

Abstract

During the process of manufacturing and sterilizing disposable pipette tips, the tips are subjected to various methods and treatments that have the potential to alter tip characteristics and properties. The goal of this study was to explore the characteristic of liquid retention after an aspirate and dispense step with various types of previously treated pipette tips. Using dual-dye ratiometric photometry and gravimetric methods, we studied the degree of liquid droplet retention of a dye-based aqueous solution, a dye-based dimethyl sulfoxide (DMSO) solution, and pure deionized water for different pipette tip manufacturing processes, including low retention molding and electron beam (e^- beam) sterilization. The measured photometric and gravimetric data for the retained liquids in the pipette tips are compared and discussed.

Introduction

Plastic pipette tips are ubiquitous pieces of labware commonly used in life science, pharmaceutical, chemistry and many other types of laboratories. Because of their use in such diverse fields, certain qualities have been implemented to meet specific needs. Some of these qualities include sterilization of the tips for biological work, the addition of filters inside the tip to reduce aerosol contamination of the pipette cavity, and reducing the volume of drops retained by the tip's surface.

While lab personnel may be accustomed to selecting tips based on their particular needs, certain tip types may possess unexpected characteristics. One quality that may be commonly overlooked is the degree of liquid retention associated with a particular tip type versus another, or from one manufacturer versus another.

Initiated by an observation that Artel dye solutions were 'sticking' to, or remaining in, pipette tips after a dispense from a liquid handler, an investigation into the various properties of these tips and their interaction with different solutions was conducted. The presence of remaining droplets of dye solution in/on the tips, along with a high degree of variability in the dispensed volume prompted Artel to undertake this study.

The goal of this tip evaluation study was to determine if the observed droplet retention was due to some chemical/physical property of solution or of the tips. The types of tips tested herein included commercially available tips as well as some non-sterile samples which are currently unavailable for purchase. Using an electronic pipette to imitate an ALH dispense method, solution retention was measured by: *i*) the Artel PCS® Pipette Calibration System, and *ii*) a gravimetric method. A selection of tips was purchased from different manufacturers, including sterile, non-sterile, filter and low retention tips. The same experimental protocol was conducted for each tip type using aqueous dye solution, deionized water and DMSO. Experimental testing focused on tip sterilization as a possible factor affecting droplet retention.

Experimental Section

Summary of Pipette Tips

The pipette tips used in this study were obtained from four different manufacturers (labeled A thru D; see **Table 1**). Each tip was assigned a Tip ID and the specific characteristics of each tip were recorded. The characteristics for the tips included; *i*) standard, *ii*) low retention, *iii*) filter, *iv*) sterile, and *v*) some combination thereof. All tip types tested herein were manufactured from polypropylene, which is the material used for most commercially available tips. As a qualitative test of the polypropylene material used by different manufacturers, the presence of visible fluorescence was noted when the tips were exposed to a 15 watt black light. The sterilization method used for all 'sterile' tips was exposure to an electron beam.

Table 1. Summary of pipette tips tested in this study.

Manufacturer	Tip ID	<i>n</i> ^a	Style	Visibly Fluorescent? ^b
A	A1	25	Standard	Yes
	A2	25	Standard, Filter	Yes
	A3	25	Standard, Filter, Sterile	Yes
	A4	25	Low Retention	Yes
	A5	25	Low Retention, Filter	Yes
	A6	44	Low Retention, Filter, Sterile	Yes
B	B1	42	Standard	No
	B2	77	Standard, Sterile	No
	B3	42	Low Retention	No
	B4	42	Low Retention, Sterile	No
C	C1	84	Standard	No
	C2	32	Standard, Filter, Sterile	No
D	D1	40	Standard	Yes

^a Herein *n* represents the total number of tips tested, one test per tip.

^b Refers to a qualitative observation of visible fluorescence in the presence of a black light.

Pipetting Method used to Deliver All Solutions

All solutions tested in this study were delivered using a 5-100 μ L BioHit Proline electronic pipette. Care was taken to ensure each tip was firmly seated on the pipette mandrel before use. The pipetting program used for this study had four stages, each stage initiated by depressing the pipetting trigger: *i*) Press one draws up solution, *ii*) Press two dispenses a pre-sample volume of solution, *iii*) Press three dispenses the sample volume of interest, and *iv*) Press four dispenses the remaining liquid from the tip and resets the pipetting plunger for the next sample. This protocol was repeated for every tip tested.

PCS Method used to determine retained dye droplet volume

The Artel PCS is a system used for pipette calibration over a volume range of 100 nL up to 5 mL, which is based upon dual-dye ratiometric photometry. Details of this system are described in Reference (1). For this test, a 5 μ L aliquot of red dye (PCS Range 5 Sample Solution) was aspirated from, and dispensed back into an aliquot container using the four stage delivery protocol for the Proline electronic pipette with the tip of interest affixed, as described above. After performing this protocol, the volume of any remaining red dye droplets was determined by immersing the tip, while still attached to the pipette, into a vial inserted in the PCS instrument which contained a known volume of blue dye solution (PCS Blank Solution). The pipetting program described above was repeated, which effectively washed the red dye droplet off the tip and into the blue dye solution. This process was repeated a second time to ensure all the red dye had been washed out of the tip. The PCS then mixed the solutions in the vial, measured the absorbance of both dyes in the vial, and calculated the volume of any remaining red dye droplet using a ratiometric relation based upon Beer's Law.

Gravimetric Method used to determine retained water droplet mass

A comparative technique was performed using a gravimetric procedure and deionized water. This method was conducted to determine if the observed droplet retention was due to some chemical property of the dye solution, or if it were more likely a physical property of the tip itself. The four stage pipetting procedure previously described was performed using deionized water. For this procedure a gravimetric method was employed using a Mettler Toledo XP205 balance (resolution = 0.01mg). Each tip was weighed dry before being loaded onto the pipette for liquid aspiration. After aspirating water from, and dispensing back into an aliquot container, each tip was re-weighed to determine the mass of any remaining water.

Experimental (continued)

Gravimetric Method used to determine retained DMSO droplet mass

As a further test of the chemical/physical nature of solution retention, a DMSO-based dye solution was tested using the same gravimetric protocol as for deionized water, with one notable exception; for each tip type, only 10 different tips were tested. Red dye was incorporated into the DMSO solution to aid in visual observation of any retained solution.

Results and Summary

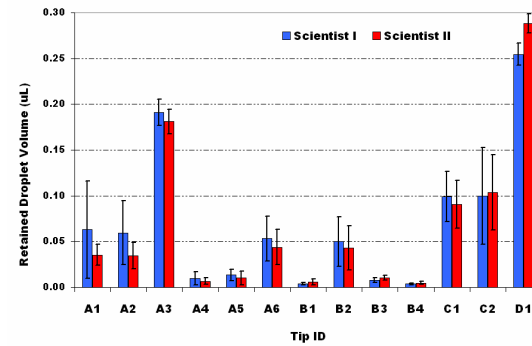


Figure 2 (at right). The average mass of retained droplets of deionized water adhering to the tips, as measured by the gravimetric procedure, is shown in this figure. The only tip from manufacturer A retaining significant quantities of water are the sterilized A6 tips. Tips A3 were not included in this study because the supply was exhausted before this specific experiment. The results are generally in agreement with those presented in **Figure 1** and seem to indicate that e^- beam sterilization appears to cause increased droplet retention (A1, A2, A4 and A5 are non-sterile). Examining B tips, the sterilized B4 tips had much higher water retention than the non-sterilized B3 tips. The C tips also acted in a similar manner to their performance with PCS Sample Solution; there appears to be no difference between pre- and post-sterilization for tips from this manufacturer. Also, as in **Figure 1**, tip D1 displayed the highest retention values.

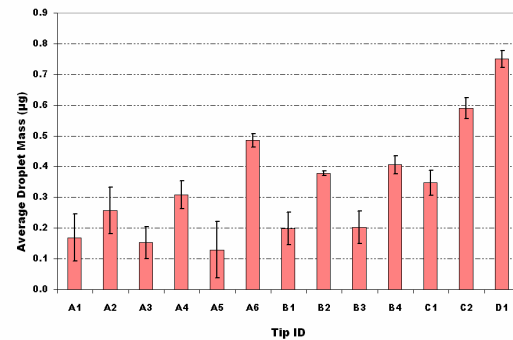


Figure 1 (at left). This figure shows the average volumes of the PCS Sample Solution droplets that were retained on each type of pipette tip. The entire experiment was conducted separately by two different scientists, and the average volumes calculated from the data collected by each scientist are shown as indicated by the figure legend. The number of tips tested for each tip type is summarized in **Table 1**. The error bars are calculated as 2X the standard error of the measurements, which approximates a 95% confidence interval. Cases where error bars do not overlap (e.g., Tip D1) show a high probability that the differences in the mean values are statistically significant.

The data in this figure demonstrate significant differences in droplet retention based upon tip type, but also based upon manufacturer. For example, comparing "standard" tips A1, B1, C1 and D1 shows a very large difference in liquid retention.

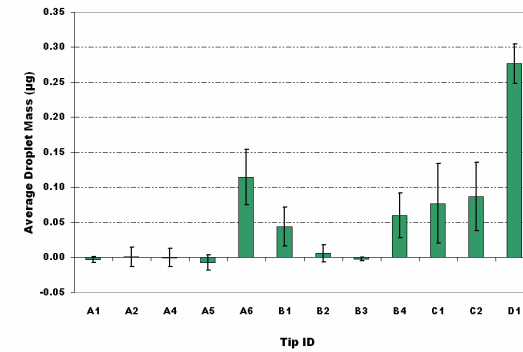


Figure 3 (at left). The average mass of DMSO solution retained by each tip is presented in this figure. The general trend observed in **Figures 1 & 2** above, where sterilized tips retain more solution, is repeated here. However a notable difference is the general increase in solution retention of the DMSO sample. A comparison of the DMSO data with data from the aqueous solutions indicates a higher retention of DMSO for most tips. Even when accounting for the difference in density of DMSO ($\rho = 1.1 \text{ g/cm}^3$) versus water ($\rho = 1.0 \text{ g/cm}^3$), most tips retained 2-5 times more DMSO than water. These data were supported by visual observations of the presence of constant, large droplets remaining on both the inside and outside of the tips which were not properly expelled from the tip during each dispense. Note: as opposed to the data in **Figures 1 & 2**, $n = 10$ for this figure.

Discussion

All of the tips tested retained some amount of solution. However, retained droplet volumes differed significantly between tip types, manufacturers and between aqueous and DMSO based solutions. It appears as if the e^- beam sterilization process increases droplet retention, most notably for aqueous solutions. Also, tips declared to be "low retention" by the manufacturer were determined to retain lower volumes of aqueous liquid, supporting those claims. In general, liquid retention seemed to trend in the same direction for both aqueous and DMSO solutions, meaning sterilized tips tended to retain more liquid than non-sterile tips.

The agreement in the droplet retention volumes for the deionized water and the Artel PCS Sample Solutions demonstrates that the initially observed 'sticking droplets of PCS dye solution' is not related to different chemical properties of the two aqueous solutions; rather that certain tips retain more liquid due to the specific characteristics of the tips themselves. It is possible that the initial concern that prompted this study was noticed due to the fact that Artel Sample Solution is much more visible to the naked eye via its red dye component versus colorless water.

The DMSO testing demonstrates that liquid retention is related to both chemical and physical interactions between the solution and the tip material.

In summary, the results of this study indicate that pipette tips have a tendency to retain various amounts of liquid post aspiration and dispense. The data demonstrates that tips sterilized via an e^- beam protocol produce higher rates of liquid retention than non-sterilized tips. Contrasting the effect of tip sterilization, tips manufactured to be low retention retain lower volumes of liquid, as would be expected. Finally, Artel Sample Solution retention volumes were comparable to deionized water volumes, which indicates that both solutions behave in a similar manner when used with identical pipette tips, no matter the method of pipette tip production (low retention, sterile, etc.).

Conclusions

The results presented herein demonstrate retention volumes for different solutions dispensed from different tip types. These results indicate that pipette tips have a tendency to retain various amounts of liquid after an aspirate and dispense step, and that the amount of liquid retained is related to the type of tip used, the process used to manufacture the tip, and the manufacturer of the tip. Liquids retained in pipette tips can cause unseen assay errors resulting from incorrect sample volumes, sample contamination or carry-over if tips are re-used. Thus an understanding of the liquid retention characteristics for a desired tip is helpful in reducing potential errors.

References

(1) Curtis, Richard, "Performance Verification of Manual Action Pipettes, Part 1 and Part 2," American Clinical Laboratory, 1994, 12 (7), 8-10.