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Elimination of PCR Carryover

Liquid Handling Consumables, Thermo Fisher Scientific

Abstract

Thermo Scientific™ ART™ self-sealing barrier tips were tested against unfiltered pipette tips to determine their effectiveness in preventing carryover contamination. Experiments using HIV-1 DNA and radioisotopes were developed to test the ability of ART barrier tips to block the passage of aerosols and liquids and prevent the contamination of subsequent samples.

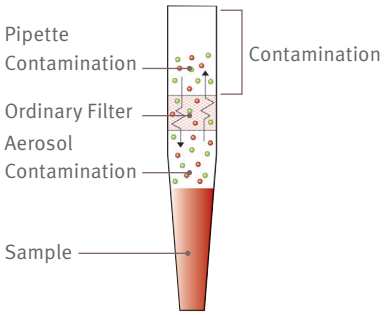
Introduction

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

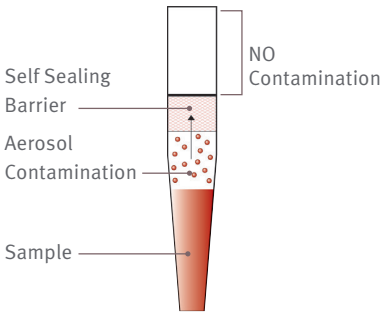
The most common cause of false signals is the carryover of previously amplified DNA from one tube to another or by other forms of sample-to-sample contamination. The action of pipetting creates aerosols that are drawn into the pipette and can then be transferred to subsequent samples by the pipette's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

To prevent this type of carryover contamination, Thermo Fisher Scientific has developed the ART self-sealing barrier tip that incorporates a barrier that is designed to block the passage of liquids and aerosols. This eliminates the possibility of contaminating the pipette through over pipetting or aerosol formation.

Pipette Tip with Generic Filter



Pipette Tip with ART Barrier



Objective

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

Materials and Methods

Experiment #1

A standard 50µL PCR cocktail mix containing the primer pair SK- 38/39 (specific oligonucleotide markers for HIV-1 gag genome). The 100µL PCR reaction was initiated by addition of 50 µl of HIV-1 cell lysate to create the positive control and the addition of 50µL of PCR reagents to create 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 µl sample of previously amplified HIV-1 DNA was pipetted up and down in an ART self-sealing barrier tip to maximize aerosol formation. The pipette barrel was then washed in 50uL of sterile water which was added to a PCR cocktail mix and was amplified for the detection of HIV-1.

The procedure was repeated with ART pipette tips using 100, 150 and 200 µl samples of HIV-1 amplified DNA and also with a 50 µl HIV-1 DNA sample using a non-filtered pipette tip.

To confirm that the reagents used to make the PCR cocktail mix were not a source of HIV-1 contamination, 50 µl PCR cocktail mix was added to 50 µl sterile water and amplified by PCR.

To demonstrate that the pipette barrel was not previously contaminated with HIV-1 from previous experiments, the shaft was immersed and washed in 50 µl sterile water which was added to a PCR cocktail mix and amplified by PCR. 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

50µL of an aqueous solution of y-labeled ATP (396,773 cpm/µL) was pipetted repeatedly up and down, generating aerosols in the pipette tip. The barrel of the pipette 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 scintillation 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 pipette barrel by fluid absorption from substances such as radioisotopes, amplified DNA, or infectious materials could occur as a result of poor pipetting technique or the use of miscalibrated pipettes. A test was developed to evaluate the effectiveness of various aerosol-blocking filters in the pipette tips. Three different insert materials were chosen: a cotton plug, a bonded cellulose fiber filter, and the ART self-sealing barrier. 20µL of y-labeled ATP/red dye mix was placed on top of the three different filters in the pipette tips.

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

The absorption of red dye into the filters 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 due to the scintillation cocktail. The remainder of the radioisotope counts was accounted for on the pipette tip and on the fluid side of the filter.

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 pipette barrel with the conventional non-filtered pipette 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 pipette and could have been carried to subsequent samples.

Lanes 2 and 3 serve as negative controls. Lane 2 indicates that the PCR reagents were not a source of HIV-1 contamination, as no HIV-1 specific sequences were found. Likewise, no HIV-1 contamination was detected in lane 3, indicating the pipette barrel was not contaminated in previous experiments with HIV-1.

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

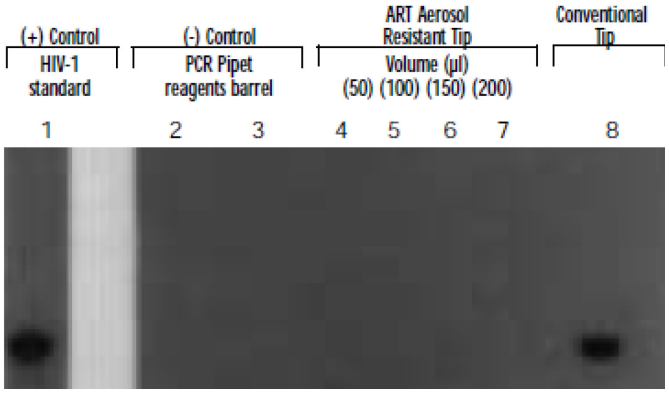


Figure 1
Evaluation of HIV-1 aerosol contamination of a conventional air displacement micropipette using ART tips.

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 and 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 pipette barrel wash (lane 3) represent negative controls; 50-200µl (lanes 4-7) of HIV-1 amplified product (lane 1) was pipetted repeatedly using Aerosol Resistant Tips. Sterile wash from the pipette barrel was amplified by PCR. When conventional non-filtered pipette tips were used (lane 8), HIV-1 contamination was observed.

Experiment #2

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

Conventional non-filtered pipette tips (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

Table 1- Measurement of radioactive contamination at the pipette barrel due to aerosolization

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 filters provided an effective barrier to these substances. The self-sealing barrier in the ART barrier tips was effective in stopping contaminants from passing through. In the case in which ART barrier tips were used, the red liquid interacted with the barrier surface to form a sealing gel that prevented the dye from penetrating.

Absorbtion 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	2,737,985	2,805,499	0
side Total CPM	7,934,723	7,438,553	7,199,755

Table 2- Measurement of radioactive dye mix in pipet tips with different physical barriers

Discussion

Filter pipette 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 Thermo Fisher Scientific, was tested using these three experiments that replicate real lab conditions. In these studies, ART barrier tips offered 100% protection from carryover contamination. These results suggest that ART barrier tips would be effective in preventing aerosol contamination, contamination due to liquid contact, and carryover contamination problems in such applications as tissue culture, serological assays, forensic studies, nucleic acid or protein gel electrophoresis, PCR, and pipetting of radioactive samples.

References

1. Erlich, H.A. and Gelfand, D.H. and Saiki, R.K. Nature 331 (1988): 461
2. Kwok, S. and Higuchi, R. Nature 339 (1989): 237-238
3. Williams, J.F. BioTechniques 7 (1989):762-766
4. Gibbs, R.A. and Chamberlain, J.S. Genes Dev 3 (1989) 1095-1098
5. Kwok, S. Procedures to minimize PCR product carryover. In: Innis MA, Gelfand, D.H., Sninsky, J.J. and White, T.J. eds. PCR Protocols: A Guide to Methods and Applications: San Diego: Academic Pres, 1990: 142-145
6. OU, C.Y. et al Science 239 (1989): 295-297