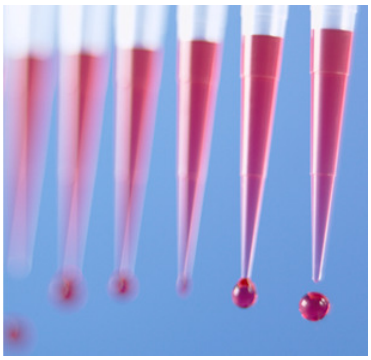

Performance Qualification of an Automated Plate Washer Using the Artel MVS®

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ARTEL

Abstract

The purpose of this application note is to demonstrate the ability of the MVS® Multichannel Verification System to test the performance of an automated plate washer. The previously written application notes, *Using the MVS to Measure Residual Volumes Remaining in a Plate After Sample Aspiration: General Approach*[1] will be cited within as these unique applications relate to this discussion involving tests for (1) bulk dispense precision and accuracy and (2) volume evacuation efficiency.

Introduction

An integral, yet often overlooked, procedure with any given assay, washing steps are vital to ensure reaction quality and integrity particularly when conducted in a microtiter plate. Whether it is complete removal of unbound material within an ELISA, effective evacuation of residual wash buffer solution, or even simple removal of supernatant such as cell media just above the surface of adherent cells, effective washes can make a significant difference in the final results.

As such, automated plate washing instruments, such as the industry leading BioTek ELx405 microplate washer, have provided research facilities with the convenience to conduct plate washes seamlessly and effortlessly. Like all automated instruments, targeted suggestions on proper care and maintenance are provided to promote long-term, error-free use of the system.

Operation and qualification performance tests are written within the ELx405 Operators Manual, describing dispense precision and evacuation efficiency tests conducted with the help of a balance and a single colorimetric, blue dye. However, results obtained through use of the balance yield the overall mean performance of the entire plate and not individual wells, making troubleshooting any problematic channel on the washer difficult to isolate. The study mentioned herein takes the advantage of the MVS technology already conducted in washable microtiter plates to efficiently and effectively perform the aforementioned tests. The MVS method of performance measurement generates more inclusive and informative output reports that present data in a well-by-well manner for ease of visualization and discovery.

Requirements

(1) BioTek ELx405 or similar plate washer
(2) MVS with Data Manager system software 2.0 or higher
(3) An understanding and administration of specific Artel application notes. *See reference 1*.

(4) MVS Sample and Diluent
(5) MVS Calibrator Plate
(6) 96-well tissue culture treated microtiter plate
(7) 96-well MVS-compatible microtiter plate(s)
(8) Pipettor or liquid handler
(9) Training on MVS operation
(10) Plate centrifuge

Methods and Discussion

The goal of this application note was to demonstrate ability of the MVS to effectively qualify a plate washer, such as the BioTek ELx405. Though measurements of washing effectiveness could be made using other available means, obtaining data would be very time-consuming and may not point out concerns with specific channels.

It is also worth noting that the results obtained reflect runs from the unoptimized BioTek software protocols. The BioTek software menu allows for a multitude of manifold placement configurations to enhance the outcome of any of its automated capabilities. Based upon the results displayed, it is recommended that the user scrutinize these settings – specifically for the experimental assay, plate type, and liquid properties to obtain the best results.

1. *Dispense Precision Test*

This first operational qualification liquid test, as described within the ELx405 Operator's Manual, requests that the dispense program "accuracy_qc_test" be conducted with the use of a 10X blue dye concentrate. From this 10X concentrate, a 1X solution is created for the washer program to dispense 300 µL into an absorbance readable plate. Performance within the manufacturer's expected tolerance is indicated if the inter-plate %CV is less than 3% or 4%, depending upon the instrument model.

Alternatively, the Artel MVS employs pre-mixed sample solutions at the appropriate concentration for the desired test. These solutions are ready for immediate use for tested volume ranges 0.1 to 350 µL in a 96-well plate. In this application, Range HV solution was used for the dispense test of 300 µL. In order to reduce the amount of dead volume required, a shortened BioTek source line tube, was inserted directly into the Range HV bottle to be used in this study.

With the MVS Data Manager software, a plate layout (see plate layout Figure 1) was created to run three consecutive 96-well plates for enhanced statistical analysis (*i.e.* to generate a greater number of data points). Because the dispense volume exceeded 200 µL, mixing as part of the MVS process was disabled. Once the sample solution was dispensed, the plates were centrifuged to release any bubbles created from the manifold dispense, and then read in the MVS plate reader.

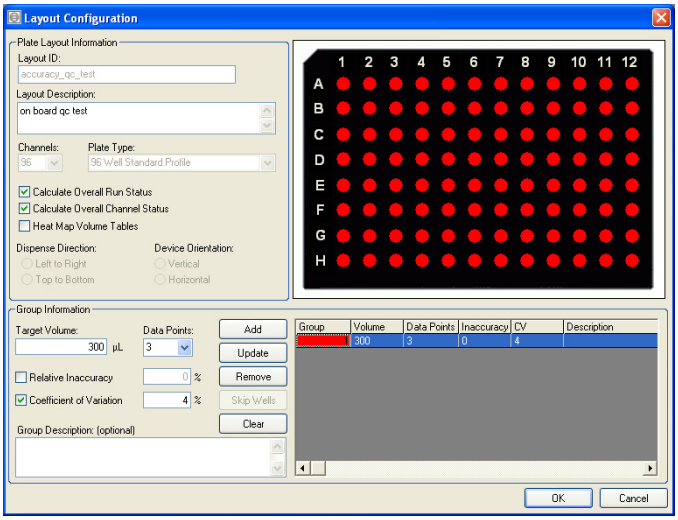

Figure 1. Dispense Precision Test Setup window for the accuracy_qc_test.

When observing the overall run statistics, the results passed the manufacturer's precision specifications of 4% for intraplate CV. As

illustrated in the results contained within *Figure 2*, three plates were run in succession and data obtained highlights a failed channel with a %CV of 8.80%. The well-by-well %CV report indicated that well E3 may require further attention to correct issues with the dispense channel.

Knowing, and thereby addressing poorer performing channels, may help to solve common problems associated with automated instruments.

Test Results for Dispense Precision Test within the MVS Data Manager software (with MVS Verification Plate):

Target Volume (µL)	300
Target Solution	Range HV
Number of data points per channel	3
Mean volume for all Channels (µL)	303.1
Relative Inaccuracy for all Channels	1.03%
Standard Deviation for all Channels (µL)	5.5
Coefficient of Variation for all Channels	1.81%
Coefficient of Variation Pass/Fail Limit	4%
Status based on channel results	Failed
Status based on run statistics	Passed

Well-by-well average volumes for the three plate replicates (all values in µL):

	1	2	3	4	5	6	7	8	9	10	11	12
A	304.5	305.9	301.6	302.8	300.2	303.4	299.3	302.2	301.3	303.5	301.0	306.6
B	310.8	305.2	309.2	311.2	300.6	305.2	306.5	307.9	309.0	308.7	307.7	304.8
C	307.2	306.9	308.5	310.6	309.9	306.2	295.2	307.5	308.5	307.9	305.8	304.2
D	307.1	308.2	295.7	307.5	305.4	305.4	307.9	307.2	305.4	311.0	306.5	306.6
E	308.2	308.1	300.8	306.5	304.9	306.5	306.2	304.0	306.2	311.6	309.3	307.5
F	306.5	309.9	305.2	307.1	303.5	309.5	310.2	310.5	311.4	304.6	303.7	297.5
G	304.1	307.5	306.2	291.1	300.8	309.1	304.7	304.4	308.1	306.3	305.6	300.7
H	298.2	302.0	306.9	287.9	290.9	300.8	282.9	299.1	290.1	302.1	299.8	306.0
	1	2	3	4	5	6	7	8	9	10	11	12

A	298.7	299.2	293.8	298.0	295.5	298.8	293.6	297.4	295.4	299.7	297.9	303.1
B	304.8	298.6	300.7	305.9	296.1	299.6	300.9	302.3	303.0	303.7	304.6	300.4
C	301.0	299.2	300.9	304.0	305.0	299.7	289.0	300.7	301.9	301.8	301.7	299.7
D	299.3	299.6	292.2	301.7	298.2	297.8	300.9	299.6	298.8	304.0	302.4	300.7
E	300.9	299.7	260.0	300.1	297.4	300.3	299.8	297.0	298.0	304.7	303.0	303.4
F	300.5	302.5	297.6	305.7	297.4	304.4	306.9	303.3	304.6	304.0	298.4	296.8
G	300.4	303.1	297.4	297.4	295.7	305.5	302.5	300.5	303.4	303.2	300.9	297.4
H	297.5	300.9	303.0	294.3	294.5	297.2	294.7	300.9	294.2	305.8	297.4	304.8
	1	2	3	4	5	6	7	8	9	10	11	12
A	305.1	304.6	300.2	302.1	299.0	301.9	297.5	301.7	300.9	303.0	301.0	306.7
B	310.0	304.4	307.5	309.6	300.0	303.7	304.8	305.8	308.7	309.1	308.1	304.4
C	305.8	304.2	304.6	308.9	308.9	304.0	293.5	305.4	308.1	307.1	300.5	303.2
D	304.8	305.0	306.7	307.2	302.6	302.3	304.0	304.2	303.4	308.7	305.3	297.2
E	318.8	301.7	307.1	304.2	300.8	304.8	290.9	300.7	303.0	309.6	305.8	305.7
F	299.3	308.1	303.2	310.6	302.6	309.0	311.1	307.5	309.2	310.4	302.6	300.5
G	308.3	307.8	305.3	303.7	301.1	310.2	309.4	305.8	309.8	308.0	305.1	302.1
H	304.9	307.3	312.6	302.6	300.7	303.4	304.6	301.1	301.3	313.5	301.8	309.6

Channel-Specific Coefficient of Variation:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.20%	1.20%	1.40%	0.90%	0.80%	0.80%	1.00%	0.90%	1.10%	0.70%	0.60%	0.70%
B	1.10%	1.20%	1.50%	0.90%	0.80%	1.00%	1.00%	0.90%	1.10%	1.00%	0.60%	0.80%
C	1.10%	1.30%	1.20%	1.10%	0.80%	1.10%	1.10%	1.10%	1.20%	1.10%	0.90%	0.80%
D	1.30%	1.40%	2.50%	1.10%	1.20%	1.30%	1.20%	1.30%	1.10%	1.20%	0.70%	1.60%
E	2.90%	1.50%	8.80%	1.10%	1.30%	1.10%	2.60%	1.20%	1.40%	1.20%	1.00%	0.70%
F	1.30%	1.30%	1.30%	0.80%	1.10%	0.90%	0.70%	1.20%	1.10%	1.10%	0.90%	0.70%
G	1.30%	0.80%	1.60%	2.10%	1.00%	0.80%	1.10%	0.90%	1.10%	0.80%	0.90%	0.80%
H	1.40%	1.10%	1.60%	2.50%	1.70%	1.00%	3.70%	0.40%	1.90%	1.90%	0.70%	0.80%

Figure 2. Data obtained from the Dispense Precision Test.

2. *Evacuation Efficiency Test*

In the second liquid test described within the BioTek manual, a manifold aspirate program named “residual_qc_test” removes fluid from

a predispensed 96-well plate. The remaining liquid is weighed on a balance to determine total weight of residual solution in the plate. The total weight is then divided by the number of employed wells to determine mean residual weight for each well. Performance within the manufacturer's expected tolerance is demonstrated if the mean residual weight is less than 2 µL or 5 µL, depending upon the instrument model.

For this application, MVS Range C sample solution was used to measure the residual volume left behind after the manifold aspirate program (see plate layout Figure 3).

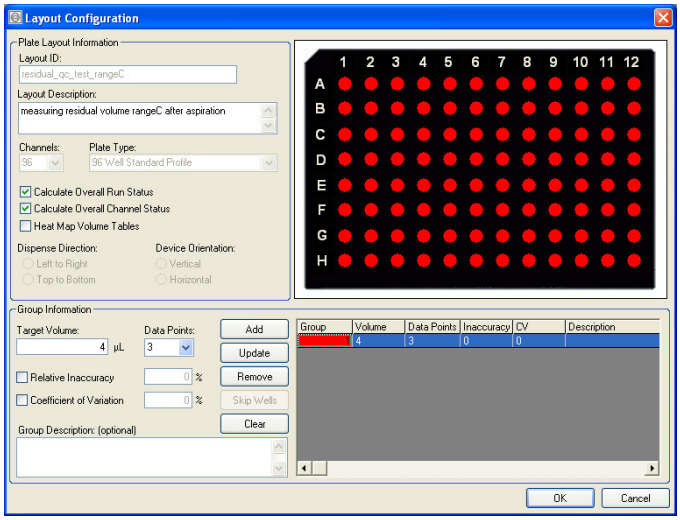

Figure 3. Evacuation Efficiency Test Setup window for the residual_qc_test.

For this test, 200 µL of Range C was pre-filled in two different 96-well plate types – an MVS Verification Plate and a tissue culture treated 96-well plate. A one-time solution removal step was conducted for each plate, as seen in *Figures 4a and 4b*. To the residual liquid remaining in all wells for each of the two plate types, 200 µL of Diluent was added with an 8-tip handheld pipette. The residual sample solution, in microliters, was measured well-by-well with the MVS after the mixingstep[1]..

This experiment not only cites particular channels that may require cleaning, as highlighted by red shaded cells representing volumes two standard deviations greater than the inter-plate mean, but serves as a reminder that not all plate types perform equally. Thus, one generic, 96-well wash program might not be sufficient to efficiently aspirate all material from the wells of any plate type.

Test Results for Evacuation Efficiency Test within the MVS Data Manager software (with MVS Verification Plate):

Plate Type	MVS Verification Plate
Target Solution	Range C
Number of data points per channel	3
Mean volume for all Channels (µL)	1.262
Standard Deviation for all Channels (µL)	0.469

Well-by-well average volumes for the three plate replicates (all values in µL):

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.664	0.077	1.165	1.249	0.073	0.893	1.579	1.214	0.888	1.025	0.941	1.011
B	0.960	1.809	1.745	1.559	1.262	0.678	1.659	1.197	1.165	0.955	0.985	0.934
C	2.141	1.792	0.928	0.950	1.505	1.214	1.371	1.024	1.077	0.858	0.862	1.204
D	1.333	3.068	1.077	1.550	1.189	1.428	1.381	0.933	0.831	0.810	0.884	0.858
E	1.342	1.297	1.393	1.354	1.395	1.345	1.147	0.905	0.961	1.095	0.725	1.067
F	1.268	1.757	1.376	1.760	1.310	1.456	1.315	0.897	0.910	1.385	0.901	1.116
G	1.244	0.919	1.323	2.422	1.107	0.914	1.204	1.204	1.544	1.020	1.143	1.413
H	1.375	1.499	1.606	1.274	1.282	1.485	1.159	1.354	1.533	1.297	1.484	1.769
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.756	3.254	1.200	1.706	1.776	0.734	1.697	1.389	1.389	1.142	1.090	1.469
B	0.889	3.874	1.779	2.018	1.160	0.717	1.482	1.342	1.112	0.954	1.311	1.270
C	1.774	3.485	1.204	1.218	1.329	1.117	1.309	1.134	1.019	0.875	1.160	1.266
D	1.310	3.731	1.134	0.047	1.323	1.357	1.407	1.051	1.124	0.836	0.914	1.557
E	1.324	1.306	0.962	1.498	1.011	1.234	1.279	0.888	1.350	0.809	0.086	1.429
F	1.323	1.716	0.940	1.341	1.121	1.429	1.570	1.301	1.060	0.949	0.861	1.024

G	1.243	1.226	0.796	0.958	1.036	1.517	1.235	1.626	0.936	1.107	1.146	1.236
H	1.388	1.256	1.209	1.124	1.111	1.445	1.216	1.437	1.233	1.455	0.980	1.314
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.801	0.034	1.214	1.095	1.716	0.744	1.434	1.223	1.249	1.395	1.518	1.462
B	1.379	1.872	1.878	2.011	1.113	1.114	1.638	1.286	1.126	0.982	0.858	1.065
C	2.010	3.366	1.021	1.017	2.091	1.219	1.368	1.183	1.096	0.982	0.907	1.250
D	1.175	1.276	0.911	1.375	1.039	1.293	1.228	0.968	0.999	0.859	0.981	1.206
E	0.854	1.025	1.016	1.289	1.043	1.038	1.157	0.823	1.003	0.836	0.823	1.377
F	1.007	1.758	1.430	0.748	1.377	1.184	0.938	1.333	1.025	1.016	1.038	1.099
G	1.218	1.140	1.360	0.656	0.890	1.118	1.400	1.187	1.219	1.166	1.377	1.173
H	1.059	1.398	1.404	3.178	1.226	1.156	1.450	1.078	1.275	1.170	1.182	1.333

Visual inspection of residual sample after evacuation:

Figure 4a. Photo of residual volume remaining after sample aspiration in an MVS verification plate.

Test Results for Evacuation Efficiency Test within the MVS Data Manager software (with generic tissue-culture treated microplate):

Plate Type	Costar TC Treated 3603
Target Solution	Range C
Number of data points per channel	3
Mean volume for all Channels (µL)	1.311
Standard Deviation for all Channels (µL)	0.444

Well-by-well average volumes for the three plate replicates (all values in µL):

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.896	0.864	0.750	0.700	0.759	0.874	0.831	1.304	1.618	1.186	1.533	1.213
B	0.984	0.932	0.480	1.002	0.937	0.923	1.340	1.629	1.871	1.885	1.994	1.564
C	1.038	0.992	0.681	0.979	0.836	1.312	1.377	1.950	1.501	1.885	1.606	1.312
D	0.955	0.951	0.851	0.983	0.928	1.908	1.865	0.714	2.314	1.784	1.607	1.628
E	0.946	0.860	0.988	0.906	0.906	1.125	1.842	1.639	0.655	2.055	1.716	1.641
F	0.900	1.047	0.731	0.792	0.764	0.590	0.901	0.906	1.154	1.676	1.784	1.253
G	0.832	0.923	0.878	0.910	0.960	0.827	0.759	1.423	1.808	1.626	1.755	1.481
H	1.045	1.022	1.119	0.822	1.000	0.996	0.914	0.886	1.467	1.649	1.631	1.425
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.106	0.823	0.809	0.814	1.011	1.016	1.005	1.024	1.144	1.020	1.268	1.066
B	1.001	1.083	0.938	0.970	1.029	0.863	0.979	1.600	1.213	1.679	1.857	1.043
C	1.015	0.961	1.043	1.262	1.029	1.074	1.277	1.940	1.460	1.693	1.656	1.459
D	1.060	1.075	0.860	1.107	1.166	1.943	2.118	2.132	2.287	1.519	1.410	1.582
E	1.001	1.079	1.116	0.544	1.061	1.157	1.154	1.427	0.572	1.995	1.771	1.591
F	1.009	1.129	1.010	0.892	0.873	1.144	1.084	0.869	0.522	1.438	1.820	1.491
G	1.088	1.073	0.955	1.079	0.942	0.983	1.029	1.455	1.620	1.039	1.718	1.687
H	1.045	1.036	0.986	1.032	1.032	1.060	0.982	1.421	1.645	1.589	1.713	1.621
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.091	1.082	1.082	1.101	1.147	1.097	1.119	1.449	1.713	1.621	2.011	1.653
B	1.092	0.932	1.033	1.102	1.102	1.106	1.413	1.652	2.009	1.889	2.026	2.021
C	1.011	1.097	0.864	1.120	1.034	1.812	1.875	2.243	1.213	2.138	2.369	1.823
D	0.983	0.992	1.130	0.704	1.638	2.122	2.401	2.173	2.102	2.297	2.256	1.981
E	1.010	1.020	1.047	1.070	1.020	2.031	1.871	2.046	2.224	1.652	1.470	1.929
F	1.074	1.125	1.047	1.083	1.604	1.467	1.021	1.529	2.494	1.543	2.154	1.847
G	1.061	0.887	0.983	0.955	1.130	1.531	1.771	2.164	1.958	2.131	2.122	1.965
H	0.818	0.813	0.790	0.946	1.009	0.950	1.366	1.810	1.957	1.784	1.793	1.637

Visual inspection of residual sample after evacuation:

Figure 4b. Photo of residual volume remaining after sample aspiration in a Costar tissue culture treated plate.

Conclusions

In the dispense precision test conducted for this application, a single sample solution, was used to measure both the precision and accuracy of volume delivery. Running three replicate plates generated statistically significant inter-plate %CV data that was vital to uncovering a mis-performing dispense channel. Knowing this information, allows attention to be given to properly remedy and therefore fix the poor dispensing channel.

The criticality of evacuation efficiency of the aspirate step within a wash cycle was discussed herein. Whether determining consistency of volume removal throughout the entire plate, or ensuring that wash buffers are not left behind to inadvertently and adversely affect assay performance, the MVS can detect small remaining volumes for each individual well without the concern of evaporation. In this application, the efficacy of the aspirate step in removing all solution from the wells was illustrated in two types of microplates highlighting the difference in performance based on the container.

As described within the plate washer's Operators Manual, proper care, programming, maintenance, and functional qualification tests are vital in securing peak performance from the instrument. For example, intermittent use with extended idle times between washes and soaks can cause channels to clog and fail, and unexpected and unexplained results to occur. Provided with the information the MVS test results offer, the appropriate corrective action can be conducted to improve performance and instill confidence in plate washer performance.

Automated plate washers, like the BioTek ELx405, do not just serve the purpose of filling microplate wells in wash step procedures within any given assay. These instruments can also independently aspirate out solutions and be used as a bulk reagent dispenser. However, if not carefully programmed and understood, the resulting data could be misinterpreted and unknowingly attributed to normal assay behavior, when in fact, there are liquid handling errors that are not detected.For example, improper and incomplete washes could create unwanted, competitive binding, because unbound and unassociated material was left behind after the wash steps. In another example, often times, a plate washer is part of a series of steps within a fully automated system. While it is common practice to invert and tap the plate to remove residual liquid when completing manual assay steps, the automated method does not include this step. Residual buffer in the wells as a result of omitting that step may be detrimental to overall assay performance.

The MVS can play a significant role in monitoring liquid handling performance as well as uncovering possible sources of error within automated assays. The examples and data discussed within this application note describe how critical information can be obtained to qualify the performance and capabilities of a plate washer. By conducting these important operational tests for a given plate washer, while understanding their relation to some of the scientific applications discussed, scientists will be more confident in their liquid handling processes and their overall assay results.

References

(1) Artel application note: “Using the MVS to Measure Residual Volumes Remaining in a Plate
After Sample Aspiration: General Approach”, Artel controlled document number: 12A5914A; http://www.artel-usa.com/resources/applications.aspx

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