WHITEPAPER

A STUDY OF ANALYTICAL & CLINICAL INTERFERENCE IN POROUS LABORATORY COMPONENTS

How advancements in analytical instrumentation led to improvements in the cleanliness of plastic consumables.

POREX
Filtration Group®

Advancements in ever-increasing sensitivity of analytical and clinical instrumentation coupled with demand for higher accuracy in many life science and diagnostic applications have led to a critical need for significant improvements in the purity of plastic consumables.

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In light of recent technological advancements and market requirements, Porex initiated its CERTIFIED PURE POREX® program in 2009 to qualify porous polymeric materials via a stringent series of analytical, clinical and life science testing procedures.

As a result of this program, POREX® porous polymers have been tested by renowned third-party national laboratories. The testing standards and methods include ISO 10993 Biocompatibility Standards – Hemolysis and Cytotoxicity, and ICP-MS (Inductively Coupled Plasma Mass Spectrometry). Porex has expanded its porous media options for its customers by adding new porous sintered polymer particles and bonded fibers.

CERTIFIED PURE POREX® materials were found to have virtually no material additives, contaminants or heavy metals that cause interferences in clinical and laboratory testing. These materials were verified to be non-cytotoxic and non-hemolytic via independent testing laboratories, complying with ISO 10993 biocompatibility testing standards.

CERTIFIED PURE POREX® materials have over 99.99978% Bacterial Filtration Efficiency (BFE) as tested by the ASTM F21012 Standard. The testing procedure of the standard provides a more severe challenge to most filtration materials than would be expected in normal use and allows for a reproducible bacterial challenge to be delivered to the test samples.

"To our knowledge, this is the first extensive qualification program for porous polymeric materials that fills a critical gap..."

To our knowledge, this is the first extensive qualification program for porous polymeric materials that fills a critical gap in the progression of developing standardized methodologies in the analysis of interference of plastic consumables with analytical and clinical tests.

Study of Rabbit Blood Hemolysis (ISO 10993-4)

The purpose of the study was to determine the potential hemolytic activity, via the induction of increased levels of free plasma hemoglobin in rabbit blood, in response to the test articles and their extracts.

The Test System

The test system was citrated (0.105M ≈ 3.2%) rabbit blood. The animal species, number, and method of test article administration were recommended by the ASTM F756 guideline. Three New Zealand white rabbits (*Oryctolagus cuniculus*) were used in the study, including one male and two females (the females were non-pregnant and nulliparous). The animals were selected from a larger pool and examined to ensure a lack of adverse clinical signs. The range of their weight was 2.87-3.25 kg, weighed to the nearest 10 grams; they were at least seven weeks old (young adults). The animals were healthy and had previously been used for other experimental procedures. They had been acclimated for at least five days prior to the removal of a blood sample.

The Standards Used in the Study

The study of hemolysis was conducted in compliance with ISO 10993-4:2017, Biological Evaluation of Medical Devices – Part 4: Selection of Tests for Interactions with Blood. It was also based on the following standards and references:

1. ASTM F756-17, Standard Practice for Assessment of Hemolytic Properties of Materials, 2017.
2. ASTM F619-14, Standard Practice for Extraction of Medical Plastics, 2014.
3. ISO 10993-12:2012, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.

Experimental Design, Control Articles and Sample Preparation

The negative control article was HDPE (high density polyethylene) equivalent to Negative Control USP HDPE Reference Standard (Negative Control Plastic). The positive control article was Nitrile. The untreated control (extraction medium) was Magnesium and Calcium-Free Phosphate Buffered Saline (PBS).

The test articles, negative control article (Negative Control Plastic, 0.06 cm thick), and positive control article (Nitrile, 0.09 mm thick) were prepared in triplicate following an ISO 10993-12/ASTM F619 ratio, as itemized in the table below. The test articles were exposed beyond their absorptive capacity (0.6 mL per 29.9 cm2) for direct contact.

Test and Control Articles for Direct Contact

Sample	Number of Replicates	Amount	Ratio
Test Article	3	29.9 cm^2	3 cm^2/mL
Negative Control	3	21 cm^2	3 cm^2/mL
Positive Control	3	42 cm^2	6 cm^2/mL

The negative control article (Negative Control Plastic, 0.06 cm thick) and positive control article (Nitrile, 0.09 mm thick) were prepared in triplicate following an ISO 10993-12/ASTM F619 ratio, as itemized in the table below.

Test and Control Articles for Indirect Contact

Sample	Number of Extracts	Amount	Volume of PBS	Ratio	Time/Temperature
Test Article	3	29.9 cm^2	10.4 mL	3 cm^2/mL	24 ± 2 hours at 70 ± 2 °C
Negative Control	3	21 cm^2	7.0 mL	3 cm^2/mL	24 ± 2 hours at 70 ± 2 °C
Positive Control	3	42 cm^2	7.0 mL	6 cm^2/mL	24 ± 2 hours at 70 ± 2 °C
Untreated Control	3	N/A	7.0 mL	N/A	24 ± 2 hours at 70 ± 2 °C

The properly prepared test article was placed in an extraction vessel and the appropriate medium was added. The medium completely covered the test article. An untreated control was also prepared for parallel extraction treatments and comparison. After the completion of the extraction, the extract was kept at room temperature and was used on the same day the extraction was completed. The test article appeared unchanged by the extraction procedure and the extract was clear and free from particulates. No storage of the extract occurred. The extract was not filtered, nor centrifuged. Each extract was agitated vigorously prior to administration. The absorbance of the extract was measured.

Dosage: Pre-Dose Procedure, Dose Administration, and Post-Dose Procedure

Blood Collection: Fresh whole rabbit blood was collected from three donors on each test day into tubes containing an anticoagulant (citrate). Approximately 10 mL of blood was then pooled together. The blood was used within four hours of collection.

Hemoglobin (Hb) Determination: A standard curve was prepared from the stock cyanmethemoglobin accommodating a range of 0.00 to 0.80 mg/mL. Drabkin's reagent was used as the zero blank in the spectrophotometer and the absorbance was measured at 540 nm (A^{540}). The calibration curve was plotted using Hb concentration (mg/mL) on the y-axis and A^{540} on the x-axis. The calibration coefficient (F) was the slope of this plot; the y-intercept was approximately zero.

Determination of Plasma Free Hemoglobin (PFH): A 3.0 mL sample of pooled blood was centrifuged at 800 g in a standard clinical centrifuge set at 4 ± 2 °C for approximately 15 minutes to obtain plasma. Plasma was added to an equal volume of Drabkin's reagent. After at least 15 minutes, the absorbance was measured at 540 nm and the concentration was calculated from the standard curve. The concentration of PFH was calculated using the formula PFH = A^{PFH} x F x 2, where F was the calibration coefficient. If the PFH of the blood sample was ≥2 mg/mL, a different blood sample would have been used.

Determination of Total Blood Hemoglobin Concentration (C): A volume of 20 µL of well-mixed, pooled whole blood was added to 5.0 mL of Drabkin's reagent. The resulting solution was allowed to stand for at least 15 minutes, and the absorbance of the solution (A^C) was read at 540 nm. The total Hb concentration of the blood (C) was calculated using the formula C = A^C x F x 251. The total Hb concentration was adjusted to 10 ±1 mg/mL by diluting with an appropriate amount of PBS. The Hb concentration was once again verified by using triplicate samples of 300 µL of diluted blood in 4.5 mL of reagent (dilution factor = 16). The Hb concentration was calculated using the formula C = A^T x F x 16, where A^T was the absorbance of the total Hb concentration.

Addition of PBS (Direct Contact Test): PBS was added to the prepared test articles in glass vials and added to the negative control articles and positive control articles in glass vials, as itemized in the table below. The untreated control (blank) was PBS without any article.

PBS and Blood Dosage

Sample	Amount	Ratio	Volume of PBS	Blood Exposure Ratio	Volume of Blood
Test Article	29.9 cm^2	3 cm^2/mL	10.6 mL	1.0 mL blood/7.0 mL PBS	1.5 mL
Negative Control	21 cm^2	3 cm^2/mL	7.0 mL	1.0 mL blood/7.0 mL PBS	1.0 mL
Positive Control	42 cm^2	6 cm^2/mL	7.0 mL	1.0 mL blood/7.0 mL PBS	1.0 mL
Untreated Control	N/A	N/A	7.0 mL	1.0 mL blood/7.0 mL PBS	1.0 mL

N/A: Not Applicable

Summary

The potential hemolytic activity in rabbit blood in response to exposure to two POREX® test articles and their extracts (direct and indirect contact) was determined. For direct contact testing, Phosphate Buffered Saline (PBS) was first added to the test articles at a ratio of 3 cm^2/mL, and then incubated with rabbit blood for three hours to three hours and 10 minutes at 37 ± 2 °C, in triplicate. For indirect contact testing, the test articles were first extracted in PBS for 24 ± 2 hours at 70 ± 2 °C, and then each extract was incubated with rabbit blood for three hours to three hours and 10 minutes at 37 ± 2 °C, in triplicate. Negative, positive, and untreated controls were analyzed in parallel. The absorbance of each sample was measured, and the average values were calculated. The percent (%) hemolysis was determined for each sample after the subtraction of the blank.

A test article led to a hemolysis index above the negative control of 0.00% via the direct contact method and 0.00% via the indirect contact method. The response obtained from the blood exposed to the positive and negative control articles and their extracts confirmed the suitability of the test system.

Based on the criteria of the protocols and the ASTM F756 guidelines, The POREX test articles meet the requirements of the test and are considered non-hemolytic.

Hemolysis Results of FORTRESS® Material

	Sample	Extract Absorbance 1	Extract Absorbance 2	Extract Absorbance 3	Supernatant Absorbance 1	Supernatant Absorbance 2	Supernatant Absorbance 3
Indirect Contact	Blank	0.0001	0.0004	0.0026	0.0039	0.0030	0.0040
	Negative	0.0007	-0.0002	0.0018	0.0028	0.0039	0.0045
	Positive	0.0450	0.0304	0.0189	0.4010	0.3363	0.3613
	Test	0.0014	0.0016	0.0008	0.0039	0.0024	0.0043

Corrected Absorbance Reading

	Sample	Absorb. 1	Absorb. 2	Absorb. 3	Average Absorb.	Blank Corrected Hemolysis [c] (%)	Blank Corrected Hemolysis [c] (%)	Blank Corrected Hemolysis [c] (%)	Mean	STD Dev	Hemolysis Above Negative (%)
Direct Contact	Blank	0.0050	0.0068	0.0056	0.0058	-0.19	0.24	-0.05	0.00	0.22	
	Negative	0.0106	0.0133	0.0133	0.0124	1.16	1.82	1.82	1.60	0.38	
	Positive	0.3363	0.4033	0.4315	0.3904	80.10	96.34	103.17	93.21	11.85	91.61
	Test	0.0062	0.0060	0.0069	0.0064	0.10	0.05	0.27	0.14	0.11	0.00
	Sample	***Corrected Absorb. 1**	**Corrected Absorb. 2**	**Corrected Absorb. 3**	**Average Absorb.**	**Blank Corrected Hemolysis (%) [c]**	**Blank Corrected Hemolysis (%) [c]**	**Blank Corrected Hemolysis (%) [c]**	**Mean**	**STD Dev**	**Hemolysis Above Negative (%)**
Indirect Contact	Blank	0.0038	0.0026	0.0014	0.0026	0.29	0.00	-0.29	0.00	0.29	
	Negative	0.0021	0.0041	0.0027	0.0030	-0.12	0.36	0.02	0.09	0.25	
	Positive	0.3560	0.3059	0.3424	0.3348	84.99	72.94	81.72	79.89	6.23	79.80
	Test	0.0025	0.0008	0.0035	0.0023	-0.02	-0.43	0.22	-0.08	0.33	0.00

Study of Cytotoxicity (ISO 10993-5)

The purpose of the study was to determine the potential biological activity of a mammalian cell culture (L929) in response to the extracts of the test articles.

The Test System

The test system was mouse fibroblast CCL-1 (NCTC clone 929) cells, also known as L929 cells. The cell line was obtained from American Type Culture Collection (ATCC) of Manassas, Virginia. The test articles were extracted and administered in vitro through a medium compatible with the test system.

The Standards Used in the Study

The study of cytotoxicity was conducted in compliance with ISO 10993-5:2009, Biological Evaluation of Medical Devices – Part 5: Tests for In Vitro Cytotoxicity. It was also based on the following standards and references:

1. ISO 10993-12:2012, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.
2. ISO/IEC 17025:2017, General Requirements for the Competence of Testing and Calibration Laboratories.

Experimental Design, Control Articles and Sample Preparation

The negative control article was HDPE (high density polyethylene) equivalent to the Negative Control USP HDPE Reference Standard (Negative Control Plastic). The positive control article was Natural Rubber. The untreated control (extraction medium) was Serum-Supplemented (complete) Minimum Essential Medium (MEM). The additive to the extraction medium was 10% of fetal bovine serum, 100 U/mL Penicillin, 0.1 mg/mL Streptomycin, and 2 mL L-Glutamine (final concentration in medium).

The test articles were prepared following an ISO 10993-12 ratio, as itemized in the table below.

Test Article, Positive, Negative, and Untreated Control Articles					
Sample	**Amount**	**Vehicle**	**Volume**	**Ratio**	**Time/Temperature**
Test Article	2.00 g	complete MEM	10.0 mL	0.2 g/mL	24 ± 2 hours at 37 ± 1°C
Positive Control	30 cm^2	complete MEM	10.0 mL	3 cm^2/mL	24 ± 2 hours at 37 ± 1°C
Negative Control	30 cm^2	complete MEM	10.0 mL	3 cm^2/mL	24 ± 2 hours at 37 ± 1°C
Untreated Control	N/A	complete MEM	10.0 mL	N/A	24 ± 2 hours at 37 ± 1°C

N/A: Not Applicable

The properly prepared test article was placed in an extraction vessel and the appropriate medium was added. The medium completely covered the test article. The positive (Natural Rubber, 0.23 cm thick) and negative (Negative Control Plastic, 0.06 cm thick) control articles were prepared following ISO 10993-12 ratios and extracted with the same medium at the same temperature and for the same duration as the test article, as itemized in the table above. An untreated control (blank) was prepared for parallel treatment and comparison. The untreated control was the extraction medium that was subjected to the same temperature and duration as the test articles, as itemized in the table above. Each extract was agitated vigorously prior to administration. After the completion of the extraction, the extracts were kept at room temperature and were used the same day that the extraction was completed. The test article appeared to be unchanged by the extraction procedure and the extract was clear and free of particulates. The test article extract was not centrifuged. No storage of the extract occurred.

Dosage: Pre-Dose Procedure, Dose Administration, Post-Dose Procedure, and Grading

Cell Culture Preparation: Cell cultures were removed from culture flasks by enzymatic digestion (trypsin/EDTA). The cells were then suspended in a culture medium and seeded at 2×10^5 cells per well in 2 mL of complete MEM in a 6-well plate. The cultures were incubated for no less than 16 hours (5 ±1% carbon dioxide (CO_2), 37 ± 1°C, >90% humidity) so that the cells formed a subconfluent monolayer. The color of the test article extract did not indicate an obvious change of pH (yellow or purple) so the pH of the extract was not adjusted. The test article extract was not filter sterilized prior to being applied to the cell monolayer.

Dose Administration: A 2 mL volume of extract of the test article and a 2 mL volume of extract of the control article, as well as the untreated control, were used to replace the maintenance medium of the cell culture. All dosing was done in triplicate. The test and control articles were tested at 100% (neat) concentration.

Post-Dose Procedure: All cultures were incubated for 48 ± 2 hours at 37 ± 1°C in a humidified atmosphere containing 5 ±1% carbon dioxide (CO_2).

Grading: The reactivity of the cells was evaluated at 24 and 48 hours. The response of the cell monolayer was evaluated under a microscope at a 10 x 10 magnification. Trypan Blue was not used in the final scoring of the cell monolayer. The biological reactivity (cellular degeneration and malformation) was rated on a scale of 0 to 4 based on the table below.

Evaluation Criteria

The reactivity of the cells was evaluated at 24 and 48 hours. The response of the cell monolayer was evaluated under a microscope at a 10 x 10 magnification. Trypan Blue was not used in the final scoring of the cell monolayer. The biological reactivity (cellular degeneration and malformation) was rated on a scale of 0 to 4 based on the table below.

Test System Stability: The test system is considered suitable if the following conditions are met:

1. The negative control article and untreated control show no signs of cellular reactivity (Grade 0).
2. The positive control article shows greater than a Mild Reactivity (Grade 2).

If the test system is not considered suitable, the test is repeated.

Determination of Cytotoxic Effect: The test article meets the requirements of the test if none of the cultures treated with the test article show greater than a Mild Reactivity (Grade 2).

Control of Bias Statement: The study employed methodology to minimize uncertainty of measurement and control of bias for data collection and analysis, which included but was not limited to concurrent control data, a system suitability assessment, and method controls such as blanks and replicates.

Reactivity Grading

Grade	Reactivity	Conditions of All Cultures
0	None	Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth.
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

Results

The Reactivity Grades are summarized in the table below.

Cytotoxicity Results of FORTRESS® Material

Time	Date Reactivity	Test Article			Controls								
					Untreated			Negative			Positive		
		A	B	C	A	B	C	A	B	C	A	B	C
24 Hours	5/8/2019	0	0	0	0	0	0	0	0	0	4	4	4
48 Hours	5/9/2019	0	0	0	0	0	0	0	0	0	4	4	4

Cytotoxicity Results of POREX® Test Article 1

Time	Date Reactivity	Test Article			Controls								
					Untreated			Negative			Positive		
		A	B	C	A	B	C	A	B	C	A	B	C
24 Hours	12/30/2017	0	0	0	0	0	0	0	0	0	4	4	4
48 Hours	12/31/2017	0	0	0	0	0	0	0	0	0	4	4	4

Cytotoxicity Results of POREX® Test Article 2

Time	Date Reactivity	Test Article			Controls								
					Untreated			Negative			Positive		
		A	B	C	A	B	C	A	B	C	A	B	C
24 Hours	12/30/2017	0	0	0	0	0	0	0	0	0	4	4	4
48 Hours	12/31/2017	0	0	0	0	0	0	0	0	0	4	4	4

Cytotoxicity Results of Competitor Test Article 1

Time	Date Reactivity	Test Article			Controls								
					Untreated			Negative			Positive		
		A	B	C	A	B	C	A	B	C	A	B	C
24 Hours	7/13/2019	4	4	4	0	0	0	0	0	0	4	4	4
48 Hours	7/14/2019	4	4	4	0	0	0	0	0	0	4	4	4

Summary of Cytotoxicity Results

The potential biological reactivity of a mammalian cell culture (mouse fibroblast L929) in response to exposure to the extract of the test articles was determined for POREX Article 1, POREX FORTRESS, and two competitor articles. The test articles were extracted in Minimum Essential Medium (MEM) with 10% Fetal Bovine Serum (referred to as complete MEM) for 24 ± 2 hours at 37 ±1 °C. Negative and positive controls were prepared similarly. The maintenance medium of L929 cells grown in 6-well plates was replaced with the 100% (neat) extracts in 3 replicates, and the cells were incubated for 48 ± 2 hours at 37 ±1 °C. The biological reactivity of the cells following the exposure to the extracts was visually observed with a microscope and graded on a scale of 0 to 4.

There was no biological reactivity (Grade 0) of the cells exposed to two POREX test article extracts. The response obtained from the positive and negative control article extracts confirmed the suitability of the test system.

Based on the criteria of the protocols and the ISO 10993-5 guidelines, the POREX test articles meet the requirements of the test and are not considered to have a cytotoxic effect. There was severe biological reaction (Grade 4) of the cells exposed to the competitor test article extract. The competitor test article does not meet the requirements of the test and is considered to have a cytotoxic effect.

Conclusion

Based on the criteria of the protocols and the ISO 10993-5 guidelines, the POREX FORTRESS and POREX test articles 1 and 2 meet the requirements of the test and are not considered to have a cytotoxic effect. There was severe biological reaction (Grade 4) of the cells exposed to the competitor test article extract. The competitor test article does not meet the requirements of the test and is considered to have a cytotoxic effect.

Study of ICP-MS – Heavy Metals & PCR Inhibitors

The test articles were analyzed by an independent polymer and analytical laboratory using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for up to 77 elements to determine purity, heavy metal interference(s), and inorganic elemental interference(s) of the test articles.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a type of mass spectrometry which is capable of detecting metals and several non-metals at concentrations as low as one part in 10^{15} (part per quadrillion, ppq) on non-interfered low-background isotopes. This is achieved by ionizing the sample with inductively coupled plasma and then using a mass spectrometer to separate and quantify those ions. Compared with other types of spectroscopy, ICP-MS has greater speed, precision, and sensitivity.

ICP-MS Sample Preparation Procedure

The samples were weighed into a pre-cleaned Teflon microwave digestion 0.5 mL vessel using 18.2 MΩ deionized water, 5 mL trace metals grade nitric acid and 0.5 mL trace metals grade hydrofluoric acid were added. The samples were then digested at 210 °C for 30 minutes. Each sample was digested in duplicate and a digestion blank was also taken through each procedure to correct for background and other possible interferences. Upon completion, all samples were brought to a final volume of 50 mL with 18.2 MΩ deioinized water. All elements subject to analysis were calibrated with a minimum calibration coefficient of 0.999. Quality control standards were run at the start and finish of the run with a minimum acceptance criterion of ± 10%. The ICP-MS results for the test articles are listed in the table below.

FORTRESS® ICP-MS Results				
Element	Measured Concentrations After Microwave Digestions (ppb)			Sample Concentration Before Digestion (µg Element/g Sample)[2,3]
	Method Blank	Measured Concentration	Corrected Concentration[1]	
Mg	< 1	111	111	58
Al	1	38	37	19
Si	215	124	< 100	< 52
Ti	< 1	224	224	117
Fe	2	26	24	13
Ni	< 1	1	1	0.52
Zn	< 1	2	2	1
Nb	2	1	< 1	< 0.52
Ta	5	3	< 1	< 0.52

ICP-MS Results of Porex Test Article 1				
Element	Measured Concentrations After Microwave Digestions (ppb)			Sample Concentration Before Digestion (µg Element/g Sample)[2]
	Method Blank	Measured Concentration	Corrected Concentration[1]	
Mg	2	3	1	0.2
Al	< 1	29	29	6.4
Si	112	818	706	155.5
Ti	< 1	59	59	13.0
Fe	< 1	2	2	0.4
Zn	< 1	1	1	0.2
Nb	5	2	< 1	< 0.2
Ta	12	6	< 1	< 0.2

ICP-MS Results of Competitor Test Article 1				
Element	Measured Concentrations After Microwave Digestions (ppb)			Sample Concentration Before Digestion (µg Element/g Sample)[2]
	Method Blank	Measured Concentration	Corrected Concentration[1]	
Na	< 100	37233	37233	21131
Mg	< 1	38	38	21
Al	< 1	93	93	53
Si	167	249	< 100	< 57
Ti	1	3953	3952	2243
Fe	13	577	564	320
Cu	< 1	2	2	1
Nb	2	1	< 1	< 0.6
Ba	< 1	2	2	1
Ta	4	1	< 1	< 0.6

ICP-MS Results of Competitor Test Article 2				
Element	Measured Concentrations After Microwave Digestions (ppb)			Sample Concentration Before Digestion (µg Element/g Sample)[2]
	Method Blank	Measured Concentration	Corrected Concentration[1]	
Mg	< 1	9	9	5
Al	< 1	34	34	20
Si	167	1773	1606	924
Ca	< 100	147	147	85
Ti	1	1063	1062	611
Fe	13	12	< 1	< 0.6
Cu	< 1	153	153	88
Zn	< 1	9	9	5
Sr	< 1	257	257	148
Nb	2	2	< 1	< 0.6
Ag	< 1	1	1	0.6
Ba	< 1	14199	14199	8170
Ta	4	6	1	0.6

Conclusion

No patterns of interference were found for the CERTIFIED PURE POREX® test articles, proving that they have a very high purity level. A significant level of Sodium ion and a very high level of Iron ion were detected in the competitor test article 1. A significant level of Barium ion was detected in the competitor test article 2. Calcium, Strontium, Copper and Aluminum ions were also detected in the competitor test article 2.

PCR Inhibitors and Antimony (Sb) in Competitors' Products

1. Heavy Metal PCR Inhibitors

Some metal ions, especially heavy metals, inhibit or interfere with PCR tests. Inhibitors are chemical and biological matrix interferences that coextract with DNA and affect downstream processing.[1] PCR inhibitors generally exert their effects through direct interaction with DNA or interference with thermostable DNA polymerases. Direct binding of agents to single-stranded or double-stranded DNA can prevent amplification and facilitate the copurification of inhibitors and DNA. Inhibitors can also interact directly with a DNA polymerase to block enzyme activity.[2] Ionized Calcium, found in competitors' filter products, has significant inhibitory effects on PCR tests.[3] Heavy metals are well known PCR inhibitors.[4] High concentration of Sodium is present in one of the PCR filter products made by a competitor that was tested in the study. Excessive amounts of Sodium and other salts can inhibit PCR.[5]

2. Antimony (Sb): PCR Interference and Human Health and Environmental Effects

Significant amounts (230 ppm) of Antimony (Sb) are present in cotton filters supplied by multiple manufacturers in products such as serological pipette tips. Antimony oxide is added to plastics and textiles to reduce flammability. Antimony interferes with spectrophotometric measurements used in PCR analysis by absorbing significant levels of UV light at 220 and 260 nm, which are the wavelengths normally used to detect and quantitate proteins and DNA.[6]

Antimony also has adverse effects on human health and the environment. He and his colleagues reviewed its biogeochemical and ecological effects.[7] The United States EPA (Environmental Protection Agency) lists Antimony's hazardous effects on its website. Furthermore, Lambert and his colleagues found that the cotton filter in a "Pasteur pipette" allows aerosol to escape upon aspiration of a bacterial culture broth and may cause cross contaminations.[8]

Study of Bacterial Filtration Efficiency (BFE)

The POREX® test articles were tested by an independent lifecycle microbiology laboratory to determine the Bacterial Filtration Efficiency (BFE) using ASTM F2101. However, they employed a test design specifically for pipette tip filters to present a greater challenge than would be experienced in normal use. No single colony was detected at the downstream of the FORTRESS filter and another filter at a challenge level of 4.6 x 105 CFU with MPS of ~3.1µm, which is a reduction of more than >99.99978%.

BFE Test Procedure

This test procedure was performed to evaluate the BFE of the test articles at an increased challenge level. A suspension of Staphylococcus aureus, ATCC#6538, was delivered to the test articles at a challenge level of greater than 10^5 colony forming units (CFU). The suspension was aerosolized using a nebulizer and delivered to the test articles at a fixed air pressure and air flow rate of 5 liters per minute (LPM).

The aerosol droplets were generated in a glass aerosol chamber and drawn through the test article into all-glass impingers (AGIs) for collection. The challenge was delivered for a one minute interval and sampling through the AGIs was conducted for two minutes to clear the aerosol chamber. The mean particle size (MPS) control was performed at a flow rate of 28.3 LPM using a six-stage, viable particle, Andersen sampler for collection. The test conditions and parameters are summarized as follows:

- Challenge Flow Rate: 5 LPM
- Area Tested: Entire Test Article
- Challenge Level: 4.6 x 10^5 CFU
- MPS: ~3.1µm
- Test Monitor Results: Acceptable

1. McCrod, B., Pionzio, A., Thompson, R., 2015, *Analysis of the Effect of a Variety of PCR Inhibitors on the Amplification of DNA using Real Time PCR, Melt Curves and STR Analysis*, Department of Justice.
2. Bessetti, J., 2007, *An Introduction to PCR Inhibitors*, www.promega.com, Promega Corporation.
3. McCrod, B., Pionzio, A., Thompson, R., 2015, *Analysis of the Effect of a Variety of PCR Inhibitors*, Department of Justice.
4. Straub, T. M., Pepper, I. L., and Gerba, C. P., 1995, *Removal of PCR inhibiting substances in sewage sludge amended soil*. Water Sci. Technol. 31:311–315.
5. Bessetti, *An Introduction to PCR Inhibitors*, www.promega.com, Promega Corporation.
6. Lewis, L. K., Robson, M. H., Vecherkina, Y., etc., 2010, *Interference with Spectrophotometric Analysis of Nucleic Acids and Proteins by Leaching of Chemicals from Plastic Tubes*, BioTechniques 48:297-302.
7. He, M., Wang, N., Long, X., etc., 2019, *Antimony speciation in the environment: Recent advances in understanding the biogeochemical processes and ecological effects*, J. of Environ. Sci. 7 5, 1 4 – 3 9.
8. Lambert, N. G., Jetté, L. P., Caprioli T., Kasatiya, S. S., 1979, *Risk of Contamination by Pipetting Bacterial Culture Broth with "Pasteur Pipettes"*. Ann. Microbiol. (Paris). 130A(3):351-4.

Discussion of Filtration Efficiency Standards

Porex worked with a renowned national laboratory to develop a test procedure to determine the bacterial filtration efficiency (BFE) of various filtration materials used in life science, biological, clinical and other analytical consumables with special emphasis on filters used in filtered pipette tips.

This test procedure complied with ASTM F2101 guidelines but provided a more severe challenge, designed specifically for filtered pipette tips, than most filtration materials would experience in normal use. This rigorous testing methodology allowed for a reproducible bacterial challenge to be delivered to the test samples.

A competitor's product literature for a consumable pipette tip states, "There is no standardized test method available yet to determine the filter efficiency of filters in pipette tips." This manufacturer references EN 1822 as an appropriate test, but it is a standard designed for the EPA/HEPA/ULPA filters used in the field of ventilation and air-conditioning—not analytical or clinical applications. It's not suitable for pipette filters. Even when using a substandard test, this manufacturer's product only achieved a BFE of 99.5% in the Filter Class E12 classification. In contrast, POREX pipette tip filters achieve a BFE of over 99.99978% even when tested according to the severe challenge protocol designed specifically for pipette tip filters that complies with ASTM F2101.

CERTIFIED PURE POREX®

This certification ensures rigorous testing by third-party analytical, clinical and life science laboratories for media purity and reproducible performance in the most demanding scientific applications.*

- No material additives or contaminants
- No heavy metal or inorganic interference
- 99.9% bacterial filtration efficiency (BFE)
- Clinical laboratory methodology compatibility

**Data on file and available upon request.*

Study Viral Filtration Efficiency (VFE)

The POREX® test articles were tested by an independent lifecycle microbiology laboratory to determine the Viral Filtration Efficiency (VFE) adapted from ASTM F2101 specifically for pipette tip filters. The filters were assembled into 1 ml pipette tips and challenged with over 104 plaque-forming units (PFU) ΦX174 bacteriophage aerosols with Mean Particle Size (MPS) of ~3.0µm at a flow rate of 1 liter per minute (LPM). Various POREX and competitive materials were tested with VFE ranging from over 99.9% VFE to over 99.99% VFE.

VFE Test Procedure

This test was performed to evaluate the VFE of test articles at an increased challenge level. A suspension of ΦX174 bacteriophage was delivered to the test article at a challenge level of greater than 104 plaque-forming units (PFU) to determine the filtration efficiency. The challenge was aerosolized using a nebulizer and delivered to the test article at a fixed air pressure and flow rate of 1 liter per minute (LPM). The aerosol droplets were generated in a glass aerosol chamber and drawn through the test article into all glass impingers (AGIs) for collection. The challenge was delivered for a one-minute interval and sampling through the AGIs was conducted for two minutes to clear the aerosol chamber. The mean particle size (MPS) control was performed at a flow rate of 28.3 LPM using a six stage, viable particle, Andersen sampler for collection. The VFE at an Increased Challenge Level test procedure was adapted from ASTM F2101.

The test conditions and parameters are summarized as follows:

- Challenge Flow Rate: 1 LPM
- Area Tested: Entire Test Article
- Challenge Level: 2.3 x 104 PFU CFU
- MPS: ~3.0µm
- Test Monitor Results: Acceptable

Porex worked with a renowned national laboratory to develop a test procedure to determine the Viral filtration efficiency (VFE) of various filtration materials used in life science, biological, clinical and other analytical consumables with special emphasis on filters used in filtered pipette tips. This test procedure complied with ASTM F2101 guidelines but provided a more severe challenge, designed specifically for filtered pipette tips, than most filtration materials would experience in normal use. This rigorous testing methodology allowed for a reproducible viral challenge to be delivered to the test samples.

Test Article, Positive, Negative, and Untreated Control Articles		
VFE	**Total PFU Recovered**	**Filtration Efficiency (%)**
Porex Material A	7.5	99.968
Competitor A	290	99.0
Competitor B	270	98.8
Porex Material B	<1- Note: no detectable plaques on any assay plates.	>99.9957

References:

- Bessetti, J., 2007, *An Introduction to PCR Inhibitors,* www.promega.com, Promega Corporation.
- He, M., Wang, N., Long, X., etc., 2019, *Antimony speciation in the environment: Recent advances in understanding the biogeochemical processes and ecological effects*, J. of Environ. Sci. 7 5, 1 4 – 3 9.
- Lambert, N. G., Jetté, L. P., Caprioli T., Kasatiya, S. S., 1979, *Risk of Contamination by Pipetting Bacterial Culture Broth with "Pasteur Pipettes"*. Ann. Microbiol. (Paris). 130A(3):351-4.
- Lewis, L. K., Robson, M. H., Vecherkina, Y., etc., 2010, *Interference with Spectrophotometric Analysis of Nucleic Acids and Proteins by Leaching of Chemicals from Plastic Tubes*, BioTechniques 48:297-302.
- McCrod, B., Pionzio, A., Thompson, R., 2015, *Analysis of the Effect of a Variety of PCR Inhibitors on the Amplification of DNA using Real Time PCR, Melt Curves and STR Analysis*, Department of Justice.
- Straub, T. M., Pepper, I. L., and Gerba, C. P., 1995, *Removal of PCR inhibiting substances in sewage sludge amended soil*. Water Sci. Technol. 31:311–315.
- US EPA, 2019, *Antimony Health Hazard Information*, https://www.epa.gov/sites/production/files/2016-09/documents/antimony-compounds.pdf
- Wilson, I. G., 1997, *Inhibition and facilitation of nucleic acid amplification*. Appl. Environ. Microbiol. 63(10): 3741–3751.

AMERICAS

POREX® Corporation
500 Bohannon Road
Fairburn, GA 30213 USA

Phone: + 1 770 964 1421
Fax: + 1 770 969 0954
Email: Info.POREX.amrs@filtrationgroup.com

EMEA

POREX® Technologies GmbH
Strangenhäuschen 30
52070 Aachen, Germany

Phone: + 49 241 9105250
Fax: + 49 241 91052516
Email: Info.POREX.emea@filtrationgroup.com

ASIA PACIFIC

POREX® Technologies Sdn Bhd
Lot PT 74, Jln Hulu Tinggi 26/6,
Sek. 26, Hicom Industrial Park,
40400 Shah Alam, Selangor, Malaysia

Phone: + 603 51913308
Fax: + 603 51923308
Email: Info.POREX.apac@filtrationgroup.com

CHINA

POREX® Technologies (Ningbo) Co., Ltd.
99 Huanghai Road
Ningbo Shi
315800 Zhejiang Sheng, China

Phone: + 86 574 2685 8761
Fax: + 0574 26886964
Email: Info.POREX.china@filtrationgroup.com

POREX
Filtration Group®