Molecular BioProducts

Technical Report

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Performance Evaluation of ART® Self-Sealing Barrier Tips

ABSTRACT

ART, self-sealing barrier tips, manufactured by Molecular Bio-Products, inc., were tested for overall performance in several important categories. In the areas of volume delivery, sample retention, sample retrievability and carryover contamination protection, ART tips were shown to provide accurate and consistent performance.

INTRODUCTION

Currently, many manufacturers claim to offer anti-aerosol pipet tips that provide the precision, accuracy and carryover protection that is essential in molecular biology. Based on appearance and the claims of each manufacturer, all pipet tips are assumed to be the same and the choice between brands is often a function of cost. However, given the importance of maintaining optimal reaction conditions in the lab, choosing a pipet tip should not be based on assumptions, but on tangible data that specifically defines a tip's performance.

Molecular Bio-Products, inc., takes pride in the manufacturing techniques and technology that goes into the production of ART, self-sealing barrier tips. Precision molding, unsurpassed quality control, and patented technology makes ART tips the industry standard.

OBJECTIVE

Useful data that describes the overall effectiveness of ART tips in various important categories is necessary for a comprehensive evaluation. Therefore, the following experiments testing volume delivery, sample retention, sample retrievability and carryover protection were designed to accurately evaluate the performance of ART tips using protocols that duplicate real-life conditions.

MATERIALS AND METHODS

Experiment #1
Accuracy

As precise volume delivery is essential, this study was designed to reliably determine the accuracy and consistency of ART tips. Ninety-six ART tips were sent to an independent lab to be tested for accuracy in sample transfer.

A Gilson Pipetman P-20 was used to draw a determined 20 µl volume of deionized water, which was then dispensed and measured gravimetrically on a Sartorious balance, model H110, calibrated with an ASTM approved 1 mg counter weight. The weight of the 20 µl sample was recorded and used as a reference for the following accuracy test.

The same P-20 pipettor, which was tested for accuracy using the calibrated balance and non-filtered pipet tips, was set at 20 µl and used to draw a sample with each of the 96 ART tips. The dispensed volume was weighed and compared to the weight of the control sample to precisely determine the actual volume drawn.

The results of this test show ART tips to be extremely consistent and accurate in volume delivery. In the sample of 96 tips, an average volume delivery of exactly 20 µl was recorded with a low standard deviation of 0.147 µl (table 1). This deviation is well within the pipettor manufacturer's stated 1% error margin indicating that ART tips are precise in design and construction and will consistently deliver intended volumes.

table 1

	ART tips
Mean	20.0 µl
Mode	20.0 µl
Std. Deviation	0.147 µl

Experiment #2
Sample Retention

A contributing factor to inaccurate volume delivery is sample retention. Flaws and irregularities in the pipet tip can contribute to the retention of liquid sample on the interior walls, or at the point. This is of particular concern when working with low volume samples and when proper reaction conditions depend on precise volumes.

Precision molding techniques and high levels of quality control ensure that ART tips are as flawless as possible. Smooth interior walls and the absence of imperfections means that no significant amounts of liquid will be retained within ART tips. This second study was designed to determine if any sample is retained in ART tips after dispensing.

In an independent lab, a 20 µl pipettor was used with ART tips to draw and expel samples of ^{32}PdCTP. Initial counts per minute were determined by expelling a 10 µl sample of ^{32}PdCTP into scintillation fluid.

Another 10 µl sample of the radioactive label was drawn and expelled and the now "empty" tip was rinsed three times in an in-and-out procedure with new scintillation fluid. Counts per minute were then determined to quantify the amount of ^{32}PdCTP that was retained within the pipet tip. This protocol was repeated using three different ART tips and percent retention was calculated for each.

A second experiment was performed using an identical protocol but with 324 bp labeled DNA in place of the ^{32}PdCTP. Results of both experiments are summarized below.

The three tips tested with ^{32}PdCTP were consistent in returning as much as 99.94% of the sample. This means a loss of only 0.012 µl from a 20 µl sample (table 2). Similar results were obtained in the study using radio-labeled DNA with up to 99.92% of the sample returned (table 3).

table 2 **^{32}PdCTP**

ART tips	**% Retained**
1	0.057
2	0.179
3	0.074
Mean	0.103

table 3 **Labeled DNA**

ART tips	**% Retained**
1	0.076
2	0.165
3	0.140
Mean	0.127

Experiment #3
Sample Retrievability

For normal pipetting, the barriers in ART tips are placed to accommodate the full volume draw of their compatible pipettors meaning that the barriers will not seal and delivery will not be affected. But, in the unlikely event that over-pipetting should occur, the barrier will seal to protect the pipettor. This invaluable feature is essential for carryover protection but can make it difficult to expel samples with the pipettor.

The sample can be retrieved from a sealed ART tip by cutting the tip near the point with a razor and using a syringe or small pipet tip to withdraw the sample through the enlarged opening.

This method was tested by using a 200 µl pipettor to intentionally overdraw sample using 20 µl ART tips. Each tip was weighed using a four-place, Sartorious balance before drawing the sample and again after the sample was removed. The weights before and after were compared to determine the percent of sample retained after retrieval.

Patented ART barriers are effective because they seal when penetrated by liquids or aerosols. It is this feature that facilitates the retrieval of precious samples if over pipetting should occur.

Other, sponge-like filters will absorb liquid leaving much of the sample retained within the tip. With the ART tips tested, as much as 91.1% of the sample was easily recovered (table 4). This proves that in the unlikely event that over pipetting should occur, nearly all of the sample can be retrieved.

table 4

	% Recovered
Average	88.94
Maximum	91.1
Std. Deviation	1.6

Experiment #4
Carryover Protection

Anti-aerosol pipet tips were designed expressly for the purpose of preventing carryover contamination from one sample to another. The ability to protect against contamination should be the determining factor when choosing a pipet tip, therefore the following experiments were conducted to determine the efficacy of ART barriers.

For the first test, a conventional PCR reaction was carried out using primers for mitochondrial D-loop DNA at 0.2 mM concentration and template consisting of 0.02 µg total human liver DNA (Life Technologies). $MgCl_2$ concentration was 2 mM, dNTP concentration was 2 mM and 1X PCR buffer was used. Thirty thermal cycles consisted of 56° C for 30 seconds, 72° C for 60 seconds and 94° C for 60 seconds.

An ART barrier tip was then used to intentionally overdraw 20 µl of PCR product using a pipettor deliberately set at 200 µl. Any liquid that passed through the barrier was to be collected and run on a 1% agarose gel paired with 15 µl of the same product taken from the original reaction tube.

In a second study, a master sample of ^{32}PdCTP was tested on a scintillation counter to determine initial counts per minute before three, 20 µl aliquots were separated from it. Then three 20 µl ART tips were chosen at random and used on a 200 µl pipettor set at 200 µl to intentionally overdraw the samples of radioactive label.

If any sample penetrated the barrier, it was to be retrieved from behind the filter and tested on the scintillation counter to determine counts per minute. An identical protocol was also performed using radio-labeled DNA. The results of these three studies are summarized below.

The ART barrier completely prevented the PCR product from passing to the pipettor. This initial observation proves that ART barrier tips protect the pipettor from contamination as a result of over pipetting.

To test for the penetration of aerosols, a dH_2O wash was used behind the ART barrier and then run on agarose gel. Again, ART self-sealing barrier tips proved to be effective by preventing aerosols containing contaminating DNA from passing beyond the barrier (Figure 1).

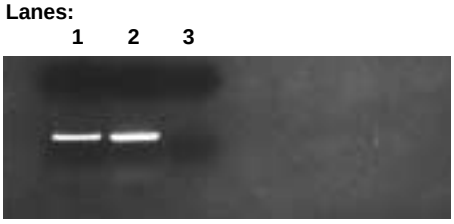

Figure 1

In *Figure 1*, Lane 1 is sample taken from behind the filter of another manufacturer's tip and lane 2 is the corresponding "unfiltered" control. Lane 3 is the dH_2O wash taken from behind the ART barrier.

ART barriers were also shown to seal completely in the studies using radio-isotope and radio-labeled DNA. In every case, the patented barriers prevented any contaminating substances from passing beyond the filters to the pipettor. It can therefore be concluded that ART, self-sealing barrier tips can be depended upon to provide complete contamination protection.

DISCUSSION

Anti-aerosol pipet tips should offer a combination of precision, consistency and protection that is equal to the efforts given to ensuring ideal reaction conditions in the lab. The consideration that goes into calibrating pipettors, careful handling of materials and maintaining a contamination free environment should be extended to choosing the right pipet tips.

ART tips are manufactured to provide the performance and consistency that labs depend on. Precise volume delivery, negligible sample retention and complete contamination protection are necessary features and should not be compromised when choosing pipet tips.

The preceding studies were specifically designed to accurately determine the effectiveness of ART tips in real-life conditions. The results show that ART, self-sealing barrier tips from Molecular Bio-Products, inc., consistently perform to the highest standards, and most importantly, provide assurance of complete carryover protection.

All pipet tips are not the same and choices between brands must be based on tangible data about the tip's performance. With the data presented in this report, no assumptions need be made about the ability of ART tips to perform as required. ART tips combine patented technology and unparalleled quality control in aerosol barrier tips that are unequaled in the industry. With this data, ART, self-sealing barrier tips are the clear choice.

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Mark Brolaski, MO BIO Laboratories, San Diego, CA.

Doreen Hom, M.S., UCSD, San Diego, CA.

Robert Enns, Ph.D., Molecular Bio-Products, inc., San Diego, CA.