Creating and Testing Custom Alternative Test Solutions with the MVS: General Approach

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Introduction

Custom test liquids using assay-specific reagents can be prepared and employed to assess volume transfer performance of a liquid handler using the Artel MVS® Multichannel Verification System. Described herein are general methods for creating and testing custom test solutions, referred to as alternative test solutions[1]. In general, an aqueous dye-based MVS Stock Solution is combined with a solvent-of-interest to create the alternative test solution (**Figure 1**)[2]. The volume range for testing an alternative solution in specific plate types is shown in column three of **Table 1**.

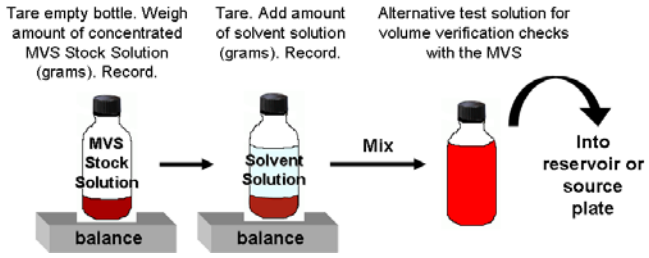

Figure 1. Overview for creating an alternative test solution by gravimetrically combining a solvent-of-interest and an MVS Stock solution.

Requirements

(a) MVS with system software v2.0 or higher; (b) training on MVS operation; (c) MVS Diluent, Stock, and Baseline Solutions; (d) solvent-of-interest with known density; (e) MVS Calibrator plate; (f) Alternative Solution Helper software v1.1 or higher; (g) 96-well or 384-well microtiter plates; (h) pipettor or liquid handler; (i) analytical balance; (j) sample container, preferably amber bottle with screw cap; and (k) transfer pipettes or small funnels.

Table 1. MVS Testable Volume Range Per Plate Type

Plate Type	Aqueous Volume Range (µL)	Alternative Solution Volume Range (µL) [b]
96-well MVS Verification Plate	0.1 – 350 [a]	0.4 – 49.9
384-well MVS Verification Plate	0.03 – 55 [a]	0.1 – 9.9
384-well (round well) low volume	0.019 – 28	0.05 – 3.9
384-well low profile	0.01 – 20	0.04 – 3.9

[a] NIST-traceable measurement for aqueous solutions.
[b] Non-aqueous or custom test reagents can be tested with the MVS, but the results are not traceable to NIST.

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Preparing an Alternative Test Solution using Alternative Solution Helper Software

(a) Launch the Alternative Solution Helper software from the desktop icon or through the installation folder. **Figure 2** represents items *a* through *h*.

(b) Select **New Solution File** from the File menu. **Select Plate Type**.

(c) **Enter the Target Volume** to test.

(d) Refer to the **Table 1** for the approximate Target Volume limits for each Plate Type.

(e) **Enter the Lot Volume** – the amount of test solution you plan to prepare. Artel recommends a Lot Volume of at least 30 mL.

(f) **Enter the Solvent Density**. *This value must be known* and it will also need to be entered into the MVS system software before testing begins. Some solvent densities can be found in reference 3.

(g) **Click Done** and a report will be generated detailing the target weights of both the appropriate MVS Stock Solution and the solvent in order to achieve the Target Volume entered for the specific plate type.

(h) Print the report or write down the "Desired Weight (g)" values in the report for the MVS Stock Solution and solvent solution.

(i) At the balance, gravimetrically combine the two solutions. Tare the balance before each component is added. Refer to **Figure 1**. Following each component addition, allow the balance to reach equilibrium before recording weight. Once all components are added, mix well. Allow the newly prepared test liquid to come to thermal equilibrium with the lab environment before use. The actual weights must be entered into MVS system software before use (see below).

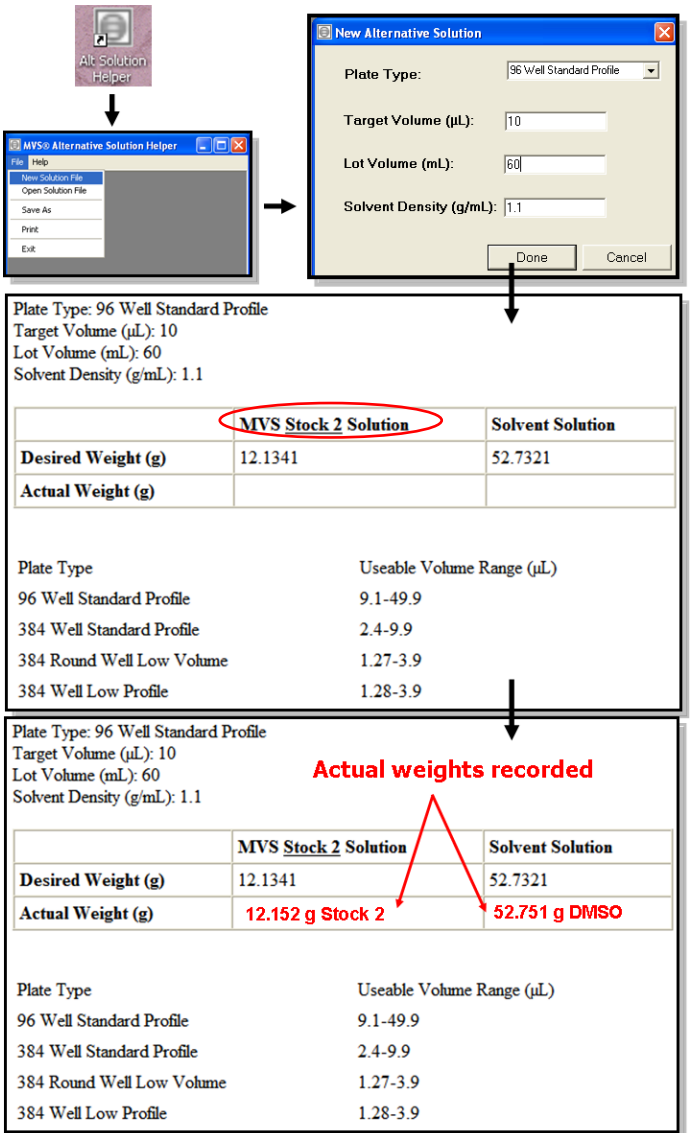

Figure 2. Schematic showing the process of opening and using the Alternative Solution Helper software. A report is created detailing how much solvent-of-interest to combine with MVS Stock Solution based on four variables (plate type, desired target volume, how much to prepare and the density value for the solvent-of-interest). In the example shown, Desired and Actual Weights (g) are both shown for preparing a 60-mL batch of a DMSO alternative test solution for a target volume of 10 µL in a 96-w microplate.

Prepared Alternative Test Solutions are Composed of 75% to 90% (by volume) of the *Original* Solvent

Final concentrations, or final composition, of the solvent-of-interest in the prepared alternative test solution will be 75 – 90% by volume (see **Table 2**). The concentration depends on the volume range and plate type. For instance, testing 100% organic solvent, such as dimethyl sulfoxide, with MVS is *not* possible because the Artel Stock Solutions are aqueous dyes and, depending on the target volume and plate type, will contribute 10 – 25% (by volume) to final alternative test solution.

Table 2. The Amount of Solvent in the Alternative Test Solution is based on Desired Test Volume and Plate Type

Concentration of Solvent in Final Test Solution (v/v)	Approx. Test Volumes in a **96-w standard plate**	Approx. Test Volumes in a **384-w standard plate**
75% solvent (v/v) "dye-based" alternative test solution, i.e., 25% aqueous (v/v) component from MVS Stock 1	0.4 – 4.3 µL	0.1 – 1.1 µL
90% solvent (v/v) "dye-based" alternative test solution, i.e., 10% aqueous (v/v) component from MVS Stock 1	1.8 – 11.0 µL	0.5 – 2.9 µL
80% solvent (v/v) "dye-based" alternative test solution, i.e., 20% aqueous (v/v) component from MVS Stock 2	9.1 – 49.9 µL	2.4 – 9.9 µL

Adding the New Test Solution to the Alternative Solution Library in MVS System Software

(a) Open MVS Data Manager Software.

(b) Select **Alternative Solution Library** from the File menu and click **Add**. Refer to **Figure 3** for items *b* through *h*.

(c) **Enter a unique ID and Description**.

(d) Click **Select Stock Solution** and scan the appropriate MVS Stock Solution with the Bar Code Reader.

(e) **Enter the Solvent Solution density**.

(f) **Enter the actual weights** of the solutions used to prepare the Alternative Solution into the Stock Solution and Solvent Solution fields.

(g) Click **Calculate** to determine the appropriate volume ranges for the solution.

(h) Click **OK** twice to save changes. Each newly prepared solution only has to be entered into the MVS system software once, *i.e.*, the test solution information is stored for repetitive testing.

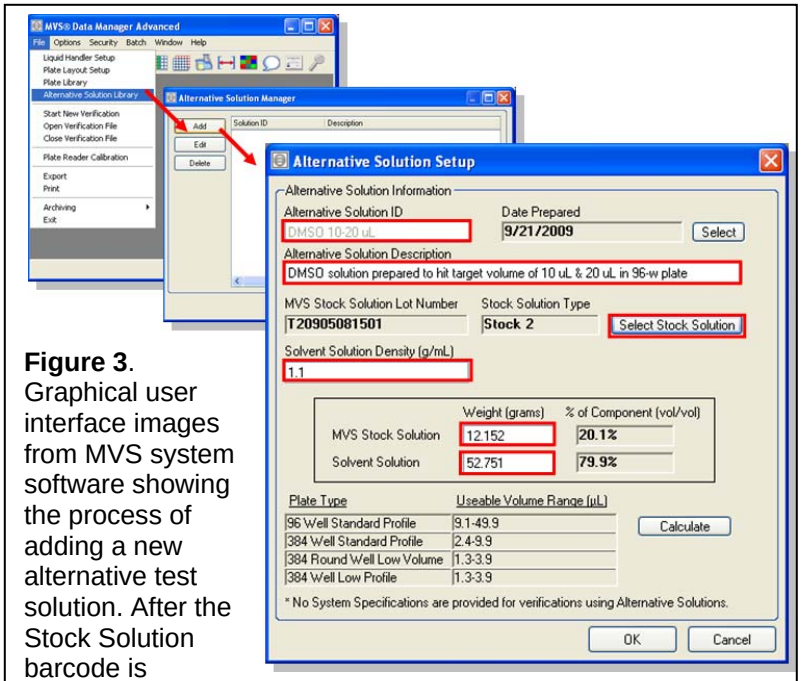

Figure 3. Graphical user interface images from MVS system software showing the process of adding a new alternative test solution. After the Stock Solution barcode is scanned, solvent density is entered, and the actual weights are entered, the software calculates a "Usable Volume Range (µL)" for the new Alternative Test Solution.

Performing a Volume Verification with an Alternative Test Solution

Once an alternative test solution is entered into MVS system software, it can be used to assess liquid handler performance. Make sure the liquid handler ID is entered into the software as well as the Plate Layout. The target volume within the Plate Layout(s) must be within the Usable Volume Range for the defined solution in the Alternative Solution Library (as shown on the right graphical interface in **Figure 3**).

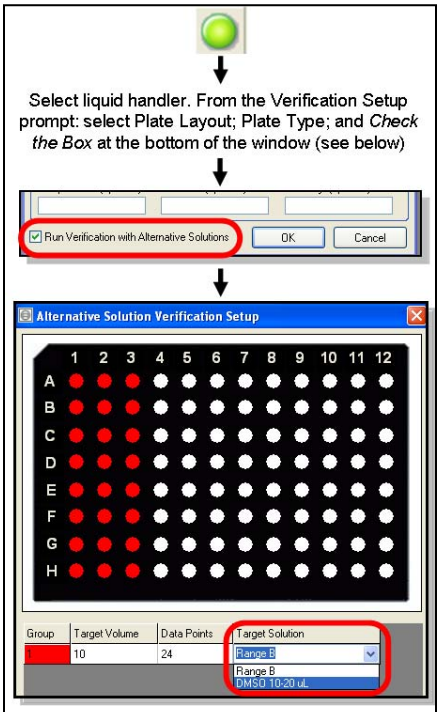

Figure 4. The process for testing a liquid handler with an alternative test solution requires one additional software interface. The user checks the box for "Run Verification with Alternative Solutions" and a new window appears where each test solution is defined per group via the drop down menu(s). This window is not required when testing only MVS Sample Solutions.

Once entered, the process for testing an alternative test solution is nearly identical to running the MVS Sample Solutions:

(a) In the MVS system software, start a volume verification. Hit the green "**Go**" button, or select "**File | Start Verification**". Refer to **Figure 4** for steps *a through c*. Select liquid handler to test, select plate layout and plate type.

(b) At the very bottom of the "**Verification Setup**" window, Select "**Run Verification with Alternative Solutions**" (check box). Hit "OK".

(c) In next *new* window, **use the drop-down menu(s) to select the appropriate Alternative Test Solution** for each of the different groups, *i.e.*, different target volumes. This window will only open if the check box is selected. Different aqueous and/or alternative test solutions can be defined and tested within the same Plate Layout where applicable.

(d) Prepare the liquid handler for testing the alternative test solution by pouring the alternative test solution into a reservoir or preparing a source plate (**Figure 1**).

(e) Proceed with testing and follow the MVS system software prompts for scanning barcodes, Diluent addition, plate shaking, etc.

Important Considerations

Custom test liquids can be prepared to assess liquid handler performance for assay-specific reagents. There are, however, <u>many considerations</u> when preparing and testing alternative test solutions:

(a) NIST-traceability for an MVS measurement with alternative test solutions is waived. The total uncertainty of measurement (error budget) when using a custom test liquid is not defined at all levels and therefore it cannot be traceable back to NIST.

(b) The test volume range is more limited compared to aqueous dye testing (**Table 1**).

(c) Trial and error experimentation might be required for different solvents: (a) the Artel dye must <u>not</u> precipitate when added to the solvent-of-interest; and (b) solvent-of-interest must <u>not</u> have measureable absorbance at/near 520 nm and 730 nm. For exploratory work, it

might be best to create an alternative test solution in a transparent container vial so that any dye precipitation or clumping can be visually observed. Additionally, centrifugation may help determine if the dye is precipitating, *i.e.*, a pellet is observed.

(d) DMSO-based alternative test solutions have been evaluated and work very well when tested with MVS[1, 4].

(e) Some work has been done with Glycerol-based aqueous solutions[4] (~20% glycerol in water (vol/vol) and up to 50% ethanol-water mixtures[4].

(f) Solutions known to precipitate Artel dyes – MVS Stock Solutions added directly to 100% methanol, ethanol or acetonitrile will precipitate. Some experiments have shown that 50% solvent-water mixtures for the above mentioned solvents seem to work well and do not precipitate dyes. Some cell lysis reagents (unknown contents), with either strongly acidic or basic components, have resulted in MVS stock dye either immediately precipitating or precipitating after an hour or more.

(g) Additional limited trials have been conducted using the MVS Alternative Solution platform: (a) table vinegar (5% acetic acid) works well; (b) some mastermix solutions (with unknown contents) have worked well and others have not worked well; (c) aqueous solutions comprised of 5% sorbitol (v/v) work well; (d) aqueous solutions with Tween (<2% v/v) cause meniscus issues (see below) and (e) some BSA and serum reagents have been shown to be rather troublesome (absorbance at 520 nm and/or 730 nm; meniscus issues – see below; as well as bubbling/frothing in the plate).

(h) A test solution causing a 'deep' or 'strong' meniscus in the well (i.e., protein-based solutions like BSA, some MasterMix and some detergent solutions like Tween) *are possible to use but may not be easy* to measure with the MVS – due to the deep meniscus and potential of overlapped absorbance bands at either 520 or 730 nm (or both). The MVS calculations do not account for these types of menisci (**Figure 5**), *i.e.*, the MVS "thinks" the meniscus is flat and therefore does not measure the solution on the sides of the well and lower-than-expected volume measurement(s) result. In some brief R&D trials, target volumes for protein-based solutions were calculated low by 20% in a 96-w plate, i.e., the MVS reported target volume was low, and therefore incorrect, by 20%. With a little effort, correlation experiments are possible. For instance, some users employ a post-measurement correction to the MVS output value whereby the MVS output report value is scaled up or down by a determined factor (the reported volume was scaled up by 20% in the above noted example).

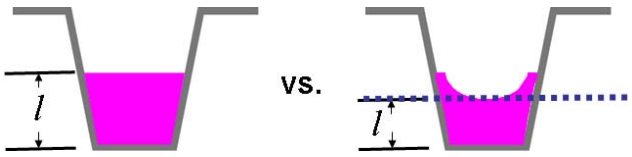

Figure 5. Schematic showing one well of a 96-w plate with pathlength (l) defined as the depth of the solution. With MVS Sample Solutions, the meniscus is essentially flat resulting in a normal pathlength measurement and a true volume calculation per well (left). With solutions that cause a deep meniscus, a shorter pathlength value is measured resulting in a smaller target volume calculation (right). In other words, MVS calculations do not *count* the portion of liquid above the dotted line and post-measurement corrections might be required.

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) 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. 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.

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

(4) Artel On-Line Learning Center and Resource Library at http://www.artel-usa.com

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