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Poster Presentation

Precision™XS

Automating a Lowry Protein Assay with BioTek's New Microplate Sample Processor

Poster shown at Miptec 2004, Basel, Switzerland and LabFusion 2004, Boston, MA, USA

Abstract

The automation of routine pipetting tasks in biomedical research and screening requires the use of both single-channel and multi-channel pipetting. In response to these requirements, BioTek Instruments has developed the Precision™ XS Microplate Sample Processor. The Precision XS is a low-cost pipetting system that uses both a single-channel and a multi-channel pipette head in its compact workstation for transferring samples. This allows pipetting to and from single tubes, bottles, reagent reservoirs, microplates or a variety of other labware. The Precision XS' flexible platform layout with single- and 8-channel pipette heads accommodates applications from hit picking to serial dilutions. Using carbon filled tips, the single channel pipette is capable of liquid level sensing, allowing transfer of sample from unevenly filled sample tubes. Pipetting is highly accurate and precise. Dispense accuracy at 100 µl is within 2% with 2% CVs. Precision Power™ Software provides complete control of experimental design through an intuitive user interface. For larger reagent transfer volumes, the Precision XS is also equipped with an autoclavable and 100% DMSO compatible bulk reagent dispenser. The Precision XS easily interfaces with the Bio-Stack™ Microplate Stacker, and for more complete automation robotics, interfaces can be developed using ActiveX® software commands. The Precision XS' small size, with a 15- x 25-inch footprint and a height of 16 inches, allows it to be used almost anywhere including most biological safety cabinets or chemical fume hoods. Details regarding speed, accuracy and precision of pipetting will be provided along with information regarding instrument software and hardware design.

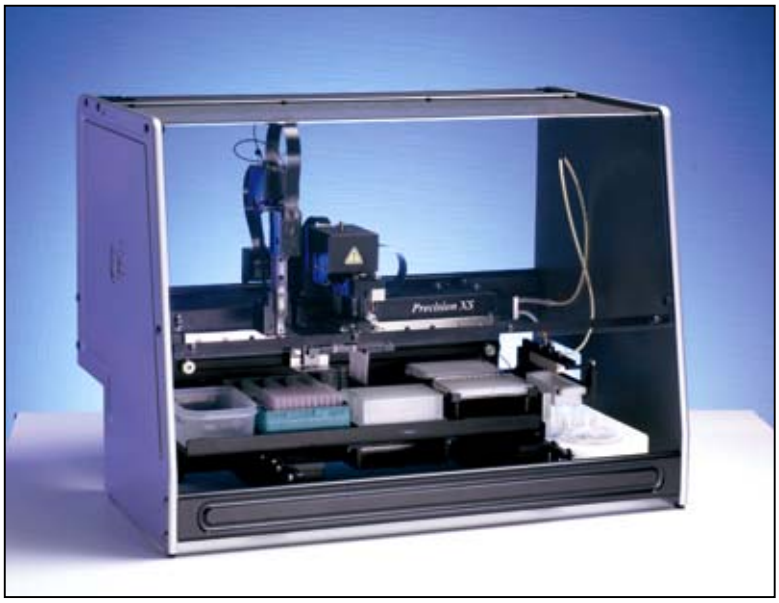

Figure 1. Precision XS Microplate Sample Processor with protective aerosol cabinet.

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Introduction

Quantitative and qualitative assays of all sorts are common to many applications in basic science, drug discovery and clinical research. Most all of these assays require the movement, either addition or subtraction, of liquid reagents. Historically these assays were performed in test tubes. However, regardless of the assay method employed, laboratories requiring high throughput have often adapted the described protocol to a 96-well and more recently a 384-well format. Despite the adaptation, processing large numbers of samples generally represents a large investment in manpower, with automation primarily taking place at the endpoint of reading the plate. The microplate assays, while reliable, still represent a very tedious and time-consuming task. Assays that are run in a microplate format use multi-channel pipettes for fluid delivery as a means to increase efficiency. However, the first step often involves the movement of sample from primary test tubes to a microplate. While test tubes have been utilized for decades, they do not conform to the standardized format of microplates and movement of fluids into and out of these tubes requires a single channel pipette capability. In order to address these needs, BioTek has developed the Precision™ XS Microplate Sample Processor. The Precision XS expands upon the proven Precision™ 2000 platform to provide single channel pipetting capabilities in conjunction with all the multi-channel features currently provided.

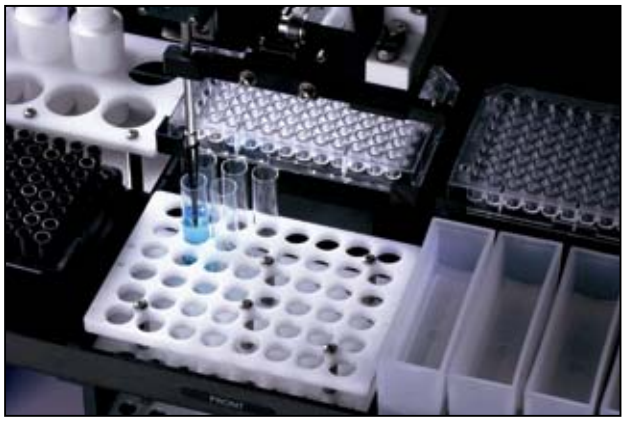

Figure 2. Single-Channel Pipette using its liquid level-sensing feature to aspirate fluid from a sample tube.

The Precision XS has a completely configurable six-station platform to hold the required pipette tips, reagent reservoirs, and microplates for fluid transfer (Figure 1). The platform is removable, allowing for multi-user friendliness, easy cleaning, and setup of the instrument. The 8-channel pipette moves up and down as well as side-to-side, while the platform moves front to back to provide complete access to all locations on the work platform and complete configurability. The pipette uses a proprietary technology to reliably pick up and seal any standard tip with individual, free-floating barrels that compensate for tips out of position. In addition, the Precision XS has a single channel probe that is capable of using disposable pipette tips to aspirate and dispense samples from tubes (Figure 2) or microplates (Figure 3). The single-channel sample processor head uses a syringe and fluid filled tubing to aspirate and dispense fluids with pipette tips.

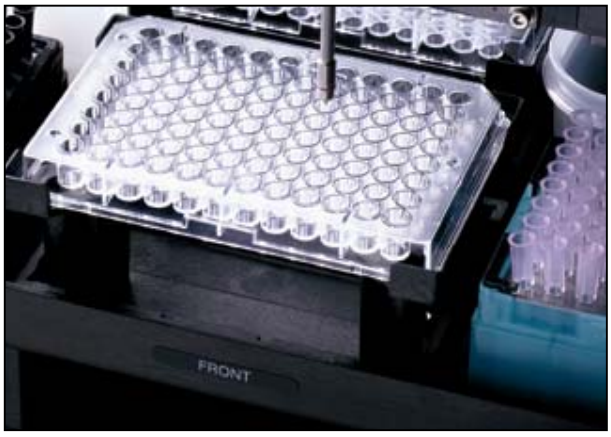

Figure 3. Single-Channel Dispenser filling an isolated well of a 96-well microplate.
The single channel probe is also be used without tips as a reagent dispenser to deliver reagent to any type of vessel.

Because the fluid path is contiguous with a fluid reservoir, the single channel probe can also be used as a dispenser without needing to pick up a pipette tip (Figure 4). An autoclavable, solvent resistant 8-channel dispenser, which uses a precise bi-directional syringe pump to accurately and rapidly dispense fluids from a large unpressurized reservoir, is also available (Figure 5).

Materials and Methods

Pipetting accuracy and precision was determined using a combination of a gravimetric method and the absorbance of dye solutions. Gravimetric determinations were performed by weighing plates using a Sartorius A 120S analytical balance, while the absorbance of a dye solution (FD&C Blue No. 1) was used to estimate the dispense precision of the Precision™ XS Microplate Sample Processor. The dispense-volume accuracy into 96-well microplates was determined by weighing an empty plate before and the same plate after pipetting with the Precision XS. The average weight per well was calculated by dividing difference between initial and final weights (Delta) by the total number of wells (96). Using the specific gravity of water (1 g/ml) a conversion from weight to volume was then made. In order to provide sufficient liquid volumes to the microplate wells, deionized water was added to each well of the microplate such that the final volume would be 300 µl per well. Note that the volume added varied depending on the intended dispense-volume programmed. The absorbance at 630 nm (450 nm reference) of all the wells in the 96-well microplate were measured using a Synergy™ HT Multi-detection Reader (BioTek Instruments, Winooski VT) and then exported to Microsoft® Excel™. Using Excel, the total absorbance of all 96 wells was calculated by summing the values. A plate-specific factor was then calculated by dividing the total absorbance by the total dispense volume previously calculated from the change in plate weight (Delta). This factor was then used as a conversion factor to calculate the dispense volume of each well from its absorbance. Plate statistics such as mean, standard deviation and %CV were calculated using Excel.

Figure 4. 8-Channel Bulk Dispenser filling a 384-well microplate. The Precision XS is available with an autoclavable and 100% DMSO compatible bulk reagent dispenser.

Table 1. Accuracy and Precision of the Single-Channel Pipette

Expected (µl)	Observed (µl) [#]	%CV
5	5.29±0.21	4.01
10	9.94±0.31	3.14
25	24.92±0.40	5.81
50	49.6±0.99	1.85
100	98.39±1.71	1.74
200	190.40±1.59	0.84

[#] Note that these data represent the mean and standard deviation of 48 wells.

The Lowry assay performed on the Precision XS is essentially the micro Lowry assay [1] that has been adapted to microplates and automated. The reagents can be purchased in a kit (Catalog No. 23240; Pierce Endogen, Rockford, IL) or obtained as individual components. Working protein stock solution was prepared with sterile deionized water to a concentration of 250 µg/ml. Dilutions of the protein standards were then made automatically as described below. The working protein stock solution was placed into a 13- x 100-mm tube located at position A1 of the 48-tube rack of the Precision XS, along with seven empty tubes in positions B1 to H1. Protein samples were prepared by diluting stock solutions of bovine serum albumin (BSA) to various concentrations, and 1 ml was distributed into 13- x 100-mm sample tubes and the tubes placed in the remaining spaces of the 48-tube rack of the Precision XS.

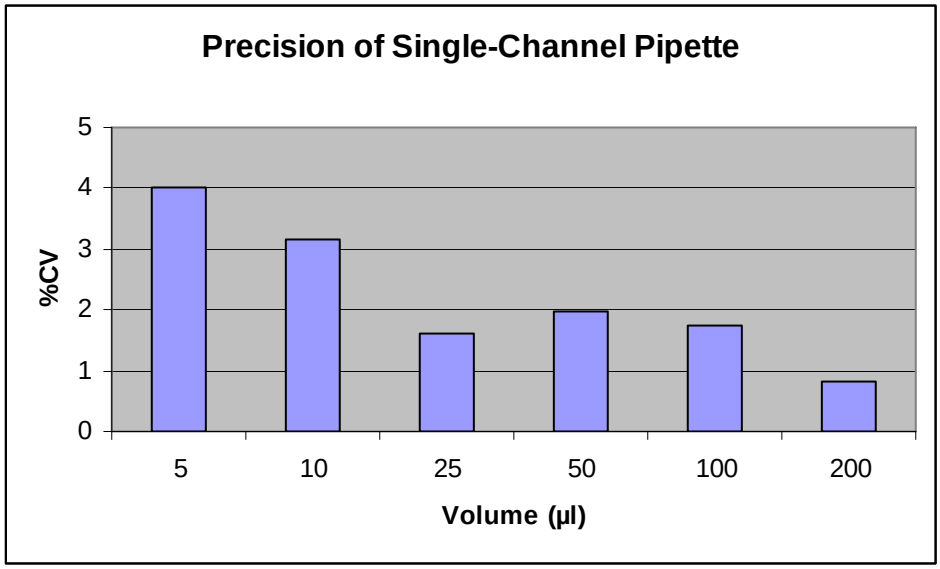

Figure 5. Precision of the Single-Channel Pipette. Using the single-channel pipette, various volumes of dye solution were pipetted into microplates. Using a combination of gravimetric and absorbance measurements, dispense volumes for each well of a 96-well plate were calculated along with the plate mean, standard deviation and %CV. Each data point represents the %CV of 96 determinations.

The initial portion of the assay involves the dilution of protein standards to generate a standard curve. The single-channel dispenser first adds 400 µl of diluent to the 7 empty tubes. Using a disposable tip, the single channel pipette transfers 400 µl of working protein stock solution from the tube in location A1 to the tube located at position B1, effectively making a 1:2 dilution. A series of 1:2 dilutions are then made into the tubes of the first column, with the final tube only containing diluent. After completion of the standards dilutions, 100 µl aliquots of standards and samples are transferred in duplicate to the assay plate located in position D of the deck.

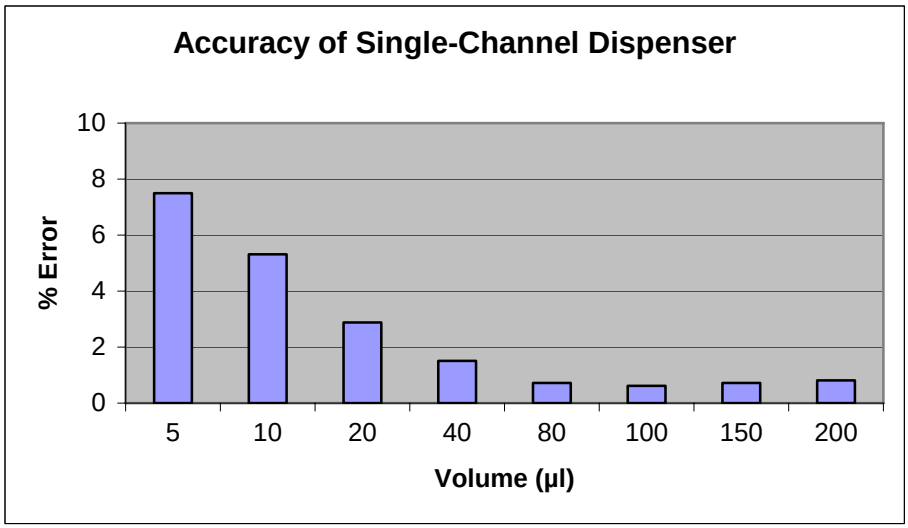

Figure 6. Accuracy of the Single-Channel Dispenser. The reagent dispenser was used to add dye solution to each well of a 96-well microplate. Afterward the average per well dispense was calculated by measuring the change in weight from before and after dispensing then dividing by the number of wells. The percent error of the determined dispense volume from the expected volume was calculated and expressed as an absolute value.

Once all of the samples and standards have been transferred to the assay plate, the 8-channel pipette adds 200 µl of freshly prepared biuret reagent to each well. Biuret reagent consists of 1 ml of 1% cupric sulfate + 1 ml of sodium potassium tartrate + 100 ml of 2% sodium carbonate in 0.1 N sodium hydroxide. Samples are allowed to incubate for 15 minutes prior to the addition of 20 µl on 1 N Folin & Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis, MO) to each well. Mixing is accomplished by three intrawell pickups and dispenses immediately after dispensing the Folin & Ciocalteu reagent. Samples are then allowed to incubate at room temperature for 30 minutes and their absorbance measured at 660 nm using a Synergy™ HT Multi-Detection Reader (BioTek Instruments, Winooski, VT).

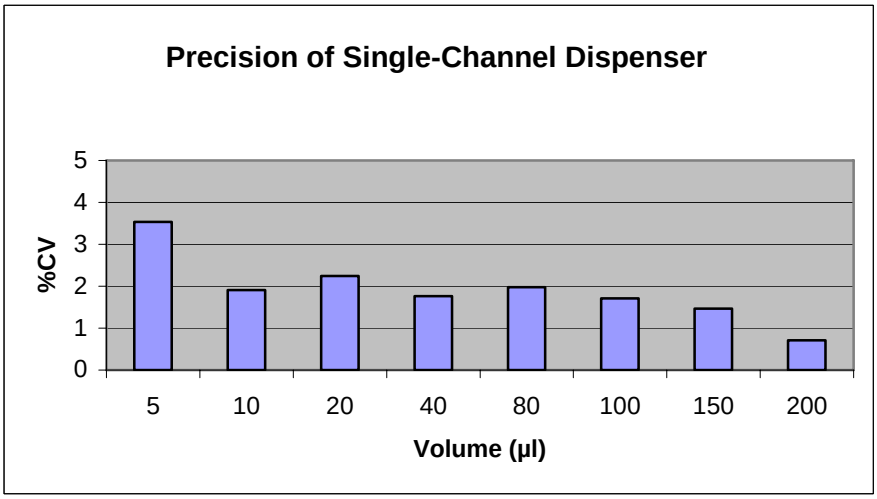

Figure 7. Precision of the Single-Channel Dispenser. Various volumes of blue dye solution were dispensed into microplates using the single channel sample probe as a dispenser. Using a combination of gravimetric and absorbance measurements, dispense volumes for each well of a 96-well plate were calculated along with the plate mean, standard deviation and %CV.

Results

The accuracy and precision of the various subcomponents of the Precision™ XS were examined. As demonstrated in Table 1, the single-channel pipette of the Precision XS is accurate across a range of volumes when dispensing fluids into a dry well. While the pipette dispenses slightly less volume than expected, it does so in a very consistent fashion. The precision data presented in Figure 5 demonstrate that the %CV of calculated dispense volume across an entire plate is generally less than 4% regardless of the volume selected. While the smaller volumes have slightly higher %CV, they are not remarkably greater. These data are very similar to those observed with the 8-channel pipette. As demonstrated in Table 2, this is also quite accurate. When the linearity of dispense of the 8-channel pipette is examined, there is a very high degree of correlation between the expected volume and the determined volume. As seen in Figure 9, the linear regression shows a correlation coefficient of greater than 0.9999. Similar studies were performed using the 8-channel bulk reagent dispenser. As demonstrated in Figure 10, the accuracy and precision when using the 8-channel dispenser is quite good at the volumes tested (25-200 µl). Only at the lowest volume tested is there any significant deviation from the expected volume. Regardless of the volume tested, the precision, as measured by %CV, is very good. The %CV was typically less than 3% at all volumes tested (Figure 10).

Table 2. Pipetting Accuracy of 8-Channel Pipette.

Expected Volume (µl)	Determined Volume (µl)[#]	%CV
5	3.75 ± 0.26	6.99
10	8.88 ± 0.33	3.68
20	18.92 ± 0.50	2.65
40	38.76 ± 0.72	1.86
60	58.48 ± 1.09	1.88
80	78.18 ± 1.53	1.95
100	97.97 ± 1.68	1.72

[#] Note that these data represent the mean and standard deviation of 96 wells.

While the single-channel pipette is most often used to aspirate and dispense fluids, in addition it can be used without tips as a reagent dispenser from a reservoir to either microplates or tubes. As shown in Figures 6 and 7, when the single-channel probe is used as a pipette, it is both accurate and precise. Typically the error from expected decreases from around 7% at the lowest volume setting to less than 2% when volumes greater than 20 µl are tested (Figure 6). The precision of dispense, as measured by %CV, was routinely 3% or less (Figure 7).

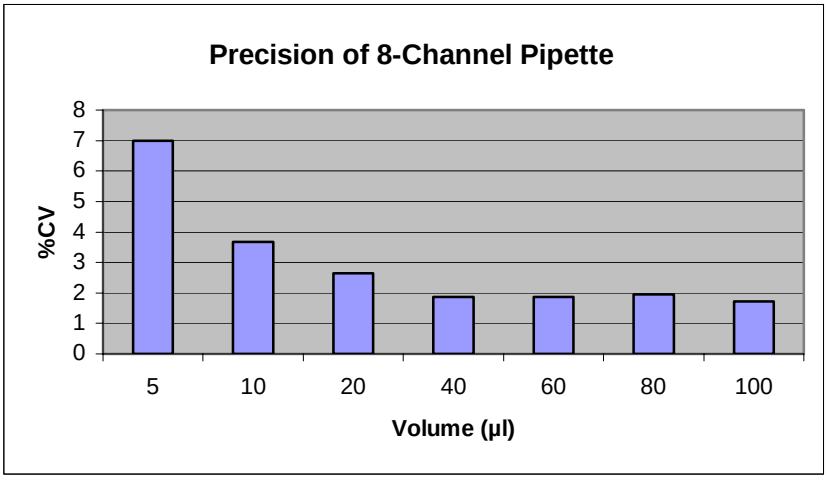

Figure 8. Precision of the 8-Channel Pipette when pipetting into dry 96-well plates at various dispense volumes. Various volumes of blue dye solution were dispensed into microplates using the 8-channel pipette. Using a combination of gravimetric and absorbance measurements, dispense volumes for each well of a 96-well plate were calculated along with the plate mean, standard deviation and %CV.

Complex assays can be programmed and processed using the Precision™ XS. As demonstrated in Figure 11, colorimetric assays such as the Lowry assay for total protein can be performed using the Precision XS. Standards that were first diluted from a stock solution, transferred to a microplate and then assayed according to the method of Lowry [1] demonstrate a very linear relationship. Table 3 demonstrates the Precision XS' ability to reliably perform these tasks, as the observed results agree quite well with expected values. Besides routine reagent additions, the Precision XS diluted the standards from a concentrated stock solution, transferred standards and samples from tubes to the assays plate, and controlled incubation time. The end user need only load samples and a stock standard solution, reagents and an empty assay microplate, and the Precision XS will complete the task. At the completion of the assay, the absorbance of each well is determined using a microplate reader.

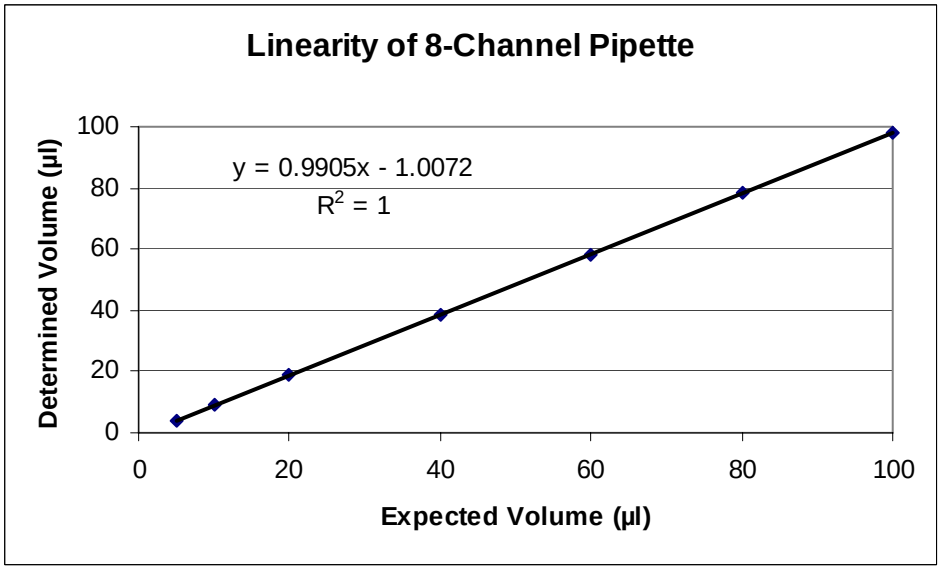

Figure 9. Linearity of the 8-Channel Pipette. The mean absorbance value from a 96-well plate at each volume was plotted against the expected volumes and a linear regression analysis performed on the data.

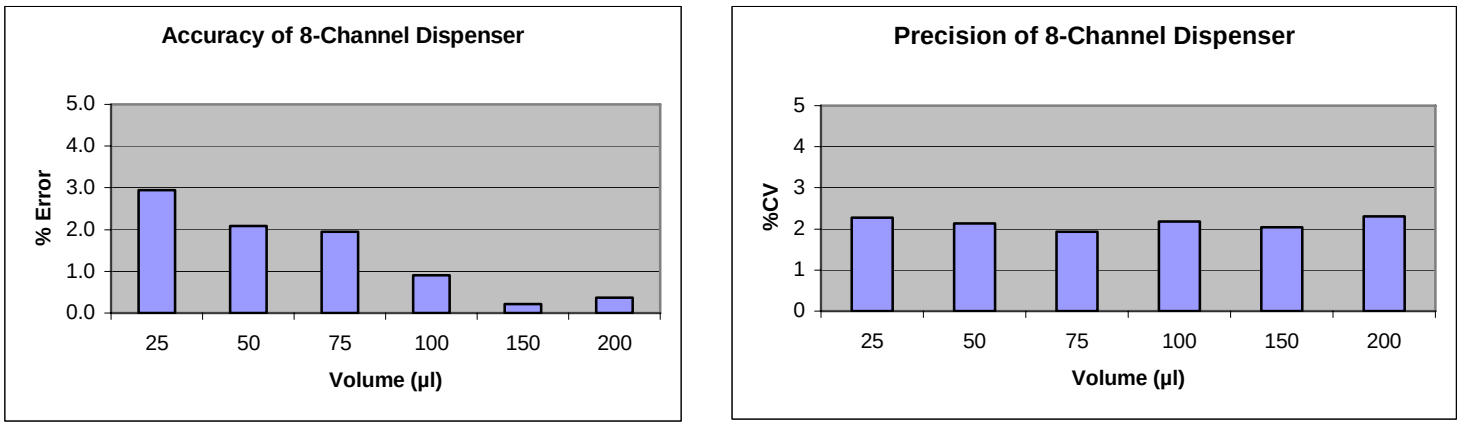

Figure 10. Dispense Accuracy and Precision of the 8-Channel Dispenser at Various Volumes. Various volumes of blue dye solution were dispensed into microplates using the bulk reagent dispenser. Using a combination of gravimetric and absorbance measurements, dispense volumes for each well of a 96-well plate were calculated along with the plate mean, standard deviation and %CV. The percent error of the determined dispense-volume from the expected volume was calculated and expressed as an absolute value.

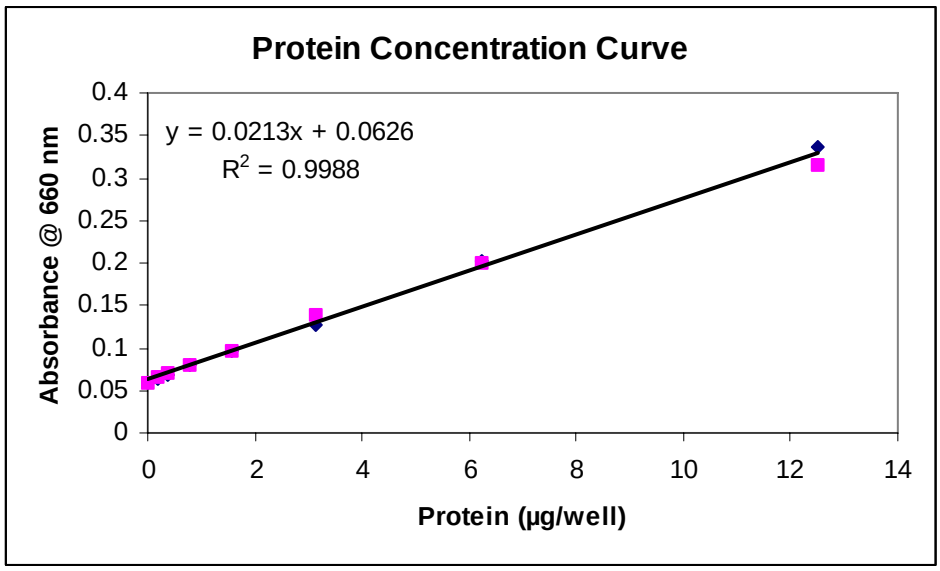

Figure 11. Protein Concentration Curve. Serial dilutions of a known concentration of BSA stock solution were prepared using the Precision™ XS and a Lowry assay for total protein performed. Data represents the individual data points as well as a linear regression of the data.

Table 3. Accuracy and Precision of Samples and Standards of a Typical Lowry Protein Assay.

Standards			***Samples***	
Expected (µg/well)	Determined (µg/well)[#]		Expected (µg/well)	Determined (µg/well)[&]
12.50	12.33±0.70		10.00	9.30±0.78
6.25	6.49±0.07		7.50	7.48±1.37
3.13	3.30±0.40		5.00	5.07±0.75
1.56	1.59±0.03		3.00	2.78±0.28
0.78	0.79±0.03		2.50	2.47±0.23
0.39	0.32±0.03		1.00	0.87±0.15

0.20	0.14±0.03		0.50	0.28±0.09
0.00	0.00		0.00	0.01±0.02

Data represents the mean and standard deviation of duplicate determinations.
& Five different tubes for each concentration were assayed in duplicate, for a total of ten determinations.

Discussion

These data presented here demonstrate the performance and the prowess of the Precision™ XS in regards to performing fluid transfers. The multimode design, in regards to pipetting of the instrument, allows the end user the flexibility to perform complex assays. The single-channel pipette allows for the transfer of fluids into and out of tubes, a feature that multi-channel designs normally cannot perform. The ability to address tubes provides the capability to transfer samples from specimen tubes into microplates for processing, as well as the dilution of standards within tubes to be treated in the same fashion as samples after dilutions. These types of features in conjunction with incubation control allows for turnkey-type assay processing for many biochemical assays. At the completion of the assays, the end-user needs only perform the necessary measurement be it fluorescence, absorbance or luminescence to name a few.

References

[1] Lowry, O. H, N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein Measurement with the Folin Phenol Reagent, J. Biol. Chem. 193:265-275.

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