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High Throughput "Direct" Serial Dilution

by Phil Farrelly and P. Robert Reed

What if a solution could be found that would produce accurate, verifiable dilutions across an entire plate in less than 1 minute per target plate?

Dilution pitfalls

Recent discussions of problems associated with serial dilution have highlighted the need to improve throughput and accuracy so that increased demands for better and more complete IC_{50} dose-response analyses and other measures of activity can be met. What if a solution could be found that would produce accurate, verifiable dilutions across an entire plate in less than 1 minute per target plate, and which could run multiple target and source sample plates in a single setup? This report describes one possible answer to this need.

Serial dilutions are a time-honored means of providing response data over a regular logarithmic series of sample concentrations to aid in predicting the response of reactions to an agent in varying settings.

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Because desired dose concentrations of the agent compound are usually very small, the relative inaccuracy or inconsistency of traditionally available pipetting tools at such small volumes has required that the small concentrations be achieved by a series of sequential dilutions. This way, the capabilities of the pipetting tool are better fit to the size of the actual aliquot being handled. If done correctly, even nanoliter sample quantities can be correctly dispensed by ordinary pipettors when mixed in a large enough aliquot of solution.

However, the word "correctly" has serious implications and pitfalls. The normal process, which uses a microplate, is started by adding a volume of sample large enough to be adequately handled by the pipettor being used, to a volume of diluent in a well. Then, the two are mixed (typically by repeated aspiration/dispense actions), followed by aspirating part of the mixture and adding it to an adjacent well.

The process is then repeated for this and subsequent wells, ulti-

Figure 1. The nano25 provides CVs of 10% at 25 nL to <2% at 1 μL.

mately resulting in a logarithmic series of increasingly dilute sample concentrations across a row of wells, with a very small quantity of sample in the final well. Correctly accomplishing this is not easy. There are a number of potential problems which often result in incomplete or inaccurate sample distribution. The first concern is simply adequate mixing of each sample solution before aspirating it for the next well. This can vary by the solubility, viscosity, and temperature of the sample and its relative concentration in that well. Any incompleteness here will be compounded as each subsequent well is processed. There are also often components which incompletely disssolve in the diluent and may not mix at all, further skewing results of the analysis. If a robotic pipettor is being used, the user must assume that the volumes being aspirated/dispensed are within desired bounds, with no assurance that a clogged tip or air leak in the system has not caused a sample-handling error. Finally, the process usually takes a great deal of time, up to 20 minutes for a 96-well plate.

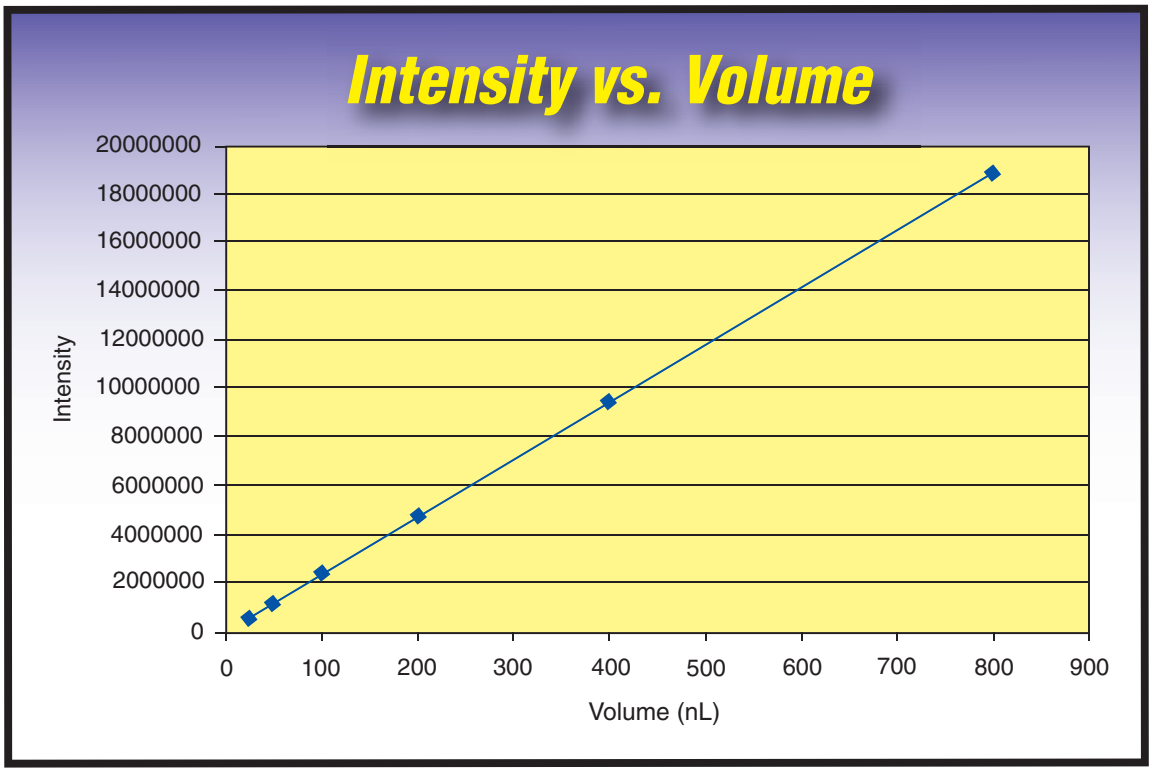

Figure 2. **Typical results for a serial dilution are illustrated below, where the correlation factor is greater than 0.9996. The sample dispensed was in 100% DMSO. The graph shows Fluorescence units as a function of requested volume.**

By feeding target plates while the pipettor is washing its tips and aspirating sample, a real throughput of one target plate per minute has been achieved.

Dispense directly into wells

A recent trial by a major pharmaceutical research lab of a new product from Hudson Control Group (Springfield, New Jersey), the nano25, has shown that these potential problems can be solved by direct addition of prescribed, decreasing amounts of source sample into pre-dispensed volumes of diluent in each well. Direct addition of the sample itself into the series of wells eliminates the need for the mixing necessary to traditional serial dilutions. Sample components that may be insoluble are not lost in the selected diluent. This way the entire desired amount of sample is placed into each well. Sample-handling errors are eliminated by a flow feedback system that keeps a log of the actual amount of sample that was dispensed. Even if an incorrect amount is dispensed, the dose-response curve would still be accurate because that amount would be known and would produce a valid activity measurement. However, because of this feedback loop, variations in volume caused by viscosity or temperature differences among different samples are overcome automatically by the system, producing consistent results regardless of these factors with no special actions required from the user. Finally, the time required to complete a 96-well plate is reduced to approximately 15 seconds dispensing time. Taking account of the time for washing its tips and aspirating the sample from its source plate, the system can produce a fully-diluted 96-well plate in under 1 minute.

The system being tested comes equipped with an automated stacker that can feed target and source plates directly onto the deck of the nano25. By feeding target plates while the pipettor is washing its tips and aspirating sample, a real throughput of one target plate per minute has been achieved, as the plate feeding and removal time is very nearly equal to the wash/aspirate time, resulting in little net impact from plate movements. Scanning each plate's barcode, then tying that into the nano25's dispense data log, produces a documented record of the actual sample amounts that were dispensed into each target plate, providing the IC_{50} analysis with the valid data it needs.

Dispensing accuracy is shown in the linearity of the test results from absorbance readings and weight analyses as shown in Figure 2.

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More information about serial dilution and related technologies is available from:

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