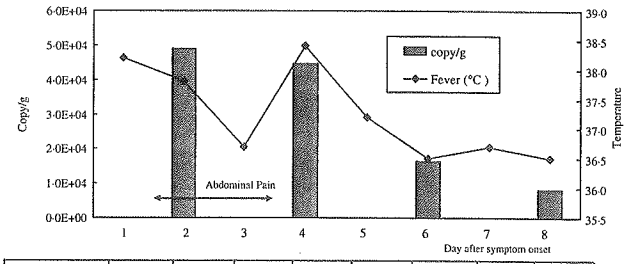
Table 1. Results of AH1pdm influenza virus detection and isolation in case 1

Days after symptom onset	1	2	3	4	5	6
Rapid antigen test result	+		+		−	
Collection of nasal swab						
Virus isolation (MDCK)		+				
Real-time RT-PCR Ct		33·42				
Collection of stool specimen						
Virus isolation (MDCK)		+		−		ND
Real-time RT-PCR Ct		33·92		37·99		39·22

MDCK, Madin–Darby canine kidney.



Negative strand RNA[1)]		+		+		+		-
Positive strand RNA[2)]		-		-		+		-
Ttal RNA[3)]		+		+		+		+

1) Negative stranded RNA ; genomic RNA 2) Positive stranded RNA ; replicating RNA, mRNA
3) Total RNA ; +/-

Figure 1. Persistent viral shedding of AH1pdm in stool specimens and results of strand-specific RT-nested PCR.

symptom onset. However, viral shedding was still present 8 days after symptom onset. Positive stranded RNA was detected 6 days after symptom onset from the stool specimen (Figure 1). Above two AH1pdm strains (isolated from nasal swab and stool specimen) bound exclusively to human type receptor, Neu5Acα2-6Gal. Sequence analysis demonstrated that isolated virus from stool samples was identical with that from nasal swabs in comparison of HA gene (990 bp).

Discussion

AH1pdm influenza virus was isolated from the stool and nasal swab samples in the same patient simultaneously by using MDCK cells. Our results suggests the detection of viral RNA and viable AH1pdm influenza virus from stool samples may serve as a potential mode of transmission and has important implications in understanding the context of AH1pdm influenza virus.

References

1 Dilantika C, Sedyaningsih ER, Kasper MR *et al.* Influenza virus infection among pediatric patients reporting diarrhea and influenza-like illness. BMC Infect Dis 2010; 10:3.

2 Tamura D, Fujino M, Ozama M *et al.* Significance of Seasonal Influenza Viruses in the Stool of Pediatric Patients. Pediatr Infect Dis J 2010; 29:578–579.

3 To KK, Chan KH, Li IW *et al.* Viral load in patients infected with pandemic H1N1 2009 influenza A virus. J Med Virol 2010; 82:1–7.

4 Yamada S, Yamada S, Suzuki Y, Sizuki T *et al.* Haemagglutinin mutations responsible for the binding of H5N1 influenza A viruses to human-type receptors. Nature 2006; 444:378–382.

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

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Keywords Acid polymers, Actiprotect, antiviral coating, antiviral masks, infectivity assay, respirators.

Please cite this paper as: Prince *et al.* (2011) Inactivation of influenza viruses by coated respirators: *in vitro* infectivity assays. Influenza and Other Respiratory Viruses 5 (Suppl. 1), 301–327.

Introduction

Strategies to prevent transmission of influenza include use of respirators. FFP2 and N95 respirators are certified to filter at least 95% of particles (0·3 μm in diameter), and many guidelines have recommended that healthcare workers wear respirators in certain healthcare settings to protect against infection from patients with pandemic influenza.[1–3]

We have developed a proprietary acid-polymer formulation to coat a standard FFP2 respirator with an antiviral layer. 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.

Materials and methods

We tested human, swine, and avian influenza viruses, including influenza A and B viruses. Influenza A subtypes were the A/H1N1 2009 pandemic strain, seasonal H1N1, H5N1, H3N2, H5N9, and H2N2. In each test, 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. One set of respirators was maintained at 40°C and 75% relative humidity for 24 hours before the viral challenge, and repeatedly sprayed with HEPES buffer to simulate respiratory secretions.

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 applied with a pipette, sprayed, or aerosolised to create airborne droplets. After 1 minute at room temperature (on a shaker), the respirator samples were assayed for the presence of infectious viruses using standard methods.[4] In one test, after a 1 minute contact time of the respirator with the virus, nitrile gloves were applied with light pressure to the outer surface of inoculated respirator samples and then assayed after 1 minute.

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 10-fold dilutions in MEM. 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 embryonated 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}.[4] 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.

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 $\log_{10}$ $TCID_{50}$).[1] Tables 1–2 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 ≤1·5 $\log_{10}$ $TCID_{50}$ (1·0 $\log_{10}$ EID_{50}). Therefore, the relative antiviral efficacy of the coating ranged from ≥2·7 to 6·4 $\log_{10}$. Table 1 shows that the relative antiviral efficacy of the coated mask remained high in simulated-use conditions such as organic contaminants and repeated saturation at high temperature and humidity.

In the experiment to test transfer of viruses from respirators, the gloves applied to regular uncoated inoculated respirators had a viral load of 3·5 $\log_{10}$ EID_{50} (Table 1). By contrast, no viruses were detected on either the coated respirators or the gloves applied to them. The relative reduction in contamination was therefore ≥2·5 $\log_{10}$.

Table 1. Viral loads on respirator surfaces

	Uncoated respirators	Coated respirators	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 $\log_{10}$
Viral load after heat, moisture, and simulated secretions**	5·1	0·8	4·3 $\log_{10}$
Viral load transferred to glove**	3·5	≤1·0	≥2·5 $\log_{10}$ EID_{50}

*Influenza subtype was A/H5N1, and strain was VNH5N1-PR8/CDC-RG.
**Influenza subtype was A/H3N2, and the strain was Hong Kong/8/68. Results are mean $\log_{10}$ $TCID_{50}$, unless specified otherwise.

Table 2. Antiviral activity against different types and subtypes of influenza

Influenza subtype	Influenza strain	Uncoated respirators	Coated respirators	Mean relative reduction in viral load
A/H1N1	NYMC-X-179A*	5·2	≤1·5	≥3·7 $\log_{10}$
A/H1N1	Mexico/4108/2009*	6·7	≤0·9	≥5·8 $\log_{10}$
A/H1N1	Swine/Iowa/15/30	5·6	≤0·5	≥5·1 $\log_{10}$
A/H1N1	JPN/35/2007	6·5	≤1·4	≥5·1 $\log_{10}$
A/H1N1	JPN/36/2007	6·7	≤0·5	≥6·2 $\log_{10}$
A/H2N2	A2/JP/305/57	5·0**	≤1·0**	≥4·0 $\log_{10}$
A/H3N2	JPN/12/2007	6·9	≤0·5	≥6·4 $\log_{10}$
A/H3N2	JPN/31/2007	5·1	≤0·5	≥4·6 $\log_{10}$
A/H3N2	Hong Kong 8/68	5·0**	≤1·0**	≥4·0 $\log_{10}$
A/H5N1	VNH5N1-PR8/CDC-RG	4·8	≤0·5	≥4·3 $\log_{10}$
A/H5N9	TurkeyA/Wisc/68	5·0**	≤1·0**	≥4·0 $\log_{10}$
A/H5N9	MynaA/Mass/71	5·0**	≤1·0**	≥4·0 $\log_{10}$
B	JPN/128/2007	5·2	≤0·8	≥4·4 $\log_{10}$
B	JPN/143/2007	5·7	≤0·8	≥4·9 $\log_{10}$
B	JPN/85/2007	5·2	≤0·9	≥4·3 $\log_{10}$
B	JPN/115/2007	3·2	≤0·5	≥2·7 $\log_{10}$

Results are mean $\log_{10}$ $TCID_{50}$, unless specified otherwise, based on an infectivity assay in triplicate. Limits of detection varied.
*2009 pandemic strains.
**Results are mean $\log_{10}$ EID_{50}, based on a haemagglutinin assay in duplicate.

Discussion

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.

Acknowledgements

We thank all authors for their participation in data gathering and analysis, and in writing this manuscript. The studies were funded by GSK Consumer Healthcare, and GSK investigators were involved in all stages of the study conduct and analysis.

Disclosure

PY, PO, DW and KB are employees of GlaxoSmithKline. This study was funded by GlaxoSmithKline.

References

1 World Health Organization Writing Group. Nonpharmaceutical interventions for pandemic influenza, international measures. Emerg Infect Dis 2006; 12:81–87 Available at http://www.cdc.gov/ncidod/EID/vol12no01/05-1370.htm (Accessed 22 April 2010).
2 World Health Organization. Avian influenza, including influenza A (H5N1), in humans: WHO interim infection control guideline for health care facilities. Revised February 2006. Available at http://www.wpro.who.int/NR/rdonlyres/177DBE1C-4E3C-4C7E-B784-4124E6C600CF/0/InfectionControl.pdf (Accessed 22 April 2010).
3 Weber TP, Stilianakis NI. Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. J Infect 2008; 57:361–373.
4 Organisation for Economic Co-operation and Development. Quantitative Method for Evaluating Virucidal Activity of Biocides Used on Hard Surfaces. OECD. December 2009 draft, 2009.