Appendix: Modified Dot-Blot Method to Evaluate Personal Protective Equipment (PPE)

Appendix A

The original unmodified apparatus is currently commercially available. The image depicted in Figure 1 is of the dismantled apparatus after it has been modified. The modification, for the purposes of this method, consists of drilling out the 1 mm holes in the filtration plate (not the sample well plate) to expand the diameter to 5 mm and thus increase the surface area of the PPE sample exposed to the vacuum.

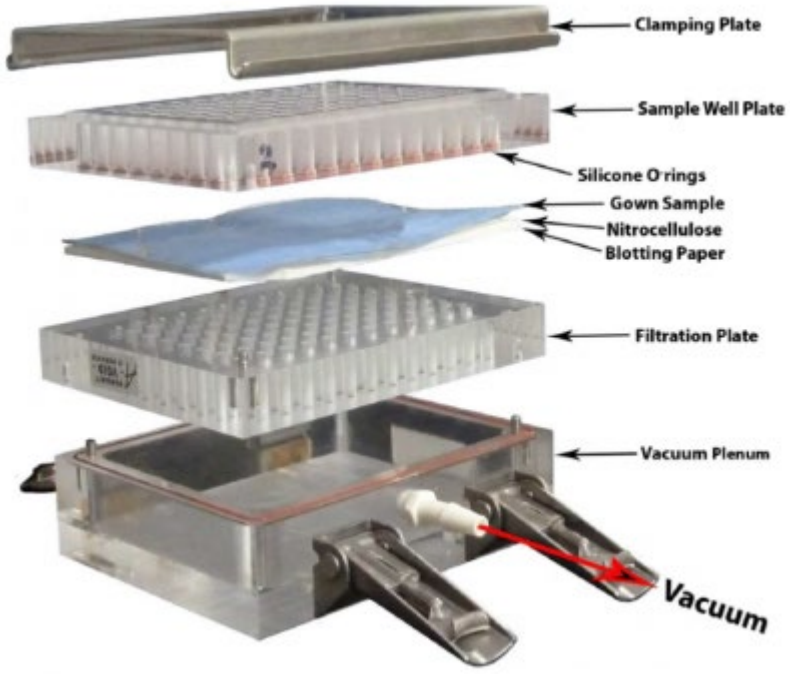

Figure 1

The Modified Dot-Blot Apparatus. In this expanded view, the apparatus layers from top to bottom are shown: clamping plate; sample well plate containing a silicone o-ring in each well; a gown sample, nitrocellulose and blotting paper layered together; a filtration plate; and at the bottom a vacuum plenum with the vacuum port showing.

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Appendix B. Protocols for Testing PPE Materials

To execute the Modified Dot-Blot Method as it pertains to viral penetration, as a screening test before undertaking the more resource-intensive ASTM F1671/ F1671M-13, the steps are listed below.

1. Cut PPE, nitrocellulose membrane and blotting paper into rectangles of 12cm x 9cm.
2. Soak blotting paper in PBS for a minimum of 2 minutes; prewet nitrocellulose membrane in PBS for a minimum of 1 minute.
3. Place blotting paper, then the nitrocellulose membrane and then PPE on the filtration plate. Be sure the face (outside) of the PPE is directly below the sample wells so it will directly contact test solution. Ensure that it lies flat when the apparatus is re-assembled.
4. Clamp in place according to apparatus manufacturer's instructions.
5. Using a multi-channel pipet, place 100 mL of suspension (test organism in nutrient broth) in wells (any unused wells must be blocked off).
6. Let rest for 5 minutes.
7. Apply vacuum of 0.14 bar (2 psi) via the vacuum port for one minute. Turn off vacuum and allow system to return to ambient pressure.
8. If no visual penetration or loss of test suspension from the wells is observed, continue observing for an additional 54 minutes.
9. Using a multi-channel pipet or a pipet tip attached to vacuum, remove any remaining test suspension. It is important to do this without dismantling the apparatus to avoid the test suspension contaminating non-leak areas of the nitrocellulose membrane.
10. Using a multichannel pipet, place 100ml PBS in each well, then remove all liquid from the wells. Repeat this washing step once.
11. Carefully dismantle the apparatus and use tweezers to remove the nitrocellulose membrane. Place in a shallow dish slightly bigger than the membrane.
12. Wash the nitrocellulose membrane with PBS-T twice to remove any unbound assay interferents.
13. Into the dish containing the nitrocellulose membrane, pour a solution of 0.05% BSA (bovine serum albumin) in PBS-Tween 20 and cover the nitrocellulose membrane. Rock gently for 30 minutes. The BSA blocks the unbound sites on the nitrocellulose membrane.
14. Remove solution from shallow dish, keeping the nitrocellulose membrane.
15. Rinse the nitrocellulose membrane three times with PBS-Tween 20, rocking gently for 10 min each time. Remove solution after each rinse step, keeping the nitrocellulose membrane.
16. Add solution of primary antibodies* (in PBS-Tween 20) to bind to viral surrogate. Rock gently for 30 min. Remove solution from shallow dish, keeping the nitrocellulose membrane.

17. Rinse the nitrocellulose membrane three times with PBS-Tween 20, rocking gently for 10 min each time. Remove solution after each rinse step, keeping the nitrocellulose membrane.
18. Add solution of secondary antibody* (in PBS-Tween 20) to bind to the primary antibody. Rock gently for 30 min. Remove solution from shallow dish, keeping the nitrocellulose membrane.
19. Rinse twice with PBS-Tween 20, rocking gently for 10 min each time. Remove solution after each rinse step, keeping the nitrocellulose membrane.
20. Rinse once with PBS, rocking gently for 10 min. Remove PBS solution, keeping the nitrocellulose membrane.
21. Add solution containing substrate* specific to the secondary antibody, covering the nitrocellulose membrane. The reacted substrate will change color and precipitate if the secondary antibody is present (i.e., bound to the primary antibody, in turn bound to viral surrogate, in turn bound to the nitrocellulose membrane). Color change is a qualitative indication of PPE failure.
22. Any evidence of penetration constitutes a "Fail" endpoint.

*The primary antibodies bind to the antigenic proteins of the bacteriophage. Primary antibodies specific to ΦX174 are needed. If primary antibodies are not commercially available, contract labs can produce them. Rabbits are commonly used to produce primary antibodies. Primary antibodies, while they bind to the antigenic proteins, generally do not have labels discernible by detection techniques. Such is the case with the present method. Therefore, secondary antibodies with labels are needed to bind to the primary antibodies. An appropriate secondary antibody for this method would be a goat antirabbit with HRP (horse radish peroxidase) enzyme label; it is available from commercial vendors. After the binding of the labeled secondary antibody to the primary antibody, the label is detected through the use of a commercially available substrate for the HRP enzyme. Once the substrate reacts with the enzyme, a signal occurs. In the case of the present method, the reacted substrate changes color and precipitates, allowing for visual detection. The signal-to-noise ratio is modulated by the respective concentrations and incubation times of primary and secondary antibodies; acceptable concentrations for desired signal-to-noise ratios should be determined before using the present method.

To execute the Modified Dot-Blot Method as it pertains to artificial blood, as a screening test before undertaking the more resource-intensive ASTM F1670, the steps are listed below.

1. Cut PPE and blotting paper into rectangles of 12cm x 9cm.
2. Soak blotting paper in PBS for a minimum of 2 minutes.
3. Place blotting paper and then PPE on the filtration plate. Be sure the face (outside) of the PPE is directly below the sample wells so it will directly contact test solution. Ensure that it lies flat when the apparatus is re-assembled.
4. Clamp in place according to specific manufacturer's instructions.
5. Using a multi-channel pipet, place 100 mL of test solution (artificial blood) in wells (any unused wells must be blocked off).
6. Let rest for 5 minutes.
7. Apply vacuum of 0.14 bar (2 psi) via the vacuum port for one minute. Turn off vacuum and allow system to return to ambient pressure.
8. If no visual penetration or loss of test suspension from the wells is observed, continue observing for an additional 54 minutes.
9. Using a multi-channel pipet or a pipet tip attached to vacuum, remove any remaining test suspension. It is important to do this without dismantling the apparatus to avoid the test solution contaminating non-leak areas of the blotting paper.
10. Carefully dismantle the apparatus and check if any dye penetrated the PPE and is visible on the blotting paper.
11. Any evidence of penetration constitutes "Fail" endpoint.