

Elimination of PCR Carryover

ABSTRACT

ART[®], self-sealing barrier tips, manufactured by Molecular BioProducts, inc., were tested against unfiltered pipet tips to determine their effectiveness in preventing carryover contamination. Experiments using HIV-1 DNA and radioisotopes were developed to test the ability of ART tips to block the passage of aerosols and liquids and prevent the contamination of subsequent samples.

INTRODUCTION

Performing the Polymerase Chain Reaction (PCR*) successfully depends on the careful handling of samples and reagents in order to maintain the integrity of the reaction. Any cross contamination can result in false signals or the amplification of extraneous DNA.

The most common causes of false signals is the carryover of previously amplified DNA from one tube to the other or by other forms of sample-to-sample contamination. The action of pipetting creates aerosols that are drawn into the pipettor and then transferred to subsequent samples by the pipettor's air displacement.

To prevent this type of carryover contamination, Molecular BioProducts, inc., has developed the ART, self-sealing barrier tip that incorporates a patented barrier that blocks the passage of liquids and aerosols. This eliminates the possibility of contaminating the pipettor through over pipetting or aerosol formation.

OBJECTIVE

Given the importance of complete carryover protection, it is necessary to test the efficacy of ART tips in situations that relate directly to those found in the lab. The following experiments were therefore designed to determine the level of protection that ART tips provide.

MATERIALS AND METHODS

Experiment #1

A standard 50 μ l PCR cocktail mix was used that contained the primer pair SK-

38/39 (specific oligonucleotide markers for HIV-1 gag genome). The 100 μ l PCR reaction was initiated by addition of 50 μ l of HIV-1 cell lysate for the positive control and PCR reagents only for the negative control.

After 30 cycles of PCR amplification, SK-38/39 amplified product was liquid hybridized with ³²P-labeled SK-19 probe. The hybridized mixture was then separated on a 10% PAGE gel and developed by autoradiography.

A 50 μ l sample of previously amplified HIV-1 DNA was pipetted up and down in a non-filtered pipet tip and in an ART, self-sealing barrier tip in order to maximize aerosol formation and thus compare the ability of each to prevent carryover contamination.

The pipettor barrel was then washed in 50 μ l of sterile water which was added to a PCR cocktail mix and amplified for the detection of HIV-1. The procedure was repeated with ART tips using 100, 150 and 200 μ l samples of HIV-1 amplified DNA.

To confirm that the reagents used to make the PCR cocktail mix were not a source of HIV-1 contamination, 50 μ l PCR cocktail mix was added to 50 μ l sterile water and amplified by PCR. As shown in *Figure 1* (lane 2), no HIV-1 specific sequences were detected.

To demonstrate that the pipettor barrel was not previously contaminated with HIV-1 from previous experiments, the shaft was immersed and washed in 50 μ l sterile water which was added to a PCR cocktail mix and amplified by PCR. No HIV-1 contamination was detected (lane 3). This assay is capable of detecting as few as 1-10 copies of HIV-1 in a background of one million peripheral blood mononuclear cells.

Experiment #2

50 μ l of an aqueous solution of γ -labeled ATP (396,773 cpm/ μ l) was pipetted repeatedly up and down, generating aerosols in the pipet tip. The barrel of the pipettor was then wiped with a moist filter paper disk which was placed into scintillation fluid for radioactive counting.

The data presented in table 1 represents counts minus background due to the scin-

tillation cocktail. Radioactive contamination of the two sides of the physical barrier was measured by cutting the barrier into halves and placing them into scintillation fluid for counting.

Experiment #3

Contamination of the pipettor barrel by fluid absorption from substances such as radioisotopes, amplified DNA, or infectious materials could occur as a result of poor technique or the use of miscalibrated pipettors. A test was developed to evaluate the effectiveness of various anti-aerosol inserts in the pipet tips. Three different insert materials were chosen: a cotton-plugged version, a bonded cellulose fiber plug, and the patented ART, self-sealing barrier. 20 μ l of γ -labeled ATP/red dye mix was placed on top of the three different inserts in the pipet tips.

The pipet tips indicated above were placed in a rack. Twenty microliters of an aqueous solution (γ -labeled ATP mixed with red food coloring-396,733 cpm/ μ l) was applied to the top side of the tip's insert.

The absorption of red dye into the inserts was observed and recorded. The penetration of the radioactive material was measured by cutting the inserts into halves and placing them into scintillation fluid for counting. The data in table 2 represents counts minus background. Remainder of counts were accounted for on the pipet tip and on the fluid side of the insert.

RESULTS

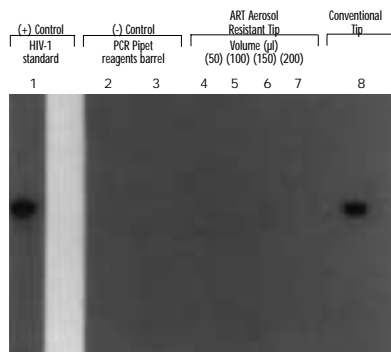
Experiment #1

As shown in *Figure 1* (lane 1) PCR amplification of HIV-1 cell lysate generates a specific product that can be visualized by autoradiography detection at 115 bp. After amplification of the sterile water used to wash the pipettor barrel with the unfiltered pipet tip, the same HIV-1-specific product was detected (lane 8). This shows that the aerosols generated by the action of pipetting did contaminate the pipettor and could have been carried to subsequent samples.

As shown in lanes 4-7, no HIV-1 contamination could be detected on the pipettor barrels that were protected by the ART tips. ART tips offered 100% protection from carryover contamination with each of the four samples.

Figure 1

Evaluation of HIV-1 aerosol contamination of a conventional air displacement micropipet using



ART tips.

HIV-1 specific product can be seen on autoradiogram after amplification of PCR reaction mixture, which contained HIV-1 gag gene primer pair SK38-39. Cell line 85-14-F2, which contained one copy of HIV-1 proviral DNA per cell, was used to generate an HIV-1 standard in a background of two million normal PBMCs. HIV-1-specific product, 115 bp in size, is marked (lane 1). PCR amplification, reagents only (lane 2 or sterile water from pipet barrel wash (lane 3) represent negative controls; 50-200 µl (lanes 4-7) of HIV-1 amplified product (lane 1) was pipetted repeatedly using Aerosol Resistant Tips. Sterile wash from the pipet barrel was amplified by PCR. When conventional pipet tips were used (lane 8), HIV-1 contamination was observed.

Experiment #2

As shown in table 1 below, when ART tips were used, no detectable radioactivity could be detected on the pipettor barrel or on the pipettor side of the barrier. However, radioactive counts could be detected on the barrier side exposed to the isotopes. These results prove that ART tips are effective in preventing aerosol contamination in laboratory applications that require the handling of radioisotopes.

Table 1.

Measurement of radioactive contamination of the pipettor barrel due to aerosolization.

Conventional Tip (no barrier)	ART (Aerosol Resistant Tip)
122,993 CPM at pipette barrel	0 CPM at pipette barrel
	0 CPM at barrel side barrier
	170,920 CPM at fluid side of barrier

Experiment #3

Absorption of red dye was observed passing through all inserts tested except the ART barrier (table 2). As can be seen, the detection of radioactivity paralleled the location of the red dye.

This confirms that neither cotton plugs nor bonded cellulose plugs provided an effective barrier to these substances. The self-sealing barrier in the ART tips was effective in stopping contaminants from passing through. In the case in which ART tips were used, the red liquid interacted with the barrier surface to form a sealing gel that prevented the dye from penetrating.

Table 2.

Absorption of contaminant	Cotton plug	Cellulose plug	ART (Aerosol Resistant Tip)
Red dye	+	+	-
CPM top side	5,196,738	4,633,054	7,199,755
CPM bottom side	2,737,985	2,805,499	0
Total CPM	7,934,723	7,438,553	7,199,755

Absorption of radioactive dye mix in pipet tips with different physical barriers.

DISCUSSION

Filter pipet tips have been developed to preserve the integrity of precious samples by preventing carryover contamination. The efficacy of ART, self-sealing barrier tips, manufactured by Molecular BioProducts, inc., was tested using these three experiments that parallel real lab conditions. In these studies, ART tips offered 100% protection from carryover contamination. These results prove that ART tips would be effective in preventing aerosol contamination, contamination due to liquid contact, and carryover problems in such applications as tissue culture, serological assays, forensic studies, nucleic acid or protein loading, PCR, and pipetting of radioactive samples.

References

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Molecular BioProducts, Inc.
 9880 Mesa Rim Road, San Diego, CA92121
 Toll-free:1/800/995-2787
 Tel:858/453-7551 • Fax:858/453-4367
 e-mail: info@mbpinc.com • www.mbpinc.com