Study on the Testing Method to Determine the Performance of Personal Respiratory Protection Equipment Against a Viral Aerosol

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Abstract

Respiratory illnesses are increasingly recognized as a growing concern for healthcare workers (HCWs) and patients. The 2003 hospital-based outbreak of Severe Acute Respiratory Syndrome (SARS) has once again highlighted the vulnerability of HCWs to aerosol-transmitted viral infections. Personal respiratory protective equipment was one of the key means for the HCWs to avoid nosocomial transmission of the virus. This article studied a testing method for determining the performance of personal respiratory protection equipment against a viral aerosol. Full-mask respirators with HEPA filters were selected for this study. Phage f2 was used as a surrogate for a viral pathogen. A viral aerosol was generated and then sampled in front of and behind the test respirators, allowing a percentage efficiency value to be calculated against viral aerosols. HEPA-filtered respirators demonstrated a high filtration efficiency of >99.99% and can protect the wearers against the viral aerosol transmission. This test methodology can be used to assess the filtration efficacy of personal respiratory protective equipment against a viral aerosol.

Keywords

Nosocomial transmission, personal respiratory protection equipment, viral aerosol, testing method

1. Introduction

After the severe acute respiratory syndrome (SARS) epidemic in 2003, the concept of aerosol transmission of the coronavirus was well accepted (Tang et al., 2006). SARS posed a mammoth challenge because of the impact of nosocomial transmission on healthcare manpower and facilities, and the resources needed for controlling and preventing further spread (Tai, 2006). Nishiyama showed that the risk for developing SARS was 12.6 times higher in individuals not using a mask than in those using a mask (Nishiyama et al., 2008). The consistent and proper use of a mask was shown to be crucial for constant protection against small virions (Balazy et al., 2006). This suggests that N95 or N99 respirators may not have good protection against viral aerosol infection. As a result, in this study HEPA equivalent filter cartridges (similar to filters in N95 or N99 respirators) were selected from full-face respirators and challenged with aerosolized virus.

Personal respiratory protective equipment is used to protect against viral aerosol-mediated infection and is rated by test methods using physical particles, but not by a standard viral aerosol testing method (AQSIQ, 2003; BS EN, 2001; CFR, 1995). Few research papers have focused on respirator (N95, N99) filtering efficiency against viral aerosols (Balazy et al., 2006). There is a need to develop and use standard methodologies to test such materials, using reliable viral aerosol generating and sampling techniques, to assess their filtration efficiency against airborne viruses.

The aerosolization of pathogenic viruses requires a very high level of containment to prevent uncontrolled release. Due to aerosol safety issues involved with the generation of high viral concentrations within the aerosol, the testing method selected used the non-pathogenic virus phage f2. The Balazy method, with some modifications, was used in this study to evaluate the filtration efficiency of personal respiratory protective equipment against viral aerosol (Balazy et al., 2006).

2. Materials and Methods

2.1 Personal Respiratory Protection Equipment

Full-mask respirators with a replaceable particulate filter (Tangshan Chemistry Co., Ltd., China) which were HEPA equivalent filters were provided by the manufacturer.

2.2 Particulate Filter Filtration Efficiency Against Physical Particle Aerosol

Particulate filter filtration efficiency against 0.3 μm Dioctylphalate (DOP) aerosol was determined by the manufacturers using a Laser particle counter. The testing flow rate was 28.3 L/min.

2.3 Test Organism

Bacteriophage f2 was used as the model virus. Phage f2 (24 nm) consists of a protein icosahedron and a single-strand RNA genome and is considered to be one of the smallest viruses.
2.4 Preparation of the Test Suspension

Preparation of the Viral Suspension—An overnight culture of *E. coli* at 37°C was inoculated into 10 ml of nutrient broth in a conical flask and incubated for 4 hours at 37°C with shaking. This logarithmic phase culture was then inoculated (10 ml) into 100 ml of nutrient broth and 1 ml of an f2 stock preparation (approximately 1.0×10^7 pfu/ml) was added. The culture was incubated overnight at 37°C. After incubation, the culture was centrifuged (5,000 g, 10 minutes) and the supernatant filtered through a 0.22 μm filter.

Preparation of the Test Suspension—The actual concentration of phage f2 in this preparation was then determined by titration. A fresh preparation was made for each series of tests.

2.5 Titration of the Virus

Phage f2 was titrated by preparing 10-fold dilutions of the sample in phosphate buffered saline (PBS) (Shanghai Shengwu Biotechnology, China). A culture of *E. coli* (0.5 ml) was added to 10 ml of 0.7% agar while still molten at a temperature of approximately 45°C. This was poured over a nutrient agar base and allowed to set. Each sample dilution (100 μl) was spotted onto the overlay and the plates inoculated at 37°C for 16 hours. The number of plaques was counted and the concentration expressed as plaque-forming units (PFU) per ml.

2.6 Size of the Viral Aerosol Particles

A TSI 3321 (TSI, Shoreville, MN) aerodynamic particle size spectrometer was connected to the cylinder aerosol chamber (113 cm²×50 cm, bottom area×height) for sampling the phage f2 aerosol size distribution. The test was performed at 5L/min for 30 seconds at the control position (Figure 1).

2.7 The Test Rig

The test rig is shown diagrammatically in Figure 1. Filtered air can be drawn through the rig at 60 L/min by an air pump. The DV40 nebulizer (Qindao Zhongrui Intelligent Instruments Co., Ltd., China) was supplied with a 30 mL suspension of phage f2. The operating principle of the DV40 nebulizer is the same as a collision nebulizer. The compressed air shears the liquid into droplets. The larger fraction of the droplet is removed and the smaller fraction is ejected by the nebulizer. Phage f2 is aerosolized by supplying compressed air to the nebulizer at 10 L/min. The aerosolized phage f2 aerosol is passed into the aerosol chamber and mixed uniformly in the chamber. At the downstream end of the chamber, another length of duct is connected and linked to the fan units via a back-up high-efficiency particulate air filter assembly designed to prevent the escape of any viral aerosol into the environment. Multistage Andersen samplers (Andersen, 1958) were used to sample the air at two positions. The first was at the control position to obtain a control sample, and the second was in the test position to obtain a test sample. This allows a determination of the viral concentration within the aerosol before filtration and after the filter filtration, respectively. The flow was 28.3 L/min and sample time was 1 minute at the control position, and the flow was 28.3 L/min and sample time was 30 minutes at the test position. The collecting agars were cultured and the plaque numbers counted. The filtration efficiency was determined by the ratio of aerosol concentration at the control and test sample.

2.8 Culturing the Collected Samples

Collected samples of phage f2 were covered by a layer of 0.5 ml *E. coli* and 10 ml of semi-solid culture and incubated at 37°C for 16 hours. The number of plaques on each plate was determined as described in the reference (Andersen, 1958).

Figure 1

Rig for testing the filtration efficiency of respiratory protection equipment against viral aerosols.
2.9 Calculating Performance Efficiency

By taking pre- and post-filter viral aerosol samples with sampling devices, the authors were able to measure the viral aerosol concentration before and after filtration. The percentage efficiency of the particulate filter was calculated using the following formula, where A is the concentration of viral aerosol challenging the filter and B is the concentration of viral aerosol after filtration. Phage f2 aerosol was determined in terms of pfu/m$^3$.

\[
\text{Efficiency (\%) } = \left(1 - \frac{B}{A}\right) \times 100\%
\]

3. Results

3.1 The Filtration Efficiency of Filters of the Full-Mask Respirator Against DOP Aerosol

The filtration efficiency for four filters on full-mask respirators against a 0.3 μm DOP aerosol is shown in Table 1. The average filtration efficiency of the filters was 99.9957±0.0015%.

3.2 Size of the Viral Aerosol Particles

The viral aerosol particles size distribution is shown in Figure 2. The geometric mean size of an aerosol particle containing phage f2 was 0.765 μm, with a geometric standard deviation of 1.29.

3.3 The Filtration Efficiency of Filters of Full-Mask Respirator Against Viral Aerosol

The same four filters were selected to test filtration efficiency against a viral aerosol. The calculation of filtration efficiency against viral aerosol is shown in Table 2. A multistage Andersen sampler (Andersen, 1958) was used to collect the air after filtration by the filters, using a flow rate of 28.3 L/min. The sampling time was set at 30 minutes so the testing limit was 1 pfu/m$^3$. If no phage f2 plaques were observed on the collected agar of the tested samples, the result was determined to be <1 pfu/m$^3$. All of the tested masks demonstrated a filtration efficiency for a phage f2 aerosol of >99.9971%.

4. Discussion

Because of the special aerosol properties of bioaerosols, respiratory protective equipment used to prevent aerosol-transmitted diseases should require a bioaerosol protection test (Rengasamy et al., 2004). However, there is no standardized method to test for high-risk respiratory protection equipment filtration efficiency against a viral aerosol. Several studies have reviewed the role of respiratory protective devices in the control of TB in healthcare settings (Hodous et al., 1994; Jarvis et al., 1995; McCullough et al., 1999; Schaefer, 1997). Studies on respiratory protection against TB were carried out with nonpathogenic bacteria having

Table 1

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Flow Rate (L/min)</th>
<th>Filtration Efficiency (%)</th>
<th>Filtration Efficiency (Mean +/-Standard Deviation) (%)</th>
</tr>
</thead>
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<tr>
<td>002</td>
<td>28.3</td>
<td>99.9961</td>
<td></td>
</tr>
<tr>
<td>011</td>
<td>28.8</td>
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<td>28.9</td>
<td>99.9944</td>
<td></td>
</tr>
<tr>
<td>032</td>
<td>28.9</td>
<td>99.9947</td>
<td>99.9957±0.0015</td>
</tr>
</tbody>
</table>

Figure 2

The size distribution of phage f2 aerosol particles.
The primary concern of this study was the filtration capability of the filter in the full-face respirator when challenged with viral aerosol. A number of important characteristics, such as face fit factor, skin hypersusceptibility, maintenance, and storage, were excluded. In this study, the filters had a high filtration efficiency >99.99% against a Bacteriophage f2 aerosol. Phage f2 is considered to be one of the smallest viruses; therefore, the filters would demonstrate better filtration efficiency against larger viruses. The filters can protect the wearers against a viral aerosol transmission. The filters’ filtration efficiency against 0.3 μm DOP particles aerosol was >99.99% for all. The result of the viral aerosol test was similar to the physical DOP particles results.

Research on respiratory protection against biological agents is needed to address concerns in areas such as occupational safety and prevention against terrorist attacks. However, test methodologies and protocols have not been fully developed and documented in the literature. The paucity of literature on various aspects of respiratory protection against bioaerosols is a limiting factor in drawing conclusions (Rengasamy et al., 2004). This test method has been designed to introduce an aerosol challenge to the test specimens at a flow rate of 28.3 L/min. This flow rate is within the range of normal respiration. This test method was used to measure the viral aerosol filtration efficiency of specific respiratory protection equipment, employing a ratio of the aerosol concentration before and after testing samples to determine filtration efficiency of respiratory protection materials. This is a quantitative method that allows filtration efficiency for respiratory protection materials to be determined. The flow rate can be adjusted if other flow rate air samplers are used, and the aerosol concentration can be adjusted by increasing the dilution air so it can be used in evaluating the respiratory protection materials filtration efficiency against viral aerosols with larger test flow rates.

Table 2
Filtration efficiency of replaceable particulate filter against f2 aerosol.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Aerosol Concentration Before Filtrating (PFU/m³)</th>
<th>Aerosol Concentration After Filtrating (PFU/m³)</th>
<th>Filtration Efficiency (%)</th>
<th>Average Filtration Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>002</td>
<td>34613±4800</td>
<td>&lt;1</td>
<td>&gt;99.9971</td>
<td>&gt;99.9971</td>
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<tr>
<td>011</td>
<td>34613±4800</td>
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<td>&gt;99.9971</td>
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<tr>
<td>030</td>
<td>34492±6345</td>
<td>&lt;1</td>
<td>&gt;99.9971</td>
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<td>34492±6345</td>
<td>&lt;1</td>
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</tbody>
</table>

Acknowledgments

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References


**NIH Office of Biotechnology Activities: U.S. Government Issues Policy on the Oversight of Dual Use Research of Concern**

On March 29, 2012, the Federal government issued a policy for the oversight of life sciences “dual use research of concern” (DURC). The purpose of this policy is to establish regular federal review of U.S. government funded or conducted research with certain high-consequence pathogens and toxins for its potential to be DURC in order to mitigate risks where appropriate and collect information needed to inform the development of an updated policy, as needed, for the oversight of DURC.

The policy applies to research with 15 pathogens and toxins, all of which are also covered by the current Select Agent Regulations. The policy requires that, within 60 days of issuance of the policy, all Federal departments and agencies that conduct or fund life sciences research undertake a review to identify all current or proposed, unclassified intramural or extramural life sciences research projects with these 15 agents that could meet the definition of DURC. DURC is defined as research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public.

If an agency identifies any DURC involving any of the 15 listed agents in its portfolio, the agency will determine whether a risk mitigation strategy is warranted, and then contact the institutions and investigators conducting the research as necessary.

A copy of the full policy may be found at: [http://oba.od.nih.gov/biosecurity/bio_usg_activities.html](http://oba.od.nih.gov/biosecurity/bio_usg_activities.html) and [www.phe.gov/s3/Pages/default.aspx](http://www.phe.gov/s3/Pages/default.aspx). Further educational material on DURC may be found at: [http://oba.od.nih.gov/biosecurity/biosecurity.html](http://oba.od.nih.gov/biosecurity/biosecurity.html)