Dispensing Precision and Accuracy for the JANUS Modular Dispense Technology (MDT)™ Automated Workstation

Introduction

Today’s biotech, pharmaceutical, environmental, clinical research, and agricultural laboratories employ a diverse array of applications and depend upon automated systems to address the dynamic pipetting needs for these assays. Regardless of the application, users demand a high degree of accuracy and precision of the pipetting platform to ensure confidence in the final results.

The JANUS™ Automated Workstation is a robotic liquid handling system designed for flexible and efficient high performance pipetting. In addition to the versatility of 4- and 8-tip Varispan pipetting arms, JANUS multichannel dispense heads featuring MDT (Modular Dispense Technology™) further expand JANUS liquid handling flexibility. MDT allows for automated swapping of multichannel dispense heads without user intervention. The JANUS MDT Workstation may be configured with a combination of four multi-channel dispense heads capable of pipetting into 96-, 384-, and high density 1536-well plates, with volume ranges of 0.05 uL - 200 uL.

Pipetting accuracy and precision of automated liquid handling systems is essential for data quality and integrity. JANUS was specifically designed with pipetting performance in mind. WinPREP® is a flexible, powerful, and intuitive software application that controls the JANUS system. WinPREP has the unique capability to calibrate pipetting performance based upon liquid type, volume ranges, tip type, and dispense mode. WinPREP software uses Performance Files, which contain optimized settings for various parameters which affect the variability of the volume transferred. These parameters include aspirate and dispense speeds, air gap sizes, waste & blowout volumes, and movement delays.

Results reported in this application note demonstrate typical performance characteristics for an aqueous liquid transfer with physical properties similar to water. Reported performance was characterized by specific tip type, dispense mode, and dispense technique for each requested volume. Additionally, examples of data generated using the JANUS MDT Automated Workstation are discussed.

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Materials and Methods

Liquid handling protocols were created in WinPREP to aspirate and dispense the appropriate Sample Solution and Diluent for a requested volume into wells of a microtiter plate.

Measurement of the JANUS performance characteristics was accomplished with the Artel Multi-channel Verification System™ (MVS). The MVS utilizes ratiometric photometry to rapidly and simultaneously measure the accuracy and precision of independent dispensing channels. The system is a standardized platform and the measurement results are traceable to the National Institute of Standards and Technology. The MVS is composed of various components including aqueous-based dye solutions, dimensionally-characterized microtiter plates and a microtiter plate reader. The operating principles of the MVS involve dispensing the target volume of a sample solution into the wells of a characterized microtiter plate. This dispense is carried out by the liquid handling device that is being tested. Measurements were tabulated and summarized using MVS Data Manager software.

Pipetting performance was evaluated using contact dispense (see below), with the dispense mode dependent on the requested volume and tip type. Diluent was dispensed into empty MVS microtiter plate wells followed by transfer of the appropriate Sample Solution volume. The dispense height for the Sample Solution, was a minimum of 1 mm below the Diluent volume surface for each transfer.

JANUS liquid delivery calibrations are based on the use of performance files. Performance files ensure accurate and precise pipetting by defining system parameter values specific to tip type, volume requested, mode of operation, and liquid type. JANUS includes several performance files with WinPREP for dispensing various liquid types. The MDT Water performance file was used in this study which contains the necessary system parameters. Below are a few useful definitions:

<table>
<thead>
<tr>
<th>wastes mode</th>
<th>definition</th>
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<tr>
<td>Waste Mode</td>
<td>The requested transfer volume plus an excess volume is aspirated. The requested volume is dispensed to the appropriate container and the excess volume is discarded in a waste container.</td>
</tr>
<tr>
<td>Blowout Mode</td>
<td>A volume of air is aspirated prior to the requested transfer volume. The total volume (sample + air) is expelled during the dispense step. The air volume is used to force any remaining sample from the tip.</td>
</tr>
<tr>
<td>Dispense Back Volume</td>
<td>Volume of liquid to dispense back into source container after an aspirate operation. Used to reduce the effect of mechanical backlash on pipetting variability.</td>
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</table>

Pipetting performance data were collected on a single JANUS™ MDT Workstation. Two replicate dispense heads were used to collect data for each head type. Six replicate sample plates were dispensed per head for each individual volume. This results in 1152 data points per volume for a 96 channel head and 4608 data points per volume for a 384 channel head. Volume data sets were used to calculate the mean & sample standard deviation for the dispense volume, followed by calculations of inaccuracy (%) and coefficient of variation (% CV). The reported values for accuracy and precision were determined using the default system parameters outlined in the MDT Water Performance File.

Results & Discussion

MDT Liquid Handling Performance

Performance files provide a powerful and convenient method of automatically customizing liquid handling parameters for different liquid types. In the experiments performed, optimized system parameters for water were utilized to minimize dispense variability for all combinations of tip types and dispense modes (Table 1). Liquid transfers with a variety of dispense heads remained accurate across the entire volume dynamic range of 0.5 uL to 235 uL. Certain tip/volume/head combinations were more effective for pipetting precision as demonstrated by the 5 uL transfer data. For example, by using a P50 head with a P20 tips, variability was reduced by a factor of three, as compared to the P200 head and P235 tips. The P50 head is designed to pipette smaller volumes, and the P20 tips are well suited to low volume transfers due to their small inner diameter. In previous studies it has been determined that blowout mode is better suited for small volumes while waste mode is better utilized with larger volume liquid transfers. Precision of 0.5 uL transfers with the P30 384-channel dispense head fell under 5%
CV. Performance values observed for other volumes and dispense heads were even more impressive.

**JANUS MDT Application Case Studies**

**Case Study #1: Serial dilutions for AlphaLISA [1]**

**Materials and Methods:** Automated sample preparation for the AlphaLISA™ Insulin Detection Assay was performed on a JANUS Mini MDT, equipped with a P30 384-channel dispense head loaded with P30 clear disposable tips. Using the on-deck tip wash station, disposable tips were cleaned between transfers. Movement of labware (e.g. plate lids) was performed with the MDT Gripper Tool. For comparison purposes, manual liquid transfers were performed with a handheld multichannel pipettor. Assay setup was executed in accordance to the AlphaLISA product insert.

**Results and Discussion:** Single row or column pipetting may be performed using 96- or 384-channel MDT pipetting heads. This pipetting flexibility enables the end user to generate standard curves with an MDT dispense head partial tip load, followed by assay completion (i.e. multi-channel reagent dispensing) with all 96 or 384 tips. Utilizing this high throughput liquid handling flexibility, the MDT P30 384-channel dispense head was used to automate the PerkinElmer AlphaLISA Insulin Detection Assay. Comparing standard curves generated with either JANUS automation or manual multichannel pipetting, indicate very similar results (Figure 1).

**Case Study #2: Automation of Homogeneous Cytotoxicity Assays [2]**

**Materials and Methods:** ATPlite 1step cell viability assays were automated on a JANUS Cellular Workstation, equipped with an MDT P30 384-channel dispense head. Additional workstation equipment included a Variomag Teleshake, PerkinElmer EnVision 2100 Multilabel plate reader, Thermo CataLyst Express arm, and a Cytomat 2C incubator. U937 cells (American Type Culture Collection, Manassas, VA) were dispensed into 384-well plates (2,000 cells/well) offline with a PerkinElmer FlexDrop PLUS Precision Reagent Dispenser. Staurosporine and Actinomycin D were tested for cytotoxicity. Serial dilutions of these
agents, as well as transfers to cells were performed with the MDT head. Following 24 to 48 hours incubation under appropriate growth conditions, ATPlite 1step reagent was dispensed with the MDT head and plates were transferred to the EnVision for luminescence measurements. Data are reported as Relative Luminescence Units (RLU).

**Results and Discussion**

The U937 human cell line (diffuse histiocytic lymphoma) was used as a model system for cytotoxicity testing against Staurosporine (non-selective tyrosine kinase inhibitor) and Actinomycin D (DNA transcription inhibitor). U937 cells treated with serially diluted Staurosporine displayed a concentration dependent decrease in ATPlite assay fluorescence over a 48 hr incubation period (Figure 2). Additionally, ATPlite fluorescence for Actinomycin D treated cells was 3% of basal values at all compound concentrations, thus indicating negligible viability (Figure 3). Delivery of fixed concentrations of Staurosporine (25 uM) or Actinomycin D (5 uM) to 32 wells of cells with the MDT head produced consistent results across all wells (Figure 4). To expand upon this, Z’ measurements were performed in 32 wells between basal and compound treated wells. Z’ values for ATPlite measurements were calculated to verify the quality of cytotoxicity results after a 48 hr treatment over a series of 10 plates. These calculations indicated assay robustness and little variation from plate to plate (Table 2). Using the P30 384-channel MDT head ATPlite 1step assays were efficiently automated providing more than 30,000 data points within an 8 hr workday, representing an 8-fold increase in productivity over manual methods.

<table>
<thead>
<tr>
<th>Plate:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>Z’ 48 h Exposure of U937 Cells to Staurosporine</td>
<td>0.85</td>
<td>0.83</td>
<td>0.81</td>
<td>0.82</td>
<td>0.81</td>
<td>0.78</td>
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<td>0.79</td>
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<tr>
<td>Z’ 48 h Exposure of U937 Cells to Actinomycin</td>
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<td>0.83</td>
<td>0.80</td>
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<td>0.82</td>
<td>0.83</td>
<td>0.89</td>
<td>0.88</td>
<td>0.87</td>
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</table>

Table 2. Multiple 384-well plate Z’ measurements for Staurosporine (25 uM) and Actinomycin D (5 uM) treated U937 cells over a 48 hr period. Z’ values were calculated using ATPlite measurements from 32 wells of untreated cells (basal) and 32 compound-treated.
Case Study #3: Automation of sample preparation for sequence specific oligonucleotide probe (SSOP) hybridization - intermediate HLA typing [3]. This work was performed by Barone et. al at the Penn Jersey Region of the American Red Cross.

Material and Methods: Human Leukocyte Antigen (HLA) allele or allele groups (HLA-A, -B, and –DRB1) were determined for a collection of samples. A JANUS Automated Liquid Handling Workstation equipped with a P50 96-channel MDT dispense head was used for all liquid handling in:

- Reverse sequence specific oligonucleotide (rSSO) target DNA amplification with biotinylated primers.
- SSOP denaturing & neutralization procedures.
- Selected xMAP bead hybridization & labeling steps.

Amplification of SSO targets was performed using the One Lambda LABType® rSSOP kit reagents and HLA group-specific primer sets. Purified human genomic DNA (gDNA) served as template to generate biotinylated PCR products. These labeled products (approximately 800 bp in length) were then denatured and re-hybridized to sequence-specific oligo probes conjugated to Luminex® MAP® microspheres specific for polymorphic HLA regions.

Results and Discussion: Nucleic acid-based Luminex xMAP SSOP hybridization technology has been utilized for intermediate HLA typing procedures by the American Red Cross. Using purified genomic DNA from 96 test samples, biotinylated rSSO target DNA products were generated and examined for quality via agarose gel electrophoresis. The PCR primer sets for HLA-A and HLA-B screens were designed to produce two amplification products, whereas HLA-DRB1 primers yielded only one amplification product. UV transilluminated agarose gels containing PCR products for 96 samples amplified for HLA-A, -B, and –DRB1 intermediate screens display high quality reaction results (Figure 5).

Figure 6. HLA typing utilizing SSOP hybridization on xMAP microspheres. Data are HLA-B typing 100-plex fluorescence intensities from a single well for JANUS automated and manually prepared procedures. Data courtesy of the Penn Jersey Region of the American Red Cross [3].
Intermediate HLA typing utilizing Luminex xMAP microspheres produces a large amount of data per plate of analyzed samples. For example, 96 samples tested in the HLA-B allele group (100-plex) yielded 9,600 data points. Due to space constraints, only a single sample and short result comparison summary may be published in this application note. For comparison purposes several HLA typing screens were performed manually as well as on the JANUS Automated Workstation. Fluorescence intensities values closely matched between the two methods (Figure 6). Validation results demonstrate that data from automated HLA typing protocols are comparable to manual data (Table 3). Additionally, studies indicated that automated protocols are faster than manual setups. Automation of SSOP HLA typing resulted in reduced hands-on assay setup time, decreased user errors, and increased throughput improving laboratory productivity.

**Conclusion**

From assay development to medium and high throughput screening, scientists seek to improve throughput and reduce reagent/sample consumption, while maintaining a high level of data integrity. In all applications, pipetting dynamic range, precision, accuracy, and system flexibility are critical success factors. The JANUS MDT Automated Workstation demonstrates exceptional pipetting performance and provides high liquid handling precision and accuracy required for assay miniaturization and reagent conservation.

Case studies outlining specific applications using the JANUS MDT Automated Workstation demonstrate the utility and performance of high-density pipetting. From detection of nucleic acids and proteins, to cell cytotoxicity studies and more, the JANUS MDT Automated Workstation meets the demands of the high-throughput and high-content screening communities.

<table>
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<tr>
<th>Manual LABType</th>
<th>JANUS LABType</th>
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<tr>
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**References**

