Rapid Accuracy and Precision Assessment of DMSO Compound Solutions Transferred with PocketTips®

KEITH J. ALBERT[a] DOUG KRONCKE[b]

ABSTRACT

Performing assays with liquid handling equipment to rapidly test and screen compounds is essential to drug discovery and other biopharma processes. In order to increase the productivity and integrity of such screenings, it is important to understand how accurately and precisely target volumes are being transferred, especially for critical and assay-specific volumes of DMSO-based compounds from compound management libraries. Accurate volume transfer is even more important for small volume transfers (nanoliter) where discrepancies or errors in transferred volume can have a significant impact on the validity of an assay. Screeners currently use many different liquid handling methods to transfer nanoliter target volumes of DMSO solutions to their assay, one of which is through the use of disposable PocketTips®. In this study, the MVS® Multichannel Verification System is employed to objectively and rapidly assess PocketTip transfer performance for nanoliter target volumes of DMSO solutions into microtiter wells filled with an aqueous-based buffer (diluent). The MVS uses a dual-dye, dual-wavelength absorbance method to simultaneously measure both the accuracy and precision of volumes transferred without the need for preparing standard solutions or calibration curves. The accuracy and precision results for DMSO nanoliter volume transfers with PocketTips into 384-well microtiter plates are presented and discussed. Some of the performance results for the PocketTips are also compared for two different solution types. Because environmental exposure and/or DMSO storage conditions can result in the uptake of water, many DMSO solutions have a substantial water component. In this study, a DMSO solution with approximately 75% DMSO (vol/vol) and another with approximately 95% DMSO (vol/vol) were transferred and the performance results are compared.

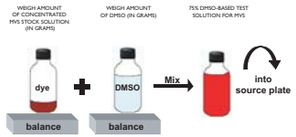
INTRODUCTION

The need to ensure quality in a laboratory process has become increasingly important, especially as it relates to the measurement of critical volume transfers of DMSO within microtiter plate-based assays. Knowing the exact volume transferred will inherently lead to confidence in the experiment, *i.e.*, the results can be trusted. Too often, however, the importance of liquid delivery is overlooked. In these situations, imprecise or inaccurate liquid delivery may not be diagnosed and may lead to a false sense of performance (for either the assay or the liquid handler). As throughput increases and assay volumes decrease, there are more demands for accuracy and precision of each volume transfer task. Most assays depend on proper volume delivery, but in many situations, the liquid handler is never calibrated nor verified for the critical transfer volumes. An alternative approach to dispensing accurate, nanoliter volumes of DMSO solutions to an assay is via PocketTips, which are disposable tips manufactured with a capillary channel on the internal portion of the tip. Due to surface tension differences between DMSO and aqueous reagents, the capillary channel can be filled with DMSO solution and then delivered to the assay in a repeatable fashion. The size of the capillary channel therefore dictates the amount of DMSO sample delivered.

MVS was used to rapidly assess the performance of PocketTips with various capillary channel sizes for DMSO-based solutions composed of approximately 75% DMSO (vol/vol) and 95% DMSO (vol/vol). MVS is a complete volume verification system consisting of a plate reader, computer with software, barcode reader, plate shaker, sample, stock and diluent solutions, a calibrator plate and a mobile cart. As discussed in this poster, the test solutions are dispensed into the destination plate via PocketTips, and the target volume is quantified well-by-well using dual-dye ratiometric photometry based on the Beer-Lambert Law.

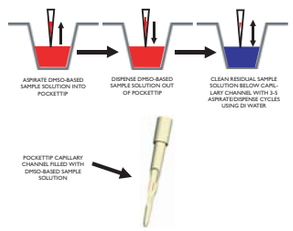
METHODS

Before testing the PocketTips for accuracy and precision, a DMSO dye-based test solution must be prepared so the MVS can photometrically measure the DMSO target volume. This procedure has been published[1] and will only briefly be discussed. Using the MVS software and/or MVS procedure guide, a concentrated dye solution (Stock Solution) is gravimetrically combined with DMSO to prepare the 75% DMSO (vol/vol) test solution.

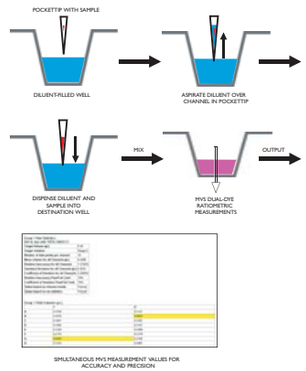


The weights of the stock solution and DMSO are entered into the MVS software. When Stock Solution and DMSO are combined, the concentration of the dye is calculated based on the dilution ratio of Stock Solution to DMSO[1]. *It should be noted that the 75% DMSO sample solution can be prepared by a current MVS user, however, the 95% DMSO solution is an Artel research-phase trial solution for proof-of-concept testing and cannot be tested by a current MVS user.* In the case for verifying dispense accuracy from PocketTips, the diluent is added to the well first, followed by the target volume of DMSO-based sample solution. The amount of target volume dispensed is quantitative and the amount of diluent dispensed is approximate, or qualitative[2]. The DMSO solutions were transferred with PocketTips using either an automated liquid handler or a handheld pipette.

Loading Tips from Source Plate



PocketTip Delivery and Measurement



MVS Target Volume Calculation

The volume verification approach is based on the Beer-Lambert Law:

$$A_\lambda = (\varepsilon_\lambda \cdot C) \cdot l = a_\lambda \cdot l$$

where a_λ represents the absorbance per unit pathlength for a solution, l is the pathlength, ε_λ is the molar absorbtivity constant, C is the concentration, and A_λ is the measured absorbance. The MVS dual-dye methodology requires absorbance readings for both dyes (dye in the DMSO solution and the dye in the diluent) to calculate the exact target volume dispensed into each well via published calculations[2]. The calculations and results are based on the concentration levels of the dyes, the measured absorbance readings, and the geometrical dimensions of the wells within the microtiter plate. Please see reference 2 for more information.

RESULTS

The results in Tables 1 and 2 show comparable performance when PocketTips are employed to transfer either the 75% or 95% DMSO-based test solution. For instance, when using one tip per transfer, the percent of DMSO in the test solution did not appear to affect the total amount of volume delivered. Results in Table 3 show that these tips can also be used for a few multiple repeat dispenses. However, it should be noted that the dye-based solutions employed for this study are known to be very water-soluble and therefore the tips and channels can be easily washed with the diluent and DI aspirate/dispense cycles. If sticky and/or complex molecules are employed in these tips and the users do not understand, or cannot validate, the potential for carryover or contamination, it is highly recommended to use one tip per transfer.

In its own laboratory, nAscent BioSciences batch tests PocketTips during quality control using a typical titration curve method incorporating DMSO and standard fluorescein dyes. This test method employed by nAscent BioSciences yielded results for the same PocketTip models tested with the Artel MVS system (Table 4). These performance results compare and correlate well to the performance data acquired with the easy-to-use MVS.

Table 1. 75% DMSO-based test solution

PocketTip type (nL)	Plate Type	n*	MVS Measurement Results: Mean (nL)	Standard Deviation (nL)	Relative Inaccuracy %	CV%
Automation - 96-ch Caliper Sciclone						
50	*384-w Corning 3711*	*n* = 96	**46.2**	**1.7**	**-7.63**	**3.75**
100	*384-w Corning 3711*	*n* = 96	**105.1**	**3.9**	**5.08**	**3.71**
250	*384-w Corning 3711*	*n* = 96	**266.1**	**13.7**	**6.45**	**5.15**
Handheld - 8-ch pipette, manual						
50	*384-w Corning 3711*	*n* = 16	**50.9**	**3**	**1.96**	**5.9**
100	*384-w Corning 3711*	*n* = 16	**102.6**	**3.2**	**2.6**	**3.12**
250	*384-w Corning 3711*	*n* = 4	**271.8**	**8.5**	**8.72**	**3.11**

* *n* = number of tips tested, *i.e.* , each tip was used only once

Table 2. 95% DMSO-based test solution

PocketTip type (nL)	Plate Type	n*	MVS Measurement Results: Mean (nL)	Standard Deviation (nL)	Relative Inaccuracy %	CV%
Automation - 96-ch Caliper Sciclone						
50	*384-w Corning 3711*	*n* = 96	**50.9**	**1**	**1.82**	**1.88**
100	*384-w Corning 3711*	*n* = 96	**100.9**	**1.3**	**0.9**	**1.29**
250	*384-w Corning 3711*	*n* = 96	**261.4**	**2**	**4.56**	**0.77**
Handheld - 8-ch pipette, manual						
50	*384-w Corning 3711*	*n* = 16	**51.2**	**0.9**	**2.4**	**1.76**
100	*384-w Corning 3711*	*n* = 16	**101.7**	**1**	**1.7**	**0.98**
250	*384-w Corning 3711*	*n* = 16	**262.2**	**3.3**	**4.88**	**1.26**
500	*96-w Artel Plate*	*n* = 16	**517.1**	**5.2**	**5.53**	**1.01**

* *n* = number of tips tested, *i.e.* , each tip was used only once

Table 3. PocketTips employed for 3 repeat dispenses*

PocketTip type (nL)	Plate Type	*DMSO (vol/vol) in test solution*	MVS Measurement Results: Mean (nL)	Standard Deviation (nL)	Relative Inaccuracy %	CV%
50	*384-w Corning 3711*	*75%*	**51.9**	**1.04**	**3.9**	**2**
50	*384-w Corning 3711*	*95%*	**55.93**	**0.76**	**11.87**	**1.35**
50	*384-w Corning 3540 LV**	*75%*	**47.5**	**1.3**	**-4.93**	**2.74**
50	*384-w Greiner 788096 LP**	*75%*	**48.6**	**1.6**	**-2.67**	**3.23**
100	*384-w Corning 3711*	*75%*	**109.2**	**0.99**	**9.2**	**0.9**
100	*384-w Corning 3711*	*95%*	**106.6**	**0.15**	**6.66**	**0.14**
100	*384-w Corning 3540 LV**	*75%*	**103**	**2**	**3**	**2.05**
100	*384-w Greiner 788096 LP**	*75%*	**106.6**	**9.7**	**6.63**	**0.91**

* *all data collected using a single-channel handheld pipette; LV = low-volume microtiter plate; LP = low-profile microtiter plate.*

Table 4. Accuracy Determination of PocketTips via Internal Method

PocketTip type (nL)	Determined Volume of Channel (nL)
50	55
100	106.1
250	253

DISCUSSION

The MVS is a standard platform that enables the volume verification process to be rapid, accurate and precise. Once the DMSO test solution is prepared, all results are instantly processed using the dual-dye dual-wavelength measurement process. Because most compound management groups store their compound libraries in DMSO solutions, verifying dispensing performance of DMSO, especially at varying DMSO concentrations, is of extreme importance, especially to the high-throughput screening community. If the dispensed volume of DMSO is inaccurate, there is a likely chance that the perceived concentrations of species in solution of the DMSO-based assay (targets, antibodies, antigens, *etc.*) will also be inaccurate, potentially leading to false-positive or false-negative intepretations.

This poster addresses some important results for understanding PocketTips performance when transferring nanoliter volumes of both 75% and 95% DMSO test solutions (vol/vol). PocketTips offer nanoliter dispensing and are compatible with existing liquid handling equipment, as shown here with the use of these tips on a 96-channel Caliper Sciclone and various handheld pipettors. Results show that the MVS system confirms the accuracy and precision performance for PocketTip transfers, which ultimately allow users to perform assays quicker, prevent compound precipitation, eliminate intermediate dilutions, conserve valuable compound, and reduce reagent costs through miniaturization. The data collected also verified that PocketTips perform consistently using either automated or handheld pipettors, allowing the scientist to accurately develop assays from the bench to automation using nanoliter transfers.

REFERENCES

(1) Albert *et al. J. Assoc. Lab. Autom.*, **2006**, *11*, *172-180.*
(2) Bradshaw *et al. J. Assoc. Lab. Autom.*, **2005**, *10*, *35-42.*

Measuring for life... precisely.

[a]Artel, Inc., 25 Bradley Drive, Westbrook, Maine 04092 www.artel-usa.com

[b]nAscent BioSciences, Inc., 25 Walnut Street, #102, Wellesley Hills, MA 02481 www.nascentbiosciences.com