Wash Protocols with the Agilent Open Bath Wash Station

Technical Overview

Summary

Wash station effectively washes tips with low carryover achievable.

Introduction

Laboratory automation systems can run cycles of liquid handling processes with disposable tips. There are two options for disposable tip processes: to change disposable tips every cycle or wash the disposable tips. Washing disposable tips can potentially reduce running costs, but it requires an effective and efficient wash process without wasting time and wash solution.

This technical overview evaluates the washing capability of the Agilent Open Wash Station (product no. G5498B/G#048). This wash station is a white polypropylene open wash bath with a 250 mL capacity. It has 5 input/output channels (see Figure 1) designed to perform different methods of washing, such as "fill & empty" and "continuous flow" wash methods. The goal of this work was to create a wash process that would have the lowest possible carryover and also minimize wash volume and process time.

Materials

- Agilent Bravo Automated Liquid Handling Platform with a 384-channel ST Disposable Tip Head
- Agilent 384ST 30 µL disposable tips (product no.11484-202)
- Open Wash Station (product no. G5498B/G#048)
- Agilent Pump Module

Figure 1. Agilent Open Wash Station.

- Agilent 384-well manual fill reservoir (product no. G5498B/G#050)
- 384-well polystyrene, black flat bottom plates (Costar 3710)
- 10 mM Fluorescein dissolved in DMSO
- 50 mM Tris-HCl pH 8.0
- Fluorescence Spectrophotometer (Tecan SPECTRAFluor)

Method

A wash station plumbed with water is placed on position 1. 60 mL of fluorescein solution was poured into a manual fill reservoir and placed on position 2 of the Bravo. A 384-well polystyrene plate pre-filled with 60 µL of 50 mM Tris-HCl is placed on location 4 (the "external" carryover plate). An empty 384-well polystyrene plate is placed on position 5 (the "internal" carryover plate). A 30 µL disposable tip box is placed on position 3. An Agilent VWorks liquid class for 11-50 µL dispense was utilized.

1. Tips on are pressed onto the head.
2. *Contamination step.* 20 µL of 10 mM fluorescein solution is mixed from the manual fill reservoir. Mix parameters are 3 mix cycles, 6 mm from the bottom of the plate, with a 6 µL pre-aspirate volume and a 2 µL blowout volume.
3. *Wash step.* Wash parameters vary on each experiment performed throughout the study as described below.

   Fill empty method: wash station is filled with wash fluid [water]. Tips are mixed a varying number of times in the bath. The wash station is emptied and refilled.

   Continuous flow method: wash station is filled and emptied with wash fluid continuously as the tips are mixed for a varying number of times in the bath.
4. Steps 2 and 3 are repeated five times.
5. After every 5th round of contamination and washing, 30 µL is transferred from an 'external' carryover plate to the 'internal' carryover plate.
6. Process from steps 2-5 is repeated four times to replicate 20 plate/contamination cycles.

Plates are centrifuged at 1800 rpm for 60 seconds to ensure consistent well menisci. Fluorescence was read at 485 nm excitation and 535 nm emission wavelength.

Fluorescence values in each well were used to determine the effectiveness of the wash protocol. Carryover was calculated based on an equation derived from a Fluorescein in DMSO calibration curve consisting of data points from 0 to 1000 part per million, compared to the actual fluorescence value in each well.

Individual results may vary, and are expected to be highly dependent on contaminating solvent and the wash solution chosen. It is highly recommended to do analogous experiments based on your application prior to adopting a wash protocol.

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Results

Fill & Empty Wash Method

In the "fill & empty" method, the wash station is configured as shown in Figure 2. In this configuration the wash bath is filled from both ends but is drained via the bottom outlet. In this method the bath can be filled and emptied one or more times between each wash cycle.

The first experiments were to determine how to minimize carryover in the shortest process time with the lowest consumption of wash buffer. The tips were mixed at full volume three times per fill & empty cycle.

Based on the results in Table 1, two fill & empty cycles are sufficient to eliminate carryover. As process time and wash buffer volume are also critical in determining an optimal wash protocol, those parameters were the next explored.

Number of Mix Cycles

Based on the results in Table 1, two fill & empty cycle were sufficient to reduce carryover to undetectable levels. As this first experiment was performed with three mix cycles per fill and empty cycle, we next determined if we could reduce the process time by reducing the number of mix cycles.

Table 2 shows the result of these experiments. Two mixes per fill and empty cycle was sufficient to reduce carryover below detectable levels.

Volume Exchange

After optimizing this method, the next step was to determine if there was a chance of long term contaminant build-up based on this protocol. The protocol was run for 20 cycles of contamination and tip washing, and on every 5th plate a liquid transfer was performed between an "external" carryover plate (the aspirate step will leave contaminating fluid from the outside of the tips) and an "internal" carryover plate (the dispense will leave contaminating fluid from the inside of the tips).

Since process time and wash buffer volumes are critical in considering an optimized wash protocol, we also included an experiment on 'used' wash buffer solution, in which case the wash station is still filled from the previous wash cycle for the first fill & empty step ['used' wash solution]. A 'fresh' wash solution is used for the second wash. This technique would reduce process time and consumption of wash solution. This method was compared to a process where the reservoir was drained and filled with fresh wash solution prior to the first wash cycle ['fresh' wash solution].

Washing with a fresh wash solution increases the likelihood of achieving low carryover on tips. Using a 'fresh' wash solution for every wash cycle may consume more wash solution and have a longer process time compared to washing with 'used' wash solution (Table 3). Washing with 'used' wash solution almost halves the process time and wash volume consumption.

Based on the results from Table 4, both 'fresh' and 'used' wash solution were about 99% effective in reducing carryover to undetectable levels over 20 processed plates. Carryover occurred randomly in about 1% of wells in a 384-well plate from random tips of a 384-channel head. Contamination in no case was greater than 30 ppm.

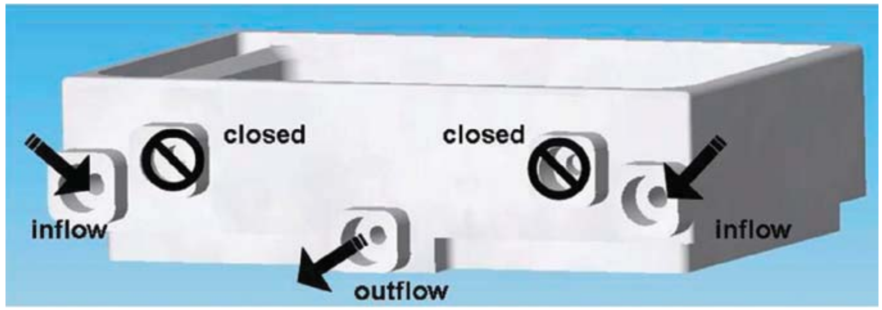

Figure 2. Configuration for a fill & empty wash method.

Number of Fill & Empty Cycles	Number of wells with carryover 1-10ppm	Number of wells with carryover >10ppm	Time (sec/plate)	Volume(mL)
1	16	11	90	250
2	0	0	180	500
3	0	0	270	750

Table 1. Number of carryover, process time and wash volume consumption on varying number of fill/empty cycles.

Number of Mixes	Number of wells with carryover 1-10ppm	Number of wells with carryover >10ppm	Time (sec/plate)	Volume (mL)
1	206	1	150	500
2	0	0	165	500
3	0	0	180	500
5	0	0	210	500

Table 2. Number of wells with carryover, process time and volume of wash solution consumed by varying the number of mix cycles per fill & empty cycle.

Wash solution for a Fill & Empty wash method	Process Time (sec)	Volume of wash solution (mL)
Fresh	180	500
Used	120	250

Table 3. Time and volume of wash solution consumed when washing with 'fresh' or 'used' wash solution for two wash cycles.

Continuous flow wash method
Another method of tip washing is the continuous flow wash method. Fresh wash solution is continuously pumped into the wash station, overflows, and is removed waste. An advantage of this method is that the process time is shorter compared to the fill & empty method, therefore improving throughput. This method does consume a larger volume of wash solution. The wash parameters of two washes and three mix cycles per wash were also applied to this method.

The results of the continuous flow wash method are shown in Table 5. Approximately 99.5% of wells had no detectable carryover. The 0.5% of wells that had measurable carryover did not exceed 8 ppm carryover. This wash method does indeed run faster (80 seconds, an improvement of 55%). The volume of wash solution is larger in comparison (800 mL vs. 500 mL) to the fill and empty method.

Plate Number	'Fresh' wash solution				'Used' wash solution			
	Wells w/external carryover		Wells w/internal carryover		Wells w/external carryover		Wells w/internal carryover	
	1-10ppm	>10ppm	1-10ppm	>10ppm	1-10ppm	>10ppm	1-10ppm	>10ppm
5th	3	2	3	1	0	0	2	0
10th	0	0	0	0	0	0	3	0
15th	0	0	0	1	0	0	4	0
20th	0	0	1	0	0	0	4	0

Table 4. Number of wells with carryover on plates processed with a "fill & empty" wash method with 'fresh' and 'used' wash solution.

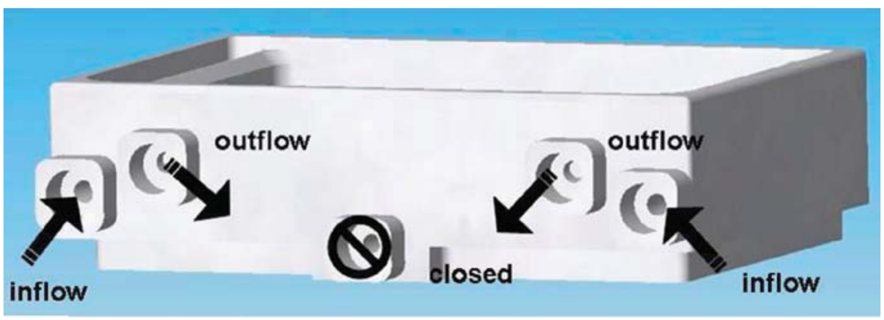

Figure 3. Configuration for a continuous flow wash method.

Plate Number	External wells with carryover		Internal wells with carryover		Process Time (sec)	Volume of Wash Solution (mL)
	1-10ppm	>10ppm	1-10ppm	>10ppm		
5th	0	0	1	0	80	800
10th	0	0	1	0	80	800
15th	0	0	0	0	80	800
20th	0	0	0	0	80	800

Table 5. Number of wells with carryover with the continuous flow wash method.

Comparison to chimney wash stations
One concern in using a wash station of this style is the possibility of cross contamination from one tip to the other. To test for this possibility, we changed our carryover plate to have alternating rows of fluorescein contaminant and no contaminant at all. By looking for carryover in the tips that had never been exposed to fluorescein, we could determine if there was a chance of cross contamination.

The previous results demonstrate that there is very low carryover from plate to plate. In testing this method, we found that there was no cross contamination from contaminated wells into uncontaminated wells, suggesting that the open bath wash station would not increase the risk of cross contamination in comparison to chimney-styled wash stations.

Conclusion

This technical overview demonstrates two feasible methods of washing tips that are capable of reducing carryover in disposable tip usage cases with the Agilent Open Wash Station. The reservoir is designed to allow multiple methods of cleaning tips.

Each of the methods studied in this technical overview has different strong characteristics. The empty and fill method results in clean tips with a minimal use of wash volume. This may be advantageous in cases where disposal costs are prohibitive, and it is an acceptable tradeoff to have a slightly longer wash time in order to minimize waste.

The other method, the continuous flow method, is useful in cases where throughput times are paramount and the cost of disposing of waste fluid is comparatively lower.

Please contact your sales representative or Agilent Applications Support if you have particular questions regarding your specific application. Supplemental information (protocol files and data analysis spreadsheets) are also available upon request.

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