Serial dilutions or titrations of analytes and other assay components are key to assembling biochemical and cell-based assays in drug discovery and life science research. These assays are used, for example, in assay development to determine appropriate concentration ranges, in secondary screening to evaluate pharmacological response, and in early Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) to determine toxicological effects. The ability to rapidly and accurately produce dilution curves in an automated system is essential to improving assay throughput and quality.

The JANUS® Automated Workstation equipped with the Modular Dispense Technology® (MDT) permits users to rapidly assemble microplate assays with a variety of 96- and 384-well dispense tools.

Optional JANUS MDT Serial Dilution Tools (SDT) enable users to perform analyte pipetting in either a single row or column pattern using a single row or column of disposable tips from a standard box of tips. Moreover, Modular Dispense Technology permits rapid, “on-the-fly” swapping between standard 96- or 384-well heads and these serial dilution heads.

Serial Dilution Tools deliver maximum flexibility in the liquid handling processes. These tools have the same dynamic pipetting range (0.5 μL – 235 μL) as the standard MDT pipetting heads, are used with the same disposable tip options, and accommodate 96-, 384-, and 1536-well plate formats.
This application note presents case studies outlining performance capabilities, as well as applications-specific results, of the Serial Dilution Tools. This data includes pipetting accuracy and precision for a range of volumes, row and column configurations, plate types, and disposable tip sizes. Experimental data from AlphaLISA® standard curves constructed using the tools demonstrate enhanced versatility.

JANUS Serial Dilution Tools

<table>
<thead>
<tr>
<th>Serial Dilution Tool</th>
<th>Plate Type</th>
<th>Tip Type</th>
<th>Volume (µL)</th>
<th>Accuracy (%)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P30 Row/Column MDT Serial Dilution Tool</td>
<td>384</td>
<td>P10</td>
<td>0.5</td>
<td>1.21</td>
<td>2.04</td>
</tr>
<tr>
<td>P30 Row and Column MDT Serial Dilution Tool</td>
<td>0.5 – 50 µL</td>
<td>P30</td>
<td>9</td>
<td>1.40</td>
<td>0.69</td>
</tr>
<tr>
<td>P200 Row/Column MDT Serial Dilution Tool</td>
<td>5 – 235 µL</td>
<td>P200</td>
<td>25</td>
<td>1.20</td>
<td>1.20</td>
</tr>
</tbody>
</table>

Case Study 1: Pipetting Precision and Accuracy

**Materials and Methods:** The pipetting accuracy and precision were evaluated for each of the Serial Dilution Tools (P30 row/column, P50 row, P50 column, and P235 row/column) installed on a JANUS Automated Workstation. Serial Dilution Tools were tested in either row and/or column format for a range of volumes using corresponding PerkinElmer disposable tip sizes.

Pipetting accuracy was evaluated gravimetrically using a Mettler Toledo AT 261 Delta Range balance under controlled temperature and humidity conditions. Destination plates were prepared with known volumes of 80% DMSO filled using a PerkinElmer FlexDrop™ IV EX Precision Reagent Dispenser. Fixed volumes of 2% Orange-G dye solution (80% DMSO, Sigma-Aldrich, St. Louis, MO) were pipetted into destination plates with the SDT. Results for each condition were reported as percent accuracy, averaged across triplicate 96- (Nunc clear 96-well flat bottom), or 384- (Greiner Bio One clear 384-well flat bottom) well plates.

Pipetting precision evaluations utilized the above-mentioned Orange-G dye-containing plates. Immediately following gravimetric accuracy measurements, dispensed Orange-G dye plates were loaded, and absorbance readings were obtained, using a PerkinElmer EnVision® Multilabel Plate Reader. Pipetting precision (% CV) was determined using optical density values across each of the triplicate (N=3) 96- or 384-well plates.

**Results and Discussion:** The ability to dispense analytes with a high level of precision and accuracy is critical for any application. We evaluated the JANUS Serial Dilution Tools to demonstrate pipetting accuracy and precision in either row and/or column pipetting format(s) at common volume ranges and plate types. Results indicated that the Serial Dilution Tools performed with very high accuracy and precision (Table 1).

Table 1. Examples of Summary of Serial Dilution Tool sample pipetting performance data.

Case Study 2: Liquid Serial Dilution Performance Analysis

**Materials and Methods:** Fully automated serial dilutions were performed using the JANUS Automated Workstation, a P235 Row/Column Serial Dilution Tool and disposable P235 pipette tips. A stock solution of 10 nM fluorescein isothiocyanate (FITC, 1X phosphate buffered saline, pH 9.0) was serially diluted (two-fold) with 80% DMSO to a final concentration of 1/64th the stock concentration. Dilutions were performed on three replicate plates. Controls included positive (undiluted 10 nM FITC dye) and negative (80% DMSO buffer).

Fluorescence readings were obtained using a PerkinElmer Envision Multilabel Pate Reader for single 96-well plate (row format) serial dilutions. Results were plotted as observed fluorescence (relative fluorescence units, RFU) versus expected RFU (based upon the signal obtained with 10 nM FITC).

**Results and Discussion:** In order to evaluate the efficiency and reproducibility of an analyte pipetting, we used a model fluorescence-based assay. Fluorescent tags are widely utilized in applications such as nucleic acid and protein quantification, cellular metabolism and separation technologies. These procedures often require serially diluted gradients to serve as standard curves, or in the case of enzymatic reactions, optimization of enzyme/substrate ratios. The use of fluorescent tags...
offers a quantitative method of evaluating targeted molecules and provides a convenient and cost-effective methodology for evaluating sample protocols prior to introducing more expensive or scarce reagents.

The SDT P235 was used to serially dilute FITC fluorescent dye in row format. Results demonstrate a highly reproducible pipetting pattern for serial dilutions (Figure 1). Reproducibility of automated procedures allows users to perform complex dilution sequences, with reduced potential for human error or potential loss of valuable compound in repeated experiments.

**Case Study 3: Performance with Immunoassay Analytes**

**Materials and Methods:** AlphaLISA® Insulin and vascular endothelial growth factor (VEGF) immunoassay kits were obtained from PerkinElmer BioSignal (Montreal, Canada). Serial dilutions of analyze standards containing insulin or VEGF were performed automatically on a JANUS with a P50 Column SDT, and manually, using a multichannel pipette.

AlphaLISA Insulin Immunoassay Serial Dilutions. A column of 8 wells (PerkinElmer half-area white OptiPlates) containing insulin stock standards (10 μUnits/μL) was serially diluted two-fold (25 μL + 25 μL) in column format with AlphaLISA diluent buffer.

AlphaLISA VEGF Immunoassay Serial Dilutions. In an identical manner as the above-mentioned insulin immunoassay, a stock concentration of vascular endothelial growth factor (VEGF) standard (1.5 x 10E-5g/ml) was serially diluted in column format with AlphaLISA diluent buffer.

**Results and Discussion:** As an example of partial plate processing capability, we used the SDT to perform single column pipetting to generate serially diluted analyze standard curves for AlphaLISA (Amplified Luminescent Proximity Homogeneous Assay) immunoassays for insulin and VEGF. AlphaLISA is a no-wash ELISA assay alternative developed by PerkinElmer [1]. Insulin and VEGF are two common biomarkers whose concentrations are monitored for research diagnostic and screening applications.

The JANUS Automated Workstation equipped with a P50 Column SDT was used to effectively construct standard curves for both analytes. Manually generated standard curves compared favorably with those automated using the SDT (Figure 2). Results were consistent with previously reported AlphaLISA data generated for insulin and VEGF standard curves performed using a JANUS 8-tip Varispan™ pipetting arm [3-4].

**Conclusions**

JANUS Modular Dispense Technology Serial Dilution Tools provide an adaptable array of row and column pipetting options for standard curves, serial and direct dilutions, and discrete row / column sample additions. These pipetting capabilities are highly utilized in a number of areas including compound library screening in drug discovery, as well as in assay development and high density combinatorial assays.
JANUS Modular Dispense Technology Serial Dilution Tools:

- Have the same dynamic pipetting range (0.5 μL – 235 μL) as other MDT pipetting heads
- Use with the same disposable tip options as current MDT heads
- Accommodate 96-, 384-, and 1536-well plate formats
- Are interchangeable with all JANUS MDT pipetting heads, providing flexibility for seamless “on-the-fly” head swap operations without need for user intervention.
- AlphaLISA standard curves generated with the JANUS Serial Dilution Tools strongly compare with those performed manually.

The JANUS Serial Dilution Tools, together with full-plate pipetting using a standard MDT head, provides uninterrupted walk-away automation for the high throughput laboratory environment.

References

1. AlphaLISA brochure at www.perkinelmer.com