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5: Validating the Performance of Your Hydra II Microdispenser

Hydra II microdispensers are capable of performing liquid handling tasks with a high degree of precision and accuracy. A *precise* system dispenses an equivalent volume from each syringe across the syringe array, within a defined range of variance. An *accurate* system dispenses the correct volume from each syringe, again within a defined range of variance. To verify that your Hydra II microdispenser is performing optimally, you will want to test the system periodically for both precision and accuracy of dispense volumes.

This chapter outlines several tests that you can use to validate the performance of the Hydra II microdispenser. As you complete the tests for precision of dispense, you will want to compare your results to the specifications listed in Table 5-1. Accuracy for dispense volumes measured by gravimetry has been specified for a Hydra II system with 96 290µL syringes with the results listed in Table 5-2.

Dispense Precision of Hydra Syringes

Syringes for the Hydra II 96 microdispenser are available in sizes ranging from 100µL to 1mL. Generally speaking, the smaller the syringe and needle, the better the performance when dispensing low volumes. Table 5-1 shows the relationship between syringe size and dispensing precision at various volumes. The results show that a Hydra II microdispenser equipped with 100µL syringes can dispense 100nL of aqueous solutions into a plate containing buffer with a coefficient of variance (C.V.) of less than 10%. The table also shows that the minimum high-precision dispense volume increases with increasing syringe size. Selection of the correct syringe size for use on the Hydra II microdispenser will depend on the requirements of your application.

Table 5-1: Dispensing Precision of Hydra Needles: Coefficient of Variance

Syringe Volume	0.1µL	0.5µL	1.0µL	5.0µL	10µL
100µL	2.3%	2.2%	1.8%	2.1%	2.1%
290µL	–	–	2.8	2.1	2.2
580µL	–	–	2.4	2.3	2.2
580µL R/B	–	–	2.4	2.5	2.4
1mL	–	–	–	2.8	2.6

The following procedure was used in our laboratories to determine dispense precision for the Hydra II microdispenser, with results from that procedure shown in Table 5-1, preceding. Sodium fluorescein solution (4µg/mL in 1M Tris-HCl, pH 8.0 buffer) was dispensed into each well of a 96-well microplate containing 1M Tris-HCl buffer. The final volume in each well after dispensing fluorescein was 100µL. The plate was read at wavelength settings of 485nm excitation, 535nm emission. Values in the table represent the %C.V. of relative fluorescence units for one 96-well plate.

Tests for Measuring Precision and Accuracy of Dispense

The following tests have been used in our laboratories to measure dispense precision and accuracy. You can use these tests to validate the performance of your Hydra II microdispenser.

- Precision and linearity of response for different dispense volumes
- Gravimetric test for measurement of dispense volume accuracy
- Comparison to a standard curve as a measurement of dispense accuracy
- Evaluation of individual syringe performance: a visual tool for mapping performance of the Hydra II syringe array

To perform these tests, you must have:

- A fluorescence plate reader
- A basic spreadsheet/graphing software package
- Solutions as described in the various tests, such as standard fluorescein stock solution and Tris-HCl, pH 8.0
- Microplates as described in the various tests: 96-well plates for Hydra II 96 systems and 384-well plates for Hydra II 384 systems
- Electronic balance for measuring microplate weights
- Calibrated hand-held pipettors

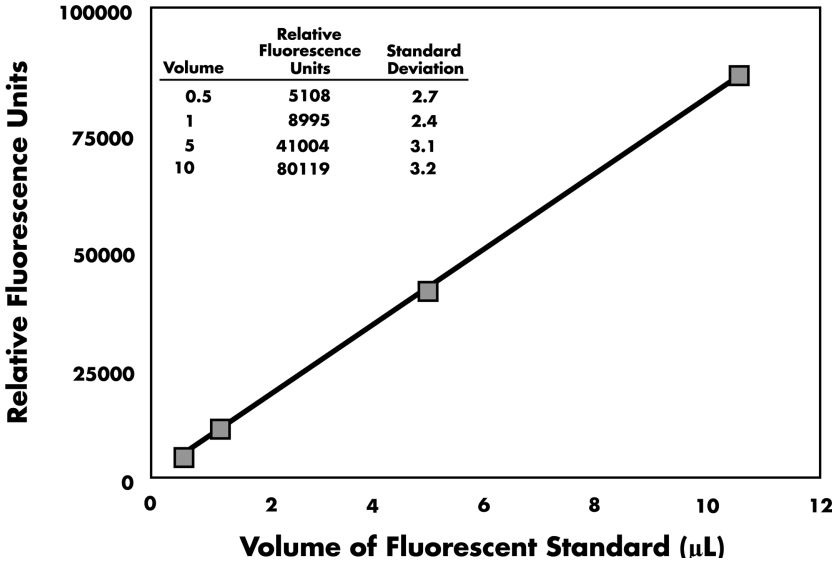
Measuring Precision and Linearity of Dispense Volumes for Hydra II Microdispensers

The precision of volumes dispensed across the array of 96 or 384 syringes of any size should have a coefficient of variance (C.V.) of less than 10% for dispense volumes of 0.1µL to 20µL. A protocol for measuring this parameter is outlined here so you can evaluate the current performance of your Hydra II microdispenser. To see detailed step-by-step instructions for this application, check the Technical Data for the Hydra microdispenser on the ApogentDiscoveries website at http://www.apogentdiscoveries.com/.

Before running the test, clean the needles and syringes of the Hydra II microdispenser thoroughly to ensure optimal performance. See Chapter 6 for information about how to clean needles and syringes.

A Hydra microdispenser equipped with 96 290µL syringes was used to dispense a 10µg/mL solution of fluorescein standard (Molecular Probes catalog #F-1300) suspended in 1M Tris-HCl, pH 8.0. For the reagent blank, each well of the 96-well plate was filled with a volume of buffer equal to 100µL minus the amount of fluorescein to be dispensed. For example, for a 5µL dispense volume, 95µL of buffer were added to each well of the microplate. This plate was then analyzed using the fluorescence plate reader (Bio-Tek® FL500) with filter settings at 485nm excitation and 530nm emission. Next, the syringes were rinsed three times with the fluorescein solution and then filled to 50µL. The desired volume was dispensed back into the reservoir three to five times to prime the syringes. The fluorescein solution was then dispensed into the plate containing the Tris buffer so that the final volume in each well was 100µL. After gentle mixing the plate was returned to the reader for analysis. This procedure was followed for each of the volumes listed in Figure 5-1.

Figure 5-1: Volume Dispensed vs. Relative Fluorescence



Each point represents the average fluorescence across all 96 wells of the microplate. The table within the figure lists the C.V. (standard deviation/mean) expressed as a percentage for each volume dispensed.

Tests for Measuring Accuracy

The next two tests describe methods for measuring dispense accuracy.

Measuring Dispense Accuracy of Hydra II Microdispensers With a Gravimetric Test

The following gravimetric test was performed to gauge the Hydra II microdispenser's dispensing accuracy. You can repeat the test to get a quick indication of your system's dispensing accuracy. For a method of obtaining a more detailed analysis of your microdispenser's dispense accuracy, see "Measuring Dispense Accuracy of Hydra II Microdispensers With a Standard Curve" on page 5-5.

The syringes of a 290µL syringe Hydra microdispenser were filled with distilled water. Syringes were primed by dispensing the desired volume into the water-filled reservoir. Water was then dispensed onto a dry, tared, MultiBlot® tray and immediately weighed. Table 5-2 shows the results of a test that measured the actual mass of water delivered onto a dry plate with test dispense volumes of 1.0µL, 2.5µL, 5.0µL, and 10µL. The results show that the instrument had an error in dispensing accuracy of less than 3% for each of the four dispense volumes.

Table 5-2: Results From Testing the Accuracy of Dispense Volume on a Hydra System* Equipped With 290µL Syringes

	Dispense Volume			
	1.0µL	**2.5µL**	**5.0µL**	**10µL**
1st dispense	0.095g	0.233g	0.470g	0.946g
2nd dispense	0.095	0.234	0.472	0.950
3rd dispense	0.096	0.234	0.469	0.949
4th dispense	0.093	0.232	0.467	0.954
Average	0.095	0.233	0.470	0.950
Std. Dev.	0.001	0.001	0.002	0.003
CV%	1.328	0.410	0.443	0.348
Expected	0.096	0.240	0.480	0.960
% Error	-1.302	-2.812	-2.187	-1.068

Note: *The results in Table 5-2 pertain only to the tests run on a particular Hydra system. To obtain dispense-accuracy values for your system, you must run similar tests on your system.

Measuring Dispense Accuracy of Hydra II Microdispensers With a Standard Curve

To ensure that the Hydra II microdispenser is dispensing accurately, a linear response curve and a standard curve should be performed periodically. For a detailed protocol on how to perform a linear response curve,check the Technical Data for the Hydra microdispenser on the ApogentDiscoveries website at http://www.apogentdiscoveries.com/.

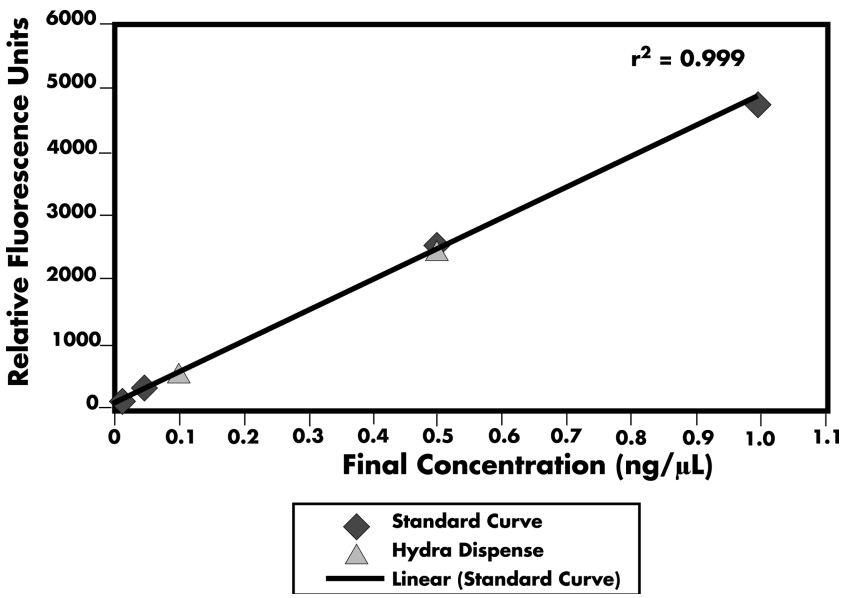
Comparing the volumes dispensed by the Hydra II microdispenser to a standard curve is the preferred method for determining the dispense accuracy for volumes less than 10µL. In the following test, calibrated hand-held Rainin pipettors (<1% error) were used to prepare standard solutions from a 10µg/mL fluorescein stock solution diluted with 1M Tris-HCl, pH 8.0. The final concentration of the standard solutions ranged from 10pg/µLto 1ng/µL. This concentration range yielded high fluorescence readings on a TECAN™ SPECTRA-Fluor™ with minimal background. One hundred microliters (100µL) of the standard solutions were transferred into microplates in triplicate wells. The plate was then read with a fluorescence plate reader and the data analyzed. The mean relative fluorescence units for all the wells of the plate were calculated for each concentration and then plotted (see Figure 5-2) against final fluorescein concentration (ng/µL). The fluorescence readings for 1µL and 5µL dispenses from a Hydra microdispenser (into buffer, total volume 100µL) were plotted and compared to the standard curve.

Table 5-3: Comparison of Predicted and Actual Fluorescence for 1µL and 5µL Dispenses

Microliters dispensed by Hydra micro-dispenser	Concentration (ng/µL) After Dispense into Buffer	Predicted Fluores-cence (from standard curve)	Actual Fluores-cence (Hydra microdispenser)	% Variation
1	0.1	5453	5160	-3.49
5	0.5	25562	24457	-0.66

The graph in Figure 5-2 shows the linear regression for the standard solutions with $r^2 = 0.999$. The relative fluorescence values of the volumes dispensed from the Hydra microdispenser are very close to the corresponding fluorescence values of the standard solutions, with a difference of -3.49% for 1µL and -0.66% for the 5µL dispense. This data supports the conclusion that the Hydra II microdispenser accurately dispenses microliter volumes in normal operation. This simple validation procedure, along with that for measuring the linearity of dispense volume, can provide you with confidence that the Hydra II microdispenser is operating according to our specification.

Figure 5-2: Relative Fluorescence of Hydra Dispense Volumes Plotted on a Standard Curve



Mapping the Performance of the Hydra II Microdispenser Syringe Array: A Visual Tool for Evaluation of Individual Syringes

A parameter that is important to examine is the variation of dispense volumes for individual syringes across multiple plates. Calculation of this indicator allows evaluation of the performance characteristics for each individual syringe and immediate identification of a syringe in need of repair or replacement.

For this test, ten 96-well plates were prepared with 99µL of 1M Tris-HCl, pH 8.0, in each well. A Hydra microdispenser equipped with 96 290µL syringes was then used to dispense 1µL of a 5µg/mL fluorescein solution (in the same Tris buffer) into all wells of each plate. Plates were read on a TECAN SPECTRAFluor fluorescence plate reader with a gain setting of 48 for all plates. The mean, standard deviation, and %C.V. (coefficient of variation) were calculated for the ten dispenses from each syringe. These results were arranged in tabular form and 3-D contour maps were generated in Microsoft Excel software to offer a quick view of overall performance.

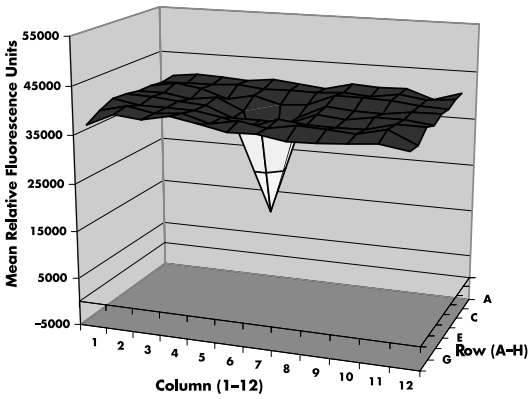


Figure 5-3: Mean Relative Fluorescence of a 1µL Dispense for Each Syringe, With a Defective Syringe in Position D6

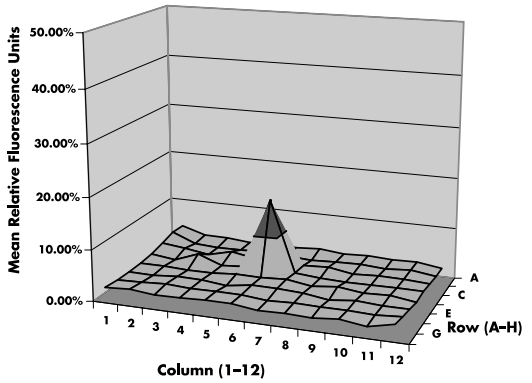


Figure 5-4: %C.V. of a 1µL Dispense for Each Syringe, With a Defective Syringe in Position D6

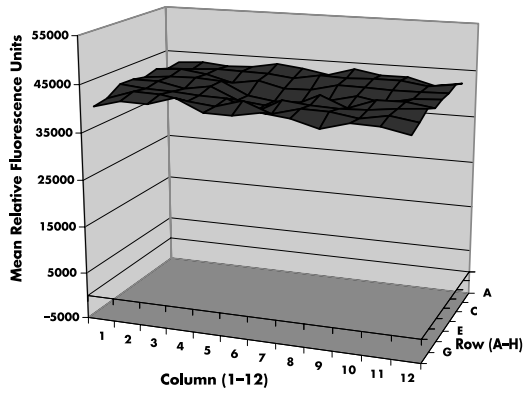
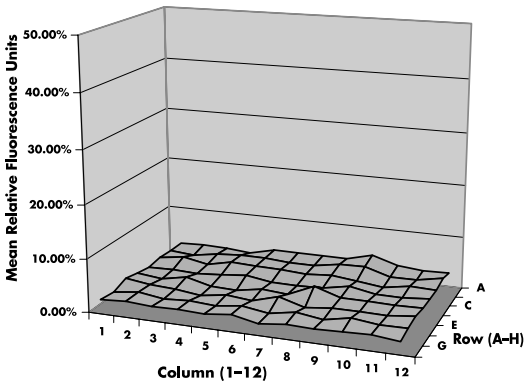


Figure 5-5: Mean Relative Fluorescence After Syringe Replacement

Figure 5-6: %C.V. After Syringe Replacement



The results in Figure 5-3 and Figure 5-4 show 3-D contour maps developed by plotting the mean and %C.V. values calculated for each syringe. The contours show minimal variation across the syringe array except for syringe D6. For this syringe the mean relative fluorescence is much lower than that for the other 95 syringes, as illustrated by the valley at D6 sinking below the overall contour for the array. This is a clear indication that the syringe is in need of repair or replacement.

Likewise, Figure 5-4 shows a peak at D6 signifying a large %C.V. for this syringe for the ten dispensings. After syringe D6 was replaced, ten plates were prepared as before and read. Figure 5-5 and Figure 5-6 show the results obtained from the same instrument after syringe D6 was replaced. The contour maps for mean relative fluorescence and %C.V. are now consistent across the syringe array.

The 3-D contour plots offer a quick, visual evaluation of two indicators representing different aspects of syringe performance. The mean relative fluorescence indicator shows whether a syringe is dispensing a greater or lesser volume than the other syringes in the array, while the %C.V. shows the variation between dispenses for an individual syringe. It is therefore possible to find that all syringes have similar values for mean fluorescence but that one syringe has a high variability (%C.V.) between dispenses. The opposite, a mean fluorescence value that consistently stands out from the rest of the syringes while the variability between dispenses is similar to other syringes, is also possible. In either case, the syringe should be repaired or replaced. You can use these indicators to evaluate and identify problems in single syringe performance and provide an accurate picture of the overall performance for the Hydra II microdispenser syringe array.