



Effectiveness of surgical masks against influenza bioaerosols

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ARTICLE INFO

Article history:

Received 9 January 2012

Accepted 4 February 2013

Available online 14 March 2013

Keywords:

Influenza virus

Pandemic preparedness

Respiratory protective device

Surgical masks

Test method

SUMMARY

Background: Most surgical masks are not certified for use as respiratory protective devices (RPDs). In the event of an influenza pandemic, logistical and practical implications such as storage and fit testing will restrict the use of RPDs to certain high-risk procedures that are likely to generate large amounts of infectious bioaerosols. Studies have shown that in such circumstances increased numbers of surgical masks are worn, but the protection afforded to the wearer by a surgical mask against infectious aerosols is not well understood.

Aim: To develop and apply a method for assessing the protection afforded by surgical masks against a bioaerosol challenge.

Methods: A dummy test head attached to a breathing simulator was used to test the performance of surgical masks against a viral challenge. Several designs of surgical masks commonly used in the UK healthcare sector were evaluated by measuring levels of inert particles and live aerosolised influenza virus in the air, from in front of and behind each mask.

Findings: Live influenza virus was measurable from the air behind all surgical masks tested. The data indicate that a surgical mask will reduce exposure to aerosolised infectious influenza virus; reductions ranged from 1.1- to 55-fold (average 6-fold), depending on the design of the mask.

Conclusion: We describe a workable method to evaluate the protective efficacy of surgical masks and RPDs against a relevant aerosolised biological challenge. The results demonstrated limitations of surgical masks in this context, although they are to some extent protective.

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Introduction

In an influenza pandemic, the range of interventions and control measures designed to protect frontline workers from infection in healthcare establishments is limited, for instance engineering controls are difficult to implement under these conditions. Reducing the potential for infection, therefore, relies heavily on procedural controls and vaccination/prophylaxis. A vaccine is unlikely to be available during the initial

stages of a pandemic and the use of antivirals can be limited due to the requirement for a timely inoculation and susceptibility of the recipient.^{1,2} Protecting the health of frontline workers may, therefore, rely heavily on procedural controls and the use of personal protective equipment including surgical masks and respiratory protective devices (RPDs) such as face-fitted respirators.^{3–5}

Surgical masks have been in use for more than a century and were originally designed to protect the patient and the operating theatre environment from infection by the surgical team.⁶ Over the last 10–20 years, surgical masks have also been advocated for protecting the wearer's mucosa from splashes of blood, which may contain infectious particles.⁷

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Surgical masks might also confer some protection against contact transmission by limiting interaction of the hands with oral/nasal mucosa.

Current UK guidance stipulates that surgical masks should be worn by healthcare workers (HCWs) in close proximity (within 1 m) to a known or suspected influenza patient, by way of reducing spread of the virus.^{8,9} Where there is an associated increased risk of viral transmission to HCWs undertaking aerosol-generating procedures (e.g. bronchoscopy) on infected patients, the guidance specifies that HCWs within 1 m of the infected patient should wear an adequate and suitable RPD.^{10,11} Unfortunately, there appears to be uncertainty regarding when and what type of RPD should be worn, resulting in the potential for confusion that surgical masks confer protection against aerosols. Nevertheless, there will be situations where HCWs are within 1 m of an infected patient and are protected from infectious droplets and particles only by a surgical mask.

Little work has been done to evaluate the level of protection afforded by surgical masks against bioaerosols.¹² Several studies have demonstrated that surgical masks are considerably less efficient than filtering facepiece respirators against inert airborne particles.^{13–20} This is probably due to the fact that surgical masks are not designed to be tight-fitting, as artificially sealing surgical masks to the wearer's face can increase their efficiency.^{14,21}

The physical characteristics of inert aerosols and bioaerosols may be comparable, but extrapolation of inert particle data to infectious bioaerosols is complicated by many factors, including infectious dose, the amount of the organism present and its viability in particles of different sizes. Most studies using bioaerosols to evaluate the performance of RPDs and/or surgical masks have concentrated on the filtration efficiency of the material, rather than on the overall protection afforded to the wearer.^{22–25} Results of filtration efficiency alone do not account for facial seal leakage, i.e. how well the mask fits the wearer.

Facial seal leakage is essential to the overall performance of RPDs, but not to the overall performance of a surgical mask as a protective device (unless being used as an RPD).²⁶ There remains a lack of scientific evidence about the protective effect of surgical masks against infectious aerosols, in particular viruses, with reference to worker safety.

Given the recent interest in the control of pandemic spread, the aim of the following study was to develop a method to test the performance of surgical masks against a relevant viral bioaerosol challenge, namely influenza virus. The techniques were based upon standard approaches for testing the efficacy of RPDs against inert aerosols (i.e. BS EN 149:2001; HSE Operational Circular OC282/28) adapted for use with live virus bioaerosols.

Methods

Viruses and cell culture

An attenuated vaccine strain of A-type influenza virus (American Type Culture Collection A/PR/8/34; LGC Promochem Ltd, Teddington, UK) was used. This strain would be expected to have similar biophysical properties to H5N1 and H1N1 strains that have caused pandemic outbreaks. High titre stocks of virus

were grown on cultured Madin–Darby canine kidney cells (MDCK; European Collection of Cell Cultures, Health Protection Agency, Porton, UK) as previously described.²⁷ The approximate titre of influenza virus in the supernatant was monitored by haemagglutination assay (HA) using chicken red blood cells (TCS Biosciences, Buckingham, UK).²⁸ When the HA titre was at a maximum (usually three or four days post infection) cellular debris was removed from the crude virus preparation by centrifugation at 1000 g for 5 min. The supernatant containing the virus was removed and stored at -80°C .

Before use in surgical mask challenge studies, influenza virus was concentrated from the crude preparation by ultracentrifugation and the viral pellet re-suspended overnight at 4°C in phosphate-buffered saline (PBS; BioWhittaker, Wokingham, UK) containing 0.2% (w/v) Fraction V bovine serum albumin (BSA; Invitrogen, Paisley, UK), the titre being confirmed as above.

Surgical masks tested in the study

A selection of surgical masks from the UK National Health Service list of products was tested. The eight products chosen for testing were those that had the highest sales for the year 2005–2006. In addition to the highest-selling products, others were selected in order to cover the range of masks available, and those likely to be employed during an outbreak of influenza. Types of mask used included the typical double-strap tie mask (Figure 1A–E), 'duckbill' elasticated double-strap mask (Figure 1F–G), double-strap tie mask with integral splash visor (Figure 1C), and moulded mask with single elastic strap (Figure 1H).

Sampling inert particles

A dummy test head attached to a breathing simulator, as described in BS EN 136:1998²⁹ for the testing of RPDs, was used. The dummy head was positioned facing, and about 700 mm away from, a pulsed compressed air atomiser (manufactured in house at HSL) within a 1200 mm-wide class II microbiological safety cabinet (MSC; Figure 2).

Test surgical masks were prepared by inserting an airtight sample port into the mask to which a sampling tube was connected. They were then fitted to the dummy head, taking care to achieve the best fit possible as per manufacturer's guidelines. The MSC airflow was switched off and the front access port closed. The breathing simulator operated at an inhalation/exhalation rate of 40 L/min (stroke volume of $2.0\text{ L} \times 20$ cycles/min), which represents a low–medium human work rate (ISO 8996:2004).³⁰

A PortaCount Plus particle-counting device (TSI Instruments Ltd, High Wycombe, UK) measured the effectiveness (goodness-of-fit and filtration efficiency) of surgical masks against a generated aerosol. This device is used extensively in the UK and the USA for fit testing of tight-fitting RPD facepieces by comparing the particle concentrations inside and outside the facepiece.³¹

A 0.5 s pulsed aerosol spray of PBS and 0.2% BSA was synchronised with the inhalation breath. The concentration of particles in the air was measured over a sample period of 1 min using a PortaCount sampling tube immediately in front of the mask. This was compared with the concentration of particles in the air immediately behind the mask via a sampling tube that

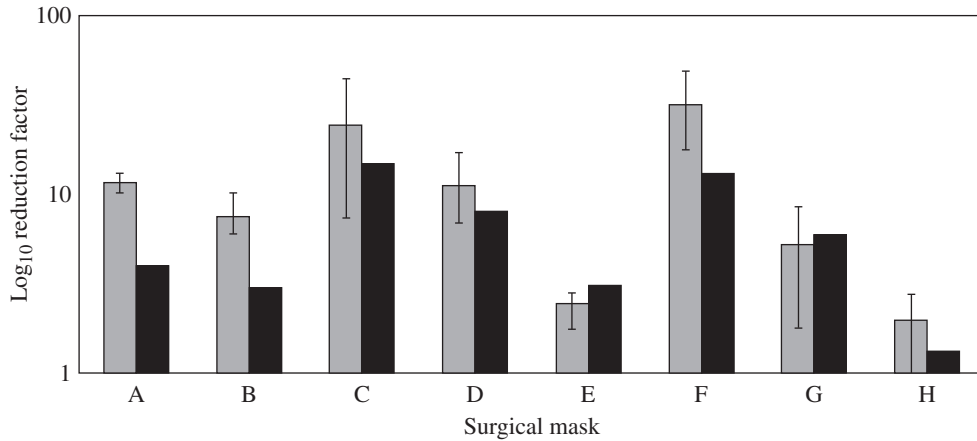


Figure 1. Mean (harmonic) influenza plaque reduction factors (grey bars) and associated inert particle reduction factors (black bars) for all surgical masks tested. Masks C, F and G were subjected to repeated testing. Error bars show 95% confidence interval.

was connected to the sample port in the mask. The fit of the surgical mask on the test head was adjusted so that it matched as far as was possible with the ‘best fit’, i.e. least leakage possible, obtained by a human volunteer.

The mean of the peak particle count both inside and outside the surgical mask for three sprays was calculated and used to

determine the reduction factor (RF). This is defined as the ratio of the particle concentration inside and outside the mask as shown below:

$$RF = \frac{\text{Particle concentration outside the mask}}{\text{Particle concentration inside the mask}}$$

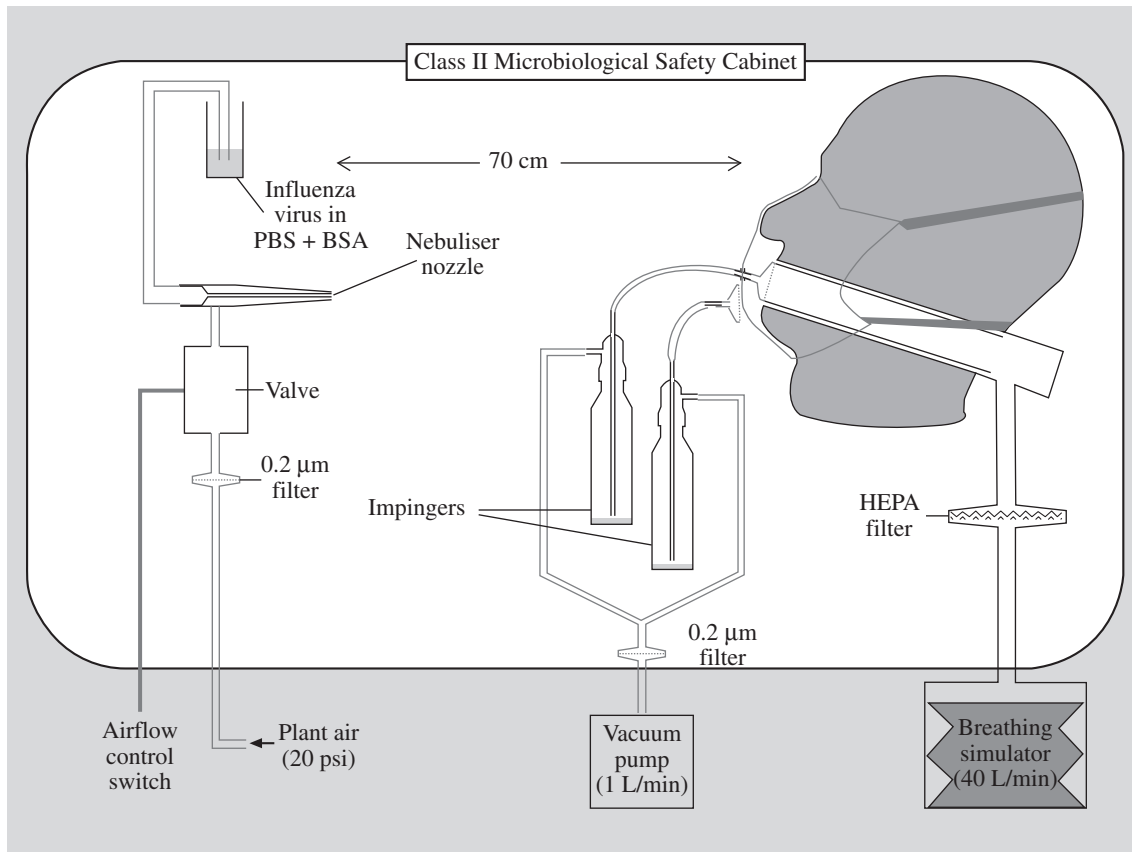


Figure 2. Influenza testing rig. For the inert particle-testing rig, the impingers and vacuum pump were replaced with a PortaCount and computer. PBS, phosphate-buffered saline; BSA, bovine serum albumin; HEPA, high-efficiency particulate air.

Influenza bioaerosol sampling

The MSC airflow was switched on to enable safe manipulation of the influenza test suspension. The atomiser was charged with 5 mL concentrated influenza virus test suspension (1×10^{12} to 1×10^{13} plaque-forming units/mL), prepared as described above, to generate an aerosol. Two midjet impingers (SKC Ltd, Blandford Forum, UK) aspirating at 2 L/min were used instead of the Portacount to sample air concurrently from in front of and inside the mask and to collect airborne particles directly into 750 μ L of virus transport medium (Eagle's minimum essential medium supplemented with 0.125% BSA, 25 mM HEPES, 100 units penicillin, 100 μ g streptomycin and 0.1 mM non-essential amino acids; Figure 2). The midjet impingers do not discriminate between particle sizes.

The MSC was switched off and sampling performed as described above, this time with a generated 0.5 s pulsed spray of influenza test suspension. The atomiser generated a poly-dispersed aerosol covering a size range $<1 \mu\text{m}$ to $>200 \mu\text{m}$, of which 50% were of a size $<60 \mu\text{m}$ and 15% $>100 \mu\text{m}$. Air was sampled for 1 min before the impingers and breathing simulator were switched off. The MSC airflow was switched back on, to remove residual bioaerosol particles and to permit safe handling of the samples. Liquid samples removed from the impingers were stored at -80°C until processed. Three separate air samples were taken inside and outside each mask. Similar air samples were taken without the influenza test suspension aerosol after bioaerosol sampling, to confirm the lack of cross-contamination between sampling runs.

The titre of influenza virus present in the air samples was determined by plaque dilution assay, as described previously.^{27,28} Monolayers of MDCK cells grown in six-well dishes, incubated at 35°C and 5% CO_2 were inoculated with 250 μL of neat sample or diluted samples. Inside- and outside-mask sample pairs were assayed using separate wells of the same dishes.

The ratio of live influenza virus sampled inside and outside masks determined the performance of the surgical masks, termed the influenza plaque reduction factor (IPRF):

$$\text{IPRF} = \frac{\text{Influenza virus titre of external air sample}}{\text{Influenza virus of internal air sample}}$$

Therefore, the IPRF is analogous to the RF measured for the inert particle tests calculated using the Portacount (except that for the inert particle tests, only the particle sizes within the Portacount's range were measured).

Results

Live influenza virus was recovered from the air in the breathing zone behind all the surgical masks tested, that is, no mask was able to completely prevent influenza virus entering the breathing zone of the dummy head under these experimental conditions. The inert particle reduction factors achieved with each mask varied greatly depending on the mask type, ranging from 1.3 to 20. The influenza plaque reduction factor also varied between masks, ranging between 1.1 and 55 (Figure 1). The performance of the surgical masks in the inert particle challenges corresponded to the performance against influenza virus bioaerosols – those surgical masks that produced a higher reduction in inert particles also demonstrated a

higher performance in the bioaerosol assays. The majority of the masks demonstrated that they would reduce the exposure to infectious influenza virus present in a direct challenge by around 10-fold on average. Notable exceptions included masks C and F, which performed best, and masks E and H performed least well in these tests. All negative controls were negative for the presence of influenza virus.

Discussion

The quantitative assay developed here measured only levels of viable virus extracted from the air immediately in front of and immediately behind the surgical mask. Masks were attached to a breathing dummy head to mimic, as closely as possible, a human wearer. This allowed for a realistic evaluation of the protective effect of surgical masks against a direct challenge with an influenza bioaerosol. The data could be compared directly to those obtained using inert particle challenges, which reflect standard testing procedures for RPDs.

Live, infectious virus was extracted from the air from behind all surgical masks tested. This suggests that influenza virus can survive in aerosol particles that are able to bypass/penetrate a surgical mask. Whether or not the surgical masks tested will offer adequate protection against infection from a viral pathogen will be dependent on the infectious dose of the virus, and its titre in secretions.

These tests were performed in a closed MSC. The proximity of the breathing dummy head and sampling apparatus to the atomiser was small ($\sim 700 \text{ mm}$) allowing greater direct interaction between the external sampler and the bioaerosol. It is likely that any bias in the sampling system would artificially increase the proportion of influenza virus recovered by the external sampler. This would result in a higher influenza plaque reduction factor and an enhanced perceived protective effect. The external sampler was positioned to face away from the direction of aerosol flow, i.e. facing the mask to reduce such bias as much as possible.

This study did not assess the relative protective capacity of surgical masks against aerosols versus large droplets, splashes and direct contact, but rather focused on their performance at protecting the wearer against a respiratory aerosol challenge. The samples taken during the influenza bioaerosol tests account for all particles that enter the samplers. Size fractions of the aerosol generated responsible for transmitting the virus were not determined, but this would be of importance for identifying influenza infection transmission and a key consideration for future work.

Of the surgical masks tested here, the performance of surgical mask C was consistently better than other surgical masks in the bioaerosol challenge. This mask has an integral visor, which may have offered additional protection to major leakage areas (e.g. around the nose), blocking the bioaerosol challenge sufficiently from reaching this area to improve overall protection. The use of a visor would also have the added value of protecting the eyes from large droplets/splashes and would discourage manual inoculation of the eyes via direct contact.

Research is needed to evaluate the ability of a visor to enhance the protective effect of a surgical mask. Further research is also required establish whether surgical masks confer adequate protection to the wearer against a bioaerosol challenge. This should also include investigating whether any

such protective effect is due to the respiratory protection afforded by the mask itself, or via minimising touch of oral/nasal mucosa with contaminated hands.

We have presented here a method, utilising standard virology techniques and RPD testing standards, to enable assessment of the protection afforded to the wearer by face-masks against a bioaerosol challenge.

Acknowledgements

The authors are grateful to Dr M. Trainor, Dr A. Curran and Dr G. Evans at the Health and Safety Laboratory for their support of the work. The authors also wish to thank C. Bailey for proofreading the manuscript.

Conflict of interest statement

None declared.

Funding sources

The Health and Safety Executive funded the scientific study work. The Health and Safety Laboratory Investment Research Programme supported manuscript preparation.

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