by Ali Safavi and Spencer Ferguson

High-Throughput Pipet Tip Washing and Laboratory Sustainability

As the number of samples processed in labs using high-throughput processes has increased, there has been a corresponding rise in the level of waste, such that in 2010, four million pounds of plastic pipet tips were disposed of after just one use. It can take 200–400 years for typical plastics to degrade under standard conditions. Furthermore, given that one ton of recycled plastic saves 5774 kWh of electricity, recycling all of these tips would conserve enough electricity to power 1354 homes or 200,486 laptops for one year.

Automated pipet cleaning and tip reuse

The TipNovus high-throughput, automated pipet tip cleaning system from Grenova LLC (Richmond, Va.) enables laboratories to safely recycle sanitized tips and thereby reduce waste output. Once tips have been re-used the maximum number of times (which is governed by the individual laboratory), they can be washed once more and recycled.

L.E.A.F. (lab equipment algorithm form) software further extends the economic and environmental benefits of TipNovus, and allows the user to intuitively create and download custom wash protocols. The appropriate wash can be selected using the instrument's touchscreen. A standard wash method was established that proved effective for most applications and sample types. Cleaning consists of a two-stage soak, multiple washes with different reagents, a final rinse and a dry cycle. A cleaning solution called GrenoClean is safe for the operator and the environment; TipNovus can accept up to four different cleaning reagents based on the type of contamination and the user's specifications.

During the eight-minute cleaning process, contaminants are eradicated from the tip surfaces through a process of soaking and pressurized washing with sonication occurring throughout. Vertical agitation removes contaminants from the inside of the tips and sends them directly to the waste drain. The insides of all tips are exposed via a UV "curtain," while the outside is sanitized via a powerful UV bulb below the wash compartment. Liquid waste produced during the wash has been verified to be environmentally safe as it consistently tests at a pH level between 7 and 8. In addition, all potential biological waste runs through ultraviolet sterilization prior to exiting through the waste drain. *Figure 1* shows the cleaning mechanisms used during the wash process. The cleaning and sanitation method was 99.9% effective for removing biologicals and rendering the tips free of DNA, RNA and other contaminants.

Pipet system validation

The devices have been validated in the testing and production phases. Multiple TipNovus systems have been in use for more than a year at Health Diagnostic Laboratory in Richmond, Virginia. The laboratory handles a large number of samples per day and its consumption of pipet tips is high. The TipNovus systems paid for themselves in months by reducing consumable expenses and the cost of biohazardous waste disposal.

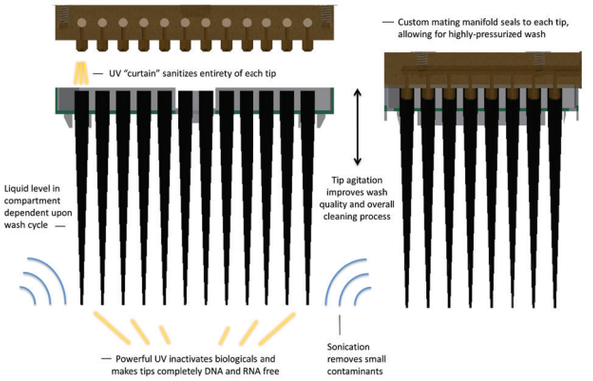

Figure 1 – Sanitation features used in the wash compartment: UV light, sonication and sealing manifold.

Experimental

The ability of TipNovus to sanitize tips was tested by comparison and validation studies of new and washed tips. All experiments were conducted using a Microlab STAR system with unfiltered CO-RE tips (Hamilton Robotics, Reno, Nev.). A TipNovus system washed up to four racks of 96 unfiltered CO-RE tips every 8–10 minutes, and the duration depended on the program. The predetermined settings were consistent with the testing parameters of each particular sample type.

Reuse of the disposable tips for red blood cell transfer was evaluated for two wash cycles. Hamilton 300-µL slim unfiltered tips were used to transfer packed (centrifuged) red blood cells from 13 × 75 mm patient sample tubes into 4 × 96 deep-well plates for fatty acid extraction. The transferred samples were extracted and analyzed for fatty acid analytes using GC/FID (flame ionization detection) to establish results for new tips. Tips were ejected into a Grenova tip carrier on the deck after transfer, which yielded four racks (96 tips per rack) of contaminated tips. Racks were marked with an "X" in the A1 position. The tips were washed using the TipNovus washer with GrenoClean reagent solution and were then moved to the TipNovus dryer for 8 minutes. To monitor carryover, washed racks of tips were rotated 180° before being returned to the Hamilton deck in their usual carrier. The tips from the rotated racks were reused during sample transfer and washed under the same protocol. Tip racks were placed back on the deck so that the third and final transfers were done using the washed tips. Four deep-well plates of samples were then extracted and run via GC/FID to generate results for comparison (see *Figure 2*).

A correlation (linear regression) was obtained to monitor reuse of the disposable tips for plasma transfer after five wash cycles. Reuse of the disposable Hamilton 300-µL slim unfiltered tips was evaluated by transferring plasma from patient blood samples to contaminate them. Tips were washed and dried after each plasma transfer using TipNovus. The wash process and rack rotation followed the same protocol as the above red blood cell study. After the fifth transfer and wash cycle, the extracted plate of patient samples was run using LC/MS/MS and analyzed for the concentration of desmosterol. The tips that had been washed five times were compared with the new filtered tips, as shown in *Figure 3*.

Gravimetric volume verification was measured for 192 tips using a Hamilton FVK2 (Field Verification Kit 2), which included a Mettler-Toledo (Columbus, Ohio) Model WXS205SDU balance. Hamilton 1-mL high-volume tips were used to transfer 100 µL of deionized water to the balance after each wash cycle for 20 consecutive washes. The Hamilton Ultimate Liquid Class Validation V4.0 method was used to establish the testing protocol (see *Figure 4*).

Results and discussion

Red blood cell transfer for new tips and washed tips was evaluated by GC/FID. Chromatography from the sample transfer using the new tips

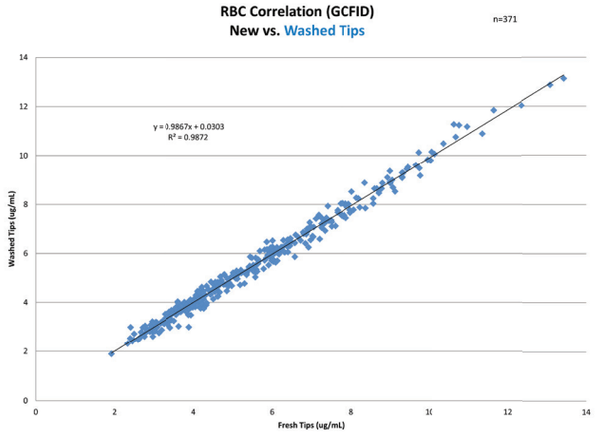

Figure 2 – Results from comparison studies of new and washed tips for packed red blood cells (RBCs).

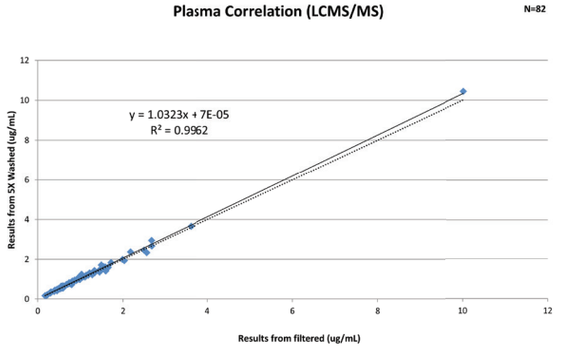

Figure 3 – Results from comparison studies of new filtered and washed tips for blood plasma when tips were reused five times (n = 82).

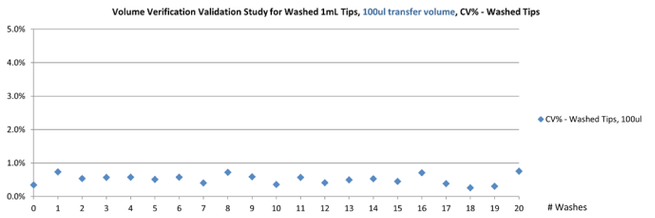

Figure 4 – Repeated gravimetric volume verification after indicated repetition of wash cycle.

was compared to that of the validation run using the washed tips. Any discordance identified during the validation was investigated further. It was found that the discrepancy in the final sample count of less than 384 was due to insufficient sample volume after multiple aliquots. A correlation was obtained via linear regression. The model explained most of the variability in the data with an R^2 value of 0.9872, indicating a strong linear relationship between results from the new and washed tips. Plasma samples transferred via the new and washed tips were run on an LC/MS/MS system.

The discrepancy in the final sample count of less than 86 was a result of two samples having insufficient volume inside the vial for transfer. An R^2 value of 0.9962 for this data demonstrated a strong linear relationship between results from the new and washed tips. Gravimetric volume verification from the transfer of 100 µL of distilled water produced a coefficient of variation below 1.0% for 20 consecutive wash cycles of the same tips.

Summary

TipNovus is a high-throughput washing device that helps reduce the cost of laboratory consumables and waste disposal by sanitizing disposable pipet tips for reuse. Test results are available at www.grenovasolutions.com.

Additional reading

1. http://utahrecycles.org/get-the-facts/the-facts-plastic/?UTAHRECYCLES=qhnhjb8ul36ucun9lj81m6pen6
2. www.forbes.com/sites/christopherhelman/2013/09/07/how-much-energy-does-your-iphone-and-other-devices-use-and-what-to-do-about-it/
3. www.labautopedia.org/mw/index.php?title=Liquid_Handling:Theory_and_practice
4. www.bioexpress.com/store/catalog/product.jsp?catalog_number=P-4170-304
5. www.the-scientist.com/?articles.view/articleNo/26845/title/Reuse--or-recycle-/

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