Inactivation of influenza viruses by coated respirators: *in-vitro* infectivity assays

Herbert N Prince[1], Karen Ramm[2], Daniel L Prince[1], S. Steve Zhou[3], Phillip J Yates[4], Phil Oths[5], Daniela Wlodarczak[5], Kimberly A Biedermann[5]*

[1]Gibraltar Laboratories, Fairfield, New Jersey, United States of America; [2]ATS Labs, Eagan, Minnesota, USA; [3]Microbiotest Division, Microbac, Sterling, Virginia, USA; [4]Clinical Virology, GlaxoSmithKline Medicines Research Centre, Stevenage, United Kingdom; [5]GSK Consumer Healthcare, Parsippany, New Jersey, USA

Background

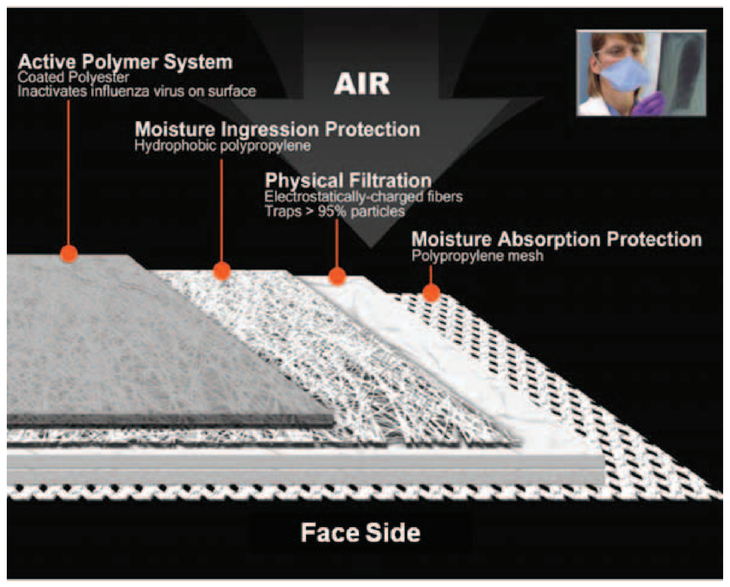
Strategies to prevent transmission of influenza include use of respirators. FFP2 and N95 respirators are certified to filter at least 95% of particles, and many guidelines recommend that healthcare workers wear these respirators to protect against infection from patients with pandemic influenza [WHO 2006; CDC 2006]. We have developed a proprietary acid-polymer formulation to coat a standard FFP2 respirator with an antiviral layer [WO 2008/009651 A1]. We aimed to test this coated respirator for antiviral efficacy against a range of influenza viruses. A series of tests compared the antiviral efficacy of coated and uncoated respirators in conditions designed to simulate real-life exposure to influenza by varying the route of inoculation, contact time, temperature, humidity, moisture, and contaminating substances. We also investigated whether infectious viruses could be transferred from contaminated respirator surfaces to gloves.

Abbreviations: FFP2=filtering facepiece certified in Europe to EN149:2001 + A1:2009; N95=certified in the USA by the National Institute for Occupational Safety and Health; $TCID_{50}$=tissue-culture infective dose (i.e. the viral load required to infect 50% of the cells); EID_{50}=embryo infective dose (i.e. the amount of virus that will infect 50% of inoculated eggs); MEM=minimum essential medium; FBS=fetal bovine serum; RMK cells= rhesus monkey kidney cells; MDCK cells=Madin-Darby canine kidney epithelial cells; HEPES= N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid; BSA=bovine serum albumin.

Materials and Methods

We tested human, swine, and avian influenza viruses, including the influenza A/H1N1 2009 pandemic strain, other influenza A subtypes (seasonal H1N1, H5N1, H3N2, H5N9, and H2N2) and influenza B viruses. Suspensions of influenza viruses were prepared to 4–8 $\log_{10}TCID_{50}$/mL in MEM. In some tests, organic contaminants (yeast, BSA, and mucin) were added [OECD 2009]. One set of respirators was maintained at 40°C and 75% relative humidity for 24 hours before viral challenge, and repeatedly sprayed with HEPES buffer to simulate respiratory secretions [GSK 2009; ATS Labs GSK01070208 2008]. For each test, three coated (GlaxoSmithKline *Actiprotect*®) and three uncoated (Sperian Willson *Easy Fit*®) FFP2 respirator samples were inoculated with 0.2 mL of a viral suspension, which was either applied with a pipette, sprayed, or aerosolised to create airborne droplets [OECD 2009]. After 1 minute at room temperature (on a shaker), the respirator samples were assayed for the presence of infectious viruses. In one test, nitrile gloves were applied with light pressure to the outer surface of inoculated respirator samples, and then assayed after 1 minute [GSK USNPD0132009; Gibraltar Labs R216131 2009]. Samples were put into test medium (MEM, supplemented with antibiotics [penicillin, gentamycin, or streptomycin] and amphotericin B or L-glutamine). The supernatants were vortexed, extracted, and used to prepare serial ten-fold dilutions in MEM [OECD 2009]. Each dilution was used to inoculate four wells of RMK cells in a multi-well plate, and these cultures were incubated and scored over 7 days for cytopathic effects, cytotoxicity, and viability. (Some tests substituted MDCK cells; others used inoculated embyonated chick eggs.) All tests included negative cell controls, cytotoxicity controls, and neutralisation controls. The Spearman-Karber formula was used to calculate viral loads as $TCID_{50}$ or EID_{50} [OECD 2009]. Antiviral efficacy was calculated from the difference between the geometric mean loads of influenza virus on the coated and uncoated respirators after 1 minute of exposure.

Figure 1: Composition of the functional layers of the *Actiprotect*® respirator



Results:

The viral loads applied to respirators in these experiments ranged from 5.5 to 8.1 $\log_{10}TCID_{50}$, and were therefore high in comparison with respiratory secretions from infected patients at the peak of influenza symptoms (range 3–7 $\log_{10}TCID_{50}$) [WHO 2006]. Tables 1–3 show that the average viral loads detected on uncoated FFP2 respirator samples remained high in all conditions tested, ranging from 3.2 to 6.9 $\log_{10}TCID_{50}$ (or 4.5–5.0 $\log_{10}EID_{50}$). In contrast, the average viral load on coated respirators after 1 minute of exposure ranged from below the limits of detection to 3.4 $\log_{10}TCID_{50}$ (1.5 $\log_{10}EID_{50}$). Therefore, the antiviral efficacy of the coating ranged from >2.7 (exceeded limits of detection) to 6.4 $\log_{10}$, which equates to surface inactivation of >99.8% to 99.99996% of viruses.

Table 1: Antiviral activity in the presence or absence of organic contaminants

	Uncoated respirator masks	Coated respirator masks	Mean relative reduction in viral load
Viral load	5.5	≤0.7*	≥4.8 $\log_{10}$
Viral load with organic contaminants	6.1	0.7	5.3 $\log_{10}$

Influenza subtype was A/H5N1, and strain was VNH5N1-PR8/CDC-RG. Results are mean $\log_{10}TCID_{50}$, unless specified otherwise. *Limit of detection was 0.7 $\log_{10}TCID_{50}$. [ATS Labs 01012308 2008; ATS Labs 01052308 2008]

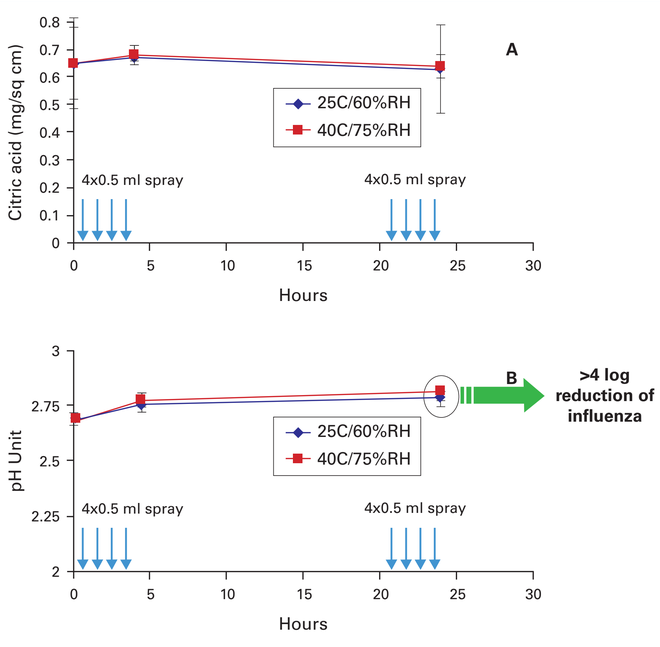


Table 2: Antiviral activity against different types and subtypes of influenza virus

Influenza subtype	Influenza strain	Uncoated respirator masks	Coated respirator masks	Mean relative reduction in viral load
A/H1N1	NYMC-X-179A+	5.2	≤1.5	≥3.7 $\log_{10}$ (≥99.9%)
A/H1N1	A/Mexico/4108/2009+	6.7	≤0.9	≥5.8 $\log_{10}$ (≥99.9998%)
A/H1N1	A/swine/Iowa/15/30	5.6	Not detected*	≥5.1 $\log_{10}$ (≥99.999%)
A/H5N1	VNH5N1-PR8/CDC-RG	4.8	≤0.5	≥4.3 $\log_{10}$ (≥99.995%)
A/H1N1	JPN/35/2007	6.5	≤1.4	≥ 5.1 $\log_{10}$ (≥99.999%)
A/H1N1	JPN/36/2007	6.7	Not detected*	≥ 6.2 $\log_{10}$ (≥99.99994%)
A/H3N2	JPN/12/2007	6.9	Not detected*	≥ 6.4 $\log_{10}$ (≥ 99.99996%)
A/H3N2	JPN/31/2007	5.1	Not detected*	≥4.6 $\log_{10}$ (≥99.997%)
B	JPN/128/2007	5.2	≤0.8	≥4.4 $\log_{10}$ (≥99.997%)
B	JPN/143/2007	5.7	≤0.8	≥4.9 $\log_{10}$ (≥99.9987%)
B	JPN/85/2007	5.2	≤0.9	≥ 4.3 $\log_{10}$ (≥99.995%)
B	JPN/115/2007	3.2	Not detected*	≥ 2.7 $\log_{10}$ (≥99.8%)

Results are mean $\log_{10}TCID_{50}$, unless specified otherwise, based on an infectivity assay in triplicate. +Represents 2009 pandemic strains. *Unless otherwise stated, the limit of detection was 0.5 $\log_{10}TCID_{50}$. [ATS Labs GSK01112408 2009; GSK 000056 2009; ATS Labs GSK01111209 2009; GSK 0000400 2009; ATS Labs GSK01042809 2009]

Table 3: Antiviral activity of coated mask against different subtypes of influenza A virus

Influenza subtype	Influenza strain	Uncoated respirator masks	Coated respirator masks	Mean relative reduction in viral load
A/H5N9	TurkeyA/Wisc/68	5.0	Not detected*	≥4.5 $\log_{10}$ (≥99.997%)
A/H5N9	MynaA/Mass/71	5.0	Not detected*	≥4.5 $\log_{10}$ (≥99.997%)
A/H2N2	A2/JP/305/57	5.0	Not detected*	≥4.5 $\log_{10}$ (≥99.997%)
A/H3N2	Hong Kong 8/68	5.0	Not detected*	≥4.5 $\log_{10}$ (≥99.997%)

Results are mean $\log_{10}EID_{50}$, unless specified otherwise, based on a haemagglutinin assay in duplicate. *Limit of detection was 1.0 $\log_{10}EID_{50}$. [Gibraltar Labs R211219 2009; Gibraltar Labs 200141RO0 2008]

Gloves applied to uncoated respirators had a viral load of 3.5 $\log_{10}EID_{50}$; none was detected on gloves applied to coated respirators [GSK USNPD013 2009; Gibraltar Labs R216131 2009]. The relative reduction in contamination was therefore >2.5 $\log_{10}$.

Figure 2: Durability of the antiviral coating after multiple challenges with simulated respiratory secretions

After repeated sprays with simulated nasal secretions (8x /24 h) at 40°C/75% RH, the coating retained effective citric acid levels (Panel A) and pH (Panel B). When challenged with influenza virus, the coated surface showed > 4 log reduction in viral titers, compared to uncoated material.

Table 4: Viral load of influenza A on mask surface and glove surface

	Uncoated respirator masks	Coated respirator masks	Mean relative reduction in viral load
Baseline load	6.3 ± 0.3	Not detected*	≥5.3 $\log_{10}$
Viral load transferred to glove	3.5	Not detected*	≥2.5 $\log_{10}$

Influenza subtype was A/H3N2, and strain was A/Hong Kong/68. Results are mean $\log_{10}EID_{50}$, unless specified otherwise. *Limit of detection was 1.0 $\log_{10}EID_{50}$. [GSK USNPD013 2009; Gibraltar Labs R216131 2009]

Conclusions

The coated respirators inactivated a broad range of influenza strains within 1 minute, including the 2009 pandemic strain and human, swine, and avian influenza viruses. Antiviral effectiveness was not reduced by hot, humid conditions or repeated saturation, which might occur during prolonged use of respirators. In contrast, infectious virions were detected on the surfaces of all uncoated FFP2 respirators, and could be transferred to glove surfaces during handling of contaminated masks.

Acknowledgments

We thank all authors for their participation in the data analysis and reporting stage of this manuscript. All authors have approved the final version. The studies were funded by GSK Consumer Healthcare, and GSK investigators were involved in all stages of the study conduct and analysis.

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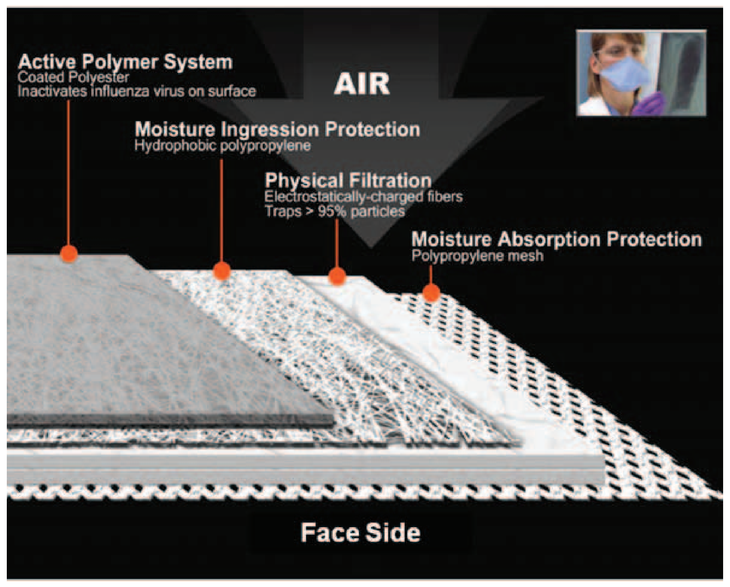

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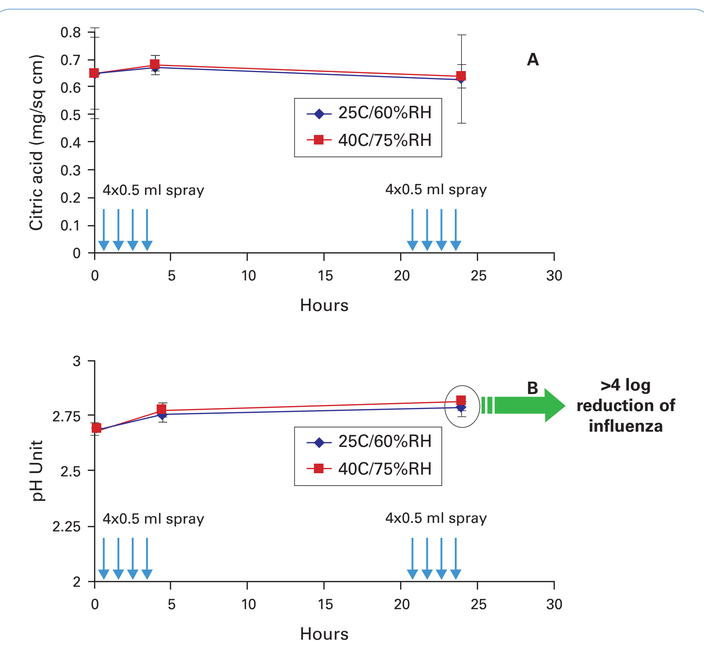

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A/H3N2	JPN/12/2007	6.9	Not detected*	≥ 6.4 $\log_{10}$ (≥ 99.99996%)
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