TPA Network
Research Consortium

Filtration, Antimicrobial and Bioburden Testing

with respect to ***Efficacy, Safety and Environmental Impact***

and the ***Stay Safer PRN95+ Protective Face Mask***

Contamination of healthcare settings and products by pathogenic microbes is a major, decades-long concern among those in the healthcare and medical industries, affecting both patient morbidity and mortality. With the emergence of CoVid-19, the limited supply of formal personal protective equipment (PPE), and the general population's increased interest in the effectiveness of face masks for personal and work-related use, consumers are looking for more and more information and data with which to make more informed purchasing decisions.

This document is intended to provide the reader with a fundamental understanding of the basic types of filtration, antimicrobial and bioburden tests that are performed on fabrics, fabric face masks and medical face masks.

Regulatory Framework and Standards

To promote transparency and the public good, government regulators require those in the medical and healthcare industries to use products that are manufactured by companies that adhere to a variety of strict regulations and testing protocols. When properly followed, these regulations, processes and protocols prevent the manufacture, distribution and use of products that are unsafe (e.g., susceptible to contamination with objectionable microbes).

These are the leading organizations that establish/issue standards for face masks, fabrics, antimicrobial testing:

ISO	The *International Organization for Standardization* is a Switzerland-based independent, non-governmental international organization with a membership of 164 national standards bodies.
NIOSH	The *National Institute for Occupational Safety and Health* is the CDC agency that conducts research and makes recommendations for the prevention of work-related injury and illness.
ATCC	The *American Type Culture Collection* collects, stores and distributes standard reference microbes, cell lines and other materials for research and development; and it creates standards.
ASTM	The *American Society for Testing and Materials* develops research, environmental safety and production standards related to products manufactured from rubber, plastics and raw materials.
BSI	The *British Standards Institution* produces technical standards on a wide range of products and services and also supplies certification and standards-related services to businesses in the UK.
EMAGMP	The *European Medicines Agency* is responsible for the scientific evaluation, supervision and safety monitoring of medicines – and the provision of scientific advice and protocol assistance – to foster scientific excellence for the benefit of public and animal health in the EU.
TGA	The Australian Government Department of Health's *Therapeutic Goods Administration* is responsible for regulating therapeutic goods including prescription medicines, vaccines, sunscreens, vitamins and minerals, medical devices, blood and blood products
OEKO-TEX	The *International Assoc. for Research and testing in the Field of Textile and Leather Ecology* consists of 18 independent worldwide research and test institutes that develop test methods that form the basis of product, production and chemical standards in the field of textile ecology.
CNS FZ/T	The *Chinese National Standards Administration* develops and issues standards and testing protocols in 26 industrial and commercial categories including spinning/textiles (FZ/T).
JISC	The *Japanese Industrial Standards Committee* establishes the standards used for industrial activities in Japan and performs certifications of various products and processes.

Filtration Efficiency Standards / Tests

There are hundreds of international standards and tests that apply to the performance, safety and eco-impact of textiles. The following are those standards that are applicable in the context of (medical) face mask filtration.

Laboratory: Nelson Laboratory (US)

Test 01: *ASTM 2100: Bacterial Filtration Efficiency (BFE) Test*

This test method is specifically designed for measuring bacterial filtration efficiency of medical face masks, using Staphylococcus aureus as the challenge organism. The use of S. aureus is based on its clinical relevance as a leading cause of nosocomial infections. It is designed to introduce a bacterial aerosol challenge to the test specimens at a defined flow rate within the range of normal respiration to measure both patient- and wearer-generated aerosols.

Laboratory: Nelson Laboratory (US)

Test 02: *ASTM 2299: Particulate Filtration Efficiency (PFE) Test: 0.1 μm*

This test evaluates the filtration efficiency of medical face mask fabric to penetration by upstream and downstream latex aerosol particles as small as 0.1 μm and establishes a basis of efficiency comparison between medical face mask materials (aka the Latex Particle Challenge).

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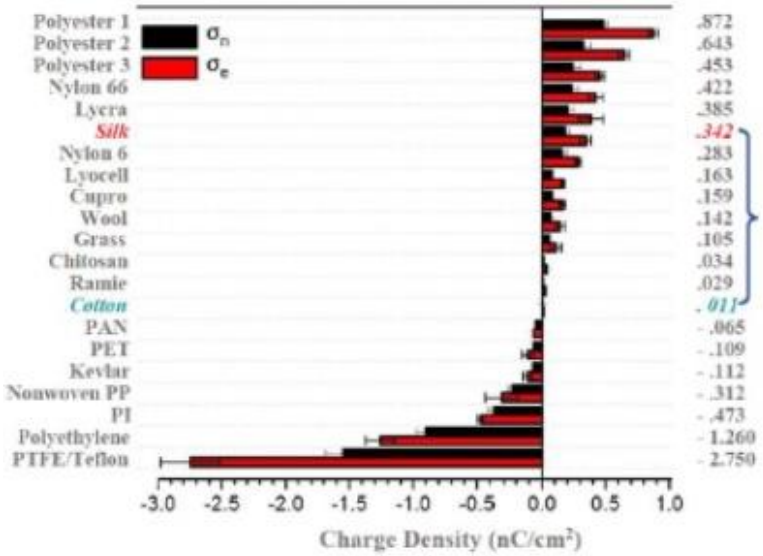
sample/fabric	<300 nm average ± error	>300 nm average ± error
N95 (no gap)	85 ± 15	99.9 ± 0.1
N95 (with gap)	34 ± 15	12 ± 3
surgical mask (no gap)	76 ± 22	99.0 ± 0.1
surgical mask (with gap)	50 ± 7	44 ± 3
cotton quilt	96 ± 2	96.1 ± 0.3
quilter's cotton (80 TPI), 1 layer	9 ± 13	14 ± 1
quilter's cotton (80 TPI), 2 layers	38 ± 11	49 ± 3
flannel	57 ± 8	44 ± 2
cotton (600 TPI), 1 layer	79 ± 23	98.4 ± 0.2
cotton (600 TPI), 2 layers	82 ± 19	99.5 ± 0.1
chiffon, 1 layer	67 ± 16	73 ± 2
chiffon, 2 layers	83 ± 9	90 ± 1
natural silk, 1 layer	54 ± 8	56 ± 2
natural silk, 2 layers	65 ± 10	65 ± 2
natural silk, 4 layers	86 ± 5	88 ± 1
hybrid 1: cotton/chiffon	97 ± 2	99.2 ± 0.2
hybrid 2: cotton/silk (no gap)	94 ± 2	98.5 ± 0.2
hybrid 2: cotton/silk (gap)	37 ± 7	32 ± 3
hybrid 3: cotton/flannel	95 ± 2	96 ± 1

ASTM 2100 and 2299 tests are in process; test results will be published by October 15, 2020. In the interim, consider the following scientific underpinnings to the triboelectric air filtration component of our face masks.

Key design elements of our mask derive from our research and a study conducted by scientists from the University of Chicago and the Argonne National Laboratory (exhibit source) which found that cotton with silk or chiffon were the best material combination for fabric face masks as they help to create a triboelectric process that filters in excess of 95% of particles.

This exhibit (by physicist/scientist Ron Kurtus) illustrates the charge differential between cotton and silk. To *optimize* performance, our face mask has two center layers, each of silk chiffon, to amplify its friction-creating capability. It gets *recharged* daily by hand rubbing for 20 seconds. It uses the aerodynamic friction of the wearer's breath to create a source of fabric abrasion to make it partially self-powered / self-sustaining.

The findings above are based solely on the (tribo-electric) air filtration results using anti-microbial-free fabrics. We anticipate that the results of our mask will meet / exceed those for the cotton/silk and cotton/chiffon combinations.



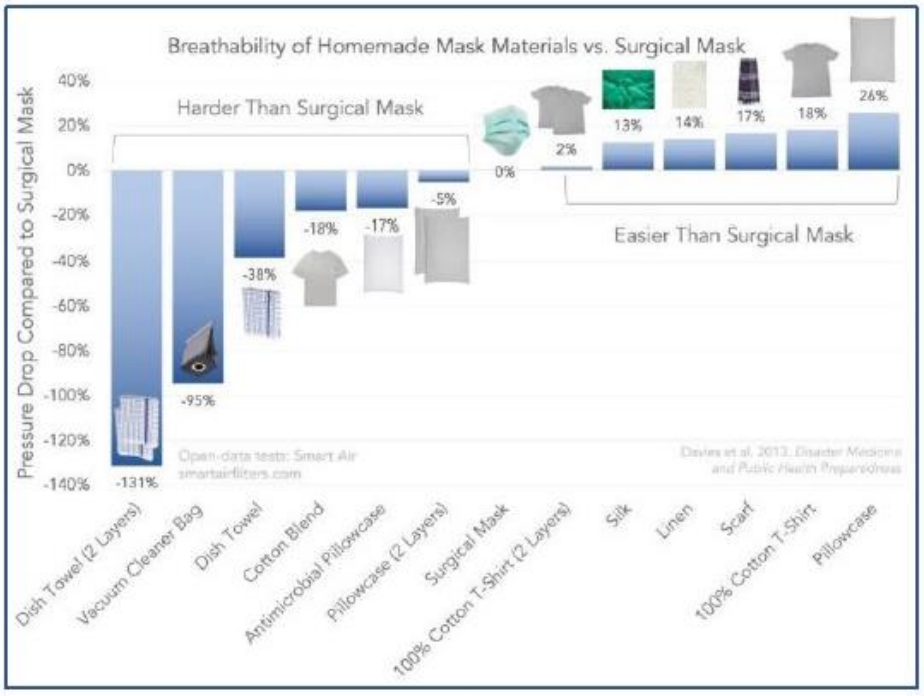
Breathability Standards / Tests

Laboratory: Nelson Laboratory (US)

Test 03: *MIL-M-36954C: Delta P Differential Pressure (Breathing Resistance) Test*

Military standard test to determine the resistance to airflow of the facemask. A lower breathing resistance indicates a better comfort level for the user. It means that the mask feels cooler and easier to breathe through, and that the mask will maintain its shape in a better way as there is less pressure on the material. There will be less unfiltered air escaping around the mask.

The MIL-M-36954C test is in process; test results will be published by October 15, 2020. In the interim, consider the results of this study on the breathability of masks made from various fabrics.



This exhibit details the relative breathability of materials often used in face masks. The data is from an article entitled "Testing the Efficacy of Homemade Masks: Would They Protect in an Influenza Pandemic?"

The study appeared in the May 2013 Cambridge University Press and was conducted by Public Health England. The findings were reformatted here by *Smart Air*.

N95 respirators are not appropriate for non-medical applications, so they were not included in the study. Had it been, it would have been at or near the bottom of the range. The surgical mask was used as the baseline measure of a mask's breathability.

Note that those mask fabrics found to be most breathable are also natural, found to be more effective at microbe filtration and less likely to create a bio-burden (linen, silk, and 100% cotton items).

Safety and Environment Standards / Tests

OEKO-TEX® STANDARD 100

OEKO-TEX® STANDARD 100 certification is a coveted textile industry mark globally. Recertified annually, STANDARD 100 labeled products are thoroughly tested for (regulated and non-regulated) harmful substances and guaranteed to be harmless in human and ecological terms throughout the supply chain. The product must show/evidence: No dermal toxicity (OECD 402); No skin irritation (OECD 404); No eye irritation (OECD 405); No sensitization (OECD 406); No oral toxicity (OECD 423); and No mutagenic properties (OECD 471)

The antimicrobial agent used on our mask is OEKO-TEX® STANDARD 100 certified.

Bluesign® Approval

The designation "*Bluesign*® approved" verifies and guarantees the elimination of substances harmful to humans and the environment from the beginning of the manufacturing process to increase product safety through the entire supply chain; conserve valuable resources; minimize risk; and ensure sustainability.

The antimicrobial agent used on our mask is Bluesign® approved.

Antimicrobial and Bioburden Standards / Tests

Many international standards and tests exist that apply to antimicrobial agents and textiles. They address their effectiveness, efficiency, kill time, safety, bioburden capacity, longevity, durability, toxicity and eco-impact on many types of microorganisms. The following are most applicable in the context of antimicrobial face masks.

Laboratory: Specialty Testing Laboratories (US)
Test 04: *AATCC 100: Antimicrobial Fabric Test*

This is the US industry standard and most commonly chosen test to evaluate the degree of anti-bacterial activity on fabric; antimicrobial fabric performance; and quantitatively measure the fabric's ability to inhibit the growth of microorganisms or kill them over a 24-hour period of.

Laboratory: Specialty Testing Laboratories (US)
Test 05: *AATCC 247: Antimicrobial Fabric Zone of Inhibition Test: Parallel Streak Method*

This test qualitatively evaluates the antibacterial activity of a diffusible antimicrobial agent on a treated fabric and the ability of the fabric to inhibit the growth / concentration of microorganisms.

Laboratory: Specialty Testing Laboratories (US)
Test 06: *Kirby-Bauer Zone of Inhibition (ZOI) Antimicrobial Test*

A qualitative test used to measure antibiotic resistance and test the ability of textiles to inhibit microbial growth. The "zone of inhibition" refers to the area where the antimicrobial is effective.

Laboratory: Hohenstein Institute (Germany
Test 07: *ISO 18184 Textiles: Determination of Antiviral Activity of Textile Products Test*

This antiviral fabric test measures virucidal antimicrobial activity on fabrics. It measures the ability of viruses to replicate in the susceptible and permissive host cell. It is designed to test the ability of fabrics to kill viruses within standard exposure times (from 2, to less than 24, hours).

Laboratory: Microchem Laboratory (US)
Test 08: *ASTM E2180: Test for Hydrophobic Antimicrobial Surfaces*

This test is designed to quantitatively measure the antimicrobial effectiveness of an antimicrobial agent in water repelling materials (which is otherwise difficult to assess without this test).

Laboratory: Microchem Laboratory (US)
Test 09: *ASTM E2149: Standard Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions Test*

This test is designed to evaluate the resistance of chemically-bonded, non-leaching antimicrobial-treated specimens to microbe growth under dynamic contact conditions. Results from 'round-robin' repeated tests can be taken to prove antimicrobial durability whereby the face mask is tested when new and after 10, 20, 50 and even more wash cycles.

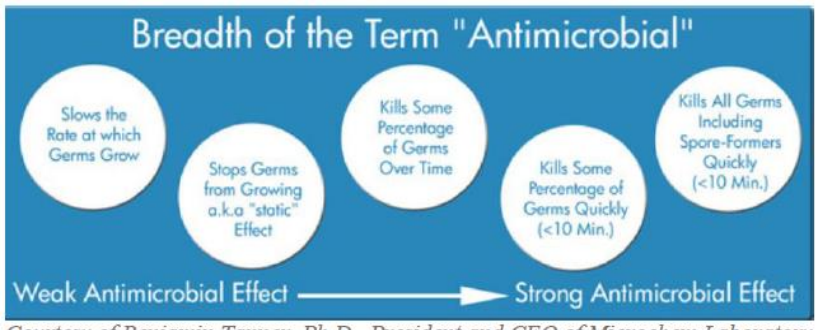
Laboratory: Hohenstein Institute (Germany
Test 10: *EN ISO 11737 (USP61/62): Bioburden (Microbial Cleanliness) for Face Masks Or Determination of a Population of Microorganisms on Product Test*

This test measures the efficiency of the antimicrobial (sonication, stomaching or vortexing) cleaning process by detecting the total number of viable microorganisms on the medical product. In the case of a reusable face mask, functionality is ensured by retesting the mask after re-processing cycles until there is no significant increase in the accumulated number of microorganisms recovered, comparing consecutive results at intervals over an extended time.

The results of tests done on the QAC-based antimicrobial agent used on our mask are detailed below.

Not All Antimicrobial-Treated Fabrics Act Alike

Not all chemical antimicrobials act alike. As the exhibit below illustrates, there are five effectiveness categories. In reviewing it, know that an antimicrobial's strength is a product of its concentration and the time that it is in contact with the microbe, i.e., the antimicrobial-treated fabrics that kill microbes faster are often the most potent.



Courtesy of Benjamin Tanner, Ph.D., President and CEO of Microchem Laboratory

At the low end of the antimicrobial-treated fabric spectrum are those that might retard the microbial growth rate, (possibly for one or a few species). This will help with odor control, but it is unlikely to slow microbial spread.

Stronger antimicrobial-treated fabrics may retard the growth or kill more microbes, but over a much longer time frame.

Most antimicrobial-treated fabrics slow the growth, or kill, a greater percentage of microbes over a longer period (under certain circumstances). This, essentially, makes them useful for only aesthetic protection, but not for infection control proposes.

Few antimicrobial-treated fabrics kill an appreciable percentage of microbes quickly (defined here as being under ten minutes). This equates their impact to that of using a low-level surface disinfectant or sanitizer.

At the upper end of the antimicrobial-treated fabric spectrum are those that employ a strong sterilant chemical; few fabric technologies can deliver such a benefit. Some QAC-based antimicrobials achieve this goal.

Our Antimicrobial Approach: QACs

The DTSACl-based quaternary ammonium compound (QAC) molecularly bonds to fabric to become a part at manufacture.

Negatively-charged microbes of all types and sizes are attracted to the fabric which carries the QAC's positive charge.

The microbe's protein-based cell membrane (or capsule), which is critical to the microbe's survival, is fatally punctured.

As a redundant measure, the microbe is *electrocuted* by the transfer of a positive electrical charge to it.

The ***Stay Safer*** ***PRN95+*** ***Protective Face Mask*** uses two means by which to attract, secure and inactivate (electrocute_ pathogenic microbes: a triboelectric air filtration (TAF) method and a chemical antimicrobial method. The chemical antimicrobial agent that we use was originally developed by Dow Chemical. It uses a quaternary ammonium compound (QAC) method, meaning it is a surface-active agent that creates a chemical reaction on the surface upon which it is applied. It is also cationic, meaning it is positively charged and highly effective at inactivating negatively-charged microbes with which it comes in contact. It does this by stabbing the target microbe's lipoprotein envelope (outer capsule or membrane) and transferring a fatal electric charge to it. QACs are able to inactivate microbes in part, as a result of this electrical interaction between the *ammonium active agent* and the microbe's negatively-charged cell membrane.

QACs are active ingredients in more than two hundred disinfectants currently approved by the US EPA for use to inactivate the CoVid-19 virus. The amounts of these compounds used in household, workplace and industry settings has increased, and usage will continue to be elevated given the pandemic's scope. QACs, especially those that use DTSACl as their active ingredient, have been found to be highly effective against *gram-negative, enveloped* pathogenic microbes, whether they are germs, fungi, bacteria or viruses. The active ingredient in the *AEM 5700* product is DTSACl.

Performance and Effectiveness Findings for DTSACl QACs

Our face mask's fabric is made with a QAC-based antimicrobial having DTSACl as its active ingredient. It was a breakthrough product created by *Dow Corning* and has a 40-year safety and efficacy profile that is unmatched by other products. Today, several QAC-based antimicrobials use DTSACl as their active ingredient. For our purposes here – providing evidence of effectiveness, safety and positive environmental impact – we reference generic research on QAC-based antimicrobials; QAC's having DTSACl as their active ingredient; and private, public and academic studies of *AEM 5700* / Aegis® Microbe Shield, a commercially marketed product.

Environmental and Sustainability Certifications

Unlike some QAC-based antimicrobials, those having DTSACl as their active ingredient have no unwanted side effects, damage or discoloration on product surfaces, toxicity to living organisms or ecological damage. They do not volatilize, dissipate or leach onto other surfaces or into the environment or off-gas after application.

- Proven to be compatible with the most stringent restricted substance lists, the *AEM 5700* product by example, and its biocidal active components, are registered with the EU Biocidal Products Regulation and the US EPA.
- The *AEM 5700* product is *Bluesign*® approved. This verifies and guarantees the elimination of substances harmful to humans and the environment from the beginning of the manufacturing process to increase product safety through the entire supply chain; conserve valuable resources; minimize risk; and ensure sustainability.
- The *AEM 5700* product is OEKO-TEX® STANDARD 100 certified, a coveted textile industry mark. Re-certified annually, STANDARD 100 labeled products are thoroughly tested for (regulated and non-regulated) harmful substances and guaranteed to be harmless in human and ecological terms through the supply chain.

AEM 5700 Product-Specific Tests

- Canada's prestigious Western University ImPaKT Facility Biosafety Level-3 Laboratory determined that the *AEM 5700* product reduces the risk of microbial transmission by as much as 99%.
- NASA's Spaceflight & Life Sciences Training Program verified *AEM 5700*'s efficacy on cotton.
- Conforming to ASTM E2149 guidelines, a study performed by Robert Monticello, Ph.D. (now serving as the Sr. Scientific Consultant to the International Antimicrobial Council) found that fabric treated with the *AEM 5700* product reduced the total population of MRSA bacteria by 99.99%. While gram-positive, *MRSA's protein capsid has a net-negative charge (like CoVid-19) that is vulnerable to QAC-antimicrobials.*
- Conforming to ATCC 9533/6538/11229 guidelines respectively, tests found the *AEM 5700* product to be 99.95% effective against Trichophyton Mentagrophytes, Staphylococcus Aureus and E-Coli, *all of which have cell walls with a net-negative charge (like CoVid-19) that is vulnerable to QAC-antimicrobials.*
- Tests conducted by the Malaysian & Thailand Government Research Lab proved the efficacy of the biocides used in the *AEM 5700* product against the 2003 H5N1 Avian Flu, *an enveloped virus having a protein capsule that carries a net-negative charge (like CoVid-19) that is vulnerable to QAC-antimicrobials.*
- Tests conducted by Hill Top Research (est. 1947) re: compliance with EPA pesticide guidelines, evidenced the solid virucidal activity of the *AEM 5700* product against the HSV-1, *an enveloped virus having a protein capsule that carries a net-negative charge (like CoVid-19) that is vulnerable to QAC-antimicrobials.*
- A landmark 1988 study of QAC-based antimicrobial agents performed by University of Michigan Professors Wang and Tsao noted the "excellent antimicrobial action" of the *AEM 5700* product and demonstrated that fabrics treated with it adsorbed both the Bacteriophage T2 and HSV-1, enveloped viruses, which it inactivated by virtue of the disruption of the viral envelope, resulting in "an irreversible loss of infectivity".
- An American Hospital Supply Corp. (now Baxter International) study of virtually all antimicrobials agents, focused on their toxicity and effectiveness against microbes and chemical and biological contaminants found on surgery fabrics; it eliminated all other antimicrobial agents except for the *AEM 5700* product.

Tests of QAC-Based Antimicrobial having the Active Ingredient DTSACl

- Conforming to the EN 14476 test standard, which quantitatively evaluates the virucidal activity of chemical disinfectant and antiseptic products in the medical area, tests performed by England's Microbial Solutions laboratory in February, 2020 determined that a QAC-based antimicrobial agent having the active ingredient DTSACl *showed a log reduction of 4.33 against the Feline Coronavirus, a member of the Coronaviridae Family and an enveloped virus having a protein capsule that carries a net-negative charge (like CoVid-19). A log reduction of 4.33 means the antimicrobial was >99.99% effective against the virus. The Feline Coronavirus is a surrogate virus used in labs as a close, but safer, alternative to human pathogenic strains of Coronavirus, for which it shares an almost identical structure. Nonetheless, it cannot be known whether CoVid-19exhibits virulence properties that are impacted by the QAC-based antimicrobial agent without testing this strain.* This same QAC-based antimicrobial was also found to be very effective against many other viruses (e.g., H9N2, H1N1, Norovirus, Reovirus Type I and Influenzas A2 (Aichi and Asian) and B).

- Tests performed by the Southern Research Institute (est. 1941) found that surfaces treated with a QAC-based antimicrobial agent having the active ingredient DTSACl showed strong virucidal activity against a non-enveloped RNA enterovirus, Poliovirus Type 1 Strain MEF-1. Note that the EPA's Emerging Viral Pathogen Guidance mandates that disinfectants and antimicrobials used against SARS and *CoVid-19* must validate efficacy against viruses of this specific type in order to gain approval, among other requirements.

- Numerous other studies, tests and papers have been written on the antiviral activity of a QAC-based anti-microbial agents having the active ingredient DTSACl (both in solution and when immobilized) evidencing the inactivation of lipid-containing viruses, some non-lipid viruses, and bacteriophages. These results are encouraging regarding the utility of this treatment at reducing doses of viruses in a variety of applications.

- A study conducted by a team of researchers from US and South Korean textile colleges, published in 2004 in *Polymer*, tested the antimicrobial effectiveness of DTSACl. It found that "excellent anti-microbial action was demonstrated for all coatings". The specific antimicrobial studied was the *AEM 5700* product.

- A study conducted by researchers from military universities in China and Augusta University in Georgia, published in 2017 in *Progress in Polymer Science*, studied the state-of-the-art of QAC-based antimicrobial agents including their anti*viral* resistance challenges, based on randomized human clinical trials. One of the study's finding was that, "in general, QACs are considered good disinfection agents for influenza viruses" and "we confirm virucidal effects of the IQACs tested against influenza viruses in our carrier tests". The study specifically noted the inactivation of the H5N1 flu virus in less than 2 minutes by (immobilized) QAC-coated surfaces. The *AEM 5700* product is such an immobilized (or bonded) product.

- The CDC's 2009 publication entitled "Interim Biosafety Guidance for All Individuals Handling Clinical Specimens or Isolates Containing 2009-H1N1 Influenza A Virus (Novel H1N1), Including Vaccine Strains" notes that "several chemical disinfectants including...quaternary ammonium compounds, are effective against human influenza viruses if used at the correct concentration for the appropriate contact time as specified in the manufacturer's recommendations". Note that the H1N1 swine flu is *an enveloped virus having a protein capsule that carries a net-negative charge (like CoVid-19), that is vulnerable to QAC-antimicrobials.*

Warning about Antimicrobials and CoVid-19 Claims

When evaluating research findings, due to individual sensitivities, the results of one test virus can't be transposed to other viruses. Based on our current knowledge, and a variety of considerations (e.g., genetic similarity, safety profile, growth time, availability, etc.) while the preferred virus for antiviral testing is either human coronavirus 229E or OC43, many laboratories are testing with H1N1 since the test method is established and faster.

The FDA and EPA have made it essentially impossible to make antiviral claims in the US. Understandably, they are concerned about misrepresentation of data from antiviral tests and whether these tests can determine whether a fabric, in use, would prevent the spread of viruses or prevent infections. As of this writing (September 2020), *no organization can justly make a claim about is effectiveness against CoVid-19.* To be clear, there has been no approval, for any form of government-sanctioned testing performed in the US or elsewhere to prove the effectiveness of any antimicrobial agent against the coronavirus. For more on this topic, see *Advisory for Visitors from the US / US Government Notice Concerning Product Claims About CoVid-19* ***here***.