Minimizing contamination

The efficacy of ART Barrier tips in preventing carryover contamination and cross-contamination

Goal

This study demonstrates the significance of the self-sealing ability of Thermo Scientific™ ART™ Barrier tips in preventing cross-contamination caused by routine pipetting, either by aerosolized particles or liquid pass-through from over-pipetting during laboratory pipetting workflows.

Background

Micropipettes and tips are some of the most ubiquitous items of equipment for laboratories. The precise pairing of pipette and tip creates an indispensable tool for the most critical work of scientists. Micropipette tips are integral in countless laboratory liquid-handling processes such as DNA isolation, sequencing, NGS applications, mammalian cell culture, microbiology applications, manufacturing and quality testing applications, RT-qPCR applications, and SARS-CoV-2 infection testing.

Successful outcomes for PCR are highly dependent on the integrity of the reaction. Any cross-contamination can result in the amplification of extraneous nucleic acids, leading to false signals. The most common causes of false signals are the carryover of previously amplified DNA from one tube to another or other forms of sample-to-sample contamination. The action of pipetting can create aerosols or liquid pass-through that are drawn into the pipette and then transferred to subsequent samples, creating carryover contamination. Carryover and cross-contamination increase the risk of erroneous results, causing costly delays requiring laborious troubleshooting. These delays can result in the loss of invaluable time and resources. If unchecked, cross-contamination can lead to reporting errors and cause irreparable damage to a lab's credibility.

Filtered pipette tips are preferred when performing PCR, cell culture, and microbiology-related workflows to prevent cross-contamination by aerosols generated during pipetting. However, not all filtered micropipette tips are created equal, and choosing the right filtered tip is necessary for reliable and repeatable results.

Self-sealing barrier

ART Barrier tips are universal tips with unique self-sealing filters that eliminate any occurrences of carryover and cross-contamination caused during pipetting. The principal feature of the ART Barrier tip is a porous, self-sealing, physical barrier that is located between the pipette tip's upper section and the tip's orifice. This feature renders it impassable to aerosol or liquid contamination and prevents any source of carryover or cross-contamination. The ART Barrier filter is instrumental in eliminating contamination even in the rare event of erroneous excess-volume pipetting by lab personnel. Thermo Fisher Scientific identifies the ART Barrier tip to be a barrier and not a filter because of the self-sealing feature.

Scientists work diligently to manage carryover and cross-contamination risks. However, it is impossible to anticipate inadvertent mistakes and human errors that can happen during laboratory workflows. Most scientists have had the unpleasant experience of erroneous pipetting and aspirating the wrong volumes using incorrect tips. Such pipetting errors are common in laboratories, especially when working under busy timelines and target deadline stresses. These errors can introduce carryover or cross-contamination in workflows and cause unreliable results and costly downtime for pipette decontamination. They can be especially difficult for laboratories working to detect low-abundance targets, such as facilities performing SARS-CoV-2 testing and laboratories performing human identification (HID) workflows. Contamination of pipettes is one of the leading causes of erroneous results for PCR-related workflows. Even after the contamination is identified, it often leads to a great deal of lost time due to the need for decontamination and recalibration of the contaminated pipettes.

The main objective of this study was to highlight the importance of the self-sealing feature of the ART Barrier tips in preventing carryover and cross-contamination. For this purpose, the Applied Biosystems™ TaqCheck™ SARS-CoV-2 Control RNA was used to evaluate ART Barrier tips against 3 tips from other manufacturers in excess-volume aspiration testing to simulate erroneous pipetting instances that may occur in real lab conditions.

Materials and methods

The products used in this study are summarized in Tables 1 and 2.

Table 1. Pipette tips tested.

Manufacturer	Product	Cat. No.
Thermo Scientific	ART 20P, filtered, sterile, hinged rack	2149P-HR
	ART 200, filtered, sterile, hinged rack	2069-HR
Manufacturer A	0.5–20 µL, filter tip, sterile, universal	
	1–200 µL, aerosol filter tip, sterile, universal	
Manufacturer B	20 µL, filtered, sterile, universal	
	200 µL, filtered, sterile, universal	
Manufacturer E*	0.2–20 µL, filtered, sterile	

* 200 µL tips were not tested for Manufacturer E on account of the supplier being unable to fulfill the order.

Table 2. Reagents and equipment.

Brand or manufacturer	Product	Cat. No.
Applied Biosystems	TaqMan Control Genomic DNA (human)	4312660
	TaqCheck SARS-CoV-2 Control Dilution Buffer	A50486
	TaqCheck SARS-CoV-2 Control RNA	956127
	TaqCheck SARS-CoV-2 Fast PCR Assay	A47693
	TaqPath 1-Step RT-qPCR Master Mix, CG	A15300
	MicroAmp Optical 384-Well Reaction Plate with Barcode	4309849
	MicroAmp Optical Adhesive Film	4311971
	MicroAmp Adhesive Film Applicator	4333183
Cytiva	Whatman Qualitative Filter Paper: Grade 1 Circles	09-805A
Thermo Scientific	Snap Cap Low Retention Microcentrifuge Tubes, Sterile, Graduated, 2.0 mL	3453PK
	Finnpipette Novus Multichannel Pipette, Yellow, 5–50 µL	46300200
	Finnpipette Novus Multichannel Pipette, Orange, 30–300 µL	46300400

Assay setup

The ART Barrier tips and filter tips from 3 other manufacturers were compared to determine their performance. This was done by performing repeat pipetting of TaqCheck SARS-CoV-2 Control RNA to establish if aerosols or liquid containing nucleic acids (RNA) pass through the filter in the pipette tip. Discs of filter paper cut to appropriate sizes were placed on top of the filter within the tip to capture any aerosols or liquid containing nucleic acids passing through the filter. A sterilized hole punch was used to create round discs from Whatman™ filter paper to fit the internal diameter of each filtered pipette tip, allowing the disc to completely cover the top of the filter within each tip. A 5/32-inch (4.0 mm) disc of filter paper was fitted into 20 µL tips, and a 3/16-inch (4.5 mm) disc of filter paper was fitted into 200 µL tips. Once the filter discs were created, they were sterilized under UV light. Next, 1 µL of human genomic DNA (10 ng/µL) was dispensed onto each sterile disc and air dried at room temperature for 15 minutes to serve as the elution control. Using sterile forceps, the discs then were placed into the 20 µL and the 200 µL filter tips directly on top of the filter. The amount of RNA that passed through the tip filters was determined by qPCR on an **Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System**. All the experimental steps were conducted in a **Thermo Scientific™ 1300 Series Class II, Type A2 Biological Safety Cabinet** to ensure sterility. All equipment, pipettes, and labware used were decontaminated using **Thermo Scientific™ RNase AWAY™ Surface Decontaminant**.

Filter tip assessment

The TaqCheck SARS-CoV-2 Control RNA is a synthetic RNA positive control that contains the target sequences for the SARS-CoV-2 S and N genes. A working solution was prepared by diluting the TaqCheck SARS-CoV-2 Control RNA 40-fold with the Applied Biosystems™ TaqCheck™ SARS-CoV-2 Control Dilution Buffer, which was used to test the tips. The 20 µL tips and 200 µL tips from the 4 manufacturers were tested in quadruplicate. An excess volume of the SARS-CoV-2 RNA working solution was aspirated into the tips. A volume of 50 µL was aspirated into the 20 µL tips using the Thermo Scientific™ Finnpipette™ Novus Multichannel Pipette, 5–50 µL. A volume of 300 µL was aspirated into the 200 µL tips using the Thermo Scientific™ Finnpipette™ Novus Multichannel Pipette, 30–300 µL. Upon aspiration, the control RNA was pipetted repeatedly 10 times by using the mixing function of the electronic pipettes at the max speed settings. This was done to create aerosols or liquids that potentially could pass through the tip filters. Previously in a separate experiment, this method of repeatedly pipetting by using the mixing function was observed to sufficiently generate aerosols (data not shown).

Quantification of RNA contamination

After each filter tip was subjected to the filter tip assessment method, the filter-paper discs were retrieved from the pipette tips. This procedure was conducted within the biosafety cabinet using sterile forceps and with extreme caution to ensure no contaminants were introduced during the process. The retrieved filter discs were placed in chilled sterile RNase/DNase-free microcentrifuge tubes and saturated with 50 µL of room temperature, sterile Tris-EDTA (TE) buffer, pH 8.0. The tubes were then vortexed and incubated at room temperature for 30 minutes to allow any nucleic acids bound to the filter paper to be eluted by the TE buffer. After incubation, the eluates were transferred into fresh cold microcentrifuge tubes.

The Applied Biosystems™ TaqPath™ 1-Step RT-qPCR Master Mix, CG (4X), and Applied Biosystems™ TaqCheck™ SARS-CoV-2 Fast PCR Assay (20X) were used to amplify any SARS-CoV-2 RNA present in the eluate that might have been present in the filter disc. The TaqCheck SARS-CoV-2 Fast PCR Assay is a multiplex RT-qPCR assay that is routinely used for the parallel detection of SARS-CoV-2 RNA, targeting the SARS-CoV-2 N and S genes (VIC™ dye) and the human RNase P *RPP30* gene (FAM™ dye). FAM dye was used to detect amplification of *RPP30* from human genomic DNA, which served as a control for nucleic acid elution.

A standard curve of the TaqCheck SARS-CoV-2 Control RNA with seven points amplified in triplicate, using 4-fold serial dilutions ranging from 1 x 10^4 copies/µL to 5 copies/µL, was prepared. A positive control, a 1:40 dilution of TaqCheck SARS-CoV-2 Control RNA, was also prepared.

Table 3. Reagents and sample volumes used for the RT-qPCR reaction setup.

Master mix setup	
Component	**Volume per reaction (µL)**
TaqPath 1-Step RT-qPCR Master Mix, CG (4X)	2.5
TaqCheck SARS-CoV-2 Fast PCR Assay (20X)	0.5
Molecular-grade water	2
Total master mix volume	5
Reaction plate setup	
Component	**Volume per reaction (µL)**
Master mix	5
Sample*	5

* Samples are the standard serial dilutions and extracted eluates from the Whatman filter discs.

As per Table 3, 5 µL of the master mix was dispensed into wells of an Applied Biosystems™ MicroAmp™ Optical 384-Well Reaction Plate. This was followed by adding either 5 µL of the test samples in five replicates or 2 µL of the standard dilution series with 3 µL molecular-grade water in triplicate. The plate was sealed using Applied Biosystems™ MicroAmp™ Optical Adhesive Film aided by the Applied Biosystems™ MicroAmp™ Adhesive Film Applicator. The sealed plate was then gently vortexed and centrifuged at 1,400 x *g* to mix the reagents and remove any air bubbles. The plate was then loaded onto the QuantStudio 5 Real-Time PCR System, and the RT-qPCR assay was performed according to the method in the Applied Biosystems™ QuantStudio™ Design and Analysis Software. The cycling parameters for the method are listed in Table 4.

Table 4. Cycling parameters for the RT-qPCR reaction.

Step	Temperature	Ramp rate	Time	Number of cycles
Reverse transcription	50°C	2.2°C/s	4 min	1
Activation	95°C	2.2°C/s	2 min	1
Denaturation	95°C	2.2°C/s	1 s	50
Annealing and extension	60°C	1.8°C/s	20 s	

Results

Filter tip assessment

During the excess-volume aspiration testing, all the tips were carefully observed for any visible signs of liquid passing through the filters. Since the volume aspirated into the pipettes was greater than the denoted volume of the tips, the liquid came in contact with the base of the filter for all tips tested. Upon further observation of the ART Barrier tips, the barrier demonstrably sealed off the liquid as designed, thereby preventing any liquid from passing through the filter. However, the filters from the 3 other manufacturers allowed the liquid to pass through and contact the filter paper placed on the top of the filter. Inherently, this allowed the SARS-CoV-2 Control RNA to contaminate the filter paper. This demonstrated that the tips from the other manufacturers did not perform well and showed visible signs of liquid pass-through in instances of erroneous pipetting.

Quantification of RNA contamination

To verify the results of the visual observations, the results obtained from the RT-qPCR reaction were analyzed using QuantStudio Design and Analysis Software to determine if the liquid passing through the filter allowed the filter paper to be contaminated with the TaqCheck SARS-CoV-2 Control RNA. The quantification cycle (C_q) values were measured for all the eluates from the filter-paper discs of each tested tip. The C_q value is the PCR cycle number at which the sample's reaction curve intersects the baseline threshold. This value corresponds to the number of cycles required for detection of a real signal from a sample. The software uses the C_q value to calculate the amount of target nucleic acid that was present in the test sample (in this case, the eluate from the discs). C_q values are inversely related to the amount of target nucleic acid and correlate to the number of copies of the target in the sample evaluated. For this assay, the baseline threshold was set at 0.01 for the SARS-CoV-2 N and S genes (VIC dye) and at 0.02 for the human RNase P *RPP30* gene (FAM dye).

The filter discs collected from the ART Barrier tips did not result in a positive signal for the SARS-CoV-2 genes. However, the filter discs collected from the tips of the other 3 manufacturers resulted in positive qPCR signals for the SARS-CoV-2 RNA genes. These results corroborated the visual observations that the ART Barrier tips were free of any contamination from the SARS-CoV-2 RNA, but the tips of the other 3 manufacturers had failed this test, which resulted in contamination of the filter discs placed on top of the tip filters. All samples demonstrated positive signals for human *RPP30*, indicating the presence of human DNA that was used as the elution control, which confirmed efficient elution of bound nucleic acids from the filter paper (Figure 1).

The RT-qPCR assay was able to provide conclusive evidence that the ART Barrier tips were successfully able to prevent the occurrence of any carryover contamination of the TaqCheck SARS-CoV-2 Control RNA, whereas the filter tips of the other 3 manufacturers failed (Table 5).

Table 5. Results of the excess-volume testing with SARS-CoV-2 RNA.

Tip	Presence of SARS-CoV-2 contamination
ART Barrier tips, 20 µL	Pass (negative)
ART Barrier tips, 200 µL	Pass (negative)
Manufacturer A tips, 20 µL	Fail (positive)
Manufacturer A tips, 200 µL	Fail (positive)
Manufacturer B tips, 20 µL	Fail (positive)
Manufacturer B tips, 200 µL	Fail (positive)
Manufacturer E tips, 20 µL	Fail (positive)

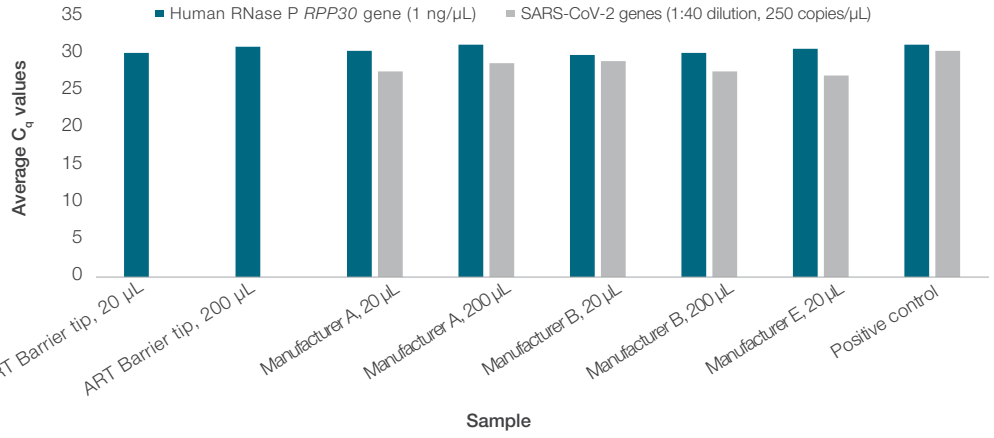

Figure 1. Average C_q values of nucleic acids in the tested eluates. Only the ART Barrier tips did not show the presence of the SARS-CoV-2 RNA, while the tips of the other 3 manufacturers showed amplification of SARS-CoV-2 genes at similar levels to the *RPP30* control.

Conclusion

Scientists must be aware of the consequences of contamination risks with the regular use of pipettes, commonly used laboratory tools. Sample contamination can be detrimental to resulting data, regardless of the area of research. Therefore, preventing carryover and cross-contamination is an imperative task for scientists worldwide.

ART Barrier pipette tips are ideal for use with critical samples and sensitive assays to prevent contamination while protecting the pipettes. ART Barrier tips can be invaluable in labs that deal with high volumes of samples, where accurate identification plays a key role, since in this study they showed up to 100% protection against aerosol and liquid contamination. ART Barrier tips are the only tips with a self-sealing barrier that seals completely to prevent liquid samples and aerosols from coming in contact with the pipette nose cone. This ensures the pipette and subsequent samples are protected from carryover contamination and cross-contamination.

- Only the ART Barrier tips demonstrated the ability to prevent SARS-CoV-2 RNA liquid and aerosol pass-through across the filter and avoid any carryover contamination, while the 3 other manufacturers that were evaluated failed.
- The self-sealing ability of the ART Barrier tips tested here proved to provide 100% security against liquid pass-through, even in cases of erroneous excess-volume pipetting.
- ART Barrier tips are a proven way for scientists to avoid potential carryover contamination and cross-contamination.
- Art Barrier tips are essential for our partners in science to help avoid delays caused by expensive troubleshooting and laborious decontamination and recalibration of instruments.
- Multiple regional manufacturing sites assure supply chain stability to better serve our partners in science.
- ART Barrier tips are manufactured following the industry's highest standards (ISO 9001 and 13485) and are certified to meet the following criteria:
  - Minimum sterility assurance level (SAL) with ISO 11137
  - Endotoxin free
  - Human DNA free
  - RNase free
  - DNase free
  - PCR inhibitor free

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