid surface, as well as the potential for the existing fluid to be projected out of the well. As demonstrated in Table 2, despite reducing the pressure to 7.5 PSI, the accuracy of the NanoQuot after a total of three dispenses (5 μl, 5 μl, and 10 μl) of a sodium fluorescein solution into a round bottom 384-well plate was quite good. The combined error of the 3 dispenses was less than 3% and the overall precision resulted in a %CV of approximately 5%. The low positive pressure was required to minimize splashing resulting from both the rounded surface of the well bottom and the partially filled nature of the well itself.

Table 2. Accuracy and Precision with Multiple Dispenses

Expected Total Volume	Accuracy		Precision	
	Actual Volume (μl)	**% Error**	**Mean**	**%CV**
20 μl	19.48*	-2.60%	95854**	5.8
20 μl	19.57	-2.15%	97775	5.2

*Accuracy measurements represent the calculated per-well volume as determined gravimetrically after a total of 3 dispenses of 5, 5, and 10 μl respectively.

**Precision measurements represent the mean of 384-well fluorescent determinations of sodium fluorescein.