March 23, 2001

Application Note 32301

Sorenson BioScience
Filtered Pipette Tip DNA and PCR Aerosol Blocking

Objective

To test the effectiveness of Sorenson BioScience Filtered Pipette Tips in blocking DNA and PCR aerosols. Techniques involving PCR amplicons are particularly sensitive to aerosol contamination. Because one contaminating molecule can be amplified and invalidate an experiment, an assay for the detection of Anthrax DNA was used to evaluate the effectiveness of the filters in blocking genomic DNA as well as PCR amplicon. Tests were performed at an independent laboratory using an ultra-sensitive assay to show that DNA and PCR amplicon aerosols do not penetrate beyond the filter to contaminate the shaft of the pipettor.

Materials and Methods

To accurately test for aerosol contamination, a new Eppendorf pipettor was used in these experiments. The assay, equipment, and reagents used were supplied by Idaho Technology, Inc. (Salt Lake City, Utah) The Anthrax identification assay is designed specifically for their R.A.P.I.D. (Ruggedized Advanced Pathogen Identification Device), which is used by military and police agencies throughout the World for PCR-based identification of pathogens. The R.A.P.I.D. is a real time quantitative PCR system based on Idaho Technology's capillary tube PCR techniques and Light Cycler technology. The assay is so sentive it can detect and amplify a single copy of target DNA.

The assay kit consists of a positive control reaction tube and a negative control reaction tube. Each reaction tube contains enough freeze-dried reagent to make 2 reactions. 40ul of ddH_2O are used to resuspend the reagent mix. 18ul of reagent are added to the capillaries for PCR and analysis. The reaction takes place within the capillary tubes. Negative control reactions were used for evaluating contamination of the pipettor.

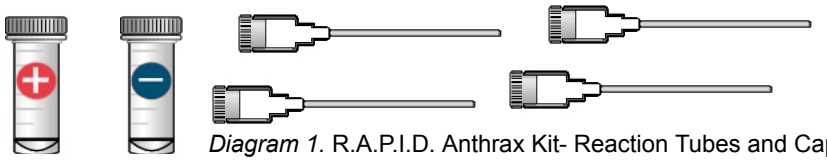


Diagram 1. R.A.P.I.D. Anthrax Kit- Reaction Tubes and Capillaries

Step 1: Negative control reactions were prepared by adding 40ul of ddH2O to the control tube. The contents were split by adding 18ul to two individual capillaries for negative controls.

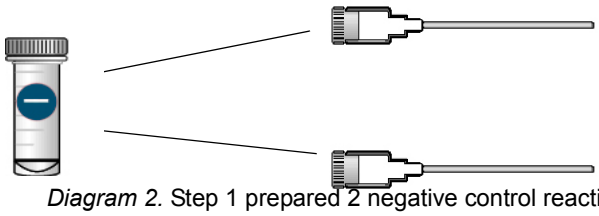


Diagram 2. Step 1 prepared 2 negative control reactions

Step 2: Positive control reaction mixes were prepared using Sorenson Filter Tips. 40ul ddH2O were pipetted in to the tube. The contents were aspirated and dispensed vigorously to promote aerosol formation. One of the positive control reactions was split by adding 18ul to two individual capillaries as positive controls. The second was saved for a later step.

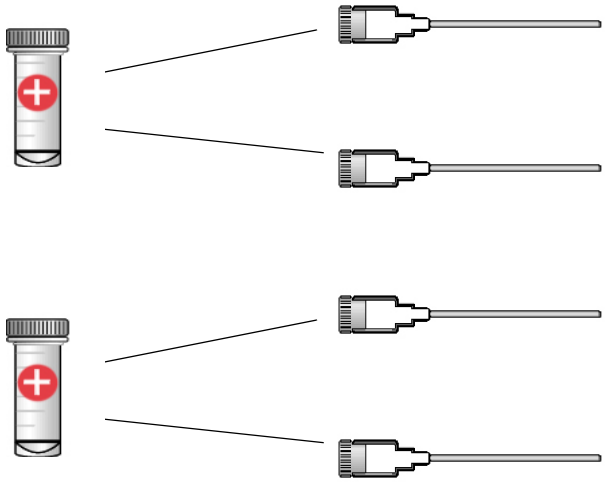


Diagram 3. Step 2 prepared 4 positive control reactions

Step 3: Another negative control tube was prepared as per step 1. After handling the positive control reaction in step 2, which contains target DNA, a new tip was used to vigorously aspirate and dispense in this second negative control. That reaction was then split by adding 18ul to two different capillaries. If DNA from step 2 contaminated the pipettor, it may blow back into a clean control reaction if the filter failed.

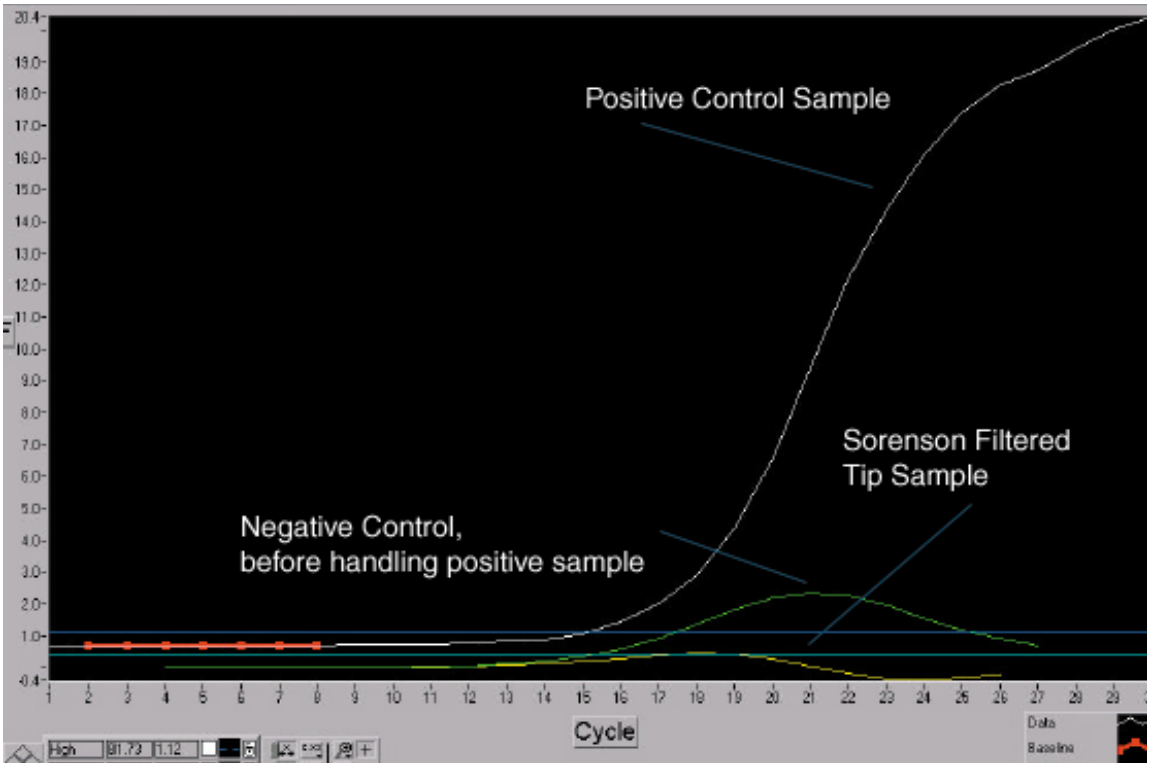
Step 4: With the tip removed from the pipettor, the end of the shaft of the pipettor was washed with 40ul of water, collected in to a 3rd negative control reaction tube. The

reaction was split in to two capillaries. If the filter failed to block DNA aerosols, the surface of the pipettor shaft would be contaminated.

Step 5: Finally, the pipettor with a Sorenson Filtered Tip was used to handle previously amplified positive control PCR product and the shaft of the pipettor was again washed with 40ul of water, collected in to a negative control reaction. This being done to detect surface contamination by PCR product. The reaction was split by adding 18ul of the reaction to two capillaries.

Results

All negative control samples were indeed negative for the presence of Anthrax DNA. The positive control reactions showed the expected Anthrax concentration. The test reactions were all negative for the presence of target DNA or amplicon. No Anthrax DNA or PCR products passed through the filter to either be blown back in to a reaction or to cause surface contamination of the pipettor. Sorenson BioScience Filtered Tips are very effective at blocking genomic DNA and PCR amplicon aerosols.



Fluorescence vs. Cycle

Diagram 4. This screen capture shows normal amplification of the positive and negative controls (shown in white and green respectively) and the Sorenson Filtered Tip Sample in yellow.