

Setting a new standard in sample delivery using VWR® Universal Aerosol Filter Pipet Tips



732-3633

INTRODUCTION

The high cost of rare biological samples, coupled with the increasing sensitivity of today's molecular methods demands high precision pipetting by laboratory technicians¹. Even minor variations in sample delivery can impact data quality for expression analysis² and next generation sequencing³ technologies. For a number of years pipet tip manufacturers have tried to market the efficiency of their tips for sample delivery through gravimetric studies using water, viscous dye comparisons and even high resolution photos showing "smoothness" of the inner tip wall. However, these methods fail to provide quantitative assessment of tip efficiency for delivery of molecules in a given substrate. In essence, are sample molecules left behind in tips due to inherent retention of plastic?

In an extensive comparison against two leading pipet tip brands on the market (**Table 1**), VWR® Universal Aerosol Filter Pipet Tips (Cat. No. **732-3633**) proved to be the most effective in preventing sample loss during pipetting. This technical article provides an overview of data generated from a comprehensive study

conducted by MRIGlobal*, a leading national research institute. The study design consisted of evaluating sample loss following dispense of three different sample types: fluorescently labeled DNA; fluorescently labeled protein; and nanoparticles utilizing nanoceria with detection by ICP-MS. An electronic pipettor was utilized to assure equivalent handling of liquids for each brand of tip, thereby limiting user introduced variance. Samples were processed in triplicate and then analyzed in duplicate.

TABLE 1: Pipet tips used in study

Manufacturer	Description
VWR	100 µl Universal Aerosol Filter Pipet Tips, Sterile
Competitor A	100 µl Filter Pipet Tips, Sterile
Competitor B	100 µl Filter Pipet Tips, Sterile

*MRIGlobal is an independent, not-for-profit research organization.
More information at www.MRIGlobal.org

DNA CHALLENGE

Methods

Human DNA diluted to 20µg/ml was used in the DNA challenge. The DNA solution was labeled with fluorescent dye as per Invitrogen’s Qubit dsDNA HS Assay Kit protocol. One hundred microliters of the fluorescent DNA solution was drawn up and down with the pipettor three full times, with a final dispense back to the original tube. One hundred microliters of molecular grade dH₂O was drawn up and down three times in the tip and dispensed into a fresh 0.5ml tube. This procedure was repeated in triplicate for each of the three brand pipet tips. DNA solutions were analyzed on the Qubit 2.0 Fluorometer for residual fluorescent signal associated with retention of DNA solutions on the pipet tip. A reading of “too low” indicates less than 0.010µg/ml dsDNA detected by the fluorometer.

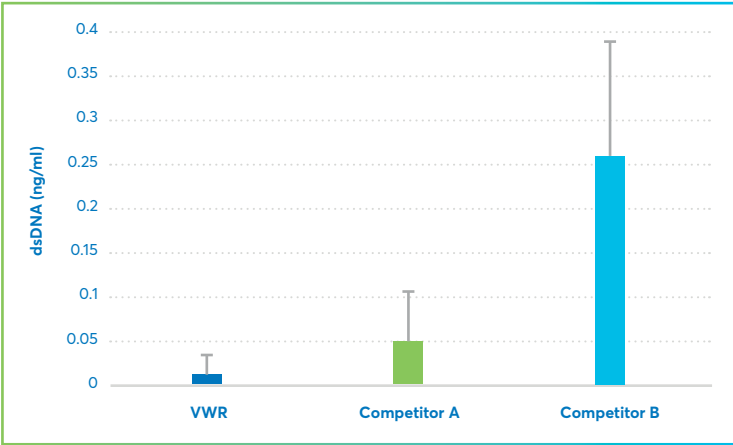
Results

There was a distinctive and measurable difference in sample loss due to residual DNA solution left in the tips following dispensing of the sample (Table 2 and Figure 1). Loss of sample due to residual DNA left was 0.25%, 0.71% and 1.75% for VWR, Competitor A, and Competitor B tips, respectively. VWR tips demonstrated the best consistency and efficiency of DNA sample delivery among the three brands.

TABLE 2: Residual DNA carryover

Sample	5 µl	10 µl	Mean (µg/ml)	SD
Negative Control	Too Low	Too Low	Too Low	Too Low
DNA 20 µg/ml	22.000	11.000	16.500	7.778
VWR – 1	0.036	0.047	0.042	0.008
VWR – 2	Too Low	Too Low	Too Low	Too Low
VWR – 3	Too Low	Too Low	Too Low	Too Low
Competitor A – 1	0.119	0.115	0.117	0.003
Competitor A – 2	Too Low	Too Low	Too Low	Too Low
Competitor A – 3	0.028	0.426	0.035	0.010
Competitor B – 1	0.130	0.448	0.289	0.225
Competitor B – 2	0.367	0.203	0.285	0.116
Competitor B – 3	0.205	0.185	0.195	0.014

FIGURE 1: Graph of residual DNA carryover on tips



PROTEIN CHALLENGE

Methods

Bovine Serum Albumin (BSA) at 5mg/ml was used in the protein challenge. The BSA solution was labeled with fluorescent dye as per Invitrogen’s Qubit Protein Assay Kit protocol. Fluorescent BSA was pipetted up and down three full times, with final dispense back to the original tube. Next, one hundred microliters of molecular grade dH₂O was pipetted up and down three times in the tip, and then dispensed into a fresh 0.5ml tube. The procedure was repeated in triplicate for each of the three brand pipet tips. The Qubit 2.0 Fluorometer was used to measure any residual fluorescent signal associated with retention of the protein solutions on the pipet tips. In the data generated, “too high” represented samples with greater than 5mg/ml, and “too low” represented samples with less than 12.5µg/ml.

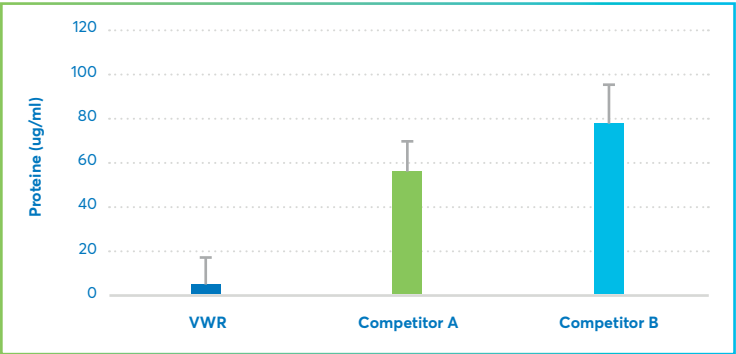
Results

As observed in the DNA analysis, there was measurable sample loss due to residual protein solution left in tips following dispensing of the sample (Table 3 and Figure 2). The greatest average measured loss of sample due to residual solution left on the tip was 0.32%, 1.47% and 1.87% for VWR, Competitor A, and Competitor B tips, respectively. The VWR tips demonstrated the least amount of protein sample loss in this analysis.

TABLE 3: Residual protein carryover

Sample	5 µl	10 µl	Mean (µg/ml)	SD
Negative Control	Too Low	Too Low	Too Low	Too Low
BSA 5mg/ml	Too High	Too High	Too High	Too High
VWR – 1	Too Low	Too Low	Too Low	Too Low
VWR – 2	Too Low	31.9	Too Low	Too Low
VWR – 3	Too Low	Too Low	Too Low	Too Low
Competitor A – 1	52.5	39.5	46	9.192
Competitor A – 2	50.8	48.2	49.5	1.838
Competitor A – 3	76.7	69.6	73.15	5.02
Competitor B – 1	83.5	83.4	83.45	0.071
Competitor B – 2	58	52.2	55.1	4.101
Competitor B – 3	97.3	89.7	93.5	5.374

FIGURE 2: Graph of residual protein carryover



NANOPARTICLE CHALLENGE

Methods

Cerium oxide nanoparticles (30 nm) at 82,000 ng/ml was used in the nanoparticle challenge. The CeO₃ solution was pipetted up and down three full times, with final dispense back to the original tube. Then using the same tip, one hundred microliters of molecular grade dH₂O was pipetted up and down three times and dispensed into a fresh 0.5ml tube. This procedure was repeated in triplicate for each of the three brands of tips. The wash solutions were analyzed on an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for residual CeO₃ associated with retention of the solution on the pipet tip.

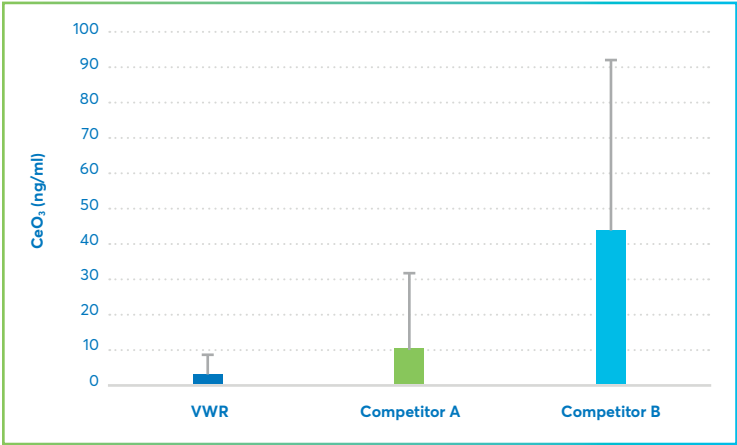
Results

There was a measurable difference in sample loss due to residual cerium nanoparticles in solution left on the tips following dispensing of the samples (Table 4 and Figure 3). The greatest average measured loss of sample due to residual solution left in the tips was 0.005%, 0.02% and 0.06% for VWR, Competitor A, and Competitor B tips, respectively. The VWR tips demonstrated the least amount of nanoparticle sample loss in this challenge.

TABLE 4: Residual CeO₃ carryover

Sample	5 µl	10 µl	Mean (µg/ml)	SD
VWR	3	4.328	4.019	0.433 – 8.49
Competitor A	3	17.287	14.556	1.36 – 29.9
Competitor B	3	44.107	48.700	9.52 – 99.8
Negative Control	2	>0.019	N/A	N/A
Positive Control	2	73545.5	8703.777	67391 – 79700

FIGURE 3: Graph of CeO₃ carryover



Testing performed by independent research laboratory, MRIGlobal, showed that the VWR® Universal Aerosol Filter Pipet Tips (Cat. No. 732-3633) to have superior performance in sample delivery as tested across three unique sample types: fluorescently labeled DNA; fluorescently labeled protein; and nanoparticles. Compared with competitive tips in this study, VWR tips consistently had the least amount of sample retention in all three challenges. When working with precious samples or using advanced technologies that are sensitive to minor variations in pipetting, choose VWR to ensure assay performance and data accuracy.

For more information visit vwr.com and search Cat. No. series 732-3709 to find all available VWR® Universal Aerosol Filter Pipet Tip volume options.

References

- Curry, D., MHale, C., and Smith, M., "Factors Influencing Real-Time RT-PCR Results: Application of Real-Time RT-PCR for the Detection of Leukemia Translocations", Molecular Biology Today, (2002) 3: 81.
- Applied Biosystems, "User Bulletin #2", ABI Prism™ 7700 Sequence Detection System, Oct. 1, 2001, pp.3.
- Illumina, Inc., "Liquid Handling", TruSeq™ Sample Preparation Best Practices and Troubleshooting Guide, June 2011, pp. 4