

# Viewpoint: Can qPCR replace the standard plaque assay in the ASTM F1671 to assess personal protective equipment barrier performance?

## Background

Personal protective equipment (PPE) is an important measure within the infection prevention hierarchy of controls to protect the wearer from hazards, including potentially harmful pathogens. Healthcare workers (HCWs) are at constant risk of exposure to pathogens and acquiring infections. Also, public safety workers such as firefighters and emergency medical service personnel who work in unpredictable environments, such as hospitals, clinical laboratories and ambulances, are also at risk of contracting infectious diseases through direct exposure to blood and other body fluids (Polder *et al.*, 1991; The National Institute for Occupational Safety and Health (NIOSH), 2019). These workers rely on PPE, such as gowns, gloves and respirators, for protection.

The protective performance of PPE is often evaluated using standard test methods. Medical gloves and isolation and surgical gowns are often evaluated for viral penetration resistance efficiency because of their role in protecting against body fluids potentially contaminated with infectious pathogens. In the United States, not all PPE are required to be assessed for viral penetration resistance effectiveness by regulatory agencies. Many manufacturers perform in-house testing of their protective clothing including the viral penetration assay and provide protection data in marketing materials to help end users and purchasers make informed decisions regarding purchases.

Surgical and isolation gowns, similar to medical gloves, are considered medical devices in the United States, and they are regulated by the Food and Drug Administration (FDA) (Administration, U.S.F.D, n.d.). In 2004, the FDA recognized the American National Standards Institute/Association of the Advancement of Medical Instrumentation (ANSI/AAMI) PB70:2003. This system of classification for gowns defines four levels of protection from 1 to 4, assigning Level 4 gowns the highest level of protection for liquid barrier performance, including protection from viral penetration.

Level 4 gowns, as well as PPE that claims viral penetration resistance, are evaluated against the American Society for Testing and Materials (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" (ASTM, 1995) to assess viral penetration resistance. This test method was originally defined to evaluate the viral penetration of hepatitis (B and C) and human immunodeficiency virus (HIV) transmitted in blood and other potentially infectious body fluids. It uses Phi-X174 bacteriophage (a virus that infects bacteria) as a surrogate for pathogenic viruses. Phi-X174 is one of the smallest viruses identified, is easy to propagate and is unharmed for humans. ASTM F1671 provides a pass or fail result based on virus detection on the other side of the exposed material. This test method is recognized by the Centers for Disease Control and Prevention (CDC) (Considerations for Selecting Protective Clothing, n.d.) and is referenced in the guidance from the Occupational Safety and Health Administration (OSHA) on the



selection of PPE for protection against exposure to Ebola ([Occupational Safety and Health Administration, n.d.](#)).

ASTM F1671 is mainly used by PPE manufacturers and third-party test laboratories as part of the product performance assessment, but because of the length of time required to complete the method (typically 5–6 days), it may not be suitable for use as a material or protective clothing quality control or quality assurance procedure. ASTM F1671 consists of two phases. During the first phase, one side of the fabric is exposed to a liquid containing Phi-X174 at a concentration between  $1 \times 10^8$  and  $3 \times 10^8$  plaque-forming units (PFU) at a pressure of 13.8 kPa (psig) for 1 min, followed by 0 kPa for 54 min. During the second phase, the other side of the fabric is assessed for the presence and eventually quantification of the infective virus that migrated through. This second phase, as well as the initial viral titer determination, is performed by a microbiology technique called standard plaque assay (hereafter referred to as SPA).

SPAs were developed as an adaptation of phage assays, which were used to calculate bacteriophage titers in plant biology ([Cooper, 1961](#)). Renato Dulbecco modified this procedure in 1952 for use in animal virology ([Dulbecco and Vogt, 1953](#)), and it has since been used for reliable determination of the titers of many different viruses. The assay consists of spreading an infecting virus on top of a lawn of susceptible cells (bacteria or animal cells) and manually counting the “plaques”—i.e. the zones of clearing within the continuous lawn of cells developed after 12–24 h incubation time. The plaques represent the site where a single virus acted as an infectious unit. The entire assay takes about 48 h to be completed.

### Standard plaque assay limitations

Since 1952, the SPA continues to represent the “gold standard” in determining viral concentration for infectious viruses ([Hartley and Rowe, 1963](#)), despite its multiple limitations. As indicated in the Edelman and Barletta report ([Edelman and Barletta, 2003](#)):

... significant limitations of this method includes (i) the requirement for extensive hands-on time ( $\geq 5$  h) for completion of the assay, at least 1.5 days to produce results, and the need for a large quantity of reagents and supplies; (ii) a limited dynamic range of one log (30–300 plaques/plate); (iii) media that are susceptible to environmental conditions (e.g. drying and aging), resulting in suboptimal bacterial growth, resulting in decreased phage infectivity; (iv) the dependence of accurate titers upon the viability of the host bacteria, with viability less than 100% causing a reduction in the true titer; (v) the inherent subjectivity and potential error when visually counting plaques; (vi) a lack of reproducibility in titer due to procedural errors from multiple dilution and pipetting steps; (vii) the detection of only functional phage rather than total phage DNA content in the preparation; and (viii) an extremely tedious and cumbersome procedure with a low throughput when assessing multiple clones.

Among these limitations, the time required to perform the SPA is a major barrier when demand for PPE is high ([Office and U.S.G.A., 2021](#)) and more PPE has to be made available, such as during pandemics. To prepare for these pandemics, and to increase PPE supply, two strategies are commonly used: (1) expedite the entrance of newly fabricated PPE into the market by reducing the time required to assess their protective performances ([Office and U.S.G.A., 2021](#)) and (2) maintain stockpiles of PPE. Both strategies may require performance assessment to ensure that both newly made and/or stockpiled products are protective when used.

PPE should be stored under controlled conditions in accordance with the manufacturer’s guidance ([Federal Register Notices, 2017](#)). Protective clothing manufacturers are not required to report the shelf life of their products, thereby bringing into question the performance of some PPE when subjected to long-term storage. The ability to quickly evaluate the

performance of stockpiled PPE, including viral penetration resistance, is essential to making informed decisions regarding the distribution of PPE.

The ASTM F1671 test method typically requires 5–6 days to be completed. About half of the time is required to propagate the virus needed for both the initial fabric exposure and the SPA post exposure. In addition, only a limited number of samples can be processed at a given time, making the assay inefficient and unsuitable in situations that require the testing of PPE on a large scale (e.g. multiple stockpiled production lots) or when results are needed quickly.

Also, before the ASTM F1671 can be initiated, a compatibility test is required to determine if the components of the PPE such as materials, adhesives and coatings are compatible with Phi-X174 bacteriophage or the host bacterium, *E. coli*. This is necessary because this method is designed to measure only infective viral particles rather than the total viral load that penetrates the fabric. Therefore, this standard test method cannot be used to assess viral penetration on fabrics containing inhibitory agents or those pretreated with antimicrobial substances – techniques that are becoming more common (Huang and Leonas, 2000; Virk *et al.*, 2024; Tafadzwa Justin Chiome, 2020; Saran *et al.*, 2020; Joe, 2009). As a consequence, potentially protective garments remain excluded by the limited applicability of the ASTM F1671, an aspect that requires additional considerations during PPE shortages.

The ASTM F1671 is also relatively expensive. Laboratories typically charge a few hundred dollars per sample, and several samples are generally required to obtain FDA approval. Among the factors that contribute to the high cost are the limited number of accredited laboratories authorized to perform the test, the time required to process the samples, the large amount of consumables required and the costs associated with the disposal of the biohazardous waste produced during the test.

Due to the numerous limitations associated with the application of the SPA for quantification of infective virus, additional techniques should be considered for inclusion in the ASTM F1671 test method. Several viral detections and quantification methodologies have been proposed to improve the ASTM F1671 (Nand *et al.*, 2020; Li *et al.*, 2019; Kisielewski *et al.*, 2000; Shimasaki *et al.*, 2016, 2020). Among the alternative methods available for viral quantification, quantitative real-time polymerase chain reaction (qPCR) appears to be a promising methodology to address the limitations of the SPA in ASTM F1671. qPCR is a laboratory technique of molecular biology that allows for the quantification of different microbial agents present in a sample by amplification and detection of their genomic material.

### **Quantitative real-time polymerase chain reaction (qPCR) benefits**

qPCR is widely used in clinical laboratory research for several applications including biomedical research, criminal forensics (Liu, 2014; Du *et al.*, 2017) and in the food industry to determine the microbial load in food or vegetable matter (Filion, 2012; Rodríguez-Lázaro, 2013; Bonilauri *et al.*, 2016). If deemed a valid technique for this application, replacing the SPA with the qPCR in the ASTM F1671 is expected to provide numerous benefits, which are summarized in Table 1. These benefits are explained in the following.

*Time requirement:* This technique is rapid and requires less sample manipulation. The results are generated in 2 to 3 h as opposed to the two days needed to analyze only a few samples when using the SPA.

*Cost per sample:* The cost of the qPCR reaction varies between \$9 and \$15 per sample. This estimate was determined by splitting the cost of the reagents (primers, probes and mastermix) and consumables for the highest number of samples that can be processed at once, a common strategy adopted to minimize the cost per reaction. This amount does not include labor costs and varies depending on several factors including the number of probes used, the volume of the reagents required by the instrument, among other factors.

*Equipment cost:* qPCR necessitates a specific instrument called a “thermocycler,” which requires an initial investment usually between \$20K and \$50K depending on the manufacturers and features such as the number of samples that can be processed simultaneously, the number of fluorescent dyes that can be detected in the reaction, etc.

*Reproducibility:* When appropriately performed, qPCR results are reproducible. Studies performed on aliquots of samples analyzed over a period of several months have shown no significant changes in results (Edelman and Barletta, 2003). Also, the lower manipulation in the sample preparation compared to the SPA reduces the risk of introducing variables that would inevitably interfere with the assay, thereby increasing the reproducibility of the qPCR compared to the SPA.

*Accuracy:* qPCR has higher accuracy than the SPA because the viral quantification is extrapolated from computer-generated data and is, therefore, less prone to human error.

*Sensitivity:* qPCR is extremely sensitive and easy to perform. In several studies, phages were detected below one PFU and from samples without prior genomic material isolation (Edelman and Barletta, 2003; Soejima *et al.*, 2016; Anderson *et al.*, 2011; Bae *et al.*, 2003).

*Material compatibility:* Use of qPCR can mitigate the concern about material compatibility associated with the SPA. qPCR can detect both infectious and noninfectious virus, which allows for the detection of virus penetration through materials or components (adhesives, coatings, etc.) that have added or inherent antimicrobial properties (Huang and Leonas, 2000; Virk *et al.*, 2024; Tafadzwa Justin Chiome, 2020; Tunon-Molina *et al.*, 2021).

*Scalability:* qPCR has a high throughput compared to SPA, and hundreds of samples can be analyzed simultaneously (Schmittgen *et al.*, 2008; Grigorov *et al.*, 2011). On the contrary, SPA is a laborious and time-consuming assay that allows for processing of only a few samples at a time.

**Limitations of qPCR and modifications to address those limitations**

There is one key limitation to quantifying virus penetration of PPE fabric when using traditional qPCR compared to the SPA. The SPA is designed to detect only infectious viruses, whereas standard qPCR is unable to distinguish between infectious and noninfectious viral particles. In recent years however, a modification of the qPCR technique involving the use of a chemical reagent called propidium monoazide (PMA) has eliminated this limitation (Leifels *et al.*, 2021; Nocker *et al.*, 2007; Koopmans and Le Guyader, 2017; Kibbee and Ormeci, 2017). The use of PMA in qPCR has been extensively documented in food and environmental applications (Zhang *et al.*, 2015; Fongaro *et al.*, 2016; Quijada *et al.*, 2016; Karim *et al.*, 2015; Rousseau *et al.*, 2019; Agusti *et al.*, 2010; Fittipaldi *et al.*, 2010), and there are already kits commercially available for the detection and quantification of infectious microorganisms like bacteria and viruses (Quimigen, n.d.; Biotium, n.d.; Biotrend, n.d.). The mechanism that allows

Factors	qPCR comparison to SPA
Time requirement	2–3 days faster than SPA
Cost per sample	40–50% less expensive than SPA
Equipment cost	\$20–\$50 k initial investment. SPA does not require specific equipment
Reproducibility	More reproducible than SPA
Accuracy	More accurate than SPA
Sensitivity	More sensitive than SPA
Material compatibility	Unaffected because the whole viral load is measured unlike SPA which is affected
Scalability	Hundreds of samples processed at a time compared to only a few for the SPA

**Source(s):** Authors’ own creation

**Table 1.**  
Comparison of the characteristics of the SPA and the qPCR

PMA to differentiate between infectious and noninfectious viruses is based on the property of the molecule to bind to the nucleic acids of viruses with damaged capsid (the protein shell surrounding the nucleic acid) and inhibit the PCR reaction. The criticism about the efficiency of PMA for infectious–noninfectious viral distinction is that the PMA is unable to differentiate viruses that have lost their infectivity due to damaged nucleic acids but whose capsid remains intact (Leifels *et al.*, 2021; Fittipaldi *et al.*, 2010). In that regard, the simultaneous detection of several portions of a genome made possible by advancement in technology (multiplex analyses) allows the qPCR to overcome the legitimate concerns (Hawkins and Guest, 2017; Stingl *et al.*, 2021). However, it should be noted that different viruses have different shapes, stability profiles and infectious potencies (Wang *et al.*, 2005); hence, claiming protective performance based exclusively on the detection of infectious PhiX-174 virus might not be representative of the protection provided in real circumstances. However, measuring total (infectious and noninfectious) penetration of PhiX-174 with the PMA reagent does allow for the adequate characterization of the barrier performance of PPE, which is the objective of the test method.

## Conclusions

The standard plaque assay as specified in ASTM F1671 to assess viral penetration resistance of PPE has the potential to be improved in relation to speed, reproducibility and material compatibility. Saving two days to assess the viral penetration during PPE shortages combined with the higher throughput of the quantitative real-time polymerase chain reaction and its larger applicability to fabrics would dramatically increase the availability of PPE, potentially reducing the spread of infection and saving lives. For these reasons, a faster, more accurate and more versatile technique such as qPCR should be considered as a valid method to assess the protective performance of PPE. Future direct-evidence studies are needed that directly compare qPCR to the gold standard SPA method for validation of the argument made by the authors in this article.

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