

Biotix Filter Tip Technology Security and protection of the highest standard

Introduction

Within the past two decades, the use of aerosol barrier pipette tips, also referred to as "filter tips", has become a best practice in protocols utilizing PCR amplification 1. Moreover, the efficiency of filter tips in preventing cross contamination of micro-particles has made it a standard consumable cited in instruction manuals for laboratories performing molecular assays in research2 and in the clinic3,4. Though generally highly effective, errors in pipetting such as overdraws and splash, can challenge even the most robust filter. Biotix has introduced a line of aerosol barrier pipette tips (Table 1) which traps aerosols most efficiently.

Aerosol Blocking Efficiency

Filter tips from Biotix and two leading brands on the market were evaluated for effectiveness in blocking aerosols. Aerosol blockade was tested on solutions containing PCR amplicon, bacterial culture (E. coli), vaccinia viral particles, and nanoceria (30nm Ce03). Amplicon contamination was detected utilizing realtime PCR, bacteria by plate counts, viral particle by realtime PCR, and nanoceria by Indicutively Coupled Plasma Mass Spectrometry (ICP-MS). Samples were processed in triplicate and analyzed in duplicate.

Table 1: Aerosol Barrier Tips Used in Study

Manufacturer	Part Number	Lot Number	Description			
Biotix	M-0100-9FC	0309 65464	100 µl Filter Pipette Tip, Sterile			
Competitor 1	TF-100-L-R-S	302-28-151	100 µl Filter Pipette Tip, Sterile			
Competitor 2	2065	09440809	100 µl Filter Pipette Tip, Sterile			

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

PCR Amplicon Challenge

Methods

A previously generated amplicon of 176 bp at a concentration of 24 ng/ml was utilized for testing filter efficiency in blocking PCR aerosols. Samples were prepared by pipetting 100 µl of the solution up and down with a 200 µl pipettor three full times, with a final dispense back into the original tube. The tips were carefully removed, and with a new pipettor and fresh tip, the inside of the used tip above the filter was washed by pipetting and dispensing 3 times with 100 µl of nuclease-free dH2O. The wash was then moved to a fresh 0.5 ml tube. This was repeated in triplicate for each of the three pipette tip brands. Ten microliters of the resulting samples were analyzed in duplicate by realtime PCR using standard cycling conditions for a total of 50 cycles on a BioRad iCycler. Positive controls were the starting amplicon at 1:10 and 1:100 dilutions. Negative controls were no template controls with molecular grade water.

Results

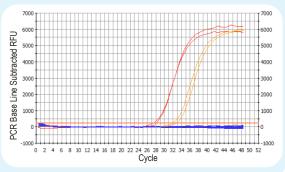
Targeted amplification showed no amplified product for all three brands, indicating equal efficiency in blocking PCR amplicon aerosols (Table 2, Figure 2). Positive controls showed expected amplification and negative controls showed no amplification.

Bacterial Challenge

Methods

A culture of Escherichia coli was grown in BHI broth to an OD of 1.0. Samples were prepared by pipetting 100 μl of the culture up





and down with a 200 μ l pipettor three full times, with a final dispense back into the original tube. The tips were carefully removed, and with a new pipettor and fresh tip, the inside of the used tip above the filter was washed by pipetting and dispensing three times with 100 μ l of sterile BHI broth. The broth was moved to a fresh 0.5 ml tube. This was repeated in triplicate for each of the three pipette tip brands. Analysis was performed by plating 100 μ l of sample in duplicate on BHI plates. Plates were incubated for 24 hours at 37°C, with bacterial colony counts taken after.

Results

Representative plates are shown in Figure 3. Control plates with the original starting E.coli stock show a lawn of growth, whereas plates containing washes from above the filters of all three tip brands showed no bacterial growth. This indicates equal effectiveness in blocking bacterial aerosols. No growth was observed even after 96 hours of incubation.

Table 2: PCR for Amplicon Aerosols

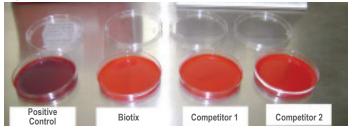
Sample	Replicates	Mean Ct	Ct SD
Positive Control - Amplicon 1:10	3	27.61	0.563
Positive Control - Amplicon 1:100	3	32.22	0.581
Biotix Tip – Amplicon Wash	6	N/A	N/A
Competitor 1 Tip – Amplicon Wash	6	N/A	N/A
Competitor 2 Tip – Amplicon Wash	6	N/A	N/A
Negative Control	2	N/A	N/A

Viral Challenge

Methods

Culture media containing 1x108 pfu/ml vaccinia virus was utilized to test filter blocking efficiency of viral aerosols. Samples were prepared by using each brand of tip to draw 100 µl of the solution up and down with a 200 µl pipettor three full times, with a final dispense back into the original tube. The tips were carefully removed, and with a new pipettor and fresh tip, the inside of the used tip above the filter was washed by pipetting and dispensing 3 times with 100 µl of sterile PBS. The PBS wash was then transferred to a new 0.5 ml tube. This was repeated in triplicate for each of the three brands of tips. DNA was isolated from each resulting sample. The starting viral stock and 10 μl of the isolated viral DNA samples were analyzed in duplicate by realtime PCR with standard cycling conditions for a total of 50 cycles on a BioRad iCycler. Positive controls were the starting viral stock at 1:10 through 1:10,000 dilutions. Negative controls were no template controls with molecular grade water and no virus control through extraction.

Figure 3: Bacterial Growth at 24 Hours



Results

Following viral DNA isolation, targeted amplification of the virus that potentially crossed the filter barrier of the pipette tips showed no amplifications for all three brands (Table 3 and Figure 4). Each tip demonstrated equal efficiency in blocking viral aerosols. Positive controls amplified as expected, and Negative controls had no amplification.

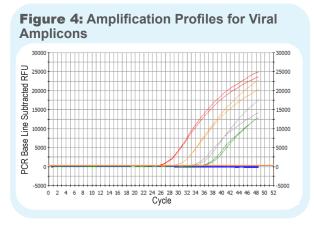
Nanoparticle Aerosol Challenge Methods

A solution containing 82,000 ng/ml CeO3 was used for testing filter blocking efficiency against nanoparticle aerosols. Samples were prepared by pipetting 100 μ l of the solution up and down with a 200 μ l pipettor three full times with final dispense back into the original tube. The tips were carefully removed, and with a new pipettor and fresh tip, the inside of each used tip above the filter was washed by pipetting and dispensing three times with 100 μ l of sterile dH2O. The wash was then moved to a fresh 0.5 ml tube. This process was repeated in triplicate for each brand of pipette tip. Detection for the presence of CeO3 was performed on an Agilent 7500 CE Inductively Coupled Plasma Mass Spectrometer (ICP-MS) with standard assay conditions. Positive controls were the starting CeO3 stock at 82,000 ng/ml. Negative controls were sterile water.

Table 3: PCR for Viral Amplicon Aerosols

Sample	Replicates	Mean Ct	Ct SD
Positive Control - Virus 1:10	2	24.92	0.004
Positive Control - Virus 1:100	2	28.23	0.035
Positive Control - Virus 1:1000	2	32.60	0.448
Positive Control - Virus 1:10000	2	34.92	0.486
Biotix Tip Wash	6	N/A	N/A
Competitor 1 Tip Wash	6	N/A	N/A
Competitor 2 Tip Wash	6	N/A	N/A
Negative control: No template	2	N/A	N/A

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Results

Analysis of washes for aerosols containing cerium nanoparticles showed no breakthrough of aerosols across the filter barriers for all three pipette tip brands (Table 4). Positive and negative controls produced the expected results.

Delta Filter Inhibition and Enhancement Testing

As a new technology innovation and a singular product of its kind on the market, it was important for Biotix to verify the suitability of their filter tips for use in biological and molecular assays. Of key focus was confirming that the color change compound was chemically inert, and would neither inhibit nor enhance the most sensitive biological assays. To test this, colorant was eluted from the filter tips into molecular grade water (MGW) and used in realtime PCR to determine any PCR inhibition; eluted into whole blood and tested in PCR and ELISA applications; and eluted into cell culture media to test for toxicity. Biotix filter tips were compared against the competitive brands broadly used in the market.

Molecular Grade Water and PCR Challenge

Methods

The filters were removed from each brand of filter tip with sterile forceps and placed into a 1.5 ml tube with 1.0 ml of molecular grade water. Filters were incubated for two hours at room temperature to allow any extractable to elute into the water. A series of dilutions were made consisting of:

- (1) 200 μl and placed in a fresh tube
- (2) 100 μl in fresh tube with 100 μl MGW
- (3) 50 μl in fresh tube with 150 μl MGW
- (4) 25 μ l in fresh tube with 175 μ l MGW
- (5) A control tube with 200 μ l MGW

PCR inhibition was measured by substituting the eluted water for the molecular grade water normally used in the set-up of realtime PCR reactions. The template used was normal human DNA isolated from whole blood and amplified with primer and probes for human RNaseP. Eight microliters of each elution sample was analyzed in duplicate by realtime PCR with standard cycling conditions for a total of 50 cycles on a BioRad iCycler. Positive controls were the starting human whole blood DNA at 1:10 to 1:10,000 dilutions. Negative controls were no template controls and an extraction control.

Results

There was no observed inhibition of PCR by eluates in molecular biology grade water from any of the filters from the three different brands of tips as compared to controls (Table 5 and Figure 5). A serial dilution of the eluates was performed, but as no dose response to the eluates was seen, data were combined

Sample	Elements	Mass	Replicates	Mean Concentration (ng/ml)	SD	Range
Positive Control	Ce	140	2	73545.5	8703.777	67391-79700
Biotix	Ce	140	3	>0.019	N/A	N/A
Competitor 1	Ce	140	3	>0.019	N/A	N/A
Competitor 2	Ce	140	3	>0.019	N/A	N/A
Negative Control	Ce	140	2	>0.019	N/A	N/A

Table 4: ICP-MS Detection of Aerosols of Cerium Nanoparticles

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and treated as replicates. All three tip brands performed equally well, with no difference in amplification as compared to each other and controls.

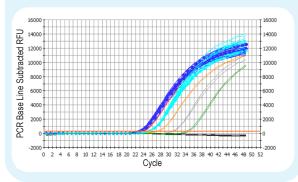
Whole Blood and PCR Challenge Methods

Filters from each brand of tip were removed with sterile forceps and placed into a 1.5 ml tube with 1.0 ml of human whole blood (WB). Filters were incubated for two hours at room temperature to allow any extractable to elute into the blood. A series of dilutions were made consisting of:

- (1) 200 µl and placed in a fresh tube
- (2) 100 μ l in fresh tube with 100 μ l WB
- (3) 50 μl in fresh tube with 150 μl WB
- (4) 25 μl in fresh tube with 175 μl WB
- (5) A control tube with 200 μ l WB

DNA was isolated from each blood sample and PCR inhibition was measured by realtime PCR. The template used was human DNA isolated from the whole blood samples and amplified with primer and probes for human RNaseP. Ten microliters of DNA from each elution sample was analyzed in duplicate by realtime PCR with standard cycling conditions for a total of 50 cycles on a BioRad iCycler. Positive controls were the starting human whole blood DNA at 1:10 to 1:10,000 dilutions. Negative controls were no template controls with molecular grade water and extraction controls.



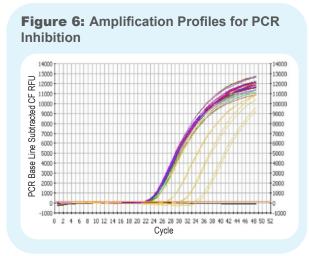


Results

There was no observed inhibition of PCR by eluates in whole blood from any of the filters from the three brands of tips as compared to controls (Table 6 and Figure 6). A serial dilution of the eluates was performed, but as no dose response to the eluates was seen, data were combined and treated as replicates. Biotix filter tips performed equally with no difference in amplification of human RNaseP as compared to the other tip brands and controls.

Table 5: PCR Inhibition Testing by Filter Eluate

Sample	Replicates	Mean Ct	Ct SD
Positive Control - 1:10 WB DNA	2	23.62	0.197
Positive Control - 1:100 WB DNA	2	27.29	0.153
Positive Control - 1:1000 WB DNA	2	30.91	0.198
Positive Control - 1:10000 WB DNA	2	34.44	0.382
Biotix Tip	8	25.00	0.157
Competitor 1 Tip	8	25.10	0.077
Competitor 2 Tip	8	25.13	0.143
Negative control – MGW	2	25.27	0.094
No Template Control – MM	2	N/A	N/A
No Template Control – SA	2	N/A	N/A



Whole Blood and ELISA Challenge

Methods

The filters from samples of each pipette tip brand were removed with sterile forceps and placed into a 1.5 ml tube with 1.0 ml of human whole blood (WB). Filters were incubated for two hours at room temperature to allow any extractable to elute into the blood. A series of dilutions were made consisting of:

- (1) 200 μl and placed in a fresh tube
- (2) 100 μl in fresh tube with 100 μl WB
- (3) 50 μl in fresh tube with 150 μl WB
- (4) 25 μl in fresh tube with 175 μl WB
- (5) A control tube with 200 μl WB

Fifty microliters of each eluted whole blood sample was analyzed for IgG2 by ELISA utilizing an Invitrogen Human IgG Subclass ELISA Kit (Cat # 99-1000). Positive controls were the starting human whole blood. Negative controls were no sample addition/PBS.

Results

There was no inhibition observed by eluates in whole blood of an ELISA for human IgG2 from any of the filter tips compared to controls (Table 7 and Figure 7). A serial dilution of the eluates was performed, but there was no dose response observed. Biotix Filter tips performed equally well with no difference in detection of human IgG2 as compared to the other tips and controls.

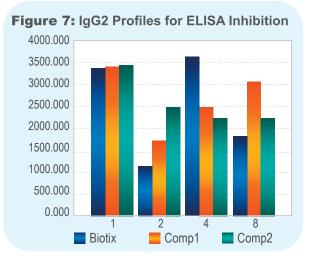


Table 6: PCR Inhibition by Filter Elutes for Whole Blood

Sample	Replicates	Mean Ct	Ct SD
Positive Control - 1:10 WB DNA	2	23.62	0.197
Positive Control - 1:100 WB DNA	2	27.29	0.153
Positive Control - 1:1000 WB DNA	2	30.91	0.198
Positive Control - 1:10000 WB DNA	2	34.44	0.382
Biotix Tip	8	22.98	0.115
Competitor 1 Tip	8	22.56	0.019
Competitor 2 Tip	8	22.98	0.169
Negative control – MGW	2	22.98	0.039
No Template Control – MM	2	N/A	N/A
No Template Control – SA	2	N/A	N/A

Cell Culture Media and Toxicity Challenge

Methods

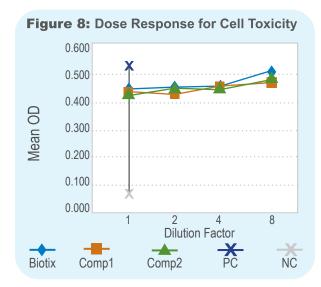
The filters from samples of each tip brand were removed with sterile forceps and placed into a 1.5 ml tube with 1.0 ml of tissue culture media. Filters were incubated for two hours at room temperature to allow any extractable to elute into the culture media (CM). A series of dilutions were made consisting of:

- (1) 200 μl and placed in a fresh tube
- (2) 100 μl in fresh tube with 100 μl CM
- (3) 50 μl in fresh tube with 150 μl CM
- (4) 25 μ l in fresh tube with 175 μ l CM
- (5) A control tube with 200 μ l CM

One hundred microliters of each elution culture media sample was added to monolayers of BSC-40 Green Monkey Kidney Cells plated at 3.94 x 103 cells/well in 96-well plates. Cells were allowed to incubate in the elution culture media for 18 hours at 37°C. Media was replaced and MTT conversion was measured to assess cell viability as per instructions from the Invitrogen Vybrant MTT Cell Proliferation Kit (Cat #V-13154). The positive control was complete culture media. Negative control was no sample addition/PBS. p

Results

There was no observed inhibition of cell viability as determined by MTT conversion by eluates in cell culture media from any of the filter tips tested compared to each other (Table 8 and Figure 8). A serial dilution of the eluates was performed and there was an observed linear dose response to the eluates with a difference of 0.5 to 1 OD unit from least to most concentrated as compared to controls. Biotix filter tips performed equally with no difference in cell toxicity as compared to the other brands.



Sample	Replicates	Mean OD	SD	lgG2 (µg/ml)
Biotix – 1	2	0.986	0.029	3375.520
Biotix – 1:1	2	0.680	0.017	1165.336
Biotix – 1:4	2	1.003	0.037	3581.320
Biotix – 1:8	2	0.806	0.013	1803.833
Competitor 1 – 1	2	0.989	0.046	3410.958
Competitor 1 – 1:1	2	0.798	0.042	1754.288
Competitor 1 – 1:4	2	0.897	0.050	2476.169
Competitor 1 – 1:8	2	0.957	0.095	3051.372
Competitor 2 – 1	2	0.992	0.008	3452.773
Competitor 2 – 1:1	2	0.898	0.002	2484.805
Competitor 2 – 1:4	2	0.867	0.042	2230.606
Competitor 2 – 1:8	2	0.870	0.004	2254.025
Positive Control	2	0.912	0.034	2613.455

Table 7: ELISA Inhibition by Filter Eluates

Sample	Replicates	Mean OD	SD	MIN	MAX
Biotix – 1	4	0.434	0.016	0.412	0.446
Biotix – 1:1	4	0.443	0.021	0.422	0.462
Biotix – 1:4	4	0.450	0.017	0.428	0.464
Biotix – 1:8	4	0.503	0.015	0.487	0.516
Axygen – 1	4	0.418	0.025	0.396	0.449
Axygen – 1:1	4	0.417	0.014	0.398	0.430
Axygen – 1:4	4	0.443	0.013	0.417	0.457
Axygen – 1:5	4	0.468	0.025	0.454	0.493
MBP ART – 1	4	0.418	0.030	0.385	0.449
MBP ART – 1:1	4	0.447	0.034	0.428	0.460
MBP ART – 1:4	4	0.443	0.014	0.438	0.452
MBP ART – 1:8	4	0.489	0.013	0.453	0.538
Positive Control	8	0.517	0.022	0.475	0.542
Negative Control	2	0.066	N/A	N/A	N/A

Table 8: Cell Toxicity of Filter Eluates in Culture Media

Conclusion

Comprehensive testing by independent research laboratory, MRIGlobal, demonstrated the ability of Delta Filter technology in Biotix pipette tips to provide excellent aerosol blocking efficiency across a broad range of highly contaminating substrates including PCR amplicons, bacteria, viruses, and nanoparticles. Delta Filter provides the security expected of leading filter tips on the market, with the added security of instant visual identification of pipetting errors with the color change filter technology. Biological testing highlighted the chemically inert Delta Filter is suitable with even the most sensitive assays.

To learn more about Biotix pipette tips, visit us at our website

References

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