Integrating tip washers into automation system waste management procedures

Diane Choi, BS; Ernest E. McGahee, III, MPH, MHSA; Christina Brosius, MS; Justin Brown, MS; Tonya Guillot, MPH; John Lee, BS; Baoyun Xia, PhD; Lanqing Wang, PhD

Centers for Disease Control and Prevention, Tobacco and Volatiles Branch – Tobacco Exposure Biomarkers Laboratory

ABSTRACT

In recent years, innovative, fully automated systems have been implemented into tobacco exposure studies in the Tobacco and Volatiles Branch, National Centers for Environmental Health, Centers for Disease Control and Prevention (CDC), which allow high-throughput sample analysis with incredible efficiency and high quality results. While automation systems have improved sample processing and increased laboratory safety, the resulting amounts of used pipette tip waste also pose significant environmental and fiscal concerns. In the Tobacco Specific Nitrosamines (TSNA) biomarkers analysis laboratory alone, 20 racks of 96 tips each are used to run one method; with a turnover rate of 6 analytical runs per week, over 11,000 tips are consumed. As a result, large amounts of autoclave waste are generated, creating environmental concerns as these tips cannot be recycled. Furthermore, a significant portion of laboratory funding must be allocated to maintain the large amount of tips required to run this method. As sample throughput increases and more laboratories turn to automation systems, there is a rising need to address the aforementioned issues. Our laboratory purchased and is utilizing the Grenova TipNovus™ tip washer systems [www.grenovasolutions.com] to improve automation systems waste management. Our aims included: (1) ensuring generated liquid waste met laboratory safety standards; (2) determining the analytical validity of reusing washed tips; and (3) performing volume verification studies on washed tips.

WASH ANALYSIS

Method:

1. In 96-well plate, distribute 190 µL of solvent to a single column.
2. Dry down plate in the Biotage TurboVap (38^{0}C, flow rate 38).
3. After washing tips, distribute the same solvent volume to the next column.
4. Once the plate is filled and dried, reconstitute with 40 µL of water for LC-MS/MS analysis.

LC-MS/MS analysis was performed on the Sciex 6500. Since we only tested those pipette tips used for solvent transfer, we did not expect to see any TSNA peak presences. We tested three plates of various solvents used in our TSNA method, and for the most part, there were no noteworthy peaks. There were some peak areas, but these were identical to those seen in water blanks. All results indicated that there was no evidence of contamination from the tip wash tests. Or, results indicated that the tips were clean after each wash.

SYSTEM FEATURES

- Four tip racks per compartment
- Fully customizable wash and dry cycles
- Four cleaning reagent feeds
- Customized mobile carts
- Tip carriers

WASH FEATURES

- High pressurized wash
- Tip agitation
- Ultrasonic cleaning
- UV sanitation
- ~ 15 minutes per wash cycle

DRY FEATURES

- Tip Shake
- Maximum heat setting of 80^{0}C
- ~ 12 minutes per dry cycle

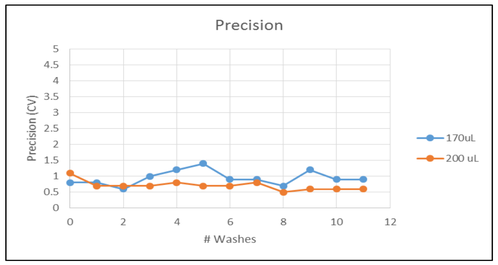
LIQUID WASTE

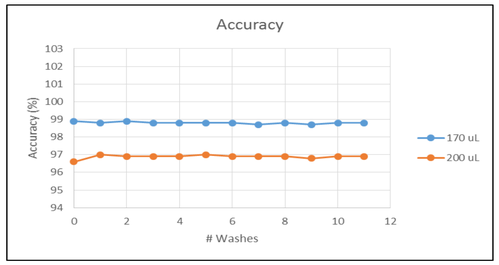
To ensure the generated liquid waste met laboratory safety standards (pH 7 – 9 and no hazardous chemicals), we determined a dilution of 12.5g of Grenoclean™ cleaning reagent to 4L of D.I. water.

Caliper Wash Program						
Wash Stage	DI Water (L)	GrenoClean (L)	Agitation	Sonication	UV	Time (S)
Soak 1	2.6	0	X	X	X	258
Soak 2	3.4	0	X	X	X	325
Prewash	0	0.3	X		X	46
Wash	3.6	0	X	X	X	213
Rinse	0.5	0	X	X	X	57
Total	10.1	0.3				899

RESULTS

Volume verifications were performed for 170 µL and 200 µL as per monthly calibrations. Results compared the precision (CV) and accuracy (%) of the pipette tips to the number of washes. Further volume verification studies show positive precision and accuracy values up to 22 washes. Studies on two automation systems also depict similar results. All obtained data was well within predetermined limits for volume verification studies.





NNAL	No peaks
NNNT	No peaks
NATT	No peaks
NABT	Some Qual peak areas < 1000 cps

TSNA analysis chart depicts peak presence for TSNA analytes. This test involved three different solvents (5% ammonia in methanol, 0.1N HCl, and toluene) to ensure cross-contamination did not occur between wash cycles.

PROJECTED SAVINGS

For the current TSNA method, approximately 47,412 tips are used per month, including tips for monthly calibrations of volume verifications. With the tip washer system washing only those tips used in solvent transfer, about 26,688 tips could be reused. Nearly 60% of tips would be saved and autoclave waste would be halved. In terms of cost savings, at approximately $8.50 per tip rack, we spend over $48,000 per year on tips. Savings are dependent on the number of times tips are washed and reused, and at 5 washes, we would save over $21,000 per year. At 10 washes, we would save over $24,000 per year.

CONCLUSION

Our studies analyzed only those tips used for solvent transfer in our method. TSNA analytes analysis results showed no evidence of contamination with washed tips, and it is safe to use these cleaned tips in our current method. Volume verification data also indicated positive results with precision and accuracy values well within our predetermined limits; we could reuse tips up to a minimum of 10 washes with little to no effect on the physical integrity or reproducibility of the washed tips. Once we apply the tip washer system to our TSNA method, we can wash and reuse nearly 60% of tips, cutting generated autoclave waste in half. Initial cost analysis also show savings of over $24,000 per year. The possibilities for tip washers extend beyond our immediate needs as at least four other laboratories involved in tobacco exposure studies utilize similar automation systems. Our results may generate interest from other automation dependent laboratories at the CDC, and the wide usage of tip washers could considerably promote campus-wide green awareness.

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CONTACT INFO

Diane Choi, B.S.
Chemist | ORISE
Email: koz0@cdc.gov
Phone: (770) 488-7191
Ernest McGahee, M.S., MPH
Lieutenant Commander | USPHS
Email: esm7@cdc.gov
Phone: (770) 488-7579

National Center for Environmental Health
Division of Laboratory Sciences

www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

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