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A Simple Method To Test Condoms for Penetration by Viruses

C. DAVID LYTLE,[1]* LICIA B. ROUTSON,[2] AND W. HOWARD CYR[1]

Division of Life Sciences, Center for Devices and Radiological Health, Food and Drug Administration,[1] and Biocon, Inc.,[2] Rockville, Maryland 20857

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A method by which virus penetration through condoms can be tested with simple, inexpensive equipment is described. The method uses φX174 bacteriophage as the challenge virus and physiologically relevant pressure. Penetration by 0.1 μl (or less) of challenge suspension can be readily detected. As examples, latex and natural-membrane condoms were examined.

There is no standard test to evaluate barrier effectiveness of condoms to penetration by viruses, although several different test methods have been described (1–4, 6, 8, 10–16). This report describes a new method which combines (i) filling a condom with a buffer containing a challenge virus and submerging the condom in collection buffer to collect for assay any virus which may penetrate the condom (8) and (ii) controlling the transcondom pressure hydrostatically and restricting expansion of the condom with a restrainer (13). This new method is easy to use, utilizes readily available equipment, and yields quantitative data on viable-virus penetration.

The method (apparatus shown in Fig. 1) consists of (i) removing each condom from its package and, if lubricant is present, rinsing the condom with buffer (Dulbecco's phosphate-buffered saline [DPBS]); (ii) attaching the empty condom and a restrainer to a Buchner funnel (described below and shown in Fig. 2) and clamping the condom-funnel assembly to a ring stand; (iii) via a top funnel and connecting tubing, filling the condom with virus challenge suspension (approximately 300 ml) and providing an 81-cm column of hydrostatic pressure (equivalent to 60 mm Hg [ca. 8,000 Pa]) above the suspended part of the condom; (iv) lowering the suspended portion (14 cm) of the condom into 1,000 ml of stirred room temperature (22 to 25°C) DPBS contained in a tall collection cylinder (a 1,000-ml Fleaker with top constriction removed) to collect any virus which penetrates the condom; and (v) assaying the DPBS in the collection cylinder for viral infectivity at 1 and 30 min.

The details of the attachment of the condom and restrainer to the Buchner funnel are shown in Fig. 2. The open end of the condom is fastened over the large end (top) of a 30-ml glass Buchner funnel (with the fritted disk removed) with rubber bands so that 14 cm of the condom hangs free. Parafilm is placed over the attached end of the condom such that it overlaps about 4 mm beyond the funnel rim, to protect the condom from abrasion when the rubber bands are positioned. Two rubber bands (6 mm wide, 180 mm in circumference, and 1 mm thick) are tightly wrapped over the Parafilm to hold the condom in place on the funnel and provide a watertight seal. When the condom is not lubricated, a piece of pH paper tape (pH range, 3.2 to 4.5) is placed around the funnel beneath the top of the condom to act as an indicator of whether any DPBS has leaked under the condom and rubber bands. A restrainer (cylindrical in shape, 12.0 to 12.5 cm in circumference, and 18 cm long, with one end closed) made of open fabric material (white organdy fabric was used, but other materials which do not adsorb viruses may also be used) is placed over the condom and held in place by one rubber band so that 15 cm of open restrainer hangs from the funnel and covers the condom. Thus, the 14 cm of condom below the rubber bands (the same length tested in the Food and Drug Administration water leak test for latex condoms [5]) may expand to 15 cm in length. The completed assembly has a 1-m length of 6-mm (inside diameter) nonreactive, sterilizable tubing (Tygon S-50-HL) connecting the small end of the Buchner funnel to the fill funnel at the top.

Bacteriophages φX174 (27-nm diameter) and PRD1 (65-nm diameter) were chosen as the challenge viruses because previous studies indicated that they are excellent choices for evaluation of barrier materials (9). The virus growth and assay systems utilizing their respective bacterial hosts, *Escherichia coli* C and *Salmonella typhimurium* LT2, have been described elsewhere (7). The virus challenge suspension consisted of φX174 alone or both viruses at titers in excess of 10^7 PFU/ml in DPBS. A nonionic surfactant, 0.1% Triton X-100, may be used in the challenge and collection buffers to produce surface tension properties similar to those of human blood. Although bacteriophage PRD1 contains an internal lipid layer, it is stable in DPBS with surfactant under the test conditions and in a refrigerator for at least a few days. The surfactant, however, interfered with the plaque assay of PRD1 (but not that of φX174) for more than 0.3 ml of virus suspension.

Virus penetration was calculated as the equivalent volume of challenge virus suspension needed to account for the amount of virus in the collection cylinder. This was done to normalize for challenge titer and to facilitate use in risk assessment. The exact relationship of that number to the actual passage of challenge buffer from inside the condom to the collection flask is unknown.

The ability of this method to detect virus penetration depends on the challenge virus titer. Furthermore, in order to have 95% confidence that an assay will find at least one virus when virus is present [i.e., $P(0) \leq 0.05$], the average number of infectious particles per total volume assayed must be at least three; e.g., there is a 95% probability that a titer of 1 PFU/ml will result in at least one plaque in a 3-ml total assay. Thus, the sensitivity of this assay can be claimed as 1 PFU/ml when 3 ml is assayed. With 1,000 ml of collection

* Corresponding author.

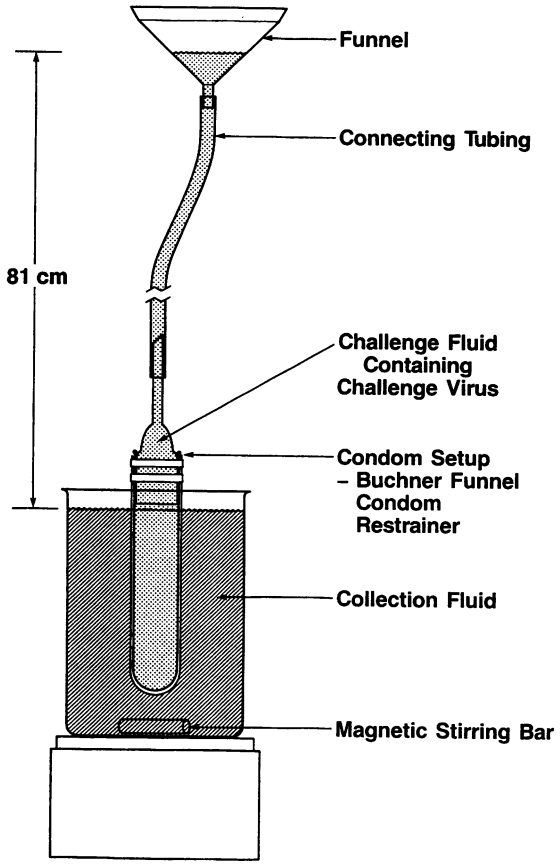

FIG. 1. Physical arrangement of a condom containing approximately 300 ml of virus-containing DPBS and a collection vessel containing 1,000 ml of DPBS. Virus penetration was determined from aliquots taken from the collection vessel. A magnetic stirring bar was used to agitate the solution in the vessel.

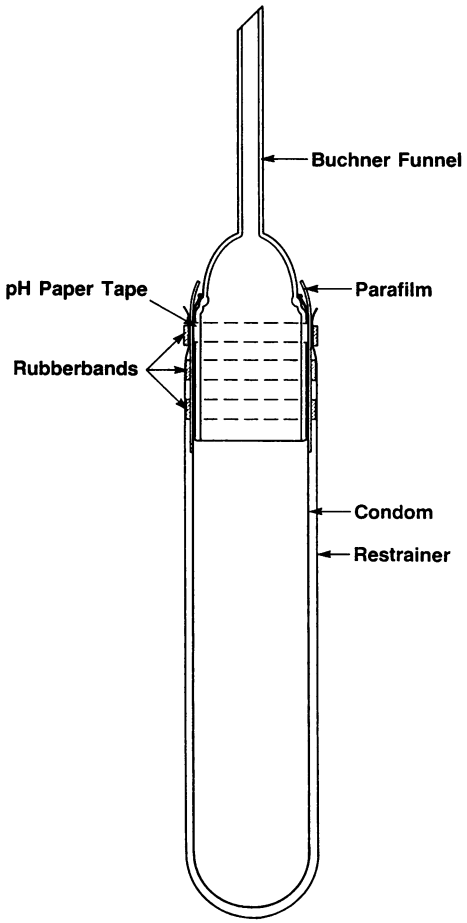

FIG. 2. Schematic representation of the attachment of the condom and restrainer to the Buchner funnel.

buffer at 1 PFU/ml (penetration by a total of 10^3 virus particles), the assay can detect penetration by challenge virus equivalent to 0.1 μl (10^{-4} ml) when the challenge virus titer is 10^7 PFU/ml. Higher sensitivity can be obtained with higher challenge virus titers or by assaying larger volumes.

The viability of the challenge virus suspension during the duration of the test period must be ensured by comparing the titer inside the condom after the 30-min test period with the initial titer. There were no statistically significant changes in ϕX174 and PRD1 titers in any condoms tested.

It is also necessary to ensure that if viruses do penetrate the condom, they remain free and viable in the collection buffer. Spiking experiments were done by (i) adding low titers of challenge viruses to the 1,000-ml collection buffer and assaying the buffer, (ii) submerging a pressurized condom (without viruses in the challenge buffer), including the restrainer, into the collection buffer for 30 min, and then (iii) assaying the collection buffer at 30 min. The titers of both challenge viruses at 30 min were not statistically different from the initial titers, indicating that no component of the collection side of the test, including the restrainer, significantly removed or inactivated any virus which might penetrate the barrier.

The method requires care so that (i) the sample condom is not harmed when it is attached to the test apparatus, (ii) the outside of the condom and the collection buffer are not contaminated with challenge virus when the condom is filled, and (iii) the challenge virus buffer cannot circumvent the rubber band seal. Thus, there is a need for appropriate tests to determine whether such confounding errors of technique occur.

Virus penetration results were obtained by this method with test fluids consisting of DPBS plus 0.1% Triton X-100. One brand of unlubricated latex condoms and two brands of natural-membrane condoms were tested. Prior to testing, the surfaces of each natural membrane condom were gently rinsed with 100 ml of DPBS to remove excess lubricant. Three of 60 latex condoms allowed ϕX174 penetration, amounting to 0.1, 0.6, or 200 μl. Thirteen of 19 natural membrane condoms (brand 1, 5 of 10; brand 2, 8 of 9) allowed ϕX174 penetration, demonstrating wide variation in the amounts of penetration (0.06 to >200 μl), in agreement with data reported previously (8). These virus penetration data confirm and extend our earlier findings (8), and those of Minuk et al. (10–12), that many samples of natural-membrane condoms permit penetration by viruses or virus-size particles. Our data also demonstrate that latex condoms are a substantial barrier to viruses, also in agreement with the findings of Minuk et al. (11, 12) and of Carey et al. (2). Thus, this method yields data consistent with those obtained in other virus and particle penetration studies.

In a separate study with one brand of natural-membrane

TABLE 1. Calculated amount of phage penetration through four natural-membrane condoms[a]

Sample[b]	Amt of virus (μl) that penetrated the condom[c]	
	φX174	PRD1
1	1.3	0.04
2	2.2	0.2
3	6.8	0.3
4	740	39

[a] The test fluid consisted of DPBS without surfactant.
[b] The sample condoms were of brand 2.
[c] Equivalent amount of challenge virus suspension needed to account for the virus which penetrated. The exact relationship of that number to the actual passage of challenge buffer from inside the condom to the collection flask is unknown.

condoms (four samples), simultaneous testing with two challenge viruses, φX174 and PRD1, confirmed that there is preferential penetration of these condoms by smaller viruses (8) (Table 1). Although there was variation in virus penetration from sample to sample with both viruses (Table 1), with every sample there was at least 10-fold more penetration by the smaller virus. This sieving effect indicates that the calculated penetration of challenge virus must be cautiously interpreted when natural-membrane condoms are tested.

In summary, the test apparatus described here is inexpensive and simple to construct. Because the primary challenge virus, φX174, is small (27-nm diameter), this test represents a conservative test for safety; i.e., if φX174 cannot penetrate a condom, then larger human viruses probably cannot penetrate it.

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