Evaluation of a Novel Tip Cleaning Technology for Simplifying and Increasing the Efficiency of siRNA Screening.

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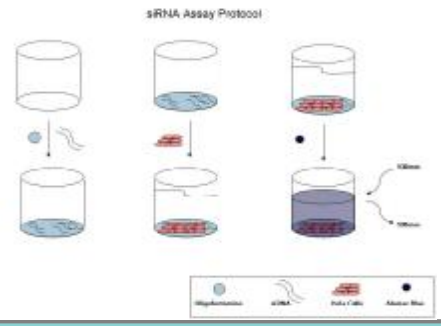
Abstract

Plasma technology has been shown to be a reliable alternative to the more typical tip wash or replacement techniques currently used with automated liquid handling devices throughout the biopharmaceutical and diagnostic industries. The TipCharger™ System utilizes cold plasma to clean disposable polypropylene micropipette tips without the use of traditional wash solvents or waste generation. The use of a dry cleaning method eliminates the problems associated with contamination in wash solutions and delivers results comparable to or better than existing washes in less than half the time. Evaluation goals in the Automated Biotechnology Lab in North Wales included simplification of tip wash processes, reduction/elimination of biological waste generation, and cost savings through reduction in assay time and consumable usage. Comparative analysis of tips cleaned with conventional DNAse/RNAse free water and TipCharger-generated plasma in siRNA assay protocols are presented.

Biological Assay Background

- The development of highly-sensitive cell-based assays are critical to HTS and HCS environments.
- siRNA assays are both sensitive and robust and have become relatively inexpensive through automation and miniaturization.
- Reducing screening timelines and costs continues to be a priority.
- The recurring costs associated with purchasing and using polypropylene pipette tips are significant.
- There is an opportunity to substitute electrically-generated atmospheric pressure plasma for current tip washing techniques and, thereby, increase operational efficiency.

Figure 1.



Goals & Results

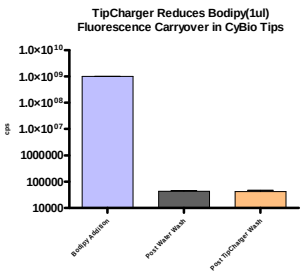
The evaluation was designed to assess how well the TipCharger System eliminates reagent carryover when compared to historical (DNAse/RNAse free water) wash protocols via a Bodipy fluorescent dye-based assay and a proprietary Merck siRNA cell-based assay.

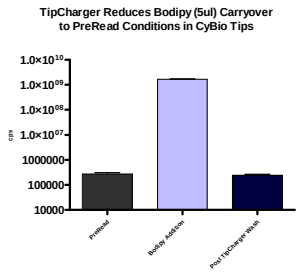
When tips are introduced into the TipCharger™ Cleaning station, an electric field is produced, ionizing the room air, resulting in a continuous plasma field around each pipette tip. The result is that solvents and organic molecules are reduced into individual atoms eliminating carryover, tip washing or tip replacement.

Comparison of the Traditional Tip Wash with Plasma Cleaning

The ability of the TipCharger System to reduce Bodipy detection is shown in Figures 2 and 3. Fluorescence was reduced to pre-read detection levels after tips were exposed to plasma for 77 seconds. Figure 2 shows that 1µL volume of Bodipy dye, at a concentration of 2.5 µM, is reduced by 24,000-fold signal-to-background ratio on average. Further, Figure 3 shows that at similar concentrations but higher volumes (5 µL at 2.5 µM), plasma cleaning reduced Bodipy signal by 6,500-fold on average. These values are comparable to historical water based wash protocols.

Figures 2 and 3. Detail carryover data from both 1µL and 5 µL transfers using TipCharger Plasma Technology

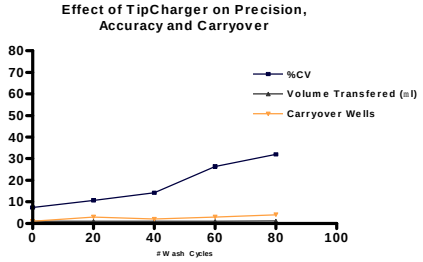




Precision, Accuracy and Carryover of Plasma Cleaning

The number of plasma cleaning cycles was measured against precision, accuracy and carryover for transfer of 1µL of Bodipy. The data in Figure 4 shows that after 20 cleaning cycles pipetting precision is mildly reduced with %CVs increasing from an initial 7.4% to 10.7% and up to 14.1% after 40 cleaning cycles. Figure 4 also shows that CyBio tip integrity is maintained through 80 cleaning cycles with no significant change in volume transfer. Finally, plasma remains effective at reducing carryover with only 0.31% wells showing ≥ 3+ St. Dev. In total, the results indicate that the TipCharger System more effectively cleans fluorescent dye coated polypropylene tips vs. historical methods.

Figure 4. Plot detailing accuracy and precision of pipetting tips over multiple wash cycles in the TipCharger for the 1µL Bodipy transfer.



Performance of the TipCharger System in siRNA Viability Assay

The siRNA assay used to evaluate the performance of the TipCharger System was a cell viability assay in which a known toxic gene was used to induce knockdown and cell death. An Alamar blue assay can then be carried out 72 hours later to measure apoptotic cells. Figure 5 details the results for a spiked gene plate, followed by a water plate. It shows that the TipCharger System effectively removes siRNA from the pipetting tips while maintaining good transfection efficiency of control siRNA.

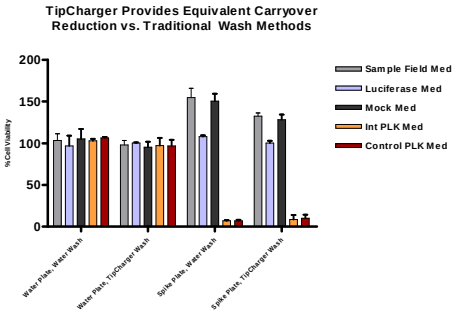


Figure 5. Shows effective knockdown of control siRNA (PLK) with both historical water wash and TipCharger plasma wash technology. siRNA is effectively cleaned from tips in both types of wash cycles.

Effect of TipCharger Plasma Technology on Assay Productivity

Through further optimization, we were able to decrease the total TipCharger wash cycle time from an initial 77 seconds to 15 seconds. This is a significant improvement from the historical water wash time of 90 seconds. Since transfection times in siRNA assays has always proved to be a bottleneck on this particular robotic system, by lowering the cycle time of the wash we are also able to lower the robotic cycle time by 20% and increase the throughput of source plates on the robotic system.

Figure 6.

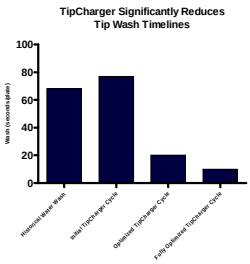
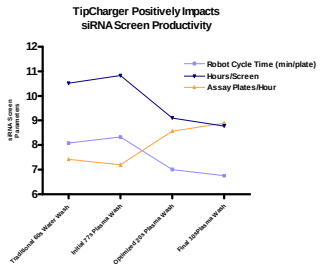


Figure 7.



Conclusions

The results demonstrate that substituting The TipCharger System for traditional tip washing significantly increases throughput, eliminates the use of DNAse/RNAse free water, significantly reduces the amount of hazardous waste being generated, and improves the overall reliability and robustness of siRNA screening campaigns. Our laboratory will save approximately 1 minute/tip wash cycle, thereby improving our daily assay run time by 20% (from the current 10.5 hours to 8.5 hours). Additionally, we eliminate the direct and indirect costs (purchase, store, dispose, etc.) associated with the costly DNAse/RNAse free water and hazardous material disposal associated with everything in contact with the cells used for siRNA screening. Based on these results, the laboratory will be installing 3 TipCharger TC-384 Systems for siRNA screening campaigns.