Performance Validation of the AVENTIX Scarlett™ 01 Volume Analysis System: High-Precision Volumetric Analysis of DMSO in 384-Well Microplates

Abstract

Precise liquid handling verification is essential for pharmaceutical screening and assay development. This application note details the formal technical validation of the AVENTIX Scarlett™ 01 Volume Analysis System (Model SVA-01). Conducted in collaboration with VTT MIKES, the National Metrology Institute of Finland, this study evaluates the system's accuracy in measuring 10 µL volumes of Dimethyl sulfoxide (DMSO) within a 384-well microplate. The results demonstrate that the Scarlett system provides SI-traceable, non-contact volume verification that meets stringent industry accuracy criteria.[1]

Introduction

The Scarlett 01 is a patent-pending, non-contact volume analysis system designed to provide rapid verification of liquid handling performance. The system determines liquid volume by optically measuring the liquid height and meniscus profile within microplate wells, converting these physical dimensions into volume via established calibration curves.

This application note describes an independent validation study conducted with VTT MIKES, the National Metrology Institute of Finland, to evaluate Scarlett's ability to measure 10 µL DMSO dispensed volumes in a 384-well microplate with an accuracy requirement of ±5%.

Measured volumes were compared against a traceably calibrated volumetric syringe, providing a reference volume linked to the International System of Units (SI) through gravimetric calibration.

Materials and Traceability

Testing was conducted using a Greiner Bio-One 384-well microplate (PN 781101). The reagents used included Dimethyl sulfoxide (DMSO, ≥99.9% purity), and White mineral oil (Vaseline oil; White Oil FU 102).

A critical component of this validation is the establishment of SI-traceability through gravimetric calibration. All reference volumes were delivered using Hamilton glass syringes traceably calibrated by RISE (Research Institutes of Sweden) according to ISO 8655 methods.[2][3] Table 1 shows the traceably

calibrated syringes that were used to establish the system calibration curve and deliver the test volumes for this study. A Chaney Reproducibility Adapter (PN: 32146) was attached to each syringe to ensure consistent, highly reproducible dispenses.

Table 1: Reference Equipment for Calibration and Testing

Equipment	Make/ Model	Serial number	Nominal Volume (µL)	Calibrated volume (µL)	Measurement Uncertainty ±
Glass syringe (1 - 10 µL)	Hamilton 801RN	84853	10	10.12	0.7%
Glass syringe (0 - 25 µL)	Hamilton 802RN	84855	20	20.06	0.5%
Glass syringe (0 - 50 µL)	Hamilton 805RN	84857	40	39.76	0.3%
Glass syringe (0 - 100 µL)	Hamilton 810RN	84859	60	59.94	0.3%
Glass syringe (0 - 100 µL)	Hamilton 810RN	84859	80	80.20	0.3%
Glass syringe (0 - 100 µL)	Hamilton 810RN	84859	100	99.56	0.2%

The calibrated dispensed volume used as the reference value in the study was 10.12 µL ± 0.07 µL (95% confidence).[2] For simplicity, this dispensed volume is referred to as a "10 µL dispense" throughout this document.

VTT MIKES and RISE are National Metrology Institutes participating in the international measurement infrastructure responsible for maintaining and disseminating SI traceability. Both were critical partners in this evaluation study.

Measurement Method

Scarlett determines liquid volume by optically measuring liquid height within each microplate well and converting the measured liquid height into a calculated volume based on either *a*) known well dimensions, or *b*) a calibration curve for the liquid/plate combination. (See Figure 1). For this study, a calibration curve was used.

Calibration Basis

Prior to testing, a calibration curve for Vaseline oil was established using traceably calibrated Hamilton syringes

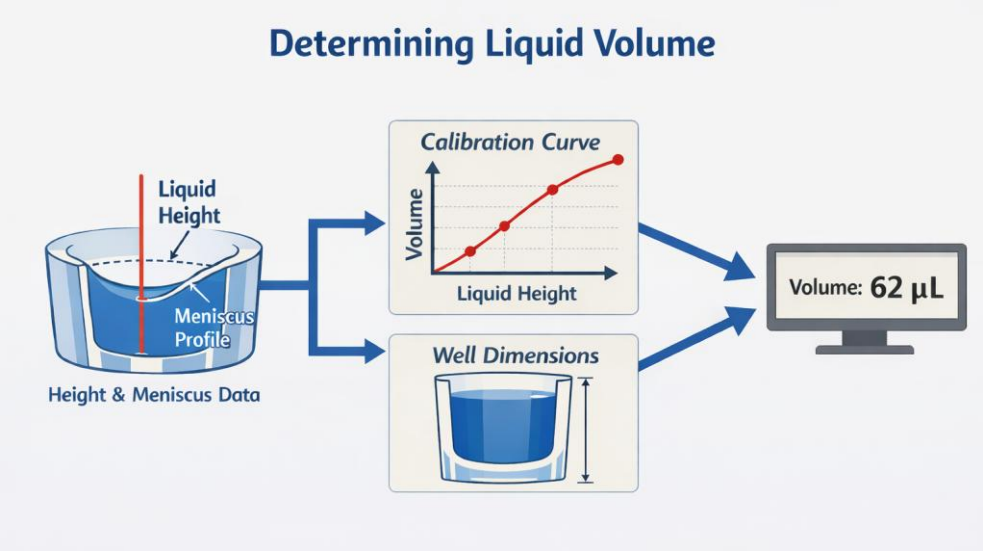

Figure 1. Scarlett determines liquid volume in a microplate well by measuring liquid height and converting the measurement to volume by either using a calibration curve for a specific liquid/plate combination, or by using the geometric well dimensions and measured height.

ranging from 20 µL to 100 µL nominal volumes. The resulting curve was fit with a polynomial, resulting in the relationship of $Y=0.0977X^2+10.667X+5.0013$ (R^2 = 0.9998), which enables the system to link measured liquid height to volume.

Test Procedure

The steps of the validation protocol monitored by VTT MIKES included:

1. **Empty Plate Measurement:** The empty 384-well test plate was measured by the Scarlett system to map the height of each empty well bottom. This information was stored by the SensVue system software.

2. **Base Layer Dispensing:** A base layer of approximately 50 µL of Vaseline oil was dispensed using a hand pipette into 18 wells across three rows (row B, I, and P). This oil layer serves two critical functions: a) it provides a stable optical interface for reliable height detection, and b) it minimizes water absorption by the DMSO during the measurement process.

3. **Oil Volume Measurement:** The volume of Vaseline oil was measured using the predetermined calibration curve, and the previously measured empty plate bottom height.

4. **Target Dispensing:** A 10 µL aliquot of DMSO was dispensed into the wells on top of the 50 µL of Vaseline oil using a calibrated Hamilton glass syringe.

5. **Repeat Volume Measurement:** The volume of the 50+10 µL of liquid was measured with the Scarlett system.

6. **Calculated Volume:** The volume of DMSO added was calculated as the difference between the pre- and post-dispense measurements.

Results

Measured DMSO dispensed volumes are summarized in Figure 2, and Tables 2 and 3. Measurements were made according to the test protocol for selected wells at the top, middle and bottom part of the well plate (selected wells highlighted in Figure 2).

All measured values were within the ±5% acceptance criterion defined in the validation protocol. The results demonstrate agreement between Scarlett measurements and the SI-traceable calibrated syringe volume, which served as the reference standard for the evaluation.

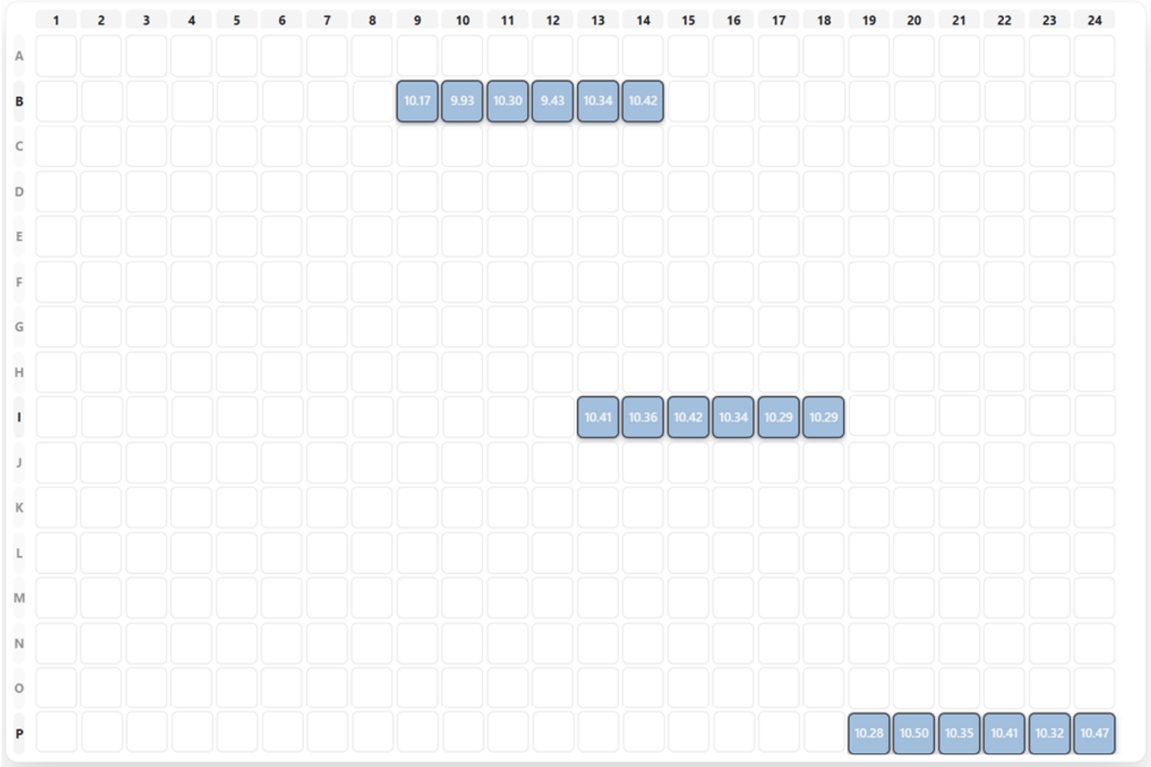

Figure 2. Final measured DMSO volume (µL) calculated as the difference between the measured well volumes before and after dispensing 10 µL onto approximately 50 µL of Vaseline oil. Image shows the calculated volumes for the wells used as part of the test process.

Table 2: Volume measurement results by the Scarlett system

Well Position	Measured Volume (µL)							
	#1	#2	#3	#4	#5	#6	**Average**	**Standard Deviation**
Row B (9-14)	10.17	9.93	10.30	9.43	10.34	10.42	10.10	0.37
Row I (13-18)	10.41	10.36	10.42	10.34	10.29	10.29	10.35	0.06
Row P (20-24)	10.28	10.50	10.35	10.41	10.32	10.47	10.39	0.09

Table 3: Measured volume of DMSO by test row and combined rows

Plate Row	n	Mean Measured Volume (µL)	Relative Error vs Calibrated Syringe	Acceptance Criteria	Statement of Conformity
Row B	6	10.10	-0.2%	±5 %	**PASS**
Row I	6	10.35	2.3%	±5 %	**PASS**
Row P	6	10.39	2.7%	±5 %	**PASS**
All Rows	18	10.28	1.57%	±5 %	**PASS**

Measurement Uncertainty Considerations

While a full uncertainty budget for the Scarlett system was not the primary goal of this study, the reference volume uncertainty was established at ±0.07 µL (k=2) at a calibrated volume of 10.12µL. The validation confirmed that the combined effects of height measurement repeatability, calibration curve uncertainty, and microplate dimensional variation remained well within the ±5% industry acceptance threshold.

Conclusion

This study demonstrates that the AVENTIX Scarlett 01 Volume Analysis System can accurately measure microliter-scale liquid volumes dispensed into microplates using non-contact optical measurements. Independent validation performed with VTT MIKES showed strong agreement between Scarlett measurements and a SI-traceably calibrated syringe reference volume.

With measured deviations ranging from -0.2% to 2.7%, the system provides a robust, non-destructive solution for auditing and verifying liquid handling performance in high-throughput laboratory environments.

Measured values differed from the reference volume by less than 3%, well within the ±5% acceptance criterion defined for the study.

These results support the use of the Scarlett system for verification of liquid handling performance in pharmaceutical screening, compound management, and assay development workflows.

References

[1] VTT MIKES – National Metrology Institute of Finland. *Validation Report VO-26-0199: Third-Party Assessment of the Aventix Scarlett System.* 10 March 2026.

[2] RISE Research Institutes of Sweden. *Calibration Certificate for Hamilton Glass Syringe 801RN (SN 84853)*. Certificate No. 105104-1351299-K06, 4 February 2026.

[3] ISO. ISO 8655-9:2022 — *Piston-Operated Volumetric Apparatus — Alternative Methods for the Determination of Measurement Error.*

[4] JCGM 100:2008. Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement (GUM).

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