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Evaluation of Apparatus Used to Test Liquid through Protective Materials: Comparison of a Modified Dot-Blot Apparatus to the ASTM Penetration Cell

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Reference

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ABSTRACT

Personal protective equipment (PPE), such as gowns used in the latest Ebola outbreak in Western Africa, are critical in preventing the spread of deadly diseases. Appropriate test systems and test soils are needed to adequately evaluate PPE. ASTM F903, *Standard Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Liquid*, has been used for decades to test fabrics' resistance to liquid penetration. However, this test apparatus requires at least 60 mL of test solutions, is labor intensive, and has problems with leakage around the gaskets. We compared the F903 test apparatus to a modified dot-blot apparatus to evaluate the visual penetration of a blood test soil. A series of commercially available gowns and drapes were tested in each apparatus. Using blood test soil at 2 psi, there was no statistically significant difference between the two methods except for in one gown. By comparing this gown in the ASTM test apparatus with and without a screen, the particular screen selected did not account for the difference between the dot-blot and F903 apparatuses; however, it is conceivable that a particular screen/fabric combination could account for this difference. The modified dot-blot apparatus was evaluated using three different test solutions: blood, vomit, and a labeled protein (goat anti-rabbit immunoglobulin G–horseradish peroxidase [GaR IgG-HRP]) in a blood test soil solution. This testing revealed significant difference in penetration for some of the PPE garments. The modified dot-blot had several large advantages over the ASTM apparatus—over six times less specimen volume and no edge or gasket leakage. In addition, nitrocellulose can be easily incorporated into the modified dot-blot apparatus,

enabling the trapping of viruses and proteins that penetrate PPE—thus permitting the use of antibodies to quickly and sensitively detect penetration.

Keywords

personal protective equipment, gowns, aprons, ASTM standards, virus (generic), penetration testing

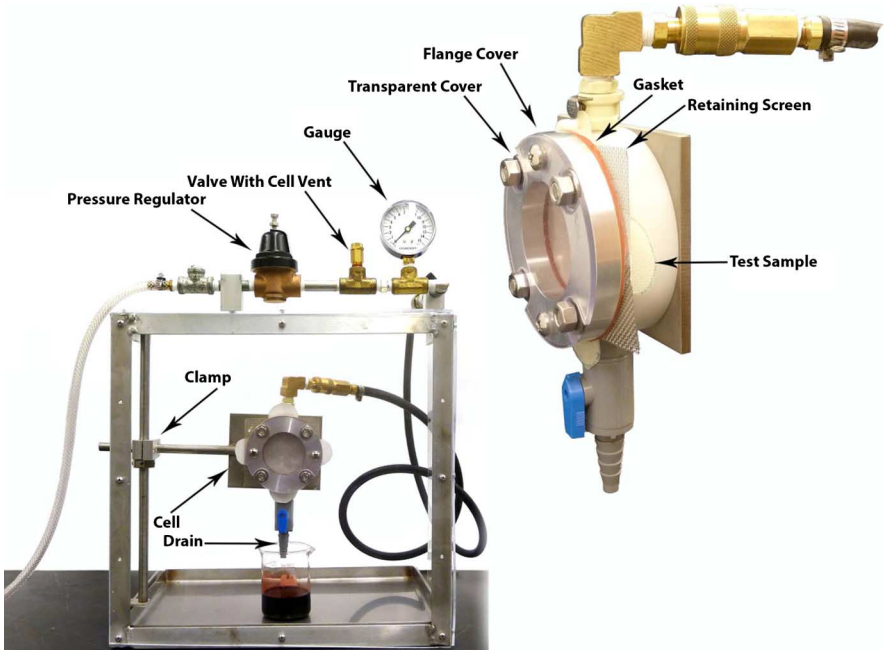
Introduction

At the time the original ASTM F903, *Standard Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Liquids*, ASTM F1670, *Standard Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Synthetic Blood*, and ASTM F1671, *Standard Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Blood-Borne Pathogens Using Phi-X174 Bacteriophage Penetration as a Test System*, standards were written, from the mid-1980s to the mid-1990s, the pressing concern was to prevent exposure to HIV in blood.[1] ASTM F903[2] is the general test system for penetration of liquids, ASTM F1670[3] is the specific application of this test system for a blood test soil, and ASTM F1671[4] is specific for bacteriophage penetration. However, concerns about infectious microbes and the physiological environment in which they may be present have expanded. For instance, in light of the recent outbreak of Ebola in Africa, testing of personal protective equipment (PPE) with viral surrogates in more clinically relevant test soils like vomit, fecal, and mucosal secretions has become a pressing need. To address these concerns, two clinically relevant test soils were selected to test in a modified dot-blot and ASTM F903 test apparatus: an existing blood soil and a newly developed vomit soil.

PPE (e.g., drapes and gowns) are in the first line of defense for health-care workers who come in close proximity to viruses with devastating outcomes, like HIV and Ebola. Therefore, safety and effectiveness of these devices are critical to meeting the labeled performance claims for impermeability. Preventing health-care worker exposure is a crucial factor in sustaining the fight against infectious diseases. There is an array of standard methods for testing PPE,[2–11] most dealing with a single isolated challenge. Additionally, ANSI/AAMI PB70, *Liquid Barrier Performance and Classification of Protective Apparel and Drapes Intended for use in Health Care Facilities*, specifies four different levels for PPE.[11] Level 1 is least protective (passing a water resistance test[9]); Level 4 is the highest level for gowns (passing a barrier test against viral challenges[4]).[11] Although viral tests developed in the 1990s in response to the HIV epidemic use a live surrogate virus, research has not confirmed the relative penetration of different types of viruses. Furthermore, the scope of the standard viral test methods (ASTM F1671 and ISO 16604, *Clothing for Protection against Contact with Blood and Body Fluids—Determination of the Resistance of Protective Clothing Materials to Penetration by Blood and Body Fluids—Test Method Using Phi-X174 Bacteriophage*) does not include comparison between different viruses. A body of relevant, standardized tests and test soils will assist both manufacturers and device regulators by providing specific standardized methods that can be used as is, saving time and the cost of developing new methods, allowing more straightforward comparison of different PPE tested using the standard methods, and ultimately speeding the entry of new devices to the market.

The liquid penetration test apparatus specified in ASTM test method F903 uses a large, heavy, and cumbersome setup (**fig. 1**). False positives can occur from fabric being pinched at the perimeter of the apparatus. Also, results depend on the type of screen used.[12] The ASTM standards specify that the screen should be a smooth finished square mesh made of either metal or plastic, with an open area of at least 50 %.[3,4] This gives a wide range of potential variation and, because of this, ASTM Committee F23 is working to standardize the screen design (ASTM ballot item WK60738, 2017). The modified dot-blot is much smaller and lightweight (**fig. 2**), has a simpler and easier setup that does not use screens, and does not have sharp edges that would cut into the test fabric. Additionally, this apparatus is not prone to leakage around the edges of the test cells because at the bottom of the

FIG. 1 The ASTM F903 setup used in this study; it was originally designed for ASTM F903 and also used in ASTM F1670 and ASTM F1671. The size of the box housing the test cell is 42 by 32 by 40 cm.



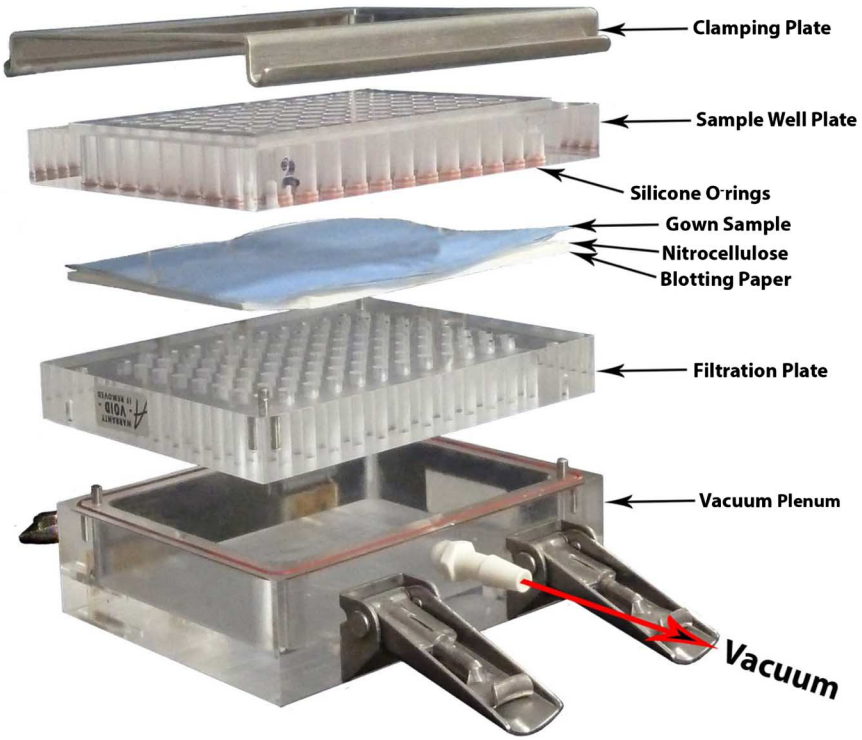
sample well is an O-ring that, when clamped into place, seals the fabric between the well and the lower layers of blotting paper (fig. 2).

Use of the ASTM F903 apparatus requires a large volume (60 mL) of test solution, whereas the modified dot-blot apparatus uses much less (9.6 mL). This difference in test volume becomes significant if microorganisms are to be used to test the PPE. Propagating and plating large volumes of bacteriophage are labor intensive, requiring technical skill to prevent contamination, as well as three- or four-day blocks of time to sanitize equipment, grow the bacteriophage, run the viral penetration test on the PPE, and plate the penetrating solution onto its host *Escherichia coli*. The modified dot-blot can enable users to more quickly and easily test PPE materials in applications in which bacteriophage or virus surrogates are used as challenge organisms, because the nitrocellulose membrane (fig. 2) traps proteins and virus, enabling quick and specific detection using antibodies rather than the more cumbersome and time-consuming traditional microbiological method.

Another drawback with the F903 apparatus is that the test result provides nonparametric, binary statistics (i.e., pass/fail). Statistical comparisons between two specimen groups are performed with a Mann–Whitney-type test that may use hundreds or thousands of specimen to demonstrate differences.[13] In contrast, the dot-blot apparatus contains 96 wells that provide a greater specimen size for statistical comparison of uniform fabrics that have few imperfections across a large area of fabric.

The goals of this research were (1) to evaluate if there is any significant difference between penetration test results for the modified dot-blot apparatus and the ASTM F903 apparatus using synthetic blood, (2) to determine if the presence of a screen in the F903 apparatus accounts for any significant difference between the two test methods, (3) to determine if a labeled protein (goat anti-rabbit immunoglobulin G–horseradish peroxidase [GaR IgG-HRP]) is more sensitive than a visualization of the red dye used in the synthetic blood, and (4) to evaluate any difference between penetration test results for synthetic blood and an alternate test soil simulating vomit. The intent of this work is to investigate the test methods and not to assess the performance of specific PPE products.

FIG. 2 Modified dot-blot apparatus. The sample well plate contains 96 wells; all wells were used for testing in this study. For trapping the labeled protein, GaR IgG-HRP, nitrocellulose was used. For simple visualization of dye penetration, the nitrocellulose was not necessary. The size of this test apparatus is 13.5 by 10 by 7 cm.



Materials and Methods

GOWNS AND DRAPES

Gowns, drapes, and one hospital curtain with different labeled levels of protection were obtained from commercially available sources. As the purpose of this study was to compare performance of test apparatus and procedures, not to evaluate different PPE materials, the materials and manufacturers are not mentioned.

PPE TESTING APPARATUS

ASTM F903 Test Apparatus

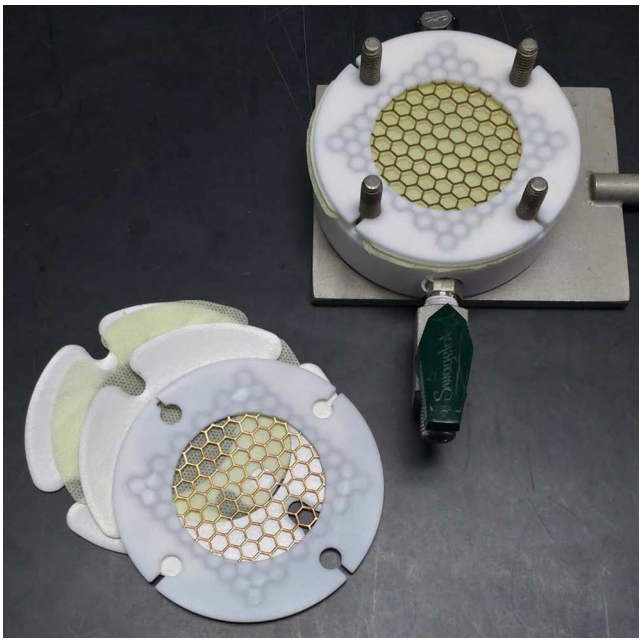
This apparatus was constructed as detailed in ASTM F903, F1670, and F1671 (fig. 1). ASTM F1670 and F1671 specify the option of using a retaining screen. Both standards describe procedure A (without a retaining screen) and procedure B (with a retaining screen). Whenever specified in our testing, a type 304 stainless steel 16 by 16 wire mesh with 0.016-in. (0.04064-cm) diameter wire and 55.4 % clear opening (L.E. Jones Wire Cloth, Baltimore, MD) was used. High-temperature silicone gasket material, Durometer 60A, 1/16-in. (0.15875-cm) thick (McMaster-Carr, Robbinsville, NJ), was used in the assembly of the test cell.

National Institute for Occupational Safety and Health Gasket/Screen

Screens may be designed to achieve different test results.[12] To help provide consistent results, National Institute for Occupational Safety and Health (NIOSH) developed a new screen that would standardize the screens used for viral penetration testing (fig. 3). This new screen consists of a hexagon hole perforated screen encapsulated within

FIG. 3

NIOSH retaining screen and gasket.



a polytetrafluoroethylene ring gasket. The screen provided the test surface, with a 79 % open area. This gasket was adopted for testing after the first three runs with all fabric specimen due to the high failure rate of some specimens.

Modified Dot-Blot Test Apparatus

A Whatman Schleicher & Schuell Minifold I Dot-Blot, acrylic 96-well plate (**fig. 2**) was modified. The filtration plate holes, initially around 1 mm in diameter, were drilled out to 5 mm, slightly smaller than the corresponding holes on the 96-sample well plate (**fig. 2**). This was done to allow a larger area of the PPE material to be under vacuum. A vacuum gauge (EN 837-1 WIKAI, Lawrenceville, GA) was used to establish and monitor the pressure on the fabric in this test setup. Otherwise, the apparatus was used as directed in the manual, with the portion of PPE to be tested placed between the nitrocellulose membrane and the sample well plate, as shown in **figure 2**. The volume of test soils per well was 100 µL. Catastrophic failures were easy to detect in the dot-blot setup. As the test solution was pulled through the fabric and the nitrocellulose and blotting paper layers, the red dye in the synthetic blood was clearly visible in the vacuum plenum, and no solution was left in the sample wells. More subtle failures could only be visualized after dismantling the apparatus. Removal of any remaining liquid in the wells must be performed first to minimize cross contamination. This was accomplished with a multichannel pipette or a small handheld vacuum plate washer held at an angle so that the tips of the device removing the liquid did not touch the material being tested. The modified dot-blot apparatus could then be dismantled for visual examination.

CHEMICALS

Unless otherwise noted, chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Water used in this experiment was purified by a Barnstead NANOpure Diamond water purification unit from Thermo Fisher Scientific (Rockford, IL).

TEST SOILS

Vomit Test Soil

The development of a vomit test soil was undertaken after discussions with health-care workers who had been in Western Africa following the 2014 Ebola outbreak. In addition to blood, vomit was identified by the health-care workers as a likely fluid for transmission of the virus. Vomiting is the involuntary, forceful expulsion of stomach contents through the mouth and sometimes the nose.[14] The objective was to develop a reasonable test soil that would reflect the clinically relevant chemical components in human vomit. In individuals who are sick and vomiting over a period of time, the undigested food content of the stomach is no longer present. The basic composition of vomitus after undigested food is expelled is the gastric juices and bile.[15,16] Previous work on simulating stomach fluid to examine changes in bioavailability of metals[17] or changes in size and surface chemistry of nanoparticles[17,18] indicated that a combination of hydrochloric acid (HCl) and glycine (both 0.4 M) at a pH of 1.5 is a good approximation of stomach fluid. Some bile acid may be expected in vomit. A study of the amount of bile acids in fasting gastric aspirates shows a large range in bile concentration. We chose to use 0.4 g/L, which is at the upper end of the concentration observed by Hoare et al.[19] As human bile contains mucin,[15] 1 g/100 mL mucin was added to vomit test soil to mimic the sticky mucus characteristic. As the mucin is above saturation in this test soil, vigorous stirring or vortexing is necessary immediately prior to use. Amaranth dye (1 mg/mL) was added to aid in visualization of any vomit test soil penetration of PPE materials.

Blood

The ISO 16603, *Clothing for Protection against Contact with Blood and Body Fluids—Determination of the Resistance of Protective Clothing Materials to Penetration by Blood and Body Fluids—Test Method Using Synthetic Blood*, blood test soil[7] was used in both the ASTM F903 and dot-blot apparatuses. This blood test soil was chosen, as the chemicals needed to make the test soil were easily commercially available (**Table 1**). Because the surface tension of synthetic blood is inconsistent with time and handling,[20] the surface tension measurements of the blood test soil (**Table 2**) should not be interpreted as exact values.

TABLE 1
Composition of test soils used in this study

Test Soils
Blood test soil[7]:
In 500 mL DD water, add slowly while stirring, 2 g carboxymethylcellulose.
Add 1.2 g KH_2PO_4 and 4.3 g Na_2HPO_4,
4 mL of 1 % Tween 20 solution (in DD water),
1 g Amaranth,
2.4 g NaCl.
Bring to 1 L using DD water.
Labeled protein test soil:
Made exactly the same as the blood test soil, but no Amaranth is added—the dye would interfere with visualizing the HRP substrate.
Prior to using this test soil, add 1:10,000 dilution of a commercially available secondary antibody, GaR IgG-HRP.
Vomit test soil:
0.4 M glycine,
0.04 g bile salt,
1 g mucin (not 100 % soluble, must mix well to make suspension before use).
Bring to about 95 mL with DD water. Stir overnight.
The next day, add 3–4 mL of concentrated HCl (about 12 M) to bring to pH 1.5.
Bring to 100 mL.

Note: DD = distilled, deionized.

TABLE 2
Test soil properties

Test Soil	pH	Conductivity	Viscosity, Pa/s	Surface Tension, dyne/cm
Synthetic Blood	7.32	10.89	2.54–2.55	62.2
Vomit	1.49	49.38	1.92–2.05	…

Note: The surface tension was measured using the capillary method immediately after mixing the synthetic blood or vomit test soil. Surface tension was not measured for vomit, as it is not a true solution.

Subset of Blood

A labeled protein, GaR IgG-HRP (Sigma-Aldrich 1:10,000) was used as an alternate to the amaranth dye in the ISO 16603 blood test soil in the modified dot-blot apparatus. The nitrocellulose membrane, used in many applications, as it traps proteins, was used to retain the GaR IgG-HRP protein (fig. 2). This protein was visualized with a one-component 3,3′,5,5′-tetramethylbenzidine membrane peroxidase substrate, which reacts with the HRP on the GaR IgG protein to create a colored precipitate, from Kirkegaard & Perry Laboratories (Gaithersburg, MD), now SeraCare (Milford, MA). Some initial work was performed in attempting to use blotting paper and nitrocellulose in the ASTM F903 apparatus, but because of expense and the bulging of some fabrics with no screen tore the nitrocellulose, this approach was abandoned.

CHARACTERIZATION OF THE TEST SOLUTIONS

Conductivity and pH

The conductivity and pH of test solutions were measured with a Mettler Toledo Seven Compact (Columbus, OH) and Hanna Instruments HI 8519N (Woonsocket, RI) meter, respectively.

Viscosity

Viscosities of the test solutions were measured at 25°C under 0.1/s to 100/s shear rate using a standard cup and din rotor system in a rheometer (model AR G2, TA Instruments, New Castle, DE).

Surface Tension

Surface tension was measured using both a capillary surface tension tensiometer (Cole-Parmer, Vernon Hills, IL) and a Du Nouy precision tensiometer (CSC Scientific Co., Fairfax, VA), used as per manufacturer instructions. Because of variable response over time with the Du Nouy tensiometer, only surface tension as measured by the capillary tensiometer is reported (Table 2).

The composition and methods to make the test soils used in this study are summarized in Table 1. Test soil pH, conductivity, surface tension, and viscosity are listed in Table 2.

STATISTICAL METHODS

Goal 1: ASTM F903 to Dot-Blot Comparison

Our first consideration was to account for the difference in area between the dot-blot and ASTM F903 apparatuses. If the fabric were perfectly uniform, a large-area test may provide the same results as a small-area test; however, fabric imperfections that manifest over a large area may not be found within a single dot-blot cell. As the diameter of the ASTM F903 apparatus (57.3 mm) is larger than a dot-blot cell (5 mm), we calculated the number of dot-blot cells having the same area as the ASTM penetration cell: π (28.65 mm)^2 / π (2.5 mm)^2 = 131 cells. ASTM F903 defines a test specimen failure as any observed penetration, regardless of quantity or intensity. To compare to the dot-blot apparatus, when considering a group of 131 cells, any observed penetration in one or more cells was counted as a singular specimen failure.

To compare statistical differences, we chose a nonparametric, two-proportion z-test. An initial specimen size of $n = 5$ was used to screen for penetrable and non-penetrable fabrics. Considering the two-proportion z-test, if 5/5 passed apparatus "a" and 5/5 passed apparatus "b," to show a significant difference between apparatuses at

$p = 0.05$, the next four tests would need to all pass apparatus "a" while four consecutive tests fail in apparatus "b." We consider this scenario sufficiently improbable; therefore, in lieu of conducting nine tests on each apparatus, if all five passed (or all five failed), the apparatuses were not significantly different.

If the first five tests had a mixture of results, the specimen size was increased to $n = 25$. This specimen size is large enough to detect a moderate difference in proportions as significant. Given that small differences are not scientifically meaningful, we deem this specimen size as appropriate, with consideration of the time and expense of the analysis.

Goal 2: Screen Effect

The ASTM F903 test method specifies that if a fabric stretches more than 5 mm during the test, a screen may be used to limit distention of the specimen. Limiting fabric stretching can improve the performance. Because the modified dot-blot apparatus is comprised of many small diameter tests with O-rings that press into the fabric, which is supported by blotting paper, a screen is not used, as there is no distension. Because of these spatial differences, we hypothesize that if the ASTM F903 and modified dot-blot apparatuses obtain different results, the difference could be mitigated by the use of a screen. Fabrics in which the test method obtained statistically different results using ASTM F903 without a screen compared with the modified dot-blot method were then evaluated with a two-proportion z-test to compare the ASTM F903 test with a screen against the dot-blot apparatus.

Goals 3 and 4: Labeled Protein (GaR IgG-HRP) and Vomit Test Soil versus Blood

The dot-blot apparatus was selected for these comparisons because it requires a smaller volume of test fluid and provides a greater number of specimens to calculate a z-score. A specimen size of seven tests (each having 96 cells) was selected for an $n = 672$. For the synthetic blood soil, 34 tests had been run for some of the fabrics. The first 7 of the 34 tests were selected for statistical analysis.

Results

APPARATUS EVALUATION

Goals 1 and 2: Comparison of ASTM and Dot-Blot Apparatuses

Table 3 details a comparison of the ASTM apparatus with the dot-blot using the ISO 10663 blood test soil at 2 psi. P (Pass) indicates that no visual dye penetration was observed and F (Fail) indicates there was visual dye penetration. Only gown #3 showed a significant difference ($p < 0.05$) between the ASTM and the dot-blot apparatus. Gowns #2 and #4 did not show significant difference ($p < 0.05$) between the ASTM and dot-blot apparatuses.

Because the type of apparatus used (i.e., ASTM or dot-blot) was different for gown #3, that gown was evaluated with the ASTM apparatus using a screen. For gown #3, 96 % of the specimens (33/34 or 24/25) passed using the dot-blot apparatus, whereas 60 % (15/25) passed using the ASTM apparatus with a screen, and only 28 % (7/25) passed using the ASTM apparatus without a screen. Although less than the dot-blot and ASTM without a screen, the difference between the dot-blot and ASTM with screen was still significant ($p < 0.05$), with a z-score of 3.0725 and p-value of 0.00214.

Table 4 shows the results of PPE testing using the dot-blot apparatus for the International Standards Organization (ISO)[7] blood test soil with dye, ISO blood test soil without dye but with the labeled protein (GaR IgG-HRP), and vomit test soils. Specimens of all fabric were run with seven 96-well plates at 2 psi except fabrics specimens 2, 3, and 4, which were run with 34 of the 96-well plates for the ISO blood soil (only the first seven of these runs were used for this comparison). The results of the different test soils used with the dot-blot apparatus were converted to the number of individual wells that passed the test soil challenge (7 tests × 96 wells per test =672 wells total). Statistical comparison of the different test soils for fabrics 1–4, 6, and 7 are delineated on the right. These data indicate that vomit test soil generates more failures than blood alone and that the labeled

TABLE 3

Comparison of the ASTM apparatus with the dot-blot using the ISO 10663 blood test soil at 2 psi for a series of gowns and drapes

Fabric	Level	ASTM No Screen	ASTM Screen	Dot-Blot		No Screen versus Screen ($p \leq 0.05$)	No Screen versus Dot-Blot[a] ($p \leq 0.05$)	Screen versus Dot-Blot[a] ($p \leq 0.05$)
Gown 1	4	5/5	5/5	7/7	(5/5)[a]	…	…	…
Gown 2	3	1/25	0/5	10/34	(5/25)[a]	…	…	…
Gown 3	4	7/25	13/25	33/34	(24/25)[a]	…	yes	yes
Gown 4	3	24/25	5/5	33/34	(24/25)[a]	…	…	…
Gown 5	2	0/5	0/5	0/7	(0/5)[a]	…	…	…
Gown 6	1	0/5	0/5	0/7	(0/5)[a]	…	…	…
Gown 7	N/A	0/5	0/5	0/7	(0/5)[a]	…	…	…
Gown 8	4	5/5	5/5	7/7	(5/5)[a]	…	…	…
Gown 9	4	5/5	5/5	7/7	(5/5)[a]	…	…	…
Drape 1	N/A	5/5	5/5	7/7	(5/5)[a]	…	…	…
Drape 2	N/A	5/5	5/5	7/7	(5/5)[a]	…	…	…

Note: PB70 levels for the materials are included, unless there was no indication of level (N/A). In cases in which there were mixed results (i.e., not all P or F for specimens 2, 3, and 4), a higher number of specimens was run to provide statistical significance. Different numbers of specimens were run for the ASTM and the dot-blot tests so that the total surface area tested was equivalent. [a] Results reported in number of dot-blot actual tests $n/7$ and converted to equivalencies with comparable area ($n/5$) to facilitate statistical comparison with ASTM data.

TABLE 4

Results of PPE testing using the dot-blot apparatus for blood test soil with dye, blood test soils without dye but with the labeled protein (GaR IgG-HRP), and vomit test soils

Fabric	Level	Blood	Labeled Protein	Vomit	Blood versus Labeled Protein ($p \leq 0.05$)	Blood versus Vomit ($p \leq 0.05$)	Labeled Protein versus Vomit ($p \leq 0.05$)
Gown 1	4	672/672	672/672	672/672	…	…	…
Gown 2	3	668/672	558/672	316/672	yes	yes	yes
Gown 3	4	671/672	669/672	665/672	…	yes	…
Gown 4	3	671/672	670/672	441/672	…	yes	yes
Gown 5	2	0/672	0/672	0/672	…	…	…
Gown 6	1	171/672	0/672	0/672	yes	yes	…
Gown 7	N/A	3/672	0/672	1/672	…	…	…
Gown 8	4	672/672	672/672	672/672	…	…	…
Gown 9	4	672/672	672/672	672/672	…	…	…
Drape 1	N/A	672/672	672/672	672/672	…	…	…
Drape 2	N/A	672/672	672/672	672/672	…	…	…

Note: All specimens were run, $n = 7$, at 2 psi except ISO blood for specimens 2, 3, and 4; $n = 34$ (Table 2). The results of the different test soils used with the dot-blot apparatus were converted to the number of individual wells that passed the test soil challenge (7 tests × 96 wells per test = 672 wells total). Statistical comparison of the different test soils for fabrics 1–4, 6, and 7 are delineated on the right.

protein (GaR IgG-HRP) can be a more sensitive indicator of material penetration than the dye in the blood test soil.

Discussion

A dot-blot is a technique initially used in molecular biology to detect proteins; it is a simplified version of western blotting. In addition to proteins, the dot-blot apparatus has been used to detect a variety of different compounds of interest, including viruses. The test apparatus is relatively simple and easy to use. There is a bottom plate, the

filtration plate, with holes that match the 96 wells of the sample well plate (fig. 2). The holes on the filtration plate are smaller than the sample well plate. Blotting paper is place on the lower filtration plate, and the PPE material is placed on the blotting paper. The apparatus is then clamped together, so that there are 96 wells with the PPE material clamped at the bottom of the well. Now, test solutions such as synthetic blood or vomit can be placed in each well, and the ability of the PPE material to resist penetration of the test solution can be evaluated. In the case in which we wish to trap proteins, such as the GaR-HRP, a sheet of nitrocellulose is placed between the blotting paper and the PPE. Any proteins (in this case, GaR-HRP) passing through the PPE material are trapped on the nitrocellulose membrane. In the absence of airflow within the dot-blot apparatus, the pressure is hydrostatic (i.e., equal in all wells, regardless of well size or position). As ASTM F1670 states, pressure was added slowly to the system[3]; the application of a vacuum to the modified dot-blot mirrored this requirement.

This initial study comparing the ASTM F903 test apparatus with a modified dot-blot apparatus (Goal 1) demonstrated that there is statistically no difference between the two test setups at 2 psi when using a blood test soil for all but one (gown #3) of the 13 tested PPE fabrics specimens. Use of a screen did not change the result for gown #3, with the dot-blot significantly different from the ASTM apparatus with the screen, also. Notably, the use of a screen for this particular gown resulted in a doubling of the number of specimens that passed the penetration test.

There is a higher percentage of failures with the ASTM F903 test apparatus than with the modified dot-blot. This could be due, at least in part, to the vertical orientation of the ASTM F903 test apparatus, in which gravitational force adds 0.1 psi of pressure to the bottom of the test specimen. An additional problem with the ASTM F903 test apparatus was the tendency of soil to penetrate at the gasket, both with and without the presence of a screen. More penetration was seen at the gasket when certain types of screen were used; thus, the failure may not be due to the material but the failure of the gasket to appropriately seal the test specimen.

In contrast, the modified dot-blot test apparatus has some significant advantages. The problems with gasket and edge leakage were not seen with the dot-blot. Previous work by Nandy et al. clearly demonstrated that visual dye alone is often not as sensitive as other detection methods, so a method that also incorporates the identification of a penetrating microorganism is highly desirable.[21] The dot-blot method uses smaller amounts of liquid, which is a great advantage when microorganisms (bacteriophage) are used in the test system, as growing large volumes of microorganisms can be very labor and time intensive. In addition, nitrocellulose can be easily incorporated into this test system, allowing for proteins and viruses to be trapped and then antibodies to be used in the detection step. The utility of having one test for drapes using a blood test soil with dye (F1670) and a separate test for gowns using a bacteriophage (F1671) needs to be reevaluated. Work addressing this issue is ongoing. It may be possible to develop one optimized method that may be used for both applications. Furthermore, both the effort and time required to use an antibody to detect the presence of a bacteriophage or viral surrogate are substantially less than what is required to plate the bacteriophage on a bacterial lawn and wait days for plaques to form. In future studies, the use of a labeled protein (GaR IgG-HRP), a fluorescent chemical, or particle may prove to be a more reliable means of detecting penetration of PPE material than either dye or viruses.

This study did have its limitations. Initial runs with a simple screen and gaskets leaked; however, when using the gaskets and screens designed by NIOSH, most of this type of problem was alleviated. Most of the work in this study had been completed prior to receiving the new screens and gaskets designed by NIOSH. Although the use of a screen for gown #3 resulted in a doubling of the number of specimens that passed the penetration test, more testing would be helpful to compare different fabrics and different screen types. Finally, to validate the dot-blot apparatus, a carefully constructed round-robin with several participants would be needed.

The test setups used here provide information on the penetration of test solutions on various PPE materials; however, the question of how well these materials may perform in the field needs to be addressed. In addition to saturation level,[22] application of realistic test soils, such as vomit, may alter the ability of viruses to penetrate PPE materials. Adding realistic pressures or stresses to the PPE material while testing for penetration is another area that needs to be investigated.[23] The work described here provides information that can be used in the development of new and revised test methods for determining viral penetration of PPE material. These methods will

provide manufacturers with standard, well-defined means of assessing PPE material penetration and assist the Food and Drug Administration (FDA) in evaluating claims for gowns and materials intended to protect against deadly viruses, enabling more straightforward comparisons and results for different gowns or other PPE, thereby reducing the time to review a new application, and providing health-care workers and the public access to new devices more quickly.

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