# Glycerol Solutions for MVS® Volume Verifications

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## Introduction

Glycerol plays an important role in many industries and because of its physical and chemical characteristics, it is used extensively in life science industries. Pharmaceutical and other life science laboratories use glycerol for innumerable sample preparations and assays. Glycerol is often combined and/or used as an additive in aqueous-based solutions and these solutions are used for many types of experiments. For purposes of this application note, the term *glycerol solution* is employed to refer to an aqueous sample solution with less than 20% glycerol by volume. Aliquots and target volumes of glycerol solutions are usually transferred to the sample holder or assay test plate using a pipettor, such as a multichannel handheld pipette or an automated liquid handler. In many cases, accurate transfer of the target volumes is vital to the integrity of the assay. More importantly, many laboratories do not have an easy and rapid way to verify the accuracy and precision of the pipetting equipment, especially for complex reagents such as glycerol solutions. Described herein is a method to employ the Artel Multichannel Verification System (MVS®) to measure the volume of glycerol solution transferred from a pipettor under test into a microtiter plate. Much of the information in this document has been explained in detail in reference 1.

## Requirements

(1) MVS, with Data Manager software 2.0 or higher
(2) Training on MVS operation
(3) MVS Diluent, Stock, and Baseline Solutions
(4) MVS Calibrator plate
(5) Glycerol solution with known density value
(6) 96-well or 384-well microtiter plates
(7) Pipettor or liquid handler
(8) Analytical balance
(9) Sample container, preferably amber bottle with cap
(10) Transfer pipettes or small funnels

More specifically, this document describes the proof-of-concept methodology employed to verify 8-µL target volumes of a ~20% glycerol solution (v/v) in a standard 96-well plate using the MVS. Additionally, this application note serves as a guide for preparing custom glycerol solutions for MVS verification at specific target volumes. It should be pointed out that glycerol solutions can be measured and quantified with the MVS between *approximately* 0.4 – 49.9 µL in a standard 96-well plate or 0.1 to 9.9 µL in a standard 384-well plate.



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## Materials & Methods

All purchased materials were used as received. Glycerol (Biochemika Ultra; 1.262 g/mL, MW 92.10 g, $C_3H_8O_3$) was acquired from Sigma-Aldrich. The MVS and all MVS-related consumables were from Artel (Westbrook, ME). A Sartorius R160D analytical balance was employed for all gravimetric determinations. All solutions were allowed to equilibrate to room temperature prior to being used. All solution components were transferred to the analytical balance with disposable transfer pipettes and subsequently weighed on the analytical balance. Following each component addition, the balance was allowed to reach equilibrium, the information was collected and the balance was tared before the next component was added. In all cases, a clean, amber glass bottle and cap were used to prepare and store the glycerol solutions. Once all components were added, the bottle was mixed by inversion at least 20 times.

## Proof-of-concept Testing

*To verify the 8-µL transfer of a ~20% glycerol solution (v/v) in a 96-well plate, four steps were performed:* (a) an initial 21% glycerol solution (v/v) was prepared; (b) this glycerol solution was gravimetrically-combined, and therefore slightly diluted, with MVS Stock Solution to prepare the glycerol (alternative) test solution; (c) the weight and density of the glycerol solution and weight of the MVS Stock Solution were incorporated into the MVS Data Manager software; and (d) the prepared glycerol test solution was dispensed with the liquid handler followed by volume verification and analysis using the MVS. These four steps are summarized in **Figure 1** and are described in more detail below.

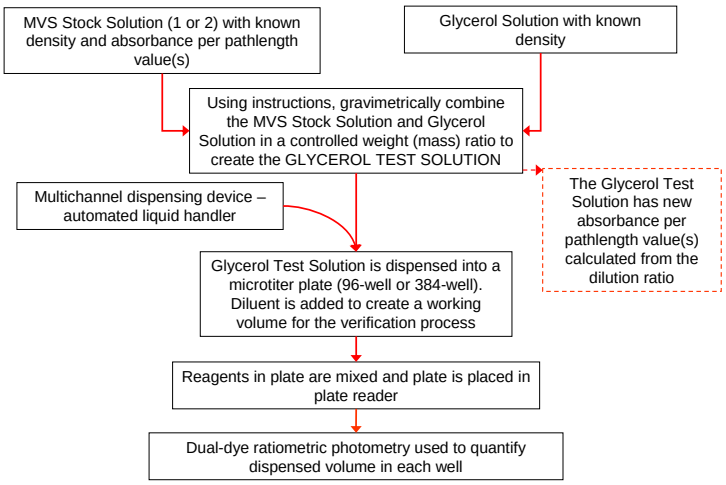


**Figure 1.** Overview of the method used to prepare and verify target volumes of glycerol solutions using the MVS

*(a) Preparation of a ~20% glycerol solution (v/v).* For proof-of-concept testing, the initial glycerol solution was simply a combination of deionized water and glycerol. In many cases, the user will have the glycerol solution readily available. A 21.4% glycerol solution (v/v), or 25.6% glycerol by mass, was prepared by combining 8.6293 g glycerol with 25.0169 g deionized water (33.6462 g total). Note, the density value of pure glycerol is approximately 1.262 g/mL and this value was used to calculate the amount of glycerol in the prepared glycerol solution. See **Figure 2** for the preparation details for the initial glycerol solution. In order for a target volume of this solution to be verified with the MVS' photometric technology, gravimetric combination with an MVS Stock Solution must be achieved, *i.e.*, MVS dye must be added to the glycerol solution to enable photometric measurement of target volumes.

For preparing other glycerol solutions at varying concentrations, or to obtain the approximate density values for solutions with 0.5% – 20% glycerol (v/v), please refer to **Table 1** at the end of this document.

| | | |
|---|---|---|
| (1) DI water added = | 25.0169 | g |
| (2) pure glycerol added = | 8.6293 | g |
| total weight of solution = (1 + 2) = | 33.6462 | g |
| % glycerol (g/g) = [(2/(1+2))*100] = | 25.6472 | % |
| (3) density of water = | 1.0 | g/mL |
| (4) density of glycerol = | 1.262 | g/mL |
| (5) vol water = (1/3) = | 25.0169 | mL |
| (6) vol glycerol = (2/4)= | 6.8378 | mL |
| (7) total volume = (5+6) = | 31.8547 | mL |
| (8) % glycerol (v/v) = [(6/(5 + 6))*100] = | 21.4656 | % |

**Figure 2.** Details for the preparation of the initial 21% glycerol solution (v/v).

*(b) Preparation of the glycerol test solution.* Using easy-to-follow instructions[3], the prepared 21% glycerol solution (v/v) was combined with MVS Stock Solution 1 to create the glycerol "alternative" test solution. The process is summarized in **Figure 3. The term *alternative* is used in the MVS Data Manager software to describe a custom test solution that is chemically different from the commercially-available, aqueous-based MVS Sample Solutions.** For purposes of this application note, the custom alternative test solution prepared with glycerol is simply referred to as the *glycerol test solution*.

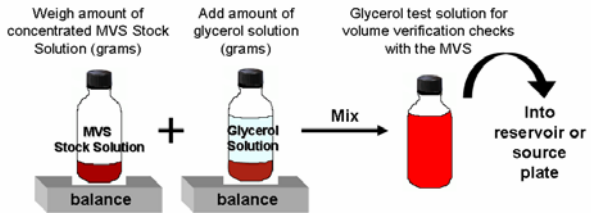


**Figure 3**. Overview of the preparation of the glycerol test solution from the glycerol solution and the MVS Stock Solution.

This glycerol test solution is then dispensed into a microtiter plate for volume verification and analysis with the MVS. For preparing other glycerol test solutions with varying amounts of glycerol, or to obtain information on the approximate amount of MVS Stock Solution 1 or 2 to add to the glycerol solution, please refer to **Table 2** at the end of this document.

*(c) Enter necessary information into MVS Data Manager software.* The density value of the prepared 21.4 % glycerol solution (v/v) is approximately 1.06 g/mL[2]. In the case presented here, all of the glycerol solution (33.6462 g) was combined with 3.5189 g MVS Stock Solution 1 in an amber glass bottle to prepare the glycerol test solution. The weight and density values of the glycerol solution and the weight of MVS Stock Solution 1 were entered into the MVS Data Manager software. The dye concentration in the final, glycerol test solution is calculated based on the dilution ratio of the MVS Stock Solution 1 to glycerol solution. In the example discussed here, as shown in **Figure 4**, the resulting glycerol test solution is comprised of 9.9 % of MVS Stock Solution 1 (v/v) and 90.1 % of the initial 21% glycerol solution (v/v).

The resulting glycerol test solution therefore had a final glycerol concentration of 19.34 % (v/v). Note, 90.1% of a 21.47 % glycerol solution is equal to 19.34 % glycerol in the test solution. This 19.34% glycerol test solution (v/v) was designed and prepared to incorporate similar absorbance per pathlength values as the commercially-available aqueous MVS Sample Solutions[1]. For example, the volume range for the custom solution described herein is similar to the aqueous-based MVS Range C Sample Solution, which covers 2.0 – 9.9 µL in a 96-well plate.

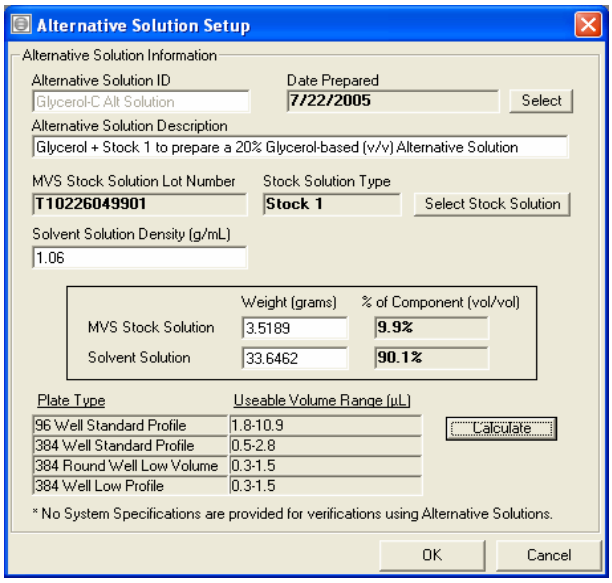


**Figure 4**. Graphical user interface for preparing custom test solutions in MVS Data Manager software. The weight and density values of the MVS Stock Solution 1 and glycerol solution are employed to calculate the usable volume range for various plate formats. For example, the proof-of-concept glycerol test solution shown can be dispensed from a liquid handler into a 96-well plate and the target volume can be assessed with the MVS for accuracy and precision between 1.8 – 10.9 µL or between 0.5 – 2.8 µL in a standard 384-well plate.

*(d) Transfer and performance analysis of prepared glycerol test solution.* After the necessary information (weights, density values) was entered into the MVS Data Manager software, 8 µL target volumes of the prepared glycerol test solution were dispensed into a microtiter plate for MVS volume verification and analysis. The proof-of-concept testing for this solution was performed with calibrated syringes. All syringes are gravimetrically-calibrated using an accredited method in a controlled test laboratory. *Because the reproducibility of the syringes has been proven, they can be used as volume transfer standards thereby removing device variability from the test procedure*. The gravimetrically-calibrated 8-µL Hamilton (Reno, NV) syringes with reproducibility adapters were used to aspirate target volume from a vial filled with the 19.34% glycerol test solution followed by a dispense into a 96-well microtiter plate. To each well containing the target volume, 192 µL of MVS Diluent solution was added to achieve a 200-µL working volume[4] in the 96-well plate. The two solutions in each well were mixed using the MVS plate shaker before the plate was placed into the MVS plate reader for measurement.

**Results and Conclusions**

The 19.34 % glycerol test solution (v/v) was employed to monitor solution stability over time. Using the calibrated 8-µL Hamilton syringe with modified reproducibility adapter, the solutions were dispensed in replicates of eight into a 96-well Artel Verification Plate on each day of testing. The stability, or shelf-life, of the prepared test solutions, as monitored by relative inaccuracy (all values are within ± 1.7 %), shows that the glycerol test solution was stable for at least four weeks. The coefficient of variation (%CV) values, or the precision data in the measurement, were within ± 0.5 % (**Figure 5**). These results indicate that it may be possible for individual users to employ this methodology to assess device performance for specific or proprietary glycerol solutions for critical volume transfers.

**Discussion**

The proof-of-concept for preparing and verifying dispensed target volumes of the 19.34% glycerol test solution (v/v) has been shown. Although this application note does not discuss the preparation or testing of other glycerol test solutions, *i.e.*,

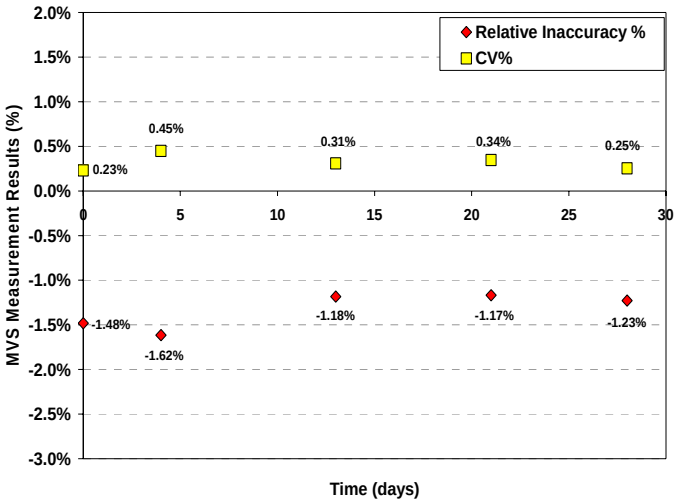


**Figure 5.** Using an 8-µL calibrated syringe, the 19.34% glycerol test solution was verified on five different days over a 28-day period. Based on the relative inaccuracy values, the shelf-life of the solution may be at least 4 weeks.

solutions with < 20 % glycerol by volume, the functionality in the MVS Data Manager software does support trial-and-error experimentation for users that wish to test their specific glycerol solutions. *Alternative test solutions containing > 20% glycerol (v/v) have not been evaluated by Artel*.

**Table 1** (below) can be used as a guide for preparing glycerol solutions when glycerol portions are < 24 % by mass (or < 20 % by volume). **Table 2** (below) can be used as a guide for adding the appropriate amount and type of MVS Stock Solution to the glycerol solution to prepare an appropriate glycerol test solution that covers a specific volume range in either 96-well or 384-well standard profile plates. For example, preparing a glycerol test solution that covers 5 µL in a standard 96-well plate is different than preparing a glycerol test solution that covers 5 µL in a standard 384-well plate.

Please note, the final glycerol test solution will have a lower concentration of glycerol as compared to the initial glycerol solution because of the addition of the aqueous-based MVS Stock Solution. For instance, a 24 % glycerol solution by mass (or 20 % glycerol by volume), may be diluted to approximately: (a) ~ 18 % glycerol by mass for target volumes between 0.4 – 4 µL in 96-well plates; (b) ~22 % glycerol by mass for target volumes between 2 – 11 µL in 96-well plates; or (c) ~ 19 % glycerol by mass for target volumes between 9 – 49 µL in 96-well plates. These values are from column *k* in **Tables 1** and **2**.

## References

(1) Albert, K.J.; Bradshaw, J.T.; Knaide, T.R.; Rogers, A.L. Verifying Liquid Handler Performance for Complex or Non-Aqueous Reagents: A New Approach. *J. Assoc. Lab. Autom.*, **2006**, *11*, 172-180.

(2) Handbook of Chemistry and Physics, 78th Edition, CRC Press, New York, David R. Lide, Editor-in-Chief, 1997-1998.

(3) Protocols and information for determining the proper amount of MVS Stock Solution to add to the corresponding amount of Solvent (or starter) Solution are detailed in multiple places, including the MVS User Guide, the MVS Help menu (MVS Data Manager software 2.0 and higher), as well as the Alternative Solution Helper software program found as part of MVS Data Manager 2.2 and higher. Additionally, Artel Technical Support can be contacted with any questions between 8 am – 5 pm EST (888.406.3463 x109) or via email at technical.support@artel-usa.com.

(4) Bradshaw, J. T.; Knaide, T.; Rogers, A.; Curtis, R. H. Multichannel Verification System (MVS): A Dual-Dye Ratiometric Photometry System for Performance Verification of Multichannel Liquid Delivery Devices. *J. Assoc. Lab. Autom.*, **2005**, *10*, 35-42.

**Table 1. Protocol to prepare 50 g of glycerol solution using only glycerol & DI water**

| *Glycerol solution ID:* | *a* | *b* | *c* | *d* | *e* | *f* | *g* | *h* | *i* | *j* | *k* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Desired mass % of glycerol in water** | **0.50** | **1.00** | **2.00** | **3.00** | **4.00** | **5.00** | **8.00** | **12.00** | **16.00** | **20.00** | **24.00** |
| contributing mass of glycerol (g) | 0.25 | 0.50 | 1.00 | 1.50 | 2.00 | 2.50 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 |
| contributing mass of DI water (g) | 49.75 | 49.50 | 49.00 | 48.50 | 48.00 | 47.50 | 46.00 | 44.00 | 42.00 | 40.00 | 38.00 |
| density of pure glycerol (g/mL) | 1.262 | 1.262 | 1.262 | 1.262 | 1.262 | 1.262 | 1.262 | 1.262 | 1.262 | 1.262 | 1.262 |
| density of DI water (g/mL) | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| theoretical contributing volume of glycerol (mL) | 0.20 | 0.40 | 0.79 | 1.19 | 1.58 | 1.98 | 3.17 | 4.75 | 6.34 | 7.92 | 9.51 |
| theoretical contributing volume of water (mL) | 49.75 | 49.50 | 49.00 | 48.50 | 48.00 | 47.50 | 46.00 | 44.00 | 42.00 | 40.00 | 38.00 |
| total theoretical volume of solution (mL) | 49.95 | 49.90 | 49.79 | 49.69 | 49.58 | 49.48 | 49.17 | 48.75 | 48.34 | 47.92 | 47.51 |
| **Theoretical volume % of glycerol in water** | **0.40** | **0.79** | **1.59** | **2.39** | **3.20** | **4.00** | **6.45** | **9.75** | **13.11** | **16.53** | **20.01** |
| approx. density of glycerol solution (g/mL, from CRC) | 0.9994 | 1.0005 | 1.0028 | 1.0051 | 1.0074 | 1.0097 | 1.0167 | 1.0262 | 1.0360 | 1.0459 | 1.0561 |

**Table 2. Protocol to prepare the glycerol test solution for volume verifications by combining the appropriate amount of MVS Stock Solution (1 or 2)* to the 50 g glycerol solution**

| *Glycerol solution ID & summary (from Table 1):* | *a* | *b* | *c* | *d* | *e* | *f* | *g* | *h* | *i* | *j* | *k* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Glycerol solution with desired mass % of glycerol in water (as above)** | **0.50** | **1.00** | **2.00** | **3.00** | **4.00** | **5.00** | **8.00** | **12.00** | **16.00** | **20.00** | **24.00** |
| **Glycerol solution with theoretical volume % of glycerol in water (as above)** | **0.40** | **0.79** | **1.59** | **2.39** | **3.20** | **4.00** | **6.45** | **9.75** | **13.11** | **16.53** | **20.01** |
| Total mass of glycerol solution (as above, g) | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 |
| Approx. density of glycerol solution (as above, g/mL) | 0.9994 | 1.0005 | 1.0028 | 1.0051 | 1.0074 | 1.0097 | 1.0167 | 1.0262 | 1.0360 | 1.0459 | 1.0561 |
| *Add this much of MVS Stock 1 or 2 (grams) to cover approximate volume ranges in either standard 96-w or 384-w plates:* | | | | | | | | | | | |
| Approx. mass of **MVS Solution Stock 1 (g)** to add to glycerol solution to measure ~ **0.4 - 4 uL in a standard 96-well plate** *or* ~ **0.1 - 1.1 uL in a standard 384-well plate** | 16.802 | 16.784 | 16.749 | 16.714 | 16.680 | 16.645 | 16.540 | 16.400 | 16.261 | 16.121 | 15.981 |
| Resulting mass % of glycerol in glycerol test solution for MVS verifications | 0.374 | 0.749 | 1.498 | 2.248 | 2.999 | 3.751 | 6.011 | 9.036 | 12.074 | 15.124 | 18.187 |
| Approx. mass of **MVS Stock Solution 1 (g)** to add to glycerol solution to measure ~ **2 - 11 uL in a standard 96-well plate** *or* ~ **0.5 - 2.9 uL in a standard 384-well plate** | 5.476 | 5.470 | 5.459 | 5.447 | 5.436 | 5.424 | 5.390 | 5.345 | 5.299 | 5.254 | 5.208 |
| Resulting mass % of glycerol in glycerol test solution for MVS verifications | 0.451 | 0.901 | 1.803 | 2.705 | 3.608 | 4.511 | 7.221 | 10.841 | 14.467 | 18.098 | 21.736 |
| Approx. mass of **MVS Stock Solution 2 (g)** to add to glycerol solution to measure ~ **9 - 49 uL in a standard 96-well plate** *or* ~ **2.4 - 9.9 uL in a standard 384-well plate** | 13.189 | 13.175 | 13.148 | 13.120 | 13.093 | 13.065 | 12.983 | 12.874 | 12.764 | 12.654 | 12.545 |
| Resulting mass % of glycerol in glycerol test solution for MVS verifications | 0.396 | 0.791 | 1.584 | 2.376 | 3.170 | 3.964 | 6.351 | 9.543 | 12.746 | 15.961 | 19.186 |

*amount and type of MVS Stock Solution are dependent on volume range and plate density

*An example in using these tables*: verify a 5-µL transfer of a 15% glycerol solution (g/g) in a 96-well plate. Refer to column *i*. In this case, to the 50 g of 16% glycerol solution (g/g), add 5.299 g MVS Stock Solution 1. The resulting test solution will be approximately 14.5% glycerol by mass. If a higher percent of glycerol is desired in the final glycerol test solution, a higher concentration of glycerol is required in the original glycerol solution.

For instance, to measure a 1-µL dispense of a 15% glycerol solution (g/g) in a 96-well plate, the solution in column *j* could be prepared. To the 50 g of the 20% glycerol solution (g/g), add 16.121 g of MVS Stock Solution 1 resulting in a 15.1% glycerol test solution (g/g).







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