APPLICATION NOTE

AUTOMATED WORKSTATIONS

Dispensing Precision and Accuracy for the JANUS Varispan Automated Workstation using VersaTip

Abstract

Today's biotech, pharmaceutical, environmental, clinical research, and agricultural laboratories employ a diversity of applications and depend more and more on 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 result. The JANUS® Automated Workstation is a robotic liquid handling system designed for the efficient and flexible automation of liquid transfer procedures with the precision and accuracy demanded. The data presented here demonstrate typical pipetting performance of the JANUS Varispan™ system equipped with the VersaTip® option.

Introduction

The JANUS Varispan Automated Workstation is a robotic liquid handling system designed for the efficient automation of sample preparation procedures used in pharmaceutical, biotech, academic and clinical research applications. The JANUS Varispan system, available with either a 4-or 8-tip pipetting arm, enables liquid transfers to be performed in multi-tipped mode from any combination of laboratory containers from test

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tubes, vials, reagent troughs to microwell plates for complete and flexible assay automation. Each sampling probe has an independent fluid path providing unique volume transfers per tip.

The VersaTip option provides enhanced versatility using a Varispan arm. The VersaTip can be used as a washable fixed tip probe or be used as a disposable tip adapter. When used as a fixed tip, this Teflon® coated probe may be washed after pipetting operations, or the tip can automatically be used as a disposable tip adapter using a selection of disposable tips with volume ranges of 0.5 µL to 1 mL.

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 and blowout volumes, and syringe delays. Performance files also include calibration parameters. A slope and offset can be obtained by linear regression analysis of dispense volumes in a specified range for a tip type. These allow the user to optimize pipetting accuracy for their specific instrument and for specific liquids.

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.

Materials and methods

Tests were created in WinPREP to aspirate and dispense the appropriate Sample Solution and Diluent for a requested volume into wells of a microtiter plate. Performance was evaluated using non-contact dispense. The dispense mode was dependent on requested volume and tip type. Sample Solution was dispensed into empty microtiter plate wells followed by the appropriate Diluent volume. The dispense height was established at a minimum of 5 mm above the final volume height for each transfer.

Liquid delivery calibration of JANUS is based on the use of performance files. Performance files ensure accurate and precise pipetting by defining ***system parameter*** values specific to a tip type, volume requested, mode of operation, syringe size, and liquid type. JANUS includes several performance files with WinPREP for non-contact dispensing of water. The Varispan arm performance files also include ***volume compensation*** settings (slope and offset), that WinPREP uses to compensate for differences between requested and actual volumes in a liquid transfer. The default system parameters were determined from a randomized sample set of JANUS instruments. Individual instrument performance may vary. For optimized results, instruments should be calibrated under expected usage conditions.

To calculate the slope and offset (y-intercept) values, consider the slope-intercept equation, shown in Eq. 1.

Eq. 1 $y = mx + b$

The component parts of the slope-intercept equation apply to WinPREP in the following way:

- y is the requested volume entered by the user
- m is slope value WinPREP reads from the performance file
- x is the actual volume delivered by JANUS
- b is the offset value WinPREP reads from the performance file

The slope and offset values are calculated by measuring requested versus actual dispense volumes over a range of volumes and then determining the linear regression line that fits those points. The slope and offset (y-intercept) values of the linear regression line are entered into the performance file. Conversely, for a single volume calibration, the difference between the actual versus requested volume can be calculated and inserted as the offset value for that volume while the slope value remains 1.

For each reported volume in this application note, a single volume calibration was performed per instrument. Performance file slope and offset values were initially set to 1 and 0 respectively. Twelve replicate uncompensated dispenses per tip were collected and the mean volume across all tips was calculated (data not shown). The difference between the mean volume and the requested volume was calculated and inserted into the offset value of the performance file. This calculated offset was used for the final data collection for the specified volume. Performance files containing the system parameters used within this study are listed in Appendix A. Below are a few useful definitions and guidelines:

Contact Dispense — The dispensed sample contacts liquid or solid interface. The adhesive and/or cohesive forces of a liquid are used to assist in removal of the liquid from the tip.

Non-Contact Dispense — Liquid is ejected into an air medium and does not contact a solid or liquid interface before being completely removed from the tip.

Waste Mode — The requested transfer volume plus an excess volume is aspirated. The requested volume is dispensed to the destination and the excess volume is discarded in a waste container. The excess volume in conjunction with an air gap significantly reduces the possibility of sample dilution by the system liquid when using fixed tips. Multiple dispense (single aspirate; multiple destinations) is only available in waste mode.

Blowout Mode — A volume of air is aspirated prior to the requested transfer volume. The total volume (sample + air) is expelled during the dispense step.

Measurement of the JANUS performance characteristics was accomplished with the Artel Multi-channel Verification System™ (MVS™). The MVS is a complete system utilizing ratiometric photometry to rapidly and simultaneously measure the accuracy and precision of independent dispensing channels for volumes between 0.01 – 200 µL. The MVS is a standardized platform and the measurement results are traceable to NIST (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. Measurement results were tabulated and summarized using MVS Data Manager software, with a final report that outputs individual dispense volumes and summary statistics for an evaluation. A detailed description for using MVS is provided in the Artel MVS user guide.

Results and discussion

Data was collected on three JANUS 8-tip Varispan systems with 500 µL syringes. Deionized water was used for system liquid. For any particular volume, 24 replicate transfers

Appendix A: Performance Files

WinPREP Software Performance Files

Tip Type	Dispense Mode	File Name
VersaTip Fixed Tip	Waste	WaterWasteFT_1 ml.prf
VersaTip Fixed Tip	Blowout	WaterBlowoutFT_1 ml.prf
1 mL Conductive Disposable Tip	Waste	WaterWaste 1 ml DT.prf
1 mL Conductive Disposable Tip	Blowout	WaterBlowout 1 ml DT.prf
200 µL Conductive Disposable Tip	Waste	WaterWaste 200 ul DT.prf
200 µL Conductive Disposable Tip	Blowout	WaterBlowout 200 ul DT.prf
20 µL Conductive Disposable Tip	Blowout	WaterBlowout 20 ul DT.prf

Appendix B: Sample Calculations and Definition of Terms:

Variable	Definition
X_i	The individual volume measurement of a single dispense
X_{ave}	The mean of all volume measurements
n	The total number of measurements taken
X_{exp}	The expected volume
ϵ	Molar Absorbtivity (L * mol^{-1} * cm^{-1})
l	Path Length (cm)
c	Concentration (mol * L^{-1})

Mean: $X_{ave} = \sum_{i=1}^{n} X_i / n$

Beer-Lambert Law: $A = \epsilon * l * c$

%CV: $\sqrt{\sum_{i=1}^{n} X_i^2 - X_{ave} \sum_{i=1}^{n} X_i / n-1)} * 100 / X_{ave}$

%Inaccuracy: $[X_{exp} - X_{ave} / X_{exp}] * -100$

were performed for each tip. This represented a total of 576 individual data points per volume. Data points were averaged to produce the mean dispense volume, followed by calculations of inaccuracy (%) and coefficient of variation (%CV) (see Appendix B). Non-contact performance files for water are included with all JANUS WinPREP installations. While default performance files typically address the performance required for many non-contact liquid transfers, we demonstrate the ease of JANUS performance optimization and report the values for accuracy and precision attained.

Utilizing the optimized system parameters for water, dispense variability is effectively maintained at a minimum level for all combinations of tip types and dispense mode. Certain tip/volume combinations are more effective for pipetting precision as demonstrated by the 1 and 5 µL blowout data. 20 µL conductive disposable tips are recommended for volumes 10 µL and less. These tips are well suited to low volume transfers due to their small inner diameter, which facilitates drop ejection from the end of the tip.

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. Each pipetting channel of the JANUS Varispan system allows for individual volumes to be pipetted on a per tip basis. Within each performance file, pipetting performance parameters are optimized for incremental volumes. The JANUS Varispan Automated Workstation with the VersaTip option demonstrated exceptional pipetting performance and provides the precision and accuracy needed for today's needs of assay miniaturization and reagent conservation.

The Artel MVS provided a fast, easy, and reliable method for testing of the JANUS instrument. The MVS is a standardized platform and the measurement results are traceable to NIST (National Institute of Standards and Technology).The MVS Data Manager software produces easy to understand reports, which allow for an immediate understanding of an instrument's performance capability.

Requested Volume (µL)	Dispense Mode	Mean Dispense Volume (µL)	Precision (%CV)	Mean Inaccuracy (± %)
VersaTip Fixed Tip				
100	Waste	100.65	0.65	0.65
10	Blowout	10.01	1.50	0.12
1	Blowout	1.04	6.66	3.64
1 mL Conductive Disposable Tip				
200	Waste	199.94	0.86	-0.03
100	Waste	100.08	1.80	0.08
50	Blowout	50.24	2.85	0.48
200 µL Conductive Disposable Tip				
175	Waste	174.96	0.47	-0.02
50	Waste	50.03	0.80	0.06
5	Blowout	5.01	2.21	0.29
20 µL Conductive Disposable Tip				
10	Blowout	9.95	1.33	-0.47
5	Blowout	5.00	1.31	0.00
1	Blowout	1.01	3.43	0.84

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