

A Case Study: Maximizing the Precision and Accuracy of a Multi-Channel Pipettor to Optimize Inhibition Data from a Kinase Assay. Development of a Low-Cost Liquid Handling Solution.

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1. Abstract

The ADP-Glo luminescence assay from Promega is best run at the 5 microliter scale and consists of three components that are added in one and two microliter additions. Such an assay cannot be run in a reproducible manner using a standard multi-channel handheld pipettor with a supported range of 2-20 microliters.

However, attaching the pipettor's head to Hudson Robotics' proprietary motion control, allows one to increase its precision an order of magnitude.

The actual pipetting method employed greatly effects the precision of low volume transfers, and a number of pipetting methods were examined using an iterative gravimetric technique developed to measure these effects. The most precise of these pipetting methods were applied to the ADP-Glo assay.

2. Hudson SOLO Automated Pipettor

We developed a robot to control the movement of a standard multi-channel manual pipettor. The robot contains stepper motors to move the pipettor in the x, y and z axes, plus an additional motor to control the movement of the plunger. This motor operates at a resolution of roughly 10,000 steps to a full stroke. This translates to 500 steps per microliter.

The unit can be connected to a computer system through a USB-to-serial convertor, and is controlled by SoloSoft software.

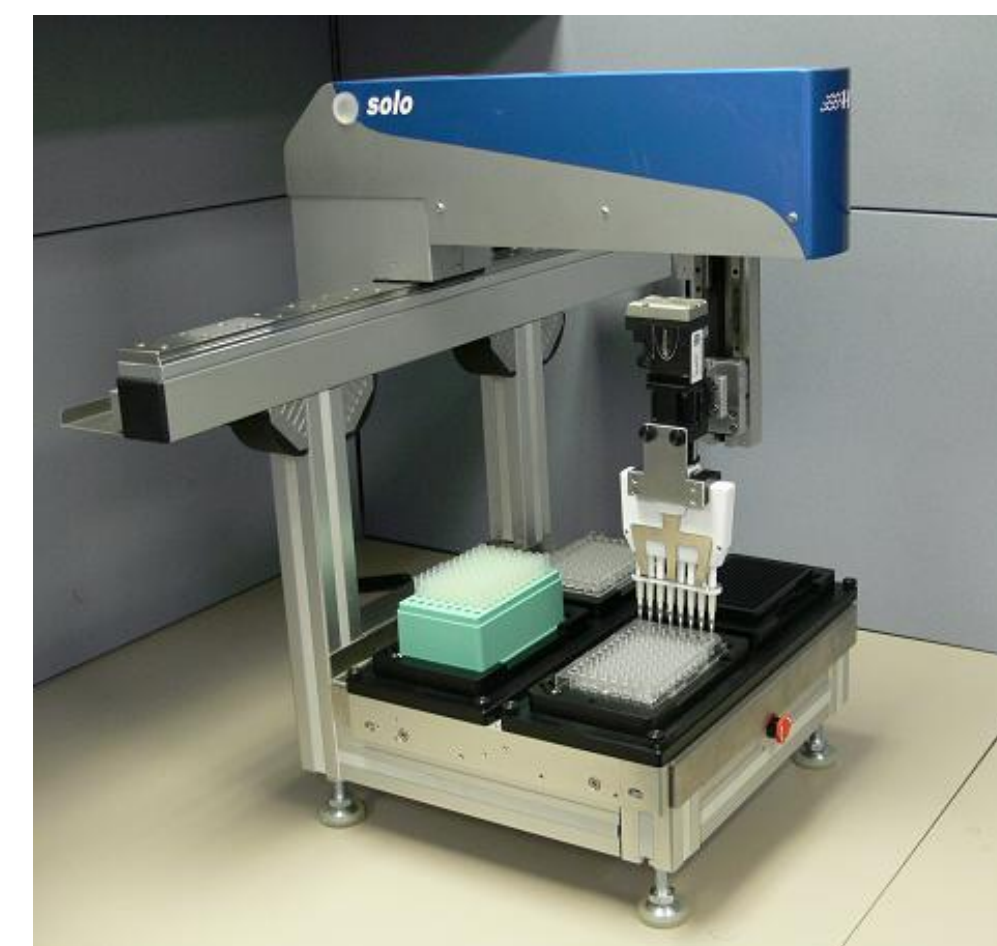


Figure 1. The Multichannel SOLO Pipettor

We were interested in developing an automated protocol for running Promega's ADP-Glo kinase assay (See next section). This assay includes a range of liquid transfers from 1 to 10uL.

We expected that the high resolution of the motor controlling the plunger would allow us to maximize the precision of all of these transfers.

3. The ADP-Glo Kinase Assay

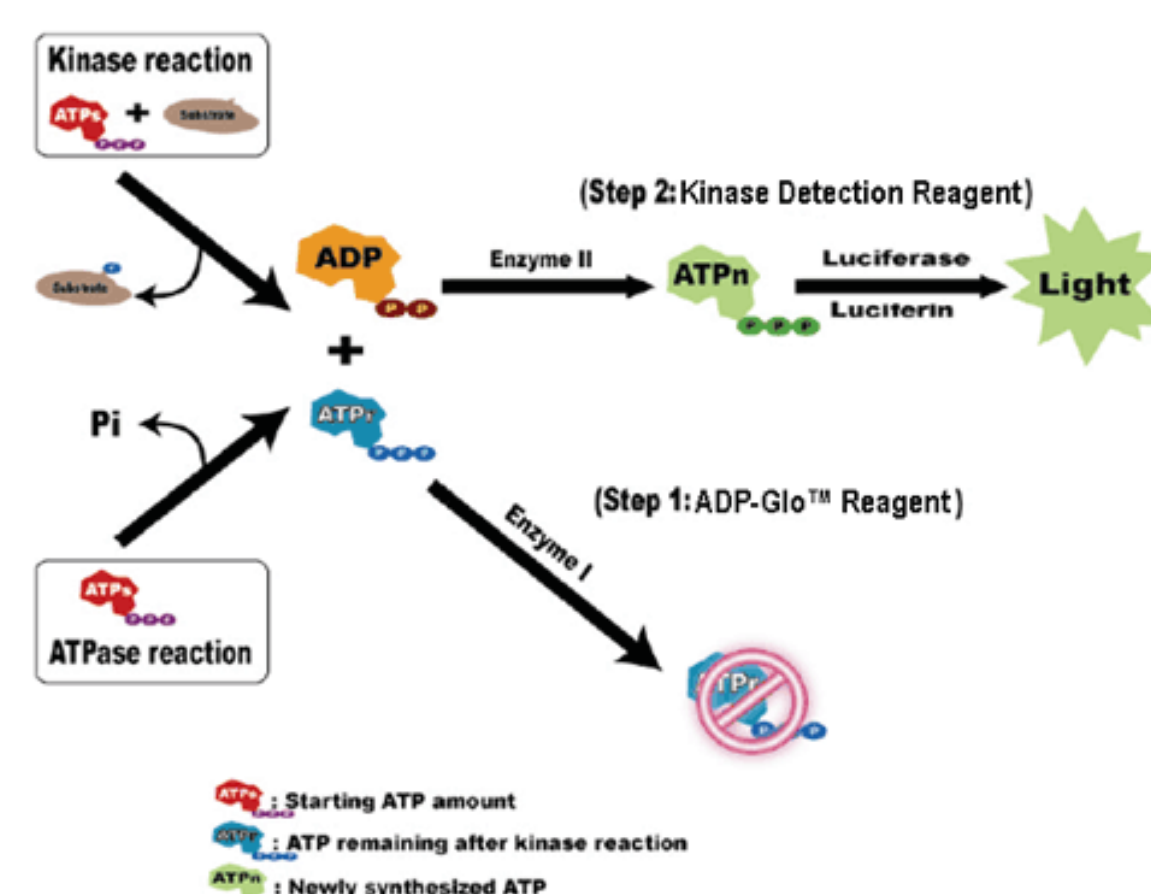


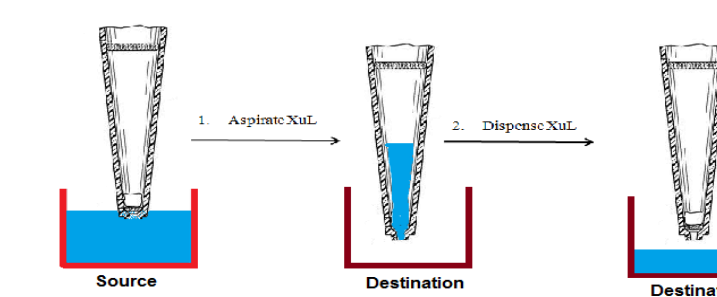
Figure 2. Overview of the ADP-Glo™ Assay.

ADP-Glo is a universal kinase inhibition assay which measures the amount of ADP generated. It follows a simple 2 step protocol in which the kinase reaction (enzyme/substrate/ATP/buffer) is quenched by the ADP-Glo reagent which also removed all unreacted ATP. A second detection reagent is then added that converts the ADP generated by the kinase to a measurable luminescence signal that directly indicates the effectiveness of the inhibitor being tested.

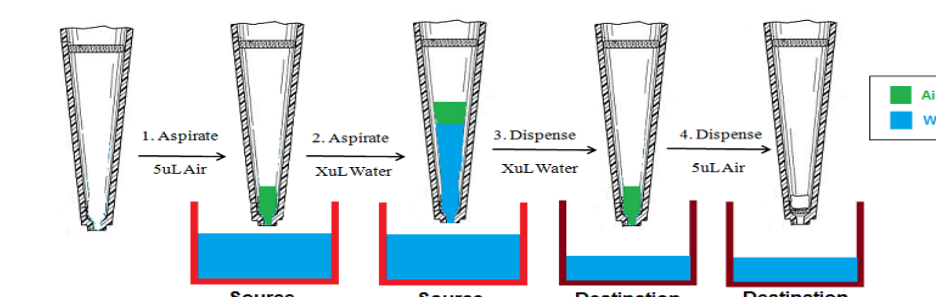
4. Pipetting Methods

We developed 4 general pipetting methods to be used on the SOLO.

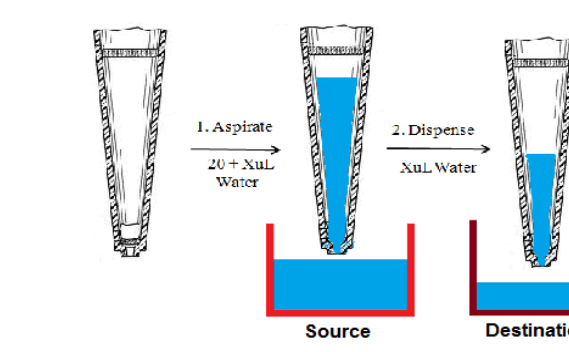
1. Direct Aspirate/Dispense– The most basic method in which the pipette aspirates and then dispenses a given amount. This accuracy of this method is problematic at low volumes, mostly due to liquid remaining in the tip.



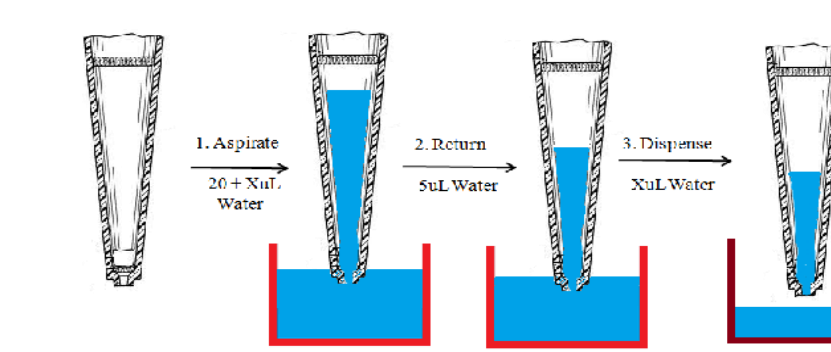
2. Pre-Aspirate/Blow-Off– The same as method 1 but preceded by aspirating a volume of air, followed by a blow-off of the same volume. The blow-off improves the accuracy of this method, but the increased ratio of air/liquid in the system results in reduced precision. This method most closely mimics that achieved with a standard hand-held pipettor.



3. Over-Aspirate – Similar to method 1 but with a larger aspirate amount. This approach combines the benefit of method 1 (decreased air vs method 2) with the benefit of method 2 (all desired liquid leaves the tip). As a result this method gives excellent accuracy and precision.



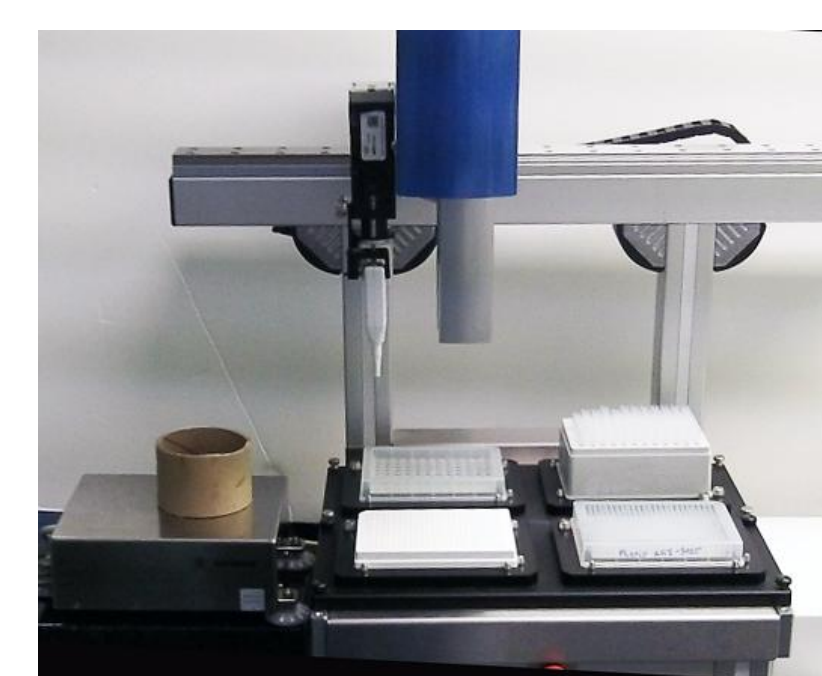
4. Over-Aspirate/Pre-Return – Similar to method 3 but with a small return of liquid to source before the actual dispense step. We have determined that the switch from upward plunger movement (aspiration) to downward movement (dispense), includes a small "backlash" effect that is only noticeable below 5uL, but can be eliminated by the initial dispense to source.



5. Gravimetric Testing

A simple automated gravimetric method was developed to determine the precision and accuracy of the SOLO using several different parameters at various volumes.

The track on which the pipette head moves extends several inches past the side of the SOLO, allowing movement of liquids beyond its deck. We have placed readers, heating/cooling/vacuum nests, washers and dispensers in this position. In this study, we placed a Sartorius microbalance in this position, as shown in figure 3.



A plug-in was written to allow communication between SoftLinX (Hudson's lab automation software) and the microbalance. This plug-in allows us tare the balance between experiments and retrieve weights after liquid is dispensed.

A SoftLinX protocol was created that iteratively zeroes the balance, runs a SoloSoft procedure, and stores the resulting weight in a table. After 20 replicates, the numbers are averaged and CV and Standard Deviations are calculated.

6. Results

This gravimetric procedure was carried out for each variation of interest. The volume dispensed, the pipetting method applied, and the type of disposable tip were varied. The results are summarized below:

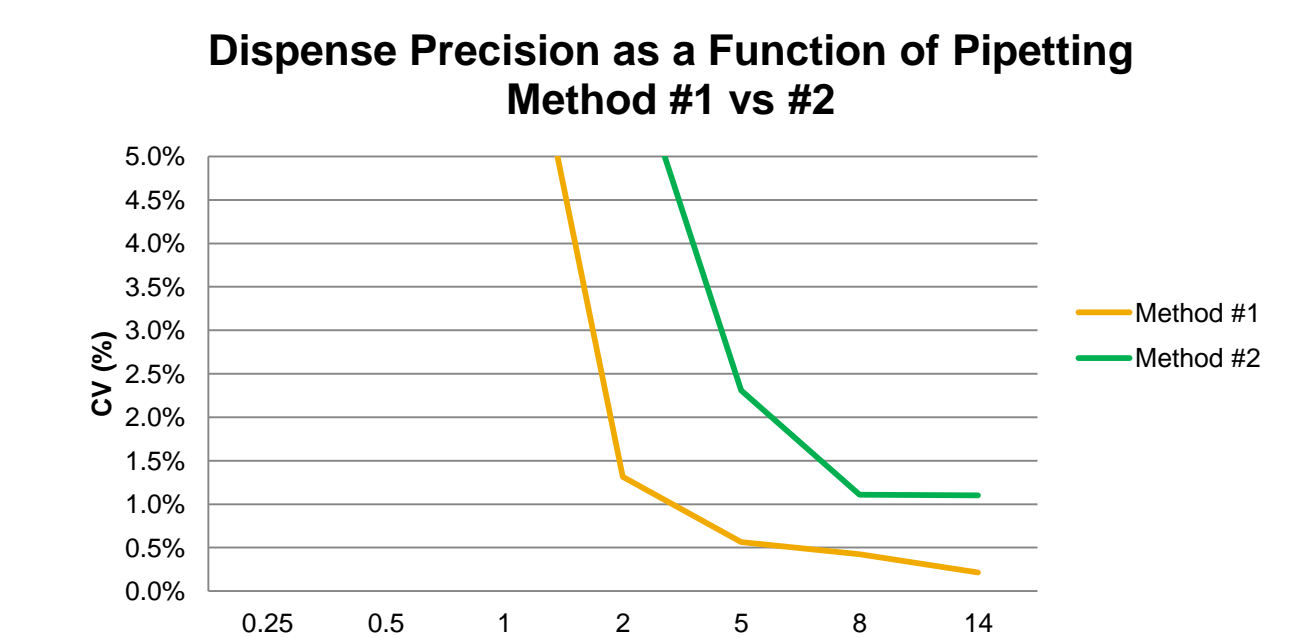


Figure 3: Shows the effect of Pre-Aspirate and Blow-Off on pipetting precision. Whereas a pre-aspirate and blow-off generally improves accuracy at low volumes, it clearly has a negative impact on precision. CVs become unacceptable using this method below 5uL, whereas direct aspirate/ dispense gives better CVs down to 2uL, both methods are unacceptable for dispensing lower volumes.

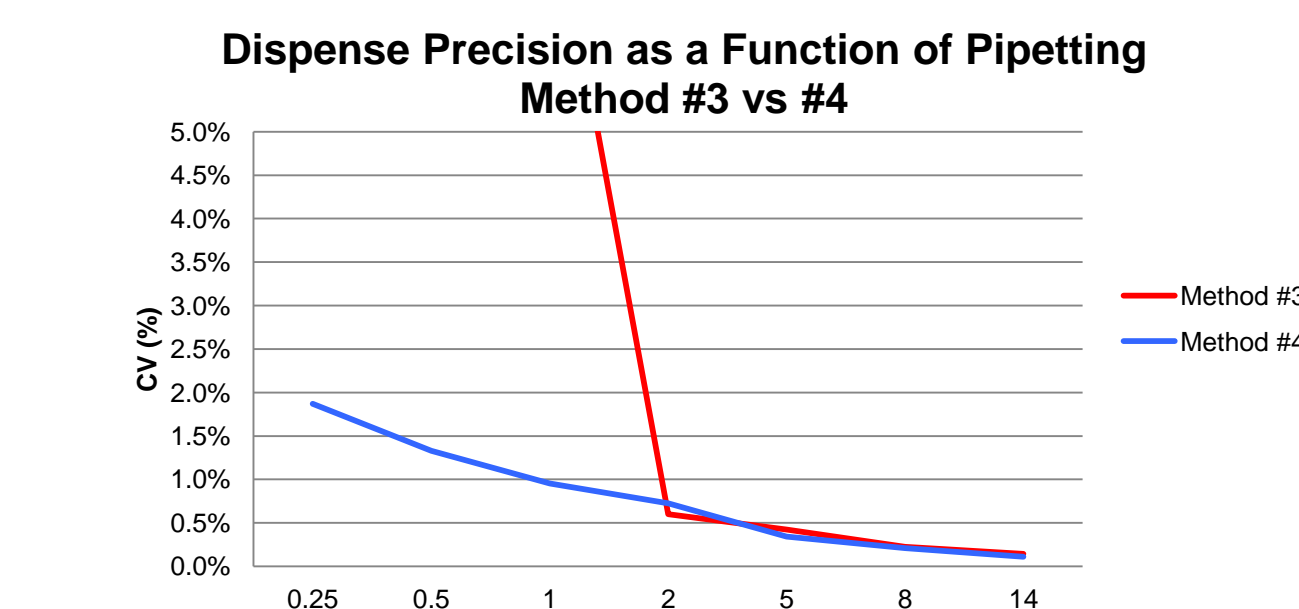


Figure 4: Shows the advantage of over-aspirating liquid before carrying out the dispense. Both methods gave improved precision through the range above 2uL (versus methods 1 or 2). However, only method #4 allows one to dispense volumes all the way down to 250nL with excellent precision (CV < 2%).

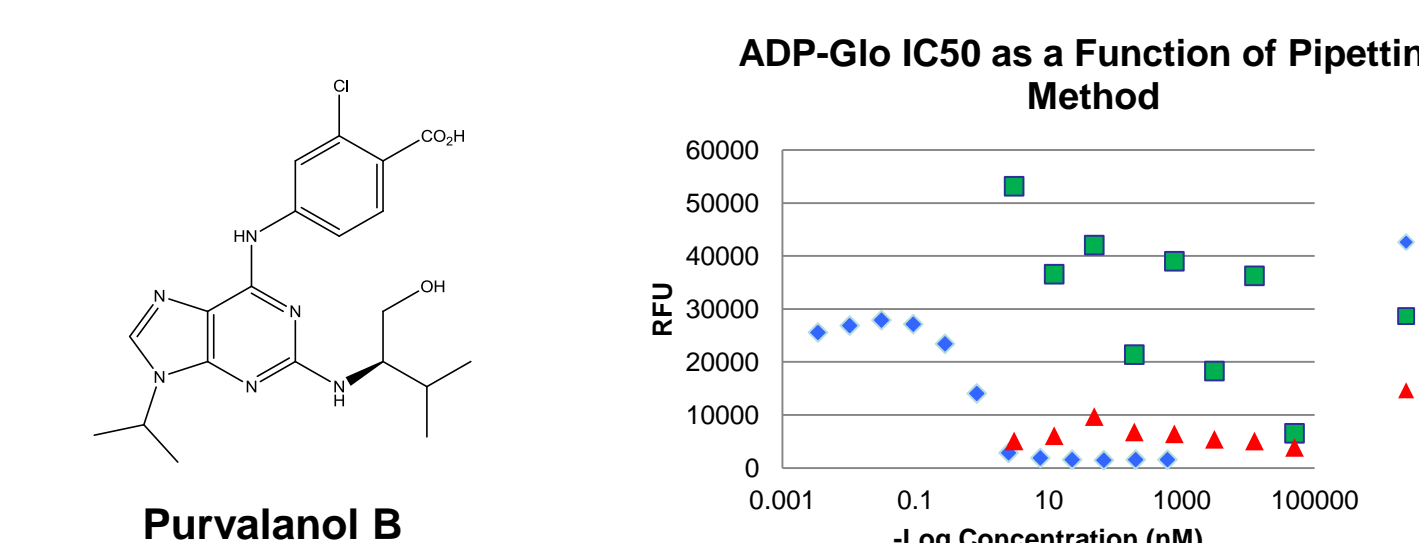


Figure 5: Shows the dose-response curves obtained using CDK2 Inhibitor Purvalanol B in the ADP-Glo assay. 1uL of the inhibitor was added to the kinase reaction mixture using methods 2, 3 and 4. Only method #4 resulted in high quality dose-response curves. Note the extreme variation in amplitude in the Method #2 experiment. This is due to the large variation associated with the pre-aspirate/blow-off method. The generally reduced values observed in the Method #3 experiment is due to incomplete dispense of the liquid resulting from the "backlash" effect, described above.

7. Conclusions

- Attaching a manual multi-channel pipettor to a motorized framework increases liquid handling precision approximately one order of magnitude (2uL down to 250nL).
- Modifications of the basic aspirate/dispense steps can greatly affect accuracy and precision. The more air in the system, the lower the observed precision.
- An automated gravimetric method was developed to determine the precision and accuracy of the new platform.
- Application of this methodology to the ADP-Glo assay resulted in highly reproducible data.