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Minimized Virus Binding for Tests of Barrier Materials

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Viruses are used to test the barrier properties of materials. Binding of virus particles during passage through holes in the material may yield misleading test results. The choices of challenge virus and suspending medium may be important for minimizing confounding effects that might arise from such binding. In this study, different surrogate viruses, as well as different support media, were evaluated to determine optimal test parameters. Two membranes with high-binding properties (nitrocellulose and cationic polysulfone) were used as filters to compare binding activities of different surrogate challenge viruses (MS2, φX174, T7, PRD1, and φ6) in different media. The media consisted of buffered saline with surfactants, serum, or culture broth as additives. In addition, elution rates of viruses that bound to the membranes were determined. The results suggest that viruses can bind by hydrophobic and electrostatic interactions, with φX174 displaying the lowest level of binding by either process. The nonionic detergents Triton X-100 and Tween 80 (0.1%) equally minimized hydrophobic interactions. Neither anionic nor cationic surfactants were as effective at nontoxic levels. Serum was effective at reducing both hydrophobic and electrostatic binding, with 2% being sufficient for eliminating binding under our test conditions. Thus, φX174 remains the best choice as a surrogate virus to test barrier materials, and Triton X-100 (0.1%) remains a good choice for reducing hydrophobic binding. In addition, binding of viruses by barrier materials is unlikely to prevent passage of blood-borne pathogens.

Viruses are being used as challenge particles to evaluate various barrier materials and barrier products (see reference 10 for additional references for testing condoms; see reference 9 for references for testing gloves). Various viruses, usually bacteriophages, often serve as surrogates for human pathogens. To date, the rationale that has guided the choice of surrogate virus has emphasized virus shape (approximately spherical) and size (small), without regard to the physicochemical properties of the virus particle. This simplistic rationale has apparently served well; however, care must be taken to avoid its shortcomings. For example, virus size may not be important in some instances, whereas binding of virus particles to the test material may be (12).

In the test protocol developed in our laboratory (10), the Poiseuille equation for fluid flow through a cylindrical hole indicates that only 1 nl (10^{-6} ml) of challenge virus suspension can penetrate a single hole with a diameter of 0.6 μm in a latex condom in 30 min. Thus, since spherical virus particles are substantially smaller in diameter than 0.6 μm, it is fluid flow and not virus size that is the limiting factor for virus penetration through a single hole.

On the other hand, virus particles typically are electrically charged. Most viruses have acidic isoelectric points and are therefore negatively charged at neutral pH (3). In addition, virus binding through hydrophobic interactions can occur with certain materials (14, 16). Thus, there are possibilities that the particles will adsorb through either or both of two basic mechanisms, electrostatic and hydrophobic, to the latex during passage through the hole. Furthermore, the chemical components of the suspending solution can also affect virus adsorption (6, 7, 15). The result might be that the test procedure does not detect a hole that could be important in real life.

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One can determine through control experiments whether loss of virus through adsorption occurs during exposure of the virus suspension to the surfaces of the test material (latex in this example) or to other components of the test apparatus (10). However, it is not practical to investigate virus adsorption resulting from the close proximity inherent during passage through small, natural, rare, randomly occurring defects in the material.

Appropriate choices of surrogate virus and, perhaps, other test conditions could minimize the occurrence and impact of virus binding during the test procedures. The goals of this study were (i) to identify which potential surrogate virus adsorbs least in saline to binding membranes and (ii) to identify conditions that minimize virus adsorption, including additions of surfactants, serum, or culture broth. The choice of additives was governed by the use of surfactants or culture broth in established tests of barrier materials (2, 10, 13) and the likely presence of serum or some other body fluid during real-life use of a barrier material (e.g., serum during use of a medical glove or serum and semen during use of a condom). Virus adsorption was determined using binding membranes as filters in a filtration protocol previously used with sizing filters (11). The results indicate that one virus has particularly low adsorption characteristics and that the presence of a surfactant may be sufficient to minimize virus adsorption.

MATERIALS AND METHODS

Viruses. The bacteriophages evaluated in this study were MS2, φX174, T7 am28, PRD1, and φ6. Two of these viruses have lipid components; PRD1 has an internal membrane layer, and φ6 has an external membrane envelope. The host bacterium for MS2 was *Escherichia coli* C-3000 (American Type Culture Collection no. 15597); descriptions of the host bacteria for the other bacteriophage and the methods of preparation and assaying of all bacteriophage have been published elsewhere (8). For most experiments, a virus cocktail containing approximately equal concentrations (1×10^3 to 3×10^3 PFU/ml) of four or five bacteriophages in Dulbecco's phosphate-buffered saline (DPBS) was used (12). The virus cocktail was used to provide direct comparative data among the different viruses for the different membrane binding conditions.

Binding membranes. BioTrace NT (Gelman Sciences, Ann Arbor, Mich.) is a nitrocellulose general-purpose binding membrane with an unspecified pore size.

TABLE 1. Relative plaque-forming abilities of viruses after exposure to surfactants for 20 min at room temperature[a]

Additive and concn	Value for the following viruses:				
	MS2	φX174	T7	PRD1	φ6
DPBS	1.00	1.00	1.00	1.00	1.00
Triton X-100 (nonionic)					
0.001%	NT[b]	NT	NT	NT	<0.002
0.01%	1.04 ± 0.10	0.81 ± 0.01	0.91 ± 0.09	1.03 ± 0.21	<0.002
0.1%	1.00 ± 0.20	0.93 ± 0.01	1.05 ± 0.04	1.12 ± 0.18	<0.002
Tween 80 (nonionic)					
0.001%	1.27 ± 0.04	1.03 ± 0.02	1.21 ± 0.03	1.12 ± 0.001	1.03 ± 0.05
0.01%	1.12 ± 0.06	0.96 ± 0.16	1.27 ± 0.08	1.11 ± 0.11	1.01 ± 0.01
0.1%	1.33 ± 0.17	0.91 ± 0.08	1.26 ± 0.07	1.08 ± 0.14	0.92 ± 0.02
SDS (anionic)					
0.1 mM	0.94 ± 0.36	1.09 ± 0.04	0.99 ± 0.02	1.03 ± 0.10	0.63 ± 0.05
1.0 mM	0.94 ± 0.14	1.01 ± 0.16	0.63 ± 0.02	1.20 ± 0.07	<0.002
10 mM	0.88 ± 0.14	1.09 ± 0.13	1.14 ± 0.06	<0.002	<0.002
FC-129 (anionic)					
0.01%	1.30 ± 0.05	1.17 ± 0.27	1.06 ± 0.02	1.18 ± 0.01	<0.001
0.1%	1.53 ± 0.09	1.07 ± 0.04	0.99 ± 0.03	1.07 ± 0.10	<0.001
FC-135 (cationic)					
0.001%	0.90 ± 0.04	1.13 ± 0.13	0.82 ± 0.02	0.96 ± 0.02	0.22 ± 0.10
0.01%	0.04 ± 0.03	0.74 ± 0.37	0.04 ± 0.00	0.96 ± 0.04	<0.002
0.1%	.02 ± .01	<0.003	<0.002	0.03 ± 0.02	<0.002
Fetal bovine serum					
1%	0.84 ± 0.16	NT	NT	1.00 ± 0.08	NT
2%	0.57 ± 0.07	NT	NT	0.97 ± 0.15	NT
5%	0.61 ± 0.07	NT	NT	1.02 ± 0.04	1.03 ± 0.05

[a] Relative to plaque-forming ability for exposure only to DPBS. This includes effects of virus inactivation and decrease of plaque formation by other interference-inhibitions. To results of two to three experiments are presented as mean values (± standard errors).
[b] NT, not tested.

BioTrace HP (Gelman Sciences) is a cationic polysulfone-based membrane with a quoted pore size of 0.45 μm. It is claimed (Gelman Sciences) that both membranes possess excellent hydrophobic binding properties and that, in addition, BioTrace HP is positively charged. Circular pieces (25-mm diameter) were used as filters in the procedure reported previously (11).

Additives to virus-suspending solution. Several surfactants were evaluated: Triton X-100, Tween 80, sodium dodecyl sulfate (SDS), FC-129, and FC-135. Triton X-100 and Tween 80 (Sigma Chemical Co., St. Louis, Mo.) are nonionic (polar) biological detergents, and SDS (Sigma) is an anionic biological detergent. All three are commonly used in biochemical research. The anionic FC-129 and the cationic FC-135 are Fluorad fluorochemical surfactants (3M, St. Paul, Minn.), which were generously provided by S. M. Retta, Center for Devices and Radiological Health, Food and Drug Administration. Triton X-100 has been used as a surfactant in a test of condoms as virus barriers (10), as have Tween 80 in the American Society for Testing and Materials test of surgical gown material as virus barriers (2) and FC-129 in the test of condoms as barriers to microspheres of the size of human immunodeficiency virus (13).

Fetal calf serum (Gibco, Grand Island, N.Y.) and a bacteriological culture medium, LC broth (which contains tryptone, yeast extract, sodium chloride, and water), were used as sources of common, important biochemicals (e.g., proteins). Serum was selected as a biological fluid of great concern for carrying viruses through a barrier material. While seminal fluid is important for condom use, the ready availability of serum made it the biological fluid of choice. While seminal fluid has greater viscosity than serum and the two fluids have similar pHs, serum has somewhat higher levels of protein and lipid to compete with the virus particles for binding sites (4, 5). LC broth was used to represent the effect of contaminating medium in the virus cocktail and because a culture medium, nutrient broth, is used as the fluid in the American Society for Testing and Materials test (2).

Procedure for passing virus through binding membranes. The procedure developed earlier to determine the filtration sizes of various viruses was used (11). Briefly, a 25-mm-diameter piece of the membrane was used as a filter in a holder connected to a syringe containing 3 ml of a virus suspension in DPBS with a selected additive. The filtration rate was controlled by atmospheric pressure by attaching a hypodermic needle to the downstream side of the filter holder and pushing the needle into a vacutainer. The filtration rates were different for the two types of membranes but were similar for the different solutions with the same membrane. Virus titers were determined before and after filtration, and the fraction of original virus in the filtrate was calculated.

Procedure for eluting virus from binding membranes. Initially, the viruses in DPBS were bound to the membranes by the procedure described above. After the filtration procedure, the filter was rinsed quickly by being dipped in 3 ml of DPBS and drained onto sterile absorbent paper to remove any unbound virus and was then submerged in 3 ml of DPBS with a selected additive for 1 h at room temperature to elute the bound virus. After removal of the membrane, the eluate was assayed to determine the fraction of bound virus that had been recovered.

RESULTS

Limits of useful concentrations of additives. Preliminary results indicated that some of the surfactants at high enough concentrations were detrimental to one or more of the viruses. Therefore, the survival of the bacteriophages in DPBS was determined after exposure to different concentrations of each of the additives (Table 1). As expected, the enveloped φ6 was most sensitive to the surfactants. The results demonstrated that for concentrations of 0.1% or less, Tween 80 was not toxic to any of the five bacteriophages, Triton X-100 and FC-129 were toxic only to φ6, SDS was toxic to PRD1 at a concentration of 10 mM (0.1%) and to φ6 at concentrations of 1.0 and 10 mM (0.01 and 0.1%), and FC-135 was toxic to three or more bacteriophages at concentrations of 0.01% and higher. It was also found that the presence of serum slightly but consistently reduced plaque formation by exposed MS2 for reasons unknown. Of course, the LC broth in which the bacteriophages were grown is not toxic. Finally, the plaque-forming ability of MS2 increased somewhat (>20%) after exposure to Tween 80

TABLE 2. Passage of viruses in DPBS with different additives through BioTrace NT binding membrane during filtration[a]

Additive and concn	Fraction of original virus in filtrate				
	MS2	φX174	T7	PRD1	φ6
None	<0.001	<0.002	<0.001	<0.002	<0.001
Triton X-100 (nonionic)					
0.001%	<0.002	<0.002	<0.001	<0.002	—[b]
0.01%	<0.002	<0.002	<0.001	<0.002	—
0.1%	0.87 ± 0.08	0.88 ± 0.05	0.96 ± 0.03	0.91 ± 0.05	—
Tween 80 (nonionic)					
0.001%	0.003 ± 0.001	<0.003	0.003 ± 0.001	0.004 ± 0.001	<0.001
0.01%	0.004 ± 0.002	0.005 ± 0.002	0.006 ± 0.004	0.011 ± 0.008	<0.001
0.1%	0.85 ± 0.13	1.16 ± 0.09	0.95 ± 0.07	1.08 ± 0.06	0.58 ± 0.02
SDS (anionic)					
0.1 mM	<0.002	<0.003	<0.002	<0.002	<0.003
1.0 mM	<0.002	<0.003	<0.003	<0.002	—
10 mM	0.71 ± 0.20	0.95 ± 0.03	0.005 ± 0.003	—	—
FC-129 (anionic)					
0.01%	<0.002	<0.004	<0.001	<0.003	—
0.1%	<0.001	<0.002	<0.002	<0.002	—
FC-135 (cationic)					
0.001%	<0.004	<0.002	<0.002	<0.002	<0.004
0.01%	<0.05	<0.003	<0.05	<0.001	—
0.1%	<0.1	—	—	—	—
Fetal bovine serum					
1%	0.50 ± 0.05	0.61 ± 0.07	0.64 ± 0.10	0.64 ± 0.14	0.67 ± 0.18
2%	0.84 ± 0.07	0.87 ± 0.07	0.89 ± 0.07	0.78 ± 0.05	0.96 ± 0.19
5%	0.72 ± 0.21	0.81 ± 0.16	0.95 ± 0.02	0.88 ± 0.12	1.01 ± 0.18
LC broth[c]					
10%	0.02 ± 0.01	0.003 ± 0.001	0.007 ± 0.003	0.02 ± 0.01	0.008 ± 0.003
33%	0.03 ± 0.01	0.10 ± 0.03	0.015 ± 0.007	0.01 ± 0.01	0.006 ± 0.001
100%	0.58 ± 0.09	0.88 ± 0.11	0.013 ± 0.005	0.01 ± 0.01	0.008 ± 0.003

[a] Results of two to three experiments presented as average values (± standard errors).
[b] —, toxicity of surfactant to virus prevented experiment.
[c] DPBS was replaced by LC broth by the percentages indicated (LC broth is 1.0% tryptone, 0.5% yeast extract, and 0.5% NaCl [wt/vol] in water).

or FC-129, with a similar increase for T7 after exposure to Tween 80. These increases may be evidence that there was some aggregation in the MS2 and T7 stocks.

Binding to nitrocellulose membrane. With the filtration procedure used in this study, all five bacteriophages were prevented from passing through the NT membrane when the viruses were in DPBS alone (Table 2). On the other hand, the presence of either of the nonionic surfactants, Triton X-100 or Tween 80, at a concentration of 0.1% was sufficient to allow essentially all of the four nonenveloped viruses to pass through the membrane; more than half the φ6 passed through in the presence of 0.1% Tween 80. In addition, 10 mM SDS allowed MS2 and φX174 to pass through the NT membrane. The other, charged surfactants did not aid virus passage at nontoxic levels.

Serum at 1% helped prevent virus binding and at 2% gave nearly total virus passage. Even at 100%, LC broth was much less effective and gave essentially complete passage only for φX174 and little passage for T7, PRD1, and φ6.

The combined data demonstrate that in the presence of Triton X-100, Tween 80, or serum, the four nonenveloped viruses were roughly equal in being prevented from binding to the NT membrane, the exception being φ6 with Tween 80. On the other hand, with LC broth as the additive, φX174 demonstrated the greatest reduction of binding, which was followed by MS2 and then by T7, PRD1, and φ6, all three of which were approximately equal.

Further, the passages of the larger viruses, T7, PRD1, and φ6, in the presence of certain surfactants or serum indicate that the effective pore size of the NT membrane is greater than the diameters of these viruses (65 to 80 nm).

Binding to cationic polysulfone-based membrane. For the cationic polysulfone-based membrane, when the viruses were in DPBS alone, φX174 was able to pass through the HP membrane at a relatively high level (Table 3). In the presence of the nonionic surfactants, 0.01 and 0.1% Triton X-100 or Tween 80, passage of all of the viruses demonstrated increased passage through the membrane; however, only φX174 and T7 neared total passage. It should also be noted that the passages of MS2 and T7 (and perhaps even of PRD1 and φ6) were not substantially increased when the concentration of Triton X-100 or Tween 80 was increased 10-fold from 0.01 to 0.1%.

The anionic surfactant SDS at a concentration of 10 mM increased the passage of the three viruses for which it was not toxic, MS2, φX174, and T7; a lower concentration, 1 mM, only increased φX174 passage. The other anionic surfactant, FC-129, did not increase passage of any virus at 0.01 or 0.1%. The cationic surfactant, FC-135, only increased passage of φX174 at nontoxic concentrations.

TABLE 3. Passage of viruses in DPBS with different additives through BioTrace HP binding membrane during filtration[a]

Additive and concn	Fraction of original virus in filtrate				
	MS2	ϕX174	T7	PRD1	ϕ6
None	0.003 ± 0.001	0.46 ± 0.08	0.013 ± 0.006	0.002 ± 0.001	<0.001
Triton X-100 (nonionic)					
0.001%	0.07 ± 0.03	0.94 ± 0.01	0.34 ± 0.07	0.004 ± 0.002	—[b]
0.01%	0.16 ± 0.04	1.07 ± 0.05	0.78 ± 0.02	0.083 ± 0.007	—
0.1%	0.19 ± 0.05	1.03 ± 0.09	0.77 ± 0.06	0.134 ± 0.013	—
Tween 80 (nonionic)					
0.001%	0.008 ± 0.005	0.93 ± 0.03	0.094 ± 0.021	0.005 ± 0.002	<0.001
0.01%	0.049 ± 0.022	1.12 ± 0.10	0.70 ± 0.08	0.039 ± 0.020	0.17 ± 0.09
0.1%	0.060 ± 0.018	1.01 ± 0.04	0.68 ± 0.09	0.060 ± 0.025	0.35 ± 0.13
SDS (anionic)					
0.1 mM	<0.002	0.42 ± 0.10	0.002 ± 0.001	<0.002	<0.002
1.0 mM	<0.002	0.79 ± 0.01	0.005 ± 0.002	<0.002	—
10 mM	0.99 ± 0.08	0.85 ± 0.01	0.95 ± 0.10	—	—
FC-129 (anionic)					
0.01%	<0.002	0.25 ± 0.12	<0.001	<0.003	—
0.1%	<0.002	0.45 ± 0.06	<0.001	<0.003	—
FC-135 (cationic)					
0.001%	<0.004	0.66 ± 0.14	<0.002	<0.002	<0.004
0.01%	<0.05	0.93 ± 0.09	<0.05	<0.001	—
0.1%	—	—	—	—	—
Fetal bovine serum					
1%	0.50 ± 0.05	1.01 ± 0.08	1.10 ± 0.13	0.59 ± 0.10	1.10 ± 0.05
2%	0.89 ± 0.16	1.14 ± 0.08	1.09 ± 0.10	0.95 ± 0.15	1.06 ± 0.07
5%	0.69 ± 0.16	1.09 ± 0.06	1.13 ± 0.09	1.12 ± 0.18	1.23 ± 0.02
LC broth[c]					
10%	0.15 ± 0.07	0.99 ± 0.15	0.46 ± 0.06	0.04 ± 0.03	0.62 ± 0.12
33%	0.39 ± 0.15	0.93 ± 0.12	0.73 ± 0.07	0.16 ± 0.01	0.89 ± 0.02
100%	0.82 ± 0.30	1.09 ± 0.003	0.76 ± 0.04	0.52 ± 0.01	0.84 ± 0.03

[a] Results of two to three experiments presented as average values (± standard errors).
[b] —, toxicity of surfactant to virus prevented experiment.
[c] DPBS was replaced by LC broth by the percentages indicated (LC broth is 1.0% tryptone, 0.5% yeast extract, and 0.5% NaCl [wt/vol] in water).

As in the case of NT membranes, serum at 1% helped prevent virus binding to HP membranes and at 2% gave nearly total virus passage. Although LC broth was more effective at allowing passage of virus through HP membranes than through NT membranes, even at 100% it was not completely effective for three of the viruses.

The combined data in this case show that ϕX174 demonstrated the least binding to the HP membrane, which was followed by T7 and then by MS2 and PRD1, both of which were approximately equal in the presence or absence of Triton X-100, Tween 80, or LC broth. In many but not all instances, ϕ6 bound less efficiently than MS2 or PRD1.

It is conceivable that the presence of divalent cations in the DPBS might affect the binding of viruses to the anionic HP membrane. Thus, passage of the viruses was ascertained using the original formulation of DPBS (with divalent cations, Ca^{2+} at 0.78 mM and Mg^{2+} at 0.49 mM) and the formulation without them. The absence of Ca^{2+} and Mg^{2+} ions from DPBS had no effect on the passage of the nonenveloped viruses, whether in the presence or absence of 0.1% Triton X-100 (data not shown).

Lack of ϕX174 binding in the presence of Triton X-100. The data presented in Tables 2 and 3 indicate that, with the techniques used in this study, one surrogate virus, ϕX174, did not demonstrate detectable binding to either membrane when 0.1% Triton X-100 was present. However, by prolonging the duration of passage of the fluid through the membrane and thereby allowing increased contact of the viruses with the membrane fibers, the extent of binding might be increased. By repeating the experiments described above using slow, hand-controlled pressure on the syringe during the filtration procedure instead of the much faster atmospheric pressure with the vacutainer, more opportunity for ϕX174 to bind to either of the filters was allowed. The amounts of virus passage under these conditions for three viruses are listed in Table 4. The data with the nitrocellulose (BioTrace NT) membrane indicate that the lower rate of fluid passage did not result in significant binding for any virus. On the other hand, the data with the cationic polysulfone (BioTrace HP) membrane indicate that the lower rate resulted in increased binding for T7 and PRD1 but in no detectable binding for ϕX174.

Elution of viruses bound to membranes. In addition to preventing binding of viruses to surfaces, the same additives may be able to elute some of the viruses which have already bound to those surfaces, although less elution would be expected from the inner structures of the filamentous membranes used in this study. An elution time of 1 h was chosen as a compromise, since it was a representative time averaging different real-life

TABLE 4. Passage of viruses in DPBS with 0.1% Triton X-100 through binding membranes at different filtration rates[a]

Membrane	Filtration rate (ml/s)	Fraction of original virus in filtrate		
		φX174	T7	PRD1
Nitrocellulose	0.53 ± 0.01[b]	0.85 ± 0.06	0.94 ± 0.01	0.96 ± 0.07
	0.13 ± 0.02[c]	0.89 ± 0.03	0.93 ± 0.03	1.05 ± 0.04
Cationic polysulfone	0.96 ± 0.05[b]	0.95 ± 0.04	0.75 ± 0.04	0.03 ± 0.02
	0.14 ± 0.01[c]	1.07 ± 0.03	0.56 ± 0.03	<0.003

[a] Results of two to three experiments presented as average values (± standard errors).
[b] Obtained with atmospheric pressure (Vacutainer).
[c] Obtained with hand pressure on syringe.

exposures to fluid-borne pathogens and durations of laboratory tests of barrier materials. Triton X-100 was used as the representative nonionic surfactant used in laboratory tests. Serum was used to evaluate the presence of blood.

For viruses that bound to the NT membrane, attempted elution with DPBS did not release significant amounts of any virus (Table 5). However, the presence of 0.1% Triton X-100 or 2 to 5% serum did. The release of the viruses was greatest for φX174, which was followed by T7, MS2, and PRD1, although the differences were often not statistically significant. φ6 was not recovered even with 5% serum.

As with viruses bound to the NT membrane, attempted elution with DPBS of viruses bound to the HP membrane did not release significant amounts of any virus (Table 6). However, the presence of 0.1% Triton X-100 or 2 to 5% serum did elute significant amounts of φX174, and 5% serum also eluted T7. Small amounts of the other viruses were eluted, except for φ6 with 2 to 5% serum and MS2 with 0.1% Triton X-100. Again, the release of the viruses was greatest for φX174, which was followed by T7, PRD1, MS2, and φ6, although the differences were often not statistically significant.

DISCUSSION

Effects of surfactants on virus binding. The nonionic surfactants Triton X-100 and Tween 80 at a concentration 0.1% (but not 0.01%) allowed total passage of the viruses through the nitrocellulose membrane. This indicates that these viruses normally bind to this membrane primarily by hydrophobic interactions, with little, if any, electrostatic interactions.

With the cationic polysulfone membrane, lower concentrations (0.01%, and, for certain viruses, even 0.001%) of the nonionic surfactants allowed increased passage. Furthermore, there were not significant increases when the surfactant concentrations were raised 10-fold from 0.01 to 0.1%. This is interpreted to mean that less nonionic surfactant was required with this membrane to overcome hydrophobic interactions than with the nitrocellulose membrane, i.e., less hydrophobic interaction was occurring. The remaining virus binding at 0.1% nonionic surfactant, therefore, is presumed to be electrostatic in nature. The cationic aspect of the membrane would be expected to attract the negatively charged virus particles. Complete virus passage for MS2 and T7 with 10 mM SDS is presumed to be from a combination of inhibition of the hydrophobic interactions by the hydrophobic part of the detergent and inhibition of the cationic aspect of the membrane by the anionic part of the detergent.

Elution of bound viruses from the nitrocellulose membrane by Triton X-100 further confirmed that at least part of the binding is hydrophobic in nature and indicated that at least part of that binding is reversible. Presumably, the lower levels of elution of MS2, T7, and PRD1 viruses from the other, cationic polysulfone membrane resulted from the lack of effect of the nonionic surfactant on electrostatic binding.

Effects of serum on virus binding. Serum at 5% allowed complete passage by all of the viruses through and partial elution of four of five viruses from both types of membranes, indicating that the presence of serum inhibits both hydrophobic and electrostatic interactions.

Serum can prevent viruses from binding to strongly binding materials. Thus, when a virus is present as a blood-borne pathogen, it is inappropriate to assume that virus binding to a barrier material may be an effective method to prevent virus passage through a hole in the material. Further, it means that it is important to eliminate binding-related false-negative results in laboratory tests of barrier materials.

Effects of culture broth on virus binding. With the nitrocellulose membrane, LC broth allowed complete passage only for φX174, with additional substantial passage for MS2. Thus, some hydrophobic properties were present. With the cationic polysulfone membrane, LC broth aided passage of all viruses. However, the combined data suggest that this culture broth, which contains both tryptone and yeast extract, may not be as useful for preventing virus binding as serum. The data further indicate that low concentrations (<1%) of culture broth together with challenge virus are not likely to confound virus binding studies of the type reported here.

Choice of surrogate virus by binding potential. One of the primary qualities required of a surrogate virus for evaluating barrier materials is low binding potential. φX174 consistently demonstrated the lowest binding potential among the five surrogate viruses, whether in binding to membranes under different conditions or in being eluted from those membranes (com-

TABLE 5. Recovery of viruses from BioTrace NT binding membrane by Triton X-100 or fetal bovine serum[a]

Additive to DPBS and concn (%)[b]	Fraction of bound virus recovered[c]				
	MS2	φX174	T7	PRD1	φ6
None	<0.003	0.003 ± 0.001	<0.005	<0.005	<0.005
Triton X-100 (nonionic)					
0.1	0.23 ± 0.07	0.35 ± 0.09	0.23 ± 0.03	0.11 ± 0.01	—[d]
Fetal bovine serum					
2	0.05 ± 0.02	0.24 ± 0.12	0.16 ± 0.07	0.09 ± 0.00	<0.005
5	0.11 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.07 ± 0.01	<0.005

[a] Viruses were bound to the BioTrace NT binding membrane by filtration as discussed in the text, under conditions in which only DPBS was used as the liquid.
[b] For virus recovery, DPBS was used alone (no additive) or with additive.
[c] Results of three to four experiments presented as average values (± standard errors).
[d] —, toxicity of surfactant to virus prevented experiments.

TABLE 6. Recovery of viruses from BioTrace HP binding membrane by Triton X-100 or fetal bovine serum[a]

Additive to DPBS and concn (%)[b]	Fraction of bound virus recovered[c]				
	MS2	φX174	T7	PRD1	φ6
None	<0.005	<0.002	<0.005	<0.005	<0.005
Triton X-100 (nonionic)					
0.1	<0.005	0.72 ± 0.09	0.04 ± 0.01	0.02 ± 0.01	—[d]
Fetal bovine serum					
2	0.01 ± 0.005	0.35 ± 0.18	0.04 ± 0.01	0.01 ± 0.007	<0.005
5	0.04 ± 0.03	0.40 ± 0.20	0.34 ± 0.05	0.07 ± 0.06	<0.005

[a] Viruses were bound to the BioTrace HP binding membrane by filtration as discussed in the text, under conditions in which only DPBS was used as the liquid.
[b] For virus recovery, DPBS was used alone (no additive) or with additive.
[c] Results of three to four experiments presented as average values (± standard errors).
[d] —, toxicity of surfactant to virus prevented experiments.

pare values in Tables 2 through 6). In particular, complete passage through the cationic polysulfone membrane in the presence of low concentrations (0.001%) of nonionic surfactant (Table 3) or during slow passage in the presence of 0.1% Triton X-100 (Table 4) indicates that it has very little, if any, electrostatic binding. This is consistent with φX174 having little negative charge at neutral pH, as reflected by its pI of 6.6 (1). Thus, φX174 ranks highly as a choice for challenging barrier materials not only because it is small and has a short assay time, but because of its low binding potential. It is less likely to yield false-negative results when holes are present in the material.

Further ordering of the binding of the other surrogate viruses depends on what conditions are being considered, since two types of binding interactions may occur. Electrostatic binding potential may be deduced from the passage of viruses through the cationic polysulfone membrane in the presence of the nonionic surfactants, where the hydrophobic interactions are minimized, giving the following order: φX174 < T7 < φ6 < MS2 ≃ PRD1. Hydrophobic binding potential may be deduced from the passage of viruses through the nitrocellulose membrane with a weakly hydrophobic interacting additive, LC broth, as follows: φX174 < MS2 < T7 ≃ PRD1 ≃ φ6. Elutions from the nitrocellulose membrane by a nonionic surfactant or with serum relate to reversible binding and yield slightly different levels of hydrophobic binding potential, respectively, φX174 ≃ T7 ≃ MS2 < PRD1, and φX174 ≃ T7 < MS2 ≃ PRD1 < φ6. Shields (14) and Shields and Farrah (16) have used more sophisticated biochemical methodologies to investigate the electrostatic and hydrophobic natures of several viruses, including φX174, T7, and MS2, and have found the same order of ranking of electrostatic binding potential for those three viruses. They also found that while φX174 was the least hydrophobic of the 15 viruses investigated, MS2 was one of the two most hydrophobic.

Aside from φX174's being the best choice for a surrogate challenge virus, T7 appears to be the least electrostatically binding of the three larger viruses, T7, PRD1, and φ6. PRD1 had strong electrostatic and hydrophobic binding potentials and thus does not make a good choice for a challenge virus. The enveloped φ6 has been considered an appropriate surrogate for human immunodeficiency virus for testing barrier materials (11); however, with intermediate electrostatic binding and strong hydrophobic binding, it no longer appears to be a good choice for a challenge virus.

Choice of additive to minimize potential binding. DPBS as the basic solution has some important properties, i.e., physiologic salinity and neutral pH. It allows hydrophobic binding of viruses; however, the neutral pH allows one surrogate challenge virus, φX174, to have minimal electrostatic binding. Thus, the presence of an additive is needed only to eliminate hydrophobic interactions when φX174 is used with DPBS. Triton X-100 at 0.1% not only fulfills that function but also provides a desirable surface tension level (31 dynes per cm, as determined by using 10-μl microcapillary tubes). This desirable combination of φX174, DPBS, and 0.1% Triton X-100 has been used to test condoms and gloves as barriers to virus penetration (9, 10).

Serum at 2 to 5% minimized all binding in these experiments. In situations in which other viruses must be used as a challenge or in which other conditions must be met, it may be necessary or desirable to use serum to minimize virus binding. Unfortunately, since serum is a natural product, its use may be difficult to standardize. On the other hand, blood-borne pathogens are often the most serious threat in the hospital setting and the greatest challenge to protective clothing. The information gathered in this study demonstrates that the presence of serum would likely provide protection to pathogenic viruses from becoming bound to surfaces through electrostatic or hydrophobic interactions.

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