Journal of Hospital Infection 104 (2020) 246–251

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Review

Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents

G. Kampf [a,*], D. Todt [b], S. Pfaender [b], E. Steinmann [b]

[a] University Medicine Greifswald, Institute for Hygiene and Environmental Medicine, Ferdinand-Sauerbruch-Straße, 17475 Greifswald, Germany
[b] Department of Molecular and Medical Virology, Ruhr University Bochum, Universitätsstrasse 50, 44801 Bochum, Germany

ARTICLE INFO

Article history:
Received 31 January 2020
Accepted 31 January 2020
Available online 6 February 2020

Keywords:
Coronavirus
Persistence
Inanimate surfaces
Chemical inactivation
Biocidal agents
Disinfection

SUMMARY

Currently, the emergence of a novel human coronavirus, SARS-CoV-2, has become a global health concern causing severe respiratory tract infections in humans. Human-to-human transmissions have been described with incubation times between 2-10 days, facilitating its spread via droplets, contaminated hands or surfaces. We therefore reviewed the literature on all available information about the persistence of human and veterinary coronaviruses on inanimate surfaces as well as inactivation strategies with biocidal agents used for chemical disinfection, e.g. in healthcare facilities. The analysis of 22 studies reveals that human coronaviruses such as Severe Acute Respiratory Syndrome (SARS) coronavirus, Middle East Respiratory Syndrome (MERS) coronavirus or endemic human coronaviruses (HCoV) can persist on inanimate surfaces like metal, glass or plastic for up to 9 days, but can be efficiently inactivated by surface disinfection procedures with 62–71% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite within 1 minute. Other biocidal agents such as 0.05–0.2% benzalkonium chloride or 0.02% chlorhexidine digluconate are less effective. As no specific therapies are available for SARS-CoV-2, early containment and prevention of further spread will be crucial to stop the ongoing outbreak and to control this novel infectious thread.

Introduction

A novel coronavirus (SARS-CoV-2) has recently emerged from China with a total of 45171 confirmed cases of pneumonia (as of February 12, 2020) [1]. Together with Severe Acute Respiratory Syndrome (SARS) coronavirus and Middle East Respiratory Syndrome (MERS) coronavirus [2], this is the third highly pathogenic human coronavirus that has emerged in the last two decades. Person-to-person transmission has been described both in hospital and family settings [3]. It is therefore of utmost importance to prevent any further spread in the public and healthcare settings. Transmission of coronaviruses from contaminated dry surfaces has been postulated including self-inoculation of mucous membranes of the nose, eyes or mouth [4,5], emphasizing the importance of a detailed understanding of coronavirus persistence on inanimate surfaces [6]. Various types of biocidal agents such as hydrogen peroxide, alcohols, sodium hypochlorite or benzalkonium chloride are used worldwide for disinfection, mainly in healthcare settings [7]. The aim of the review was therefore to summarize all available data on the persistence of all coronaviruses including emerging SARS-CoV and MERS-CoV as well as veterinary coronaviruses such as transmissible gastroenteritis virus (TGEV), mouse hepatitis virus (MHV) and canine coronavirus (CCV) on different types of

* Corresponding author.
E-mail address: guenter.kampf@uni-greifswald.de (G. Kampf).

https://doi.org/10.1016/j.jhin.2020.01.022

inanimate surfaces and on the efficacy of commonly used biocidal agents used in surface disinfectants against coronaviruses.

Method

A Medline search has been done on January 28, 2020. The following terms were used, always in combination with "coronavirus", "TGEV", "MHV" or "CCV": survival surface (88 / 10 / 25 / 0 hits), persistence surface (47 / 1 / 32 / 0 hits), persistence hand (8 / 0 / 3 / 0 hits), survival hand (22 / 0 / 3 / 1 hits), survival skin (8 / 0 / 0 / 1 hits), persistence skin (1 / 0 / 0 / 1 hit), virucidal (23 / 3 / 3 / 1 hits), chemical inactivation (33 / 0 / 6 / 1), suspension test (18 / 0 / 0 / 0 hits) and carrier test (17 / 4 / 0 / 0 hits). Publications were included and results were extracted given they provided original data on coronaviruses on persistence (surfaces, materials) and inactivation by biocidal agents used for disinfection (suspension tests, carrier tests, fumigation studies). Data with commercial products based on various different types of biocidal agents were excluded. Reviews were not included, but screened for any information within the scope of this review.

Results

Persistence of coronavirus on inanimate surfaces

Most data were described with the endemic human coronavirus strain (HCoV-) 229E. On different types of materials it can remain infectious for from 2 hours up to 9 days. A higher temperature such as 30°C or 40°C reduced the duration of persistence of highly pathogenic MERS-CoV, TGEV and MHV. However, at 4°C persistence of TGEV and MHV can be increased to $\geq$ 28 days. Few comparative data obtained with SARS-CoV indicate that persistence was longer with higher inocula (Table I). In addition it was shown at room temperature that HCoV-229E persists better at 50% compared to 30% relative humidity [8].

Inactivation of coronaviruses by biocidal agents in suspension tests

Ethanol (78–95%), 2-propanol (70–100%), the combination of 45% 2-propanol with 30% 1-propanol, glutardialdehyde (0.5–2.5%), formaldehyde (0.7–1%) and povidone iodine

Table I
Persistence of coronaviruses on different types of inanimate surfaces

Type of surface	Virus	Strain / isolate	Inoculum (viral titer)	Temperature	Persistence	Reference
Steel	MERS-CoV	Isolate HCoV-EMC/2012	10^5	20°C	48 h	[21]
				30°C	8–24 h	
	TGEV	Unknown	10^6	4°C	$\geq$ 28 d	[22]
				20°C	3–28 d	
				40°C	4–96 h	
	MHV	Unknown	10^6	4°C	$\geq$ 28 d	[22]
				20°C	4–28 d	
				40°C	4–96 h	
	HCoV	Strain 229E	10^3	21°C	5 d	[23]
Aluminium	HCoV	Strains 229E and OC43	5 x 10^3	21°C	2–8 h	[24]
Metal	SARS-CoV	Strain P9	10^5	RT	5 d	[25]
Wood	SARS-CoV	Strain P9	10^5	RT	4 d	[25]
Paper	SARS-CoV	Strain P9	10^5	RT	4–5 d	[25]
	SARS-CoV	Strain GVU6109	10^6	RT	24 h	[26]
			10^5		3 h	
			10^4		< 5 min	
Glass	SARS-CoV	Strain P9	10^5	RT	4 d	[25]
	HCoV	Strain 229E	10^3	21°C	5 d	[23]
Plastic	SARS-CoV	Strain HKU39849	10^5	22°-25°C	$\leq$ 5 d	[27]
	MERS-CoV	Isolate HCoV-EMC/2012	10^5	20°C	48 h	[21]
				30°C	8–24 h	
	SARS-CoV	Strain P9	10^5	RT	4 d	[25]
	SARS-CoV	Strain FFM1	10^7	RT	6–9 d	[28]
	HCoV	Strain 229E	10^7	RT	2–6 d	[28]
PVC	HCoV	Strain 229E	10^3	21°C	5 d	[23]
Silicon rubber	HCoV	Strain 229E	10^3	21°C	5 d	[23]
Surgical glove (latex)	HCoV	Strains 229E and OC43	5 x 10^3	21°C	$\leq$ 8 h	[24]
Disposable gown	SARS-CoV	Strain GVU6109	10^6	RT	2 d	[26]
			10^5		24 h	
			10^4		1 h	
Ceramic	HCoV	Strain 229E	10^3	21°C	5 d	[23]
Teflon	HCoV	Strain 229E	10^3	21°C	5 d	[23]

MERS = Middle East Respiratory Syndrome; HCoV = human coronavirus; TGEV = transmissible gastroenteritis virus; MHV = mouse hepatitis virus; SARS = Severe Acute Respiratory Syndrome; RT = room temperature.

Table II
Inactivation of coronaviruses by different types of biocidal agents in suspension tests

Biocidal agent	Concentration	Virus	Strain / isolate	Exposure time	Reduction of viral infectivity (log_{10})	Reference
Ethanol	95%	SARS-CoV	Isolate FFM-1	30 s	≥ 5.5	[29]
	85%	SARS-CoV	Isolate FFM-1	30 s	≥ 5.5	[29]
	80%	SARS-CoV	Isolate FFM-1	30 s	≥ 4.3	[29]
	80%	MERS-CoV	Strain EMC	30 s	> 4.0	[14]
	78%	SARS-CoV	Isolate FFM-1	30 s	≥ 5.0	[28]
	70%	MHV	Strains MHV-2 and MHV-N	10 min	> 3.9	[30]
	70%	CCV	Strain I-71	10 min	> 3.3	[30]
2-Propanol	100%	SARS-CoV	Isolate FFM-1	30 s	≥ 3.3	[28]
	75%	SARS-CoV	Isolate FFM-1	30 s	≥ 4.0	[14]
	75%	MERS-CoV	Strain EMC	30 s	≥ 4.0	[14]
	70%	SARS-CoV	Isolate FFM-1	30 s	≥ 3.3	[28]
	50%	MHV	Strains MHV-2 and MHV-N	10 min	> 3.7	[30]
	50%	CCV	Strain I-71	10 min	> 3.7	[30]
2-Propanol and 1-propanol	45% and 30%	SARS-CoV	Isolate FFM-1	30 s	≥ 4.3	[29]
		SARS-CoV	Isolate FFM-1	30 s	≥ 2.8	[28]
Benzalkonium chloride	0.2%	HCoV	ATCC VR-759 (strain OC43)	10 min	0.0	[31]
	0.05%	MHV	Strains MHV-2 and MHV-N	10 min	> 3.7	[30]
	0.05%	CCV	Strain I-71	10 min	> 3.7	[30]
	0.00175%	CCV	Strain S378	3 d	3.0	[32]
Didecyldimethyl ammonium chloride	0.0025%	CCV	Strain S378	3 d	> 4.0	[32]
Chlorhexidine digluconate	0.02%	MHV	Strains MHV-2 and MHV-N	10 min	0.7–0.8	[30]
	0.02%	CCV	Strain I-71	10 min	0.3	[30]
Sodium hypochlorite	0.21%	MHV	Strain MHV-1	30 s	≥ 4.0	[33]
	0.01%	MHV	Strains MHV-2 and MHV-N	10 min	2.3–2.8	[30]
	0.01%	CCV	Strain I-71	10 min	1.1	[30]
	0.001%	MHV	Strains MHV-2 and MHV-N	10 min	0.3–0.6	[30]
	0.001%	CCV	Strain I-71	10 min	0.9	[30]
Hydrogen peroxide	0.5%	HCoV	Strain 229E	1 min	> 4.0	[34]
Formaldehyde	1%	SARS-CoV	Isolate FFM-1	2 min	> 3.0	[28]
	0.7%	SARS-CoV	Isolate FFM-1	2 min	> 3.0	[28]
	0.7%	MHV		10 min	> 3.5	[30]
	0.7%	CCV	Strain I-71	10 min	> 3.7	[30]
	0.009%	CCV		24 h	> 4.0	[35]
Glutardialdehyde	2.5%	SARS-CoV	Hanoi strain	5 min	> 4.0	[36]
	0.5%	SARS-CoV	Isolate FFM-1	2 min	> 4.0	[28]
Povidone iodine	7.5%	MERS-CoV	Isolate HCoV-EMC/2012	15 s	4.6	[37]
	4%	MERS-CoV	Isolate HCoV-EMC/2012	15 s	5.0	[37]
	1%	SARS-CoV	Hanoi strain	1 min	> 4.0	[36]
	1%	MERS-CoV	Isolate HCoV-EMC/2012	15 s	4.3	[37]
	0.47%	SARS-CoV	Hanoi strain	1 min	3.8	[36]
	0.25%	SARS-CoV	Hanoi strain	1 min	> 4.0	[36]
	0.23%	SARS-CoV	Hanoi strain	1 min	> 4.0	[36]
	0.23%	SARS-CoV	Isolate FFM-1	15 s	≥ 4.4	[38]
	0.23%	MERS-CoV	Isolate HCoV-EMC/2012	15 s	≥ 4.4	[38]

SARS = Severe Acute Respiratory Syndrome; MERS = Middle East Respiratory Syndrome; MHV = mouse hepatitis virus; CCV = canine coronavirus; HCoV = human coronavirus.

Table III
Inactivation of coronaviruses by different types of biocidal agents in carrier tests

Biocidal agent	Concentration	Virus	Strain / isolate	Volume / material	Organic load	Exposure time	Reduction of viral infectivity (log_{10})	Reference
Ethanol	71%	TGEV	Unknown	50 µl / stainless steel	None	1 min	3.5	[39]
	71%	MHV	Unknown	50 µl / stainless steel	None	1 min	2.0	[39]
	70%	TGEV	Unknown	50 µl / stainless steel	None	1 min	3.2	[39]
	70%	MHV	Unknown	50 µl / stainless steel	None	1 min	3.9	[39]
	70%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	> 3.0	[40]
	62%	TGEV	Unknown	50 µl / stainless steel	None	1 min	4.0	[39]
	62%	MHV	Unknown	50 µl / stainless steel	None	1 min	2.7	[39]
Benzalkoniumchloride	0.04%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	< 3.0	[40]
Sodium hypochlorite	0.5%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	> 3.0	[40]
	0.1%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	> 3.0	[40]
	0.06%	TGEV	Unknown	50 µl / stainless steel	None	1 min	0.4	[39]
	0.06%	MHV	Unknown	50 µl / stainless steel	None	1 min	0.6	[39]
	0.01%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	< 3.0	[40]
Glutardialdehyde	2%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	> 3.0	[40]
Ortho-phtalaldehyde	0.55%	TGEV	Unknown	50 µl / stainless steel	None	1 min	2.3	[39]
	0.55%	MHV	Unknown	50 µl / stainless steel	None	1 min	1.7	[39]
Hydrogen peroxide	Vapor of unknown concentration	TGEV	Purdue strain type 1	20 µl / stainless steel	None	2–3 h	4.9–5.3*	[41]

TGEV = transmissible gastroenteritis virus; MHV = mouse hepatitis virus; HCoV = human coronavirus; *depending on the volume of injected hydrogen peroxide.

(0.23—7.5%) readily inactivated coronavirus infectivity by approximately 4 $\log_{10}$ or more. (Table II). Sodium hypochlorite required a minimal concentration of at least 0.21% to be effective. Hydrogen peroxide was effective with a concentration of 0.5% and an incubation time of 1 min. Data obtained with benzalkonium chloride at reasonable contact times were conflicting. Within 10 min a concentration of 0.2% revealed no efficacy against coronavirus whereas a concentration of 0.05% was quite effective. 0.02% chlorhexidine digluconate was basically ineffective (Table II).

Inactivation of coronaviruses by biocidal agents in carrier tests

Ethanol at concentrations between 62% and 71% reduced coronavirus infectivity within 1 min exposure time by 2.0—4.0 $\log_{10}$. Concentrations of 0.1—0.5% sodium hypochlorite and 2% glutardialdehyde were also quite effective with $>$ 3.0 $\log_{10}$ reduction in viral titre. In contrast, 0.04% benzalkonium chloride, 0.06% sodium hypochlorite and 0.55% ortho-phtalaldehyde were less effective (Table III).

Discussion

Human coronaviruses can remain infectious on inanimate surfaces at room temperature for up to 9 days. At a temperature of 30°C or more the duration of persistence is shorter. Veterinary coronaviruses have been shown to persist even longer for 28 d. Contamination of frequent touch surfaces in healthcare settings are therefore a potential source of viral transmission. Data on the transmissibility of coronaviruses from contaminated surfaces to hands were not found. However, it could be shown with influenza A virus that a contact of 5 s can transfer 31.6% of the viral load to the hands [9]. The transfer efficiency was lower (1.5%) with parainfluenza virus 3 and a 5 s contact between the surface and the hands [10]. In an observational study, it was described that students touch their face with their own hands on average 23 times per h, with contact mostly to the skin (56%), followed by mouth (36%), nose (31%) and eyes (31%) [11]. Although the viral load of coronaviruses on inanimate surfaces is not known during an outbreak situation it seem plausible to reduce the viral load on surfaces by disinfection, especially of frequently touched surfaces in the immediate patient surrounding where the highest viral load can be expected. The WHO recommends "to ensure that environmental cleaning and disinfection procedures are followed consistently and correctly. Thoroughly cleaning environmental surfaces with water and detergent and applying commonly used hospital-level disinfectants (such as sodium hypochlorite) are effective and sufficient procedures." [12] The typical use of bleach is at a dilution of 1:100 of 5% sodium hypochlorite resulting in a final concentration of 0.05% [13]. Our summarized data with coronaviruses suggest that a concentration of 0.1% is effective in 1 min (Table III). That is why it seems appropriate to recommend a dilution 1:50 of standard bleach in the coronavirus setting. For the disinfection of small surfaces ethanol (62—71%; carrier tests) revealed a similar efficacy against coronavirus. A concentration of 70% ethanol is also recommended by the WHO for disinfecting small surfaces [13].

No data were found to describe the frequency of hands becoming contaminated with coronavirus, or the viral load on hands either, after patient contact or after touching contaminated surfaces. The WHO recommends to preferably apply alcohol-based hand rubs for the decontamination of hands, e.g. after removing gloves. Two WHO recommended formulations (based on 80% ethanol or 75% 2-propanol) have been evaluated in suspension tests against SARS-CoV and MERS-CoV, and both were described to be very effective [14]. No in vitro data were found on the efficacy of hand washing against coronavirus contaminations on hands. In Taiwan, however, it was described that installing hand wash stations in the emergency department was the only infection control measure which was significantly associated with the protection from healthcare workers from acquiring the SARS-CoV, indicating that hand hygiene can have a protective effect [15]. Compliance with hand hygiene can be significantly higher in an outbreak situation but is likely to remain an obstacle especially among physicians [16—18]. Transmission in healthcare settings can be successfully prevented when appropriate measures are consistently performed [19,20].

Conclusions

Human coronaviruses can remain infectious on inanimate surfaces for up to 9 days. Surface disinfection with 0.1% sodium hypochlorite or 62—71% ethanol significantly reduces coronavirus infectivity on surfaces within 1 min exposure time. We expect a similar effect against the SARS-CoV-2.

Conflict of interest statement
None declared.

Funding Sources
None.

References

[1] WHO. Coronavirus Disease 2019 (COVID-19). WHO; 2020. Situation Report 23.
[2] de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nat Rev Microbiol 2016;14:523—34.
[3] Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020. https://doi.org/10.1016/s0140-6736(20)30154-9.
[4] Otter JA, Donskey C, Yezli S, Douthwaite S, Goldenberg SD, Weber DJ. Transmission of SARS and MERS coronaviruses and influenza virus in healthcare settings: the possible role of dry surface contamination. J Hosp Infect 2016;92:235—50.
[5] Dowell SF, Simmerman JM, Erdman DD, Wu JS, Chaovavanich A, Javadi M, et al. Severe acute respiratory syndrome coronavirus on hospital surfaces. Clin Infect Dis 2004;39:652—7.
[6] Geller C, Varbanov M, Duval RE. Human coronaviruses: insights into environmental resistance and its influence on the development of new antiseptic strategies. Viruses 2012;4:3044—68.
[7] Kampf G. Antiseptic stewardship: biocide resistance and clinical implications. Cham: Springer International Publishing; 2018.
[8] Ijaz MK, Brunner AH, Sattar SA, Nair RC, Johnson-Lussenburg CM. Survival characteristics of airborne human coronavirus 229E. J Gen Virol 1985;66(Pt 12):2743—8.
[9] Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH. Survival of influenza viruses an environmental surfaces. J Infect Dis 1982;146:47—51.

[10] Ansari SA, Springthorpe VS, Sattar SA, Rivard S, Rahman M. Potential role of hands in the spread of respiratory viral infections: studies with human parainfluenza virus 3 and rhinovirus 14. J Clin Microbiol 1991;29:2115—9.
[11] Kwok YL, Gralton J, McLaws ML. Face touching: a frequent habit that has implications for hand hygiene. Am J Infect Contr 2015;43:112—4.
[12] WHO. Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected. WHO; 2020. Interim guidance. 25 January 2020.
[13] WHO. Annex G. Use of disinfectants: alcohol and bleach. Infection prevention and control of epidemic-and pandemic-prone acute respiratory infections in health care. Geneva: WHO; 2014. p. 65—6.
[14] Siddharta A, Pfaender S, Vielle NJ, Dijkman R, Friesland M, Becker B, et al. Virucidal Activity of World Health Organization-Recommended Formulations Against Enveloped Viruses, Including Zika, Ebola, and Emerging Coronaviruses. J Infect Dis 2017;215:902—6.
[15] Yen MY, Lu YC, Huang PH, Chen CM, Chen YC, Lin YE. Quantitative evaluation of infection control models in the prevention of nosocomial transmission of SARS virus to healthcare workers: implication to nosocomial viral infection control for healthcare workers. Scand J Infect Dis 2010;42:510—5.
[16] Alshammari M, Reynolds KA, Verhougstraete M, O'Rourke MK. Comparison of perceived and observed hand hygiene compliance in healthcare workers in MERS-CoV endemic regions. Healthcare (Basel, Switzerland) 2018;6:122.
[17] Al-Tawfiq JA, Abdrabalnabi R, Taher A, Mathew S, Rahman KA. Infection control influence of Middle East respiratory syndrome coronavirus: A hospital-based analysis. Am J Infect Contr 2019;47:431—4.
[18] Wong TW, Tam WW. Handwashing practice and the use of personal protective equipment among medical students after the SARS epidemic in Hong Kong. Am J Infect Contr 2005;33:580—6.
[19] Wiboonchutikul S, Manosuthi W, Likanonsakul S, Sangsajja C, Kongsanan P, Nitiyanontakij R, et al. Lack of transmission among healthcare workers in contact with a case of Middle East respiratory syndrome coronavirus infection in Thailand. Antimicrob Resist Infect Control 2016;5:21.
[20] Ki HK, Han SK, Son JS, Park SO. Risk of transmission via medical employees and importance of routine infection-prevention policy in a nosocomial outbreak of Middle East respiratory syndrome (MERS): a descriptive analysis from a tertiary care hospital in South Korea. BMC Pulm Med 2019;19:190.
[21] van Doremalen N, Bushmaker T, Munster VJ. Stability of Middle East respiratory syndrome coronavirus (MERS-CoV) under different environmental conditions. Euro Surveill 2013;18.
[22] Casanova LM, Jeon S, Rutala WA, Weber DJ, Sobsey MD. Effects of air temperature and relative humidity on coronavirus survival on surfaces. Appl Environ Microbiol 2010;76:2712—7.
[23] Warnes SL, Little ZR, Keevil CW. Human Coronavirus 229E Remains Infectious on Common Touch Surface Materials. mBio 2015;6:e01697—15.
[24] Sizun J, Yu MW, Talbot PJ. Survival of human coronaviruses 229E and OC43 in suspension and after drying on surfaces: a possible source of hospital-acquired infections. J Hosp Infect 2000;46:55—60.
[25] Duan SM, Zhao XS, Wen RF, Huang JJ, Pi GH, Zhang SX, et al. Stability of SARS coronavirus in human specimens and environment and its sensitivity to heating and UV irradiation. Biomed Environ Sci 2003;16:246—55.
[26] Lai MY, Cheng PK, Lim WW. Survival of severe acute respiratory syndrome coronavirus. Clin Infect Dis 2005;41:e67—71.
[27] Chan KH, Peiris JS, Lam SY, Poon LL, Yuen KY, Seto WH. The Effects of Temperature and Relative Humidity on the Viability of the SARS Coronavirus. Adv Virol 2011;734690.
[28] Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, Doerr HW. Stability and inactivation of SARS coronavirus. Med Microbiol Immunol 2005;194:1—6.
[29] Rabenau HF, Kampf G, Cinatl J, Doerr HW. Efficacy of various disinfectants against SARS coronavirus. J Hosp Infect 2005;61:107—11.
[30] Saknimit M, Inatsuki I, Sugiyama Y, Yagami K. Virucidal efficacy of physico-chemical treatments against coronaviruses and parvoviruses of laboratory animals. Jikken Dobutsu Exp Anim 1988;37:341—5.
[31] Wood A, Payne D. The action of three antiseptics/disinfectants against enveloped and non-enveloped viruses. J Hosp Infect 1998;38:283—95.
[32] Pratelli A. Action of disinfectants on canine coronavirus replication in vitro. Zoonoses Publ Health 2007;54:383—6.
[33] Dellanno C, Vega Q, Boesenberg D. The antiviral action of common household disinfectants and antiseptics against murine hepatitis virus, a potential surrogate for SARS coronavirus. Am J Infect Control 2009;37:649—52.
[34] Omidbakhsh N, Sattar SA. Broad-spectrum microbicidal activity, toxicologic assessment, and materials compatibility of a new generation of accelerated hydrogen peroxide-based environmental surface disinfectant. Am J Infect Control 2006;34:251—7.
[35] Pratelli A. Canine coronavirus inactivation with physical and chemical agents. Vet J (London, England : 1997) 2008;177:71—9.
[36] Kariwa H, Fujii N, Takashima I. Inactivation of SARS coronavirus by means of povidone-iodine, physical conditions and chemical reagents. Dermatol (Basel, Switzerland) 2006;212(Suppl 1):119—23.
[37] Eggers M, Eickmann M, Zorn J. Rapid and Effective Virucidal Activity of Povidone-Iodine Products Against Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Modified Vaccinia Virus Ankara (MVA). Infect Dis Ther 2015;4:491—501.
[38] Eggers M, Koburger-Janssen T, Eickmann M, Zorn J. In Vitro Bactericidal and Virucidal Efficacy of Povidone-Iodine Gargle/Mouthwash Against Respiratory and Oral Tract Pathogens. Infect Dis Ther 2018;7:249—59.
[39] Hulkower RL, Casanova LM, Rutala WA, Weber DJ, Sobsey MD. Inactivation of surrogate coronaviruses on hard surfaces by health care germicides. Am J Infect Control 2011;39:401—7.
[40] Sattar SA, Springthorpe VS, Karim Y, Loro P. Chemical disinfection of non-porous inanimate surfaces experimentally contaminated with four human pathogenic viruses. Epidemiol Infect 1989;102:493—505.
[41] Goyal SM, Chander Y, Yezli S, Otter JA. Evaluating the virucidal efficacy of hydrogen peroxide vapour. J Hosp Infect 2014;86:255—9.

CORRESPONDENCE

Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1

TO THE EDITOR: A novel human coronavirus that is now named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (formerly called HCoV-19) emerged in Wuhan, China, in late 2019 and is now causing a pandemic.[1] We analyzed the aerosol and surface stability of SARS-CoV-2 and compared it with SARS-CoV-1, the most closely related human coronavirus.[2]

We evaluated the stability of SARS-CoV-2 and SARS-CoV-1 in aerosols and on various surfaces and estimated their decay rates using a Bayesian regression model (see the Methods section in the Supplementary Appendix, available with the full text of this letter at NEJM.org). SARS-CoV-2 nCoV-WA1-2020 (MN985325.1) and SARS-CoV-1 Tor2 (AY274119.3) were the strains used. Aerosols (<5 μm) containing SARS-CoV-2 ($10^{5.25}$ 50% tissue-culture infectious dose [$TCID_{50}$] per milliliter) or SARS-CoV-1 ($10^{6.75-7.00}$ $TCID_{50}$ per milliliter) were generated with the use of a three-jet Collison nebulizer and fed into a Goldberg drum to create an aerosolized environment. The inoculum resulted in cycle-threshold values between 20 and 22, similar to those observed in samples obtained from the upper and lower respiratory tract in humans.

Our data consisted of 10 experimental conditions involving two viruses (SARS-CoV-2 and SARS-CoV-1) in five environmental conditions (aerosols, plastic, stainless steel, copper, and cardboard). All experimental measurements are reported as means across three replicates.

SARS-CoV-2 remained viable in aerosols throughout the duration of our experiment (3 hours), with a reduction in infectious titer from $10^{3.5}$ to $10^{2.7}$ $TCID_{50}$ per liter of air. This reduction was similar to that observed with SARS-CoV-1, from $10^{4.3}$ to $10^{3.5}$ $TCID_{50}$ per milliliter (Fig. 1A).

SARS-CoV-2 was more stable on plastic and stainless steel than on copper and cardboard, and viable virus was detected up to 72 hours after application to these surfaces (Fig. 1A), although the virus titer was greatly reduced (from $10^{3.7}$ to $10^{0.6}$ $TCID_{50}$ per milliliter of medium after 72 hours on plastic and from $10^{3.7}$ to $10^{0.6}$ $TCID_{50}$ per milliliter after 48 hours on stainless steel). The stability kinetics of SARS-CoV-1 were similar (from $10^{3.4}$ to $10^{0.7}$ $TCID_{50}$ per milliliter after 72 hours on plastic and from $10^{3.6}$ to $10^{0.6}$ $TCID_{50}$ per milliliter after 48 hours on stainless steel). On copper, no viable SARS-CoV-2 was measured after 4 hours and no viable SARS-CoV-1 was measured after 8 hours. On cardboard, no viable SARS-CoV-2 was measured after 24 hours and no viable SARS-CoV-1 was measured after 8 hours (Fig. 1A).

THIS WEEK'S LETTERS

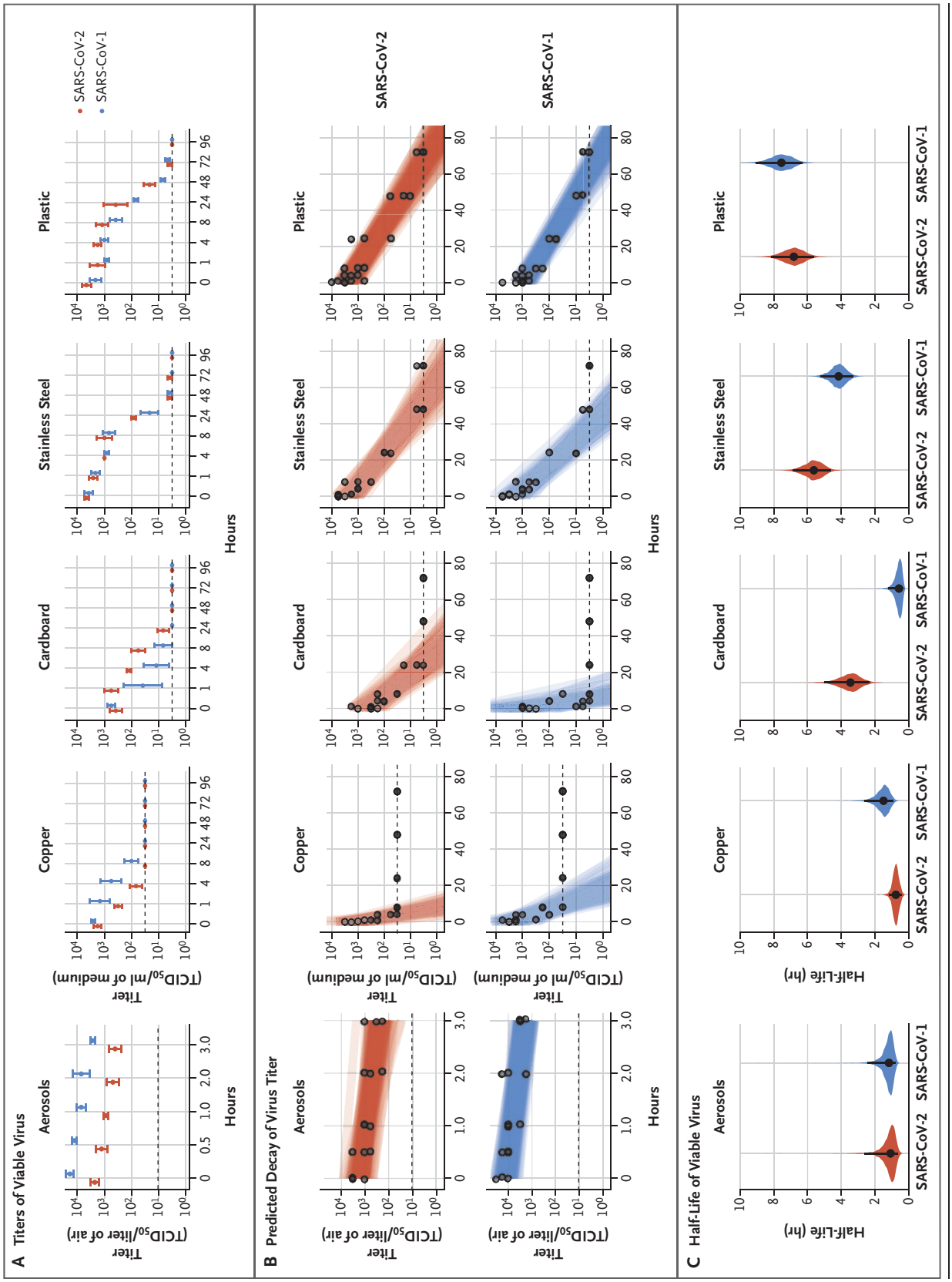

Figure 1 (previous page). Viability of SARS-CoV-1 and SARS-CoV-2 in Aerosols and on Various Surfaces.

As shown in Panel A, the titer of aerosolized viable virus is expressed in 50% tissue-culture infectious dose ($TCID_{50}$) per liter of air. Viruses were applied to copper, cardboard, stainless steel, and plastic maintained at 21 to 23°C and 40% relative humidity over 7 days. The titer of viable virus is expressed as $TCID_{50}$ per milliliter of collection medium. All samples were quantified by end-point titration on Vero E6 cells. Plots show the means and standard errors (I bars) across three replicates. As shown in Panel B, regression plots indicate the predicted decay of virus titer over time; the titer is plotted on a logarithmic scale. Points show measured titers and are slightly jittered (i.e., their horizontal positions are modified by a small random amount to reduce overlap) along the time axis to avoid overplotting. Lines are random draws from the joint posterior distribution of the exponential decay rate (negative of the slope) and intercept (initial virus titer) to show the range of possible decay patterns for each experimental condition. There were 150 lines per panel, including 50 lines from each plotted replicate. As shown in Panel C, violin plots indicate posterior distribution for the half-life of viable virus based on the estimated exponential decay rates of the virus titer. The dots indicate the posterior median estimates, and the black lines indicate a 95% credible interval. Experimental conditions are ordered according to the posterior median half-life of SARS-CoV-2. The dashed lines indicate the limit of detection, which was $3.33\times10^{0.5}$ $TCID_{50}$ per liter of air for aerosols, $10^{0.5}$ $TCID_{50}$ per milliliter of medium for plastic, steel, and cardboard, and $10^{1.5}$ $TCID_{50}$ per milliliter of medium for copper.

Both viruses had an exponential decay in virus titer across all experimental conditions, as indicated by a linear decrease in the $\log_{10}TCID_{50}$ per liter of air or milliliter of medium over time (Fig. 1B). The half-lives of SARS-CoV-2 and SARS-CoV-1 were similar in aerosols, with median estimates of approximately 1.1 to 1.2 hours and 95% credible intervals of 0.64 to 2.64 for SARS-CoV-2 and 0.78 to 2.43 for SARS-CoV-1 (Fig. 1C, and Table S1 in the Supplementary Appendix). The half-lives of the two viruses were also similar on copper. On cardboard, the half-life of SARS-CoV-2 was longer than that of SARS-CoV-1. The longest viability of both viruses was on stainless steel and plastic; the estimated median half-life of SARS-CoV-2 was approximately 5.6 hours on stainless steel and 6.8 hours on plastic (Fig. 1C). Estimated differences in the half-lives of the two viruses were small except for those on cardboard (Fig. 1C). Individual replicate data were noticeably "noisier" (i.e., there was more variation in the experiment, resulting in a larger standard error) for cardboard than for other surfaces (Fig. S1 through S5), so we advise caution in interpreting this result.

We found that the stability of SARS-CoV-2 was similar to that of SARS-CoV-1 under the experimental circumstances tested. This indicates that differences in the epidemiologic characteristics of these viruses probably arise from other factors, including high viral loads in the upper respiratory tract and the potential for persons infected with SARS-CoV-2 to shed and transmit the virus while asymptomatic.[3,4] Our results indicate that aerosol and fomite transmission of SARS-CoV-2 is plausible, since the virus can remain viable and infectious in aerosols for hours and on surfaces up to days (depending on the inoculum shed). These findings echo those with SARS-CoV-1, in which these forms of transmission were associated with nosocomial spread and super-spreading events,[5] and they provide information for pandemic mitigation efforts.

Neeltje van Doremalen, Ph.D.
Trenton Bushmaker, B.Sc.
National Institute of Allergy and Infectious Diseases
Hamilton, MT

Dylan H. Morris, M.Phil.
Princeton University
Princeton, NJ

Myndi G. Holbrook, B.Sc.
National Institute of Allergy and Infectious Diseases
Hamilton, MT

Amandine Gamble, Ph.D.
University of California, Los Angeles
Los Angeles, CA

Brandi N. Williamson, M.P.H.
National Institute of Allergy and Infectious Diseases
Hamilton, MT

Azaibi Tamin, Ph.D.
Jennifer L. Harcourt, Ph.D.
Natalie J. Thornburg, Ph.D.
Susan I. Gerber, M.D.
Centers for Disease Control and Prevention
Atlanta, GA

James O. Lloyd-Smith, Ph.D.
University of California, Los Angeles
Los Angeles, CA
Bethesda, MD

Emmie de Wit, Ph.D.
Vincent J. Munster, Ph.D.
National Institute of Allergy and Infectious Diseases
Hamilton, MT
vincent.munster@nih.gov

Dr. van Doremalen, Mr. Bushmaker, and Mr. Morris contributed equally to this letter.

The findings and conclusions in this letter are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Names of specific vendors, manufacturers, or products are included for public health and informational purposes; inclusion does not imply endorsement of the vendors, manufacturers, or products by the CDC or the Department of Health and Human Services.

Supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and by contracts from the Defense Advanced Research Projects Agency (DARPA PREEMPT No. D18AC00031, to Drs. Lloyd-Smith and Gamble), from the National Science Foundation (DEB-1557022, to Dr. Lloyd-Smith), and from the Strategic Environmental Research and Development Program of the Department of Defense (SERDP, RC-2635, to Dr. Lloyd-Smith).

Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

This letter was published on March 17, 2020, at NEJM.org.

1. Coronavirus disease (COVID-2019) situation reports. Geneva: World Health Organization, 2020 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/).
2. Wu A, Peng Y, Huang B, et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe 2020;27:325-8.
3. Bai Y, Yao L, Wei T, et al. Presumed asymptomatic carrier transmission of COVID-19. JAMA 2020 February 21 (Epub ahead of print).
4. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020;382:1177-9.
5. Chen YC, Huang LM, Chan CC, et al. SARS in hospital emergency room. Emerg Infect Dis 2004;10:782-8.

DOI: 10.1056/NEJMc2004973

Epidemiologic and Survival Trends in Amyloidosis, 1987–2019

TO THE EDITOR: Amyloidosis is a group of rare disorders caused by deposition of misfolded proteins as insoluble fibrils, which leads to progressive multiorgan failure and death.[1] The past 30 years have seen remarkable advances in diagnostic imaging, more accurate identification of fibrils, and (in recent years) the first approved treatments.[2,3]

We report here data on 11,006 patients who received a diagnosis of amyloidosis during the period from 1987 through October 2019. All data were obtained from the United Kingdom National Amyloidosis Centre database. The number of cases increased by 670% from the period 1987–1999 to the period 2010–2019 (Fig. 1A). Systemic light-chain (AL) amyloidosis remained the most common type and accounted for 55% of all cases (Fig. 1B). With the advances in therapies that target plasma cells, overall survival among patients with AL amyloidosis increased from a median of 18 months among patients who received a diagnosis before 2005 to

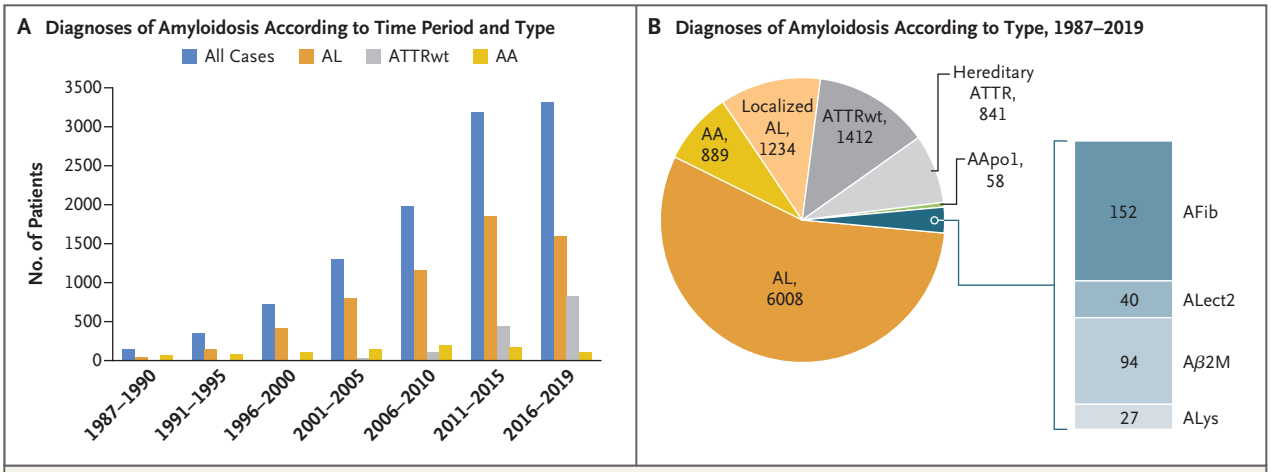

Figure 1. Diagnoses of Amyloidosis over Three Decades and Amyloidosis Types.

Panel A shows data for 11,006 cases of amyloidosis diagnosed from 1987 to 2019. Panel B shows data for the 10,755 cases for which fibril type could be determined accurately. AA denotes amyloid A, AApo1 amyloid apolipoprotein A-I, Aβ2M amyloid beta$_2$-microglobulin, AFib amyloid fibrinogen, ALect2 amyloid leukocyte chemotactic factor 2, AL light chain, ALys amyloid lysozyme, ATTR transthyretin-associated, and ATTRwt wild-type ATTR.

Efficacy of Soap and Water and Alcohol-Based Hand-Rub Preparations against Live H1N1 Influenza Virus on the Hands of Human Volunteers

M. Lindsay Grayson,[1,2,3] Sharmila Melvani,[1] Julian Druce,[4] Ian G. Barr,[5] Susan A. Ballard,[1] Paul D. R. Johnson,[1,3,4] Tasoula Mastorakos,[5] and Christopher Birch[4]

[1]Infectious Diseases Department, Austin Health, [2]Department of Epidemiology and Preventive Medicine, Monash University, [3]Department of Medicine, University of Melbourne, and [4]Victorian Infectious Diseases Reference Laboratory, Melbourne Health, and [5]World Health Organization Collaborating Centre for Influenza, Melbourne, Australia

Background. Although pandemic and avian influenza are known to be transmitted via human hands, there are minimal data regarding the effectiveness of routine hand hygiene (HH) protocols against pandemic and avian influenza.

Methods. Twenty vaccinated, antibody-positive health care workers had their hands contaminated with 1 mL of 10^7 tissue culture infectious dose $(TCID)_{50}$/0.1 mL live human influenza A virus (H1N1; A/New Caledonia/20/99) before undertaking 1 of 5 HH protocols (no HH [control], soap and water hand washing [SW], or use of 1 of 3 alcohol-based hand rubs [61.5% ethanol gel, 70% ethanol plus 0.5% chlorhexidine solution, or 70% isopropanol plus 0.5% chlorhexidine solution]). H1N1 concentrations were assessed before and after each intervention by viral culture and real-time reverse-transcriptase polymerase chain reaction (PCR). The natural viability of H1N1 on hands for >60 min without HH was also assessed.

Results. There was an immediate reduction in culture-detectable and PCR-detectable H1N1 after brief cutaneous air drying—14 of 20 health care workers had H1N1 detected by means of culture (mean reduction, 10^{3-4} $TCID_{50}$/0.1 mL), whereas 6 of 20 had no viable H1N1 recovered; all 20 health care workers had similar changes in PCR test results. Marked antiviral efficacy was noted for all 4 HH protocols, on the basis of culture results (14 of 14 had no culturable H1N1; $P < .002$) and PCR results ($P < .001$; cycle threshold value range, 33.3–39.4), with SW statistically superior ($P < .001$) to all 3 alcohol-based hand rubs, although the actual difference was only 1–100 virus copies/μL. There was minimal reduction in H1N1 after 60 min without HH.

Conclusions. HH with SW or alcohol-based hand rub is highly effective in reducing influenza A virus on human hands, although SW is the most effective intervention. Appropriate HH may be an important public health initiative to reduce pandemic and avian influenza transmission.

Although person-to-person transmission of influenza virus is due primarily to aerosol spread, transmission on the hands of patients and their caregivers is also potentially important [1–6]. Appropriate hand-hygiene (HH) practices should reduce transmission risk, but there are few in vivo data to confirm the antiviral efficacy of currently available HH protocols [7–10]. Furthermore, the long-term viability of influenza virus on unwashed human hands remains unclear, yet this has important ramifications for the risk of transmission by health care workers (HCWs) and others, should they neglect to undertake appropriate HH while caring for patients with influenza. Because of recent concerns about avian influenza and the potential for a worldwide influenza pandemic [2], we aimed to clarify theses practical issues by assessing the efficacy of various HH protocols using human volunteers cutaneously exposed to live H1N1 influenza under controlled conditions.

Received 31 May 2008; accepted 3 October 2008; electronically published 30 December 2008.

Reprints or correspondence: Dr. M. Lindsay Grayson, Infectious Diseases Dept., Austin Health, Studley Rd., Heidelberg, Victoria 3084, Australia (Lindsay.Grayson@austin.org.au).

Clinical Infectious Diseases 2009; 48:285–91

1058-4838/2009/4803-0003$15.00
DOI: 10.1086/595845

Presented in part: 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, September 2007 (abstract K-1796).

METHODS

We assessed the virological effectiveness of 4 commonly used HH protocols with use of a standardized human study protocol, and we compared the results of these protocols with any natural change in influenza viability on HCW hands when left undisturbed by HH activities.

Influenza strain. We used a live (infectious) influenza A virus strain (A/New Caledonia/20/99 [H1N1]) that was a component of the influenza vaccine administered to Australian HCWs during 2005–2006. This H1N1 strain was considered to be a suitable surrogate for H5N1 avian influenza, because their envelopes have similar physicochemical properties, but it was less likely to be associated with a significant risk of severe illness, given the ability to prevaccinate participants [4, 6]. H1N1 was originally isolated and repassaged in embryonated chicken eggs before allantoic fluid was collected, pooled, and aliquoted into 1-mL samples that contained ~1.8×10^7 tissue culture infectious dose $(TCID)_{50}$/0.1 mL live H1N1 (PCR cycle threshold [Ct] value: 17.3; World Health Organization Collaborating Centre for Influenza, Melbourne), which was stored at −70°C until used.

Participants. We recruited HCW volunteers who had undergone vaccination with the 2005 influenza vaccine (Fluvax) and had demonstrable adequate levels of antibody to influenza A before study commencement [11]. Participants were asked to avoid medicated HH products, lotions, and shampoos, as well as bathing in chlorinated pools, for 24 h before commencement of each study protocol. The hands of participants were carefully inspected, and those with dermatoses, open wounds, or other skin disorders were excluded from participation until healed. All consenting participants underwent detailed training in the correct use of each of the HH protocols and wore a fitted high-filtration (N-95) face mask, a hat, and a long-sleeved gown throughout each procedure.

All volunteers participated in the HH efficacy study, and a subset of these volunteers participated in the H1N1 viability study. Appropriate stocks of the antiviral agent oseltamivir (Tamiflu) were available to treat any participants who developed signs of clinical disease. All experiments involving humans were performed in secure negative-pressure respiratory isolation facilities at the Victorian Infectious Diseases Reference Laboratory (Melbourne, Australia), and all influenza diagnostic procedures (i.e., serological testing, PCR, and virus culture) were performed in appropriate secure laboratories at either Victorian Infectious Diseases Reference Laboratory or the World Health Organization Collaborating Centre for Influenza (Melbourne, Australia). The study was approved by the Austin Health Human Research Ethics Committee.

Procedure for hand contamination and virus sampling. To more accurately replicate likely clinical conditions experienced by HCWs who are exposed to large quantities of influenza-infected body fluids, we used a modified version of the American Society for Testing and Materials (ASTM) standard assessment protocols [12, 13] to contaminate HCWs' hands and used both direct swabs and a modified glove-juice technique to obtain culture specimens from their palms, fingertips, and entire hands (figure 1) [14, 15]. In brief, this involved the following: 1 mL of undiluted live H1N1 in allantoic fluid was placed in the participant's right palm, and the participant massaged it for 30 s using the fingertips of his or her left hand. This inoculum (1.8×10^7 $TCID_{50}$/0.1 mL) was chosen to mimic the concentrations expected in the respiratory fluids of patients with active influenza infection (usual range, 10^{3-7} $TCID_{50}$/0.1 mL) [4, 16]. Participants then allowed their hands to air dry for 2 min before undergoing assessment to determine the presence of viable H1N1, as follows: the finger pads of all 5 digits of the left hand were swabbed using a sterile cotton-tipped applicator (Defries Industries) soaked in viral transport media, which was then stored for subsequent real-time RT-PCR (PCR) analysis.

Protocols to assess the efficacy of HH products and the natural viability of influenza virus were then followed before the quantity of detectable H1N1 was again assessed by PCR and culture (immediately after drying after each HH regimen) as follows: the palm of the participant's right hand was swabbed 10 times with a sterile cotton-tipped applicator soaked in 2 mL viral transport media, which was then stored for culture and PCR analysis. The participant's entire right hand was then also sampled by the modified glove-juice technique, with use of 5-

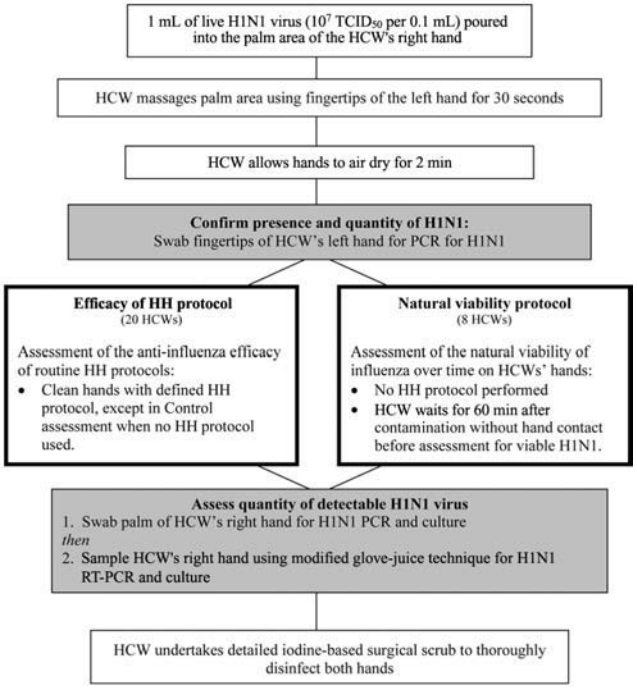

Figure 1. Study protocol. HCW, health care worker; HH, hand hygiene.

mL viral transport media, to obtain samples for both viral culture and PCR [14, 15].

After this sampling, all participants thoroughly disinfected their hands by undertaking a supervised, detailed, surgical scrub using 7.5% povidone-iodine (Orion Laboratories) [7]. All participants were provided verbal and written information with regard to the signs and symptoms of influenza and were instructed to contact the study coordinator if any problems arose. Because the study involved the assessment of a number of HH products, each participant was required to have an interval of at least 24 h between participation in each arm of the study protocol.

PCR. Viral RNA extraction, sample spiking with internal control (bovine viral diarrheal virus [BVDV]), and reverse transcription were performed using a Magnapure automated extraction robot and random primers/AMV-RT (avian myeloblastosis virus reverse transcriptase) enzyme as described elsewhere [17]. Mastermix was prepared using ABI universal fast mastermix (Applied Biosystems) with influenza A matrix protein gene primers (forward primer, FLAM-F MGAGGTCGAAACGTAYGTTCTCT; reverse primer, FLAM-R GTCTTGTCTTTAGCCAYTCCATGA) and probe (FLAM probe, FAM-CCCCCTCAAAGCCGA-MGB-3') in concentrations of 0.1 μmol and 0.2 μmol, respectively. For BVDV (internal control), the primers were BVDV-F 5'-TCAGCGAAGGCCGAAAAG, BVDV-R TGCTACCCCCTCCATTATGC-3', and BVDV-probe VIC-CTAGCCATGCCCTTAGT-MGB at concentrations of 0.02 μmol and 0.1 μmol, respectively. Two microliters of complementary DNA template was added to 18 μL of mastermix, and real-time PCR was performed on an ABI 7500 Fast real-time PCR system (Applied Biosystems) with use of ABI optimized reaction conditions.

The relationship between PCR cycle threshold (Ct) and influenza RNA copy number was assessed using a plasmid that contained a single copy of the matrix gene of influenza A virus. Serial log dilutions of the plasmid preparation containing 10^8 to 10^0 copies per input volume were tested in the described PCR assay, and a standard curve was constructed to enable estimation of the RNA copy number associated with the Ct value obtained from samples tested in the assay.

Viral culture. Viral culture was performed at the World Health Organization Collaborating Centre for Reference and Research on Influenza. In brief, 10-fold dilutions (10^{-1} to 10^{-8}) of each test sample, including the control virus, were prepared in serum-free medium, and 100 μL was added in triplicate to wells of 96-well, flat-bottomed plates (Greiner Bio One) that contained Madin-Darby canine kidney (MDCK) cells (CCL-34, ATCC) that had been seeded a day earlier with 3×10^4 cells per well. After adsorption for 2 h at 35°C in 5% carbon dioxide, the inoculums were removed, and 200 μL of serum-free medium supplemented with 4 μg/mL trypsin (JRH Biosciences) was added to all wells. The plates were then incubated for 4–5 days at 35°C in 5% carbon dioxide and were observed daily, by microscopy, for cytopathic effect, and at day 4 or 5, the presence of influenza virus was confirmed by taking 25 μL of the supernatant and mixing it with 25 μL of 1% turkey RBCs, incubating at room temperature for 30 min, and assessing whether hemagglutination was present. To determine the virus titer ($TCID_{50}$/0.1 mL), the remaining medium was removed from each well and was replaced with 200 μL of 0.036% (w/v) neutral red and was incubated and washed, and residual neutral red levels were quantified by measuring the absorbance at 490 nm [18]. The $TCID_{50}$ endpoint titrations were determined using the method of Reed and Muench [19].

Efficacy of various HH products. After initial influenza contamination and viral confirmation assessment (figure 1), each participant used 1 of the 5 HH protocols: no HH (control), hand washing with nonmedicated liquid soap (Microshield skin care cleanser [SW]; Johnson and Johnson Medical), and hand rubbing with Microshield antimicrobial hand gel (61.5% ethanol plus skin emollient [ETOH only]; Johnson and Johnson Medical), DeBug HH solution (70% isopropyl alcohol plus 0.5% chlorhexidine plus skin emollient [ISOP-CHX]; Orion Laboratories), or Avagard antiseptic hand rub (70% ethanol plus 0.5% chlorhexidine plus skin emollient [ETOH-CHX]; 3M Pharmaceuticals). This was followed by an assessment for detectable H1N1 (table 1). The control (i.e., no HH) assessment was performed immediately after the 2-min period of air drying. Each participant performed all protocols in the same sequence (as listed in table 1), and all 4 HH products were used in the recommended manner [7], followed by assessment for detectable H1N1 and the detailed surgical scrub. All HH protocols with use of alcohol-based hand rub (ABHR) products were performed with a minimum of 24 h between each protocol.

Natural viability of influenza virus. Participants in the natural viability protocol had their hands contaminated in the usual manner, followed by culture and PCR for detectable H1N1 immediately after the 2-min period of air drying (baseline). The hands were then recontaminated and allowed to air dry for the routine 2 min, followed by a further 60-min period during which the participant simply kept his or her hands suspended in the study safety cabinet, at room temperature, without contact with any other objects or HH products. Each participant's right hand was subsequently assessed for detectable virus by PCR and culture, in the described manner, to identify any change in viral concentrations during the 60 min after initial contamination.

Statistical analysis. When appropriate, PCR results were summarized as geometric mean ($\pm$SD) values and were compared by Student's *t* test before and after each intervention (i.e., after use of each HH product or after the 60-min period of air

Table 1. Hand hygiene protocols assessed, in order of testing.

Product	Composition	Hand-washing procedure [7]
Microshield skin care cleanser[a]	Nonmedicated liquid soap	Before use of liquid soap and water, hands were rinsed briefly in tap water, then ~3 mL of liquid soap was dispensed into the palm of the right hand; hands were rubbed together and washed with tap water in the prescribed manner [7] for 40 s, then dried with paper towel
Microshield antimicrobial hand gel[a]	61.5% Ethanol plus skin emollient	A single 2–3-mL volume of alcohol-based hand rub product was dispensed onto the right palm, and the hands were rubbed together in the prescribed manner [7] for 20 s, then air dried
DeBug hand-hygiene solution[b]	70% Isopropyl alcohol plus 0.5% chlorhexidine plus skin emollient	A single 2–3-mL volume of alcohol-based hand rub product was dispensed onto the right palm, and the hands were rubbed together in the prescribed manner [7] for 20 s, then air dried
Avagard antiseptic hand rub[c]	70% Ethanol plus 0.5% chlorhexidine plus skin emollient	A single 2–3-mL volume of alcohol-based hand rub product was dispensed onto the right palm, and the hands were rubbed together in the prescribed manner [7] for 20 s, then air dried

[a] Johnson and Johnson Medical.
[b] Orion Laboratories.
[c] 3M Pharmaceuticals.

drying). Because the limit of reliable PCR detection was a Ct value of 40, any specimens that had PCR Ct values >40 were arbitrarily assigned a Ct of 40.1. Results that were not normally distributed were compared using the Wilcoxon signed-rank test. A P value <.05 was considered to be statistically significant.

RESULTS

Twenty HCWs participated in the assessment of HH products, and 8 of them also participated in the natural viability study. An immediate reduction in H1N1 was noted in all participants immediately after the 2-min period of air drying, with mean ($\pm$SD) PCR Ct values of 25.2 $\pm$ 3.9 and 25.4 $\pm$ 3.8 for the palm and glove-juice specimens, respectively. Only 14 of 20 HCWs had culture-detectable virus in the palm and/or glove juice specimens at this baseline assessment, with a 3–4-log reduction noted, compared with the initial inoculum (10^7 reduced to $10^{3–4}$ $TCID_{50}$/0.1 mL) (table 2). For the remaining 6 participants, no H1N1 could be detected by culture at baseline or could be reliably detected before each HH regimen.

Among the 14 participants with baseline culture-detected H1N1, marked antiviral efficacy was noted for all 4 HH protocols on the basis of both culture (14 of 14 had no culture-detected H1N1 after HH; $P < .002$) and PCR results ($P < .001$) (table 2). All HH protocols performed well, although SW hand washing appeared to be statistically superior ($P < .001$) to all ABHR products on the basis of PCR analysis of glove-juice fluid and to be superior to ETOH only ($P = .017$) and ETOH-CHX ($P = .01$) and comparable to ISOP-CHX ($P = .08$) on the basis of palm swab assessment (table 2). However, the actual difference in virus concentrations on participants' hands with SW, compared with each ABHR, was 1–100 virus copies/μL. All 3 ABHR products were found to have similar efficacy on the basis of PCR analysis of both glove juice and palm swabs (table 2), with reduction in viral contamination to ~100 virus copies/μL. Among the 6 HCWs with baseline cultures negative for H1N1, PCR results were similar to those observed for the 14 other participants, both at baseline and after each HH protocol, which suggests that similar quantities of H1N1 were present but that virus was less viable on the skin of these individuals (data not shown).

In the assessment of natural viability of H1N1, all 8 participants had culture-detected H1N1 at baseline and had initial reductions in virus concentrations and viability after the baseline 2-min period of air drying that were similar to those found in the larger HH efficacy study. However, no further reduction in culture-detectable virus was noted after the subsequent 60-min period of noncontact air drying (not statistically significant, by Wilcoxon test). Similarly, there was no difference in the baseline-to-60-min PCR Ct values for glove-juice fluid ($P = .39$), but a slight decrease in virus concentrations was detected by the palm swab PCR ($P = .036$), which was equivalent to a reduction of <10 viral copies/μL.

DISCUSSION

To our knowledge, this is the first human study to assess the comparative efficacy of various HH products against live influenza virus in concentrations that are likely to mimic the level of cutaneous contamination encountered during an influenza

Table 2. Assessment, by PCR and culture, of the efficacy of various hand hygiene (HH) protocols against live H1N1 influenza virus on the hands of 14 human volunteers who were culture-positive at baseline.

	Real-time RT-PCR,[a] mean Ct value ± SD (range)		Culture $TCID_{50}$/0.1 mL level, mean ± SD (range)	
HH product	Palm	Glove juice	Palm	Glove juice
Control	24.0 ± 3.4[b] (19.8–32.2)	24.3 ± 3.8[b] (18.6–32.4)	3325 ± 8352[e] (0–32,000)	1041 ± 1701[e] (0–5600)
SW	37.6 ± 3.2[c] (30.9–40.1)	39.4 ± 1.1[d] (37.0–40.1)	0 (0–0)	0 (0–0)
ETOH only	34.8 ± 2.6[c] (30.4–40.1)	33.3 ± 2.1[d] (30.1–36.3)	0 (0–0)	0 (0–0)
ISOP-CHX	35.7 ± 2.2[c] (32.8–40.1)	33.5 ± 2.5[d] (30.5–39.8)	0 (0–0)	0 (0–0)
ETOH-CHX	34.4 ± 2.9[c] (28.3–38.2)	33.3 ± 3.0[d] (28.9–38.6)	0 (0–0)	0 (0–0)

NOTE. Control, no product was used; Ct, cycle threshold; ETOH-CHX, ethanol 70% plus 0.5% chlorhexidine solution; ETOH only, ethanol 61.5% gel; glove juice, right hand glove juice; ISOP-CHX, isopropanol 70% plus 0.5% chlorhexidine solution ; palm, right palm; SW, soap and water.

[a] Ct values increase with decreasing quantities of detectable virus copies per microliter. Baseline left-hand fingertip (control) RT-PCR results (mean ± SD) were similar for the assessment using no product and all HH protocol assessments: no product (control), 23.2 ± 3.5; range, 19.5–31.0; SW, 21.9 ± 2.2; range, 18.4–27.1; ETOH only, 23.3 ± 2.8; range, 19.8–30.3; ISOP-CHX, 24.8 ± 3.3, range, 20.7–30.7; ETOH-CHX, 24.4 ± 2.9; range, 20.8–31.1.

[b] Significant change in Ct values for each HH product compared with no product (control) ($P < .001$).

[c] Difference between SW and other HH protocols: ETOH only, $P = .02$; ETOH-CHX, $P = .01$; ISOP-CHX, $P = .08$.

[d] Significant difference between SW and other HH protocols, $P < .001$ for all.

[e] Significant difference between no product (control) and each HH protocol: palm culture, $P < .002$; glove juice, $P < .002$, by Wilcoxon signed-rank test.

pandemic [4, 16]. We found there to be an immediate reduction in culture- and PCR-detected H1N1 virus when the virus fluid was allowed to dry on human hands, even for brief periods of only 2 min. Among the 14 participants with culture-detected H1N1 at this baseline assessment, there was a 3–4 log reduction in virus, compared with the initial inoculum. Interestingly, 6 of 20 participants repeatedly had no culture-detected virus at baseline, suggesting that in some cases, human hands may be a naturally hostile environment for H1N1 virus and that the initial act of drying and possibly the presence of natural skin oils on the hands may also have an antiviral effect. However, among the 8 HCWs who participated in the natural viability study, little change in the concentrations of H1N1 was noted after a further 60-min period during which no HH or contact was undertaken.

We found all 4 HH protocols commonly used in Australian health care settings (i.e., SW, ETOH only, ISOP-CHX, and ETOH-CHX) were highly effective in achieving a large reduction in H1N1 from human hands, with reductions to levels undetectable by culture analysis and down to ~100 virus copies/μL by PCR analysis. Notably, SW was found to be statistically superior to all ABHR products on the basis of glove-juice analysis and superior to ethanol-containing ABHR products on the basis of direct palm swab PCR. Only isopropanol-containing ABHR was comparable to SW ($P = .08$) on the basis of this latter analysis. These potential slight differences in antiviral efficacy between ethanol- and isopropanol-containing products are consistent with the results of previous in vitro studies [8, 9]. Nevertheless, the actual difference in viral concentrations after SW washing, compared with concentrations after any ABHR use, was only 1–100 virus copies/μL. Given the additional time required to perform SW HH, compared with use of ABHRs, the latter may be preferred by some busy HCWs [20–22]. Also, skin emollients contained in ABHRs allow for repeated use without causing irritation [23].

Although, theoretically, some carry-over from each HH product could have technically affected the viral culture results, such carry-over should not have affected the PCR results. The fact that the decrease in PCR-detected virus was consistent with the culture results suggests that each HH regimen had a genuine impact on the quantity of H1N1 on participants' hands.

Our in vivo results are similar to the in vitro results reported by others for enveloped viruses [7–9, 24, 25] and are notable because of the known ability of influenza to survive in dust and on environmental surfaces [26–28]. Furthermore, our findings support the original 1919 observation by Lynch and Cumming [29] with regard to the potential role of hand contact on influenza transmission and are consistent with the in vivo study by Sickbert-Bennett et al. [30], who found that SW was highly effective in removing MS2 bacteriophage (a surrogate for non-enveloped viruses). Similar results have also been noted in human studies of ABHRs on feline calicivirus [31]. Of course, the exact role that hand and fomite contact plays in influenza transmission in hospitals and the community has been debated [1–6]. Therefore, the importance of appropriate HH as a public health and infection-control measure remains uncertain, but it is likely to be of benefit.

Our study has some limitations. First, we would have preferred to assess a larger number of participants and to randomize the order in which each HH regimen was performed. However, given the complexity and inherent potential risks associated with the study protocol, we believe that our enroll-

ment rate reflected the realities of conducting a study such as this. Furthermore, because our study design was a detailed surgical scrub after each HH regimen, randomizing the sequence of HH regimens is unlikely to have changed the final results. Second, the initial reduction in culture-detected H1N1 after simple 2-min air drying may be related to limitations in our detection methods rather than to any true decrease in virus viability. However, the fact that we found similar consistent changes by PCR suggests that our culture results and estimates of virus viability are likely to be valid. Third, we used a rather high contaminating concentration of H1N1 in an attempt to mimic a worst-case clinical scenario. Therefore, we cannot be absolutely certain that our results would be the same if lower H1N1 concentrations had been used—hence, our results cannot be generalized to all clinical situations. Finally, we cannot be sure whether HH products with higher concentrations of alcohol, such as those used in some European countries, would demonstrate the same or greater efficacy.

We believe that our findings have potentially important public health implications, because simple hand washing with unmedicated soap and water appears to be highly effective in removing influenza virus from hands and is, therefore, likely to be effective in preventing transmission of influenza, as long as HH is undertaken appropriately [1–3]. For busy HCWs for whom the number of HH opportunities is likely to be very high [32], the use of ABHR would seem to be a very suitable alternative. Future public health initiatives should highlight the importance of compliance with HH protocols for HCWs, patients, and caregivers.

Acknowledgments

We thank the many health care workers who volunteered their time to participate in this study. The Melbourne World Health Organization Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health and Ageing.

Financial support. National Health & Medical Research Council (801470), Australia.

Potential conflicts of interest. M.L.G., S.M., S.A.B., and P.D.R.J. developed DeBug (as employees of Austin Health, Heidelberg, Australia), with funding in part from the Department of Human Services (Victoria, Australia). The intellectual property for this development is held by Austin Health, which handles all patent, trademark, and licensing issues. Austin Health, but no individual author, receives a small income stream from the sale of DeBug. All other authors: no conflicts.

References

1. Hayden F, Croisier A. Transmission of avian influenza viruses to and between humans. J Infect Dis **2005**; 192:1311–4.
2. Beigel JH, Farrar J, Han AM, et al.; the Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5. Avian influenza A (H5N1) infections in humans. N Engl J Med **2005**; 353:1374–85 (erratum: N Engl J Med 2006; 354:884).
3. Collignon PJ, Carnie JA. Infection control and pandemic influenza. Med J Aust **2006**; 185:S54–7.
4. Treanor JJ. Influenza virus. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and practice of infectious diseases. 6th ed. Philadelphia: Elsevier Churchill Livingstone, **2005**:2060–85.
5. Morens DM, Rash VM. Lessons from a nursing home outbreak of influenza A. Infect Control Hosp Epidemiol **1995**; 16:275–80.
6. Hayden FG, Palese P. Influenza virus. In: Richman DD, Whitley RJ, Hayden FG, eds. Clinical virology. New York: Churchill Livingstone, **1997**:911–42.
7. World Health Organization (WHO) Consultation on Hand Hygiene in Health Care Global Patient Safety Challenge, 2005–2006: "Clean care is safer care"—WHO advanced draft guidelines for hand hygiene in health care (draft document). Geneva, Switzerland: World Health Organization, **2005**.
8. Schurmann W, Eggers HJ. Antiviral activity of an alcoholic hand disinfectant: comparison of the in vitro suspension test with in vivo experiments on hands, and on individual fingertips. Antiviral Res **1983**;3: 25–41.
9. Steinmann J. Surrogate viruses for testing virucidal efficacy of chemical disinfectants. J Hosp Infect **2004**; 56(Suppl 2):S49–54.
10. Kampf G, Kramer A. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. Clin Microbiol Rev **2004**; 17:863–93.
11. De Jong JC, Palache AM, Beyer WE, Rimmelzwaan GF, Boon AC, Osterhaus AD. Haemagglutination-inhibiting antibody to influenza virus. Dev Biol (Basel) **2003**;115:63–73.
12. American Society for Testing and Materials International. ASTM E 1838–96: standard test method for determining the virus-eliminating effectiveness of liquid hygienic handwash agents using the fingerpads of adult volunteers. **1996**. Available at: http://www.astm.org/Standards/E1838.htm.
13. American Society for Testing and Materials International. ASTM E 2011–99: standard test method for evaluation of hand washing formulations for virus-eliminating activity using the entire hand. **1999**. Available at: http://www.astm.org/BOOKSTORE/BOS/TOCS_2003/11.05_Contents.pdf.
14. American Society for Testing and Materials International. E1115–86: standard test method for evaluation of surgical hand scrub formulation. In: Annual book of ASTM standards. Easton: American Society for Testing and Materials International, **1988**:644–5.
15. Grabsch EA, Burrell LJ, Padiglione A, O'Keeffe JM, Ballard S, Grayson ML. Risk of environmental and healthcare worker contamination with vancomycin-resistant enterococci during outpatient procedures and hemodialysis. Infect Control Hosp Epidemiol **2006**; 27:287–93.
16. Murphy BR, Baron S, Chalhub EG, Uhlendorf CP, Chanock RM. Temperature-sensitive mutants of influenza virus. IV. Induction of interferon in the nasopharynx by wild-type and temperature-sensitive recombinant virus. J Infect Dis **1973**; 128:488–93.
17. Druce J, Tran T, Kelly H, et al. Laboratory diagnosis and surveillance of human respiratory viruses by PCR in Victoria, Australia, 2002–2003. J Med Virol **2005**; 75:122–9.
18. Tannock GA, Paul JA, Herd R, et al. Improved colorimetric assay for detecting influenza B virus neutralizing antibody responses to vaccination and infection. J Clin Microbiol **1989**; 27:524–8.
19. Reed LJ, Muench H. A simple method of establishing fifty percent endpoints. Am J Hyg **1938**; 27:493–7.
20. Pittet D, Hugonnet S, Harbath S, et al. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Infection Control Programme. Lancet **2000**; 356:1307–12.
21. Boyce JM, Pittet D; Healthcare Infection Control Practices Advisory Committee. Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Society for Healthcare Epidemiology of America/Association for Professionals in Infection Control/Infectious Diseases Society of America/Hand Hygiene Task Force. Infect Control Hosp Epidemiol **2002**; 23(12 Suppl):S3–40
22. Johnson PDR, Martin R, Burrell LJ, et al. Efficacy of an alcohol/chlorhexidine hand hygiene program in a university teaching hospital with

high rates of nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Med J Aust **2005**; 183:509–14.
23. Graham M, Nixon R, Burrell LJ, Bolger C, Johnson PDR, Grayson ML. Low rates of cutaneous reactions to alcohol-chlorhexidine hand hygiene solution during prolonged use in a large teaching hospital. Antimicrob Agents Chemother **2005**; 49:4404–5.
24. Kramer A, Galabov AS, Sattar SA, et al. Virucidal activity of a new hand disinfectant with reduced ethanol content: comparison with other alcohol-based formulations. J Hosp Infect **2006**; 62:98–106.
25. Kampf G, Steinmann J, Rabenau H. Suitability of vaccinia virus and bovine viral diarrhea virus (BVDV) for determining activities of three commonly-used alcohol-based hand rubs against enveloped viruses. BMC Infect Dis **2007**; 7:5.
26. Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH Jr. Survival of influenza viruses on environmental surfaces. J Infect Dis **1982**; 146:47–51.
27. Hemmes JH, Winkler KC, Kool SM. Virus survival as a seasonal factor in influenza and poliomyelitis. Nature **1960**; 188:430–1.
28. Edward DG. Resistance of influenza virus to drying and its demonstration on dust. Lancet **1941**; 241:664–6.
29. Lynch C, Cumming JG. The distribution of influenza by direct contact—hands and eating utensils. Am J Public Health (NY) **1919**; 9: 25–38.
30. Sickbert-Bennett EE, Weber DJ, Gergen-Teague MF, Sobsey MD, Samsa GP, Rutala WA. Comparative efficacy of hand hygiene agents in the reduction of bacteria and viruses. Am J Infect Control **2005**; 33:67–77.
31. Kampf G, Grotheer D, Steinmann J. Efficacy of three ethanol-based hand rubs against feline calicivirus, a surrogate virus for norovirus. J Hosp Infect **2005**; 60:144–9.
32. Swaminathan A, Martin R, Gamon S, et al. Quantifying personal protective equipment (PPE) and antiviral use during the initial acute management of suspected human cases of pandemic influenza. Emerg Infect Dis **2007**; 13:1541–7.

MAJOR ARTICLE

Survival of Severe Acute Respiratory Syndrome Coronavirus

Mary Y. Y. Lai, Peter K. C. Cheng, and Wilina W. L. Lim

Virology Division, Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Hong Kong, China

Background. The primary modes of transmission of severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) appear to be direct mucus membrane contact with infectious droplets and through exposure to formites. Knowledge of the survival characteristics of the virus is essential for formulating appropriate infection-control measures.

Methods. Survival of SARS-CoV strain GVU6109 was studied in stool and respiratory specimens. Survival of the virus on different environmental surfaces, including a laboratory request form, an impervious disposable gown, and a cotton nondisposable gown, was investigated. The virucidal effects of sodium hypochlorite, house detergent, and a peroxygen compound (Virkon S; Antec International) on the virus were also studied.

Results. SARS-CoV GVU6109 can survive for 4 days in diarrheal stool samples with an alkaline pH, and it can remain infectious in respiratory specimens for >7 days at room temperature. Even at a relatively high concentration (10^4 tissue culture infective doses/mL), the virus could not be recovered after drying of a paper request form, and its infectivity was shown to last longer on the disposable gown than on the cotton gown. All disinfectants tested were shown to be able to reduce the virus load by >3 log within 5 min.

Conclusions. Fecal and respiratory samples can remain infectious for a long period of time at room temperature. The risk of infection via contact with droplet-contaminated paper is small. Absorbent material, such as cotton, is preferred to nonabsorptive material for personal protective clothing for routine patient care where risk of large spillage is unlikely. The virus is easily inactivated by commonly used disinfectants.

In the early spring of 2003, a mysterious outbreak of severe pneumonia occurred in southern China and rapidly spread throughout the world. The causative agent was later found to be a novel coronavirus and was designated "severe acute respiratory syndrome (SARS) coronavirus" (SARS-CoV) [1–3]. As of 31 December 2003, a total of 8096 cases had been reported, of which 774 were fatal [4]. Altogether, 1706 health care workers were affected. More than 20% of the patients with SARS were themselves health care workers, which could be explained by the unique shedding pattern of SARS-CoV, with viral loads reaching a peak ~2 weeks after onset of disease, when patients were in hospital care [5]. This shedding pattern of SARS-CoV also highlights the importance of control of nosocomial spread of the disease.

Received 18 March 2005; accepted 15 June 2005; electronically published 22 August 2005.

Reprints or correspondence: Dr. Wilina W. L. Lim, Virology Div., Rm. 901, Public Health Laboratory Centre, 382 Nam Cheong St., Shek Kip Mei, Kowloon, Hong Kong, China (WIlim@pacific.net.hk).

Clinical Infectious Diseases 2005; 41:e67–71

1058-4838/2005/4107-00E1$15.00

Soon after the isolation of SARS-CoV in our laboratory, we were able to perform a survival study of the virus, and partial results were reported on the World Health Organization Communicable Disease Surveillance and Response Web site on SARS [6]. Here, we provide a full report of our study of the survival characteristics of SARS-CoV in different clinical sample matrices, as well as on various environmental surfaces in the laboratory and hospital. The risk of acquisition of SARS-CoV attributed to the inanimate environment is also discussed. We also report the virucidal effect of 3 common liquid disinfectants on SARS-CoV.

MATERIALS AND METHODS

Viruses and cell line. SARS-CoV strain GVU6109 was used in the present study. GVU6109 was isolated from a lung tissue specimen obtained from a patient during the SARS outbreak in 2003. The virus was inoculated into the Vero E6 cell line, which was grown in minimum essential medium (MEM) with 2% fetal calf serum at 37°C. All virus culture experiments were performed in a biosafety level 3 laboratory.

Survival of SARS-CoV in different stool specimens. Four different stool samples were used. The samples tested negative for SARS-CoV, norovirus, rotavirus, and other viral agents. Stool sample A was a normal stool specimen obtained from a 6-month-old baby, and samples B and C were 2 normal stool specimens obtained from adults. Sample D was a diarrheal stool specimen obtained from an adult. A 10% suspension of each stool specimen was prepared in PBS (pH, 7.4). After centrifugation at 1500 *g* for 20 min, the supernatant was collected, and the pH was checked with pH paper. Stool sample A had a pH of 6–7, sample B had a pH of 7–8, sample C had a pH of 8, and sample D had a pH of 9.

A total of 1.8 mL of each 10% stool suspension was spiked with 0.2 mL of virus stock GVU6109 (10^7 $TCID_{50}$/mL). As a control, 1.8 mL of viral transport medium was also spiked with 0.2 mL of the virus stock. The samples were incubated in closed containers at room temperature (20°C) for 0.5 h, 1 h, 3 h, 6 h, 1 day, 2 days, 4 days, 5 days, 6 days, and 7 days. Serial 10-fold dilutions of a different stool suspension and control were prepared in Earl's diluent. Fifty microliters of each dilution was inoculated into 4 wells of a 96-well plate. One hundred microliters of Vero E6 (10^5 cells/mL) was added to each well, and the plates were sealed and incubated at 37°C in 5% CO_2 for 4 days. Virus concentration (in $TCID_{50}$/50 μL) for each stool suspension at a different time was calculated on the basis of the Kärber method [7]. The whole experiment was repeated using a trivalent poliovirus vaccine to compare the effect of pH on a nonenveloped RNA virus.

Survival of SARS-CoV in different respiratory specimens. A total of 0.3 mL of virus stock GVU6109 (10^7 $TCID_{50}$/mL) was added to 2.7 mL of a nasopharyngeal aspirate (NPA) specimen, throat and nasal swab (TNS) specimens, and viral transport medium as a control. The respiratory specimens had been determined to be negative for respiratory viruses. They were then incubated in closed containers at room temperature or 4°C for 3 h, 6 h, and 1, 3, 4, 5, 7, and 10 days. The virus concentration ($TCID_{50}$/50μL) for each sample at various time points was determined as above.

Survival of SARS-CoV on paper, impervious disposable gowns, and cotton nondisposable gowns. To simulate the event of large droplets that contain SARS-CoV falling on paper and on cotton and disposable gowns, experiments were performed to determine whether SARS-CoV survived on these surfaces.

Paper. A paper laboratory request form was cut into small pieces (area, 1×1 cm), which were sterilized by autoclave at 121°C for 15 min. Stock virus GVU6109 (10^7 $TCID_{50}$/mL) was serially diluted to 10^4 $TCID_{50}$/mL with PBS. At each virus dilution, 5 μL was applied to the surface of each piece of sterilized paper. The sample was allowed to be absorbed at room temperature, and the paper pieces were left to stand for different durations (5 min, 1 h, 2 h, 3 h, 6 h, 1 day, and 2 days). Each piece of paper was then placed into a Vero E6 cell culture tube. For each virus dilution and at different intervals after absorption, 4 pieces of paper were inoculated into 4 cell culture tubes. All of the tubes were incubated at 37°C and were examined after 4 days. Sterilized paper without virus suspension was also included in the study to check for any toxicity to cell culture.

Disposable gown. For the disposable gown, the whole process used for paper was repeated, except that a disposable gown was used after treatment by irradiating it under UV light for 1 h. The gown is part of the personal protection equipment used in our laboratory when handling specimens that are potentially contaminated with SARS-CoV. It is made of polypropylene material (35 g/m^2) coated with a polyethylene film (15 g/m^2), and the waist and neck are tied when the gown is used to provide full-body protection.

Cotton gown. For testing of the cotton gown, a large piece of cloth cut from an ordinary cotton laboratory coat was soaked in distilled water overnight and was then boiled for 1 h. The whole process was repeated 3 times to remove chemical residue that was found to be toxic to the cell culture. After drying, the cloth was cut into small pieces (area, 1×1 cm). The pieces were then sterilized by autoclaving. The sterilized cotton cloth was then tested in the manner used for the paper and the disposable gown.

Effect of different disinfectants and detergents on the survival of SARS-CoV. Different dilutions of sodium hypochlorite solution (1:50 and 1:100 of the stock solution, which contains 50,000 ppm of active chlorine); a household detergent containing sodium lauryl ether sulphate, alkyl polyglycosides, and coco-fatty acid diethanolamide (1:50 and 1:100; AXE brand); and Virkon S (1%; Antec International) were made by dilution with distilled water. Fifty microliters of stock virus GVU6109 (10^7 $TCID_{50}$/mL) was added to 450 μL of different dilutions of the hypochlorite solution, household detergent, and Virkon S and to viral transport medium as a control. After standing at room temperature for 5 min, 10 min, 20 min, and 30 min, serial 10-fold dilutions of different disinfectants or controls were made in Earl's diluent. Fifty microliters of each virus dilution was added to each of 4 wells of a 96-well plate. A total of 50 μL of MEM was added to each well. After adding 100 μL of Vero E6 cells to each well, the plates were sealed and incubated at 37°C in a 5% CO_2 incubator. Cytopathic effect was recorded at day 4, and residual virus $TCID_{50}$ was calculated from wells without showing cell toxicity.

RESULTS

Survival of SARS-CoV in different stool specimens. Figure 1 shows the duration of SARS-CoV survival after incubation in stool specimens at different pHs. The virus was not recoverable within 1 day after incubation in normal adult stool specimens

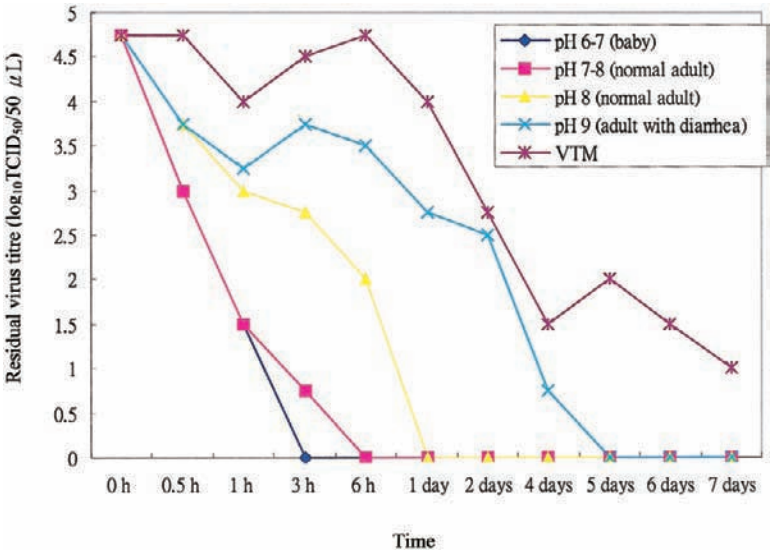

Figure 1. Survival time of severe acute respiratory syndrome coronavirus in different stool specimens at room temperature. VTM, viral transport medium.

or within 3 h after incubation in the baby stool specimen with a slightly acidic pH. However, the virus survived for 4 days in a diarrheal stool specimen with a pH of 9. Poliovirus did not show these survival characteristics. Poliovirus spiked in the same baby stool specimen survived for >4 days, and it survived for even longer in the diarrheal stool specimen (data not shown). The duration of survival for SARS-CoV in the stool suspension was retested in another 2 diarrheal stool specimens, with the same results (data not shown).

Survival of SARS-CoV in different respiratory specimens. The virus can remain alive in respiratory specimens, such as NPA or TNS specimens, for >7 days at room temperature and for >20 days at 4°C (figure 2).

Survival of SARS-CoV on paper, the impervious disposable gown, and the cotton nondisposable gown. Table 1 shows the duration of survival for SARS-CoV on different materials. Even with a relatively high virus load in the droplet, rapid loss of infectivity was observed for paper and cotton material. Inactivation on impervious surface took much longer. No cell culture toxicity was observed for the paper, disposable gown, or cotton nondisposable gown.

Effect of different disinfectants and detergents on SARS-

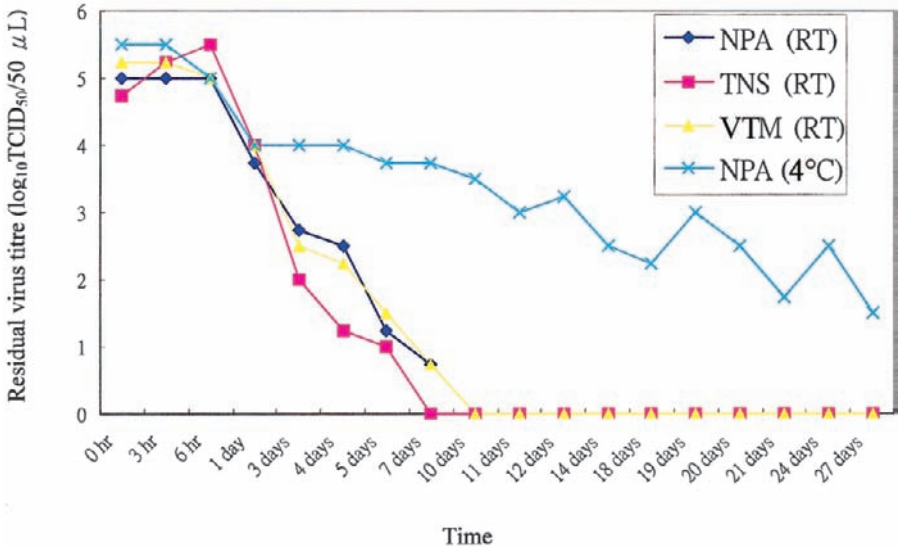

Figure 2. Survival time of severe acute respiratory syndrome coronavirus in nasopharyngeal aspirate (NPA) specimens, throat and nasal swab (TNS) specimens, or viral transport medium (VTM) at room temperature (RT) and at 4°C.

Table 1. Duration of survival of severe acute respiratory syndrome coronavirus (SARS-CoV) on paper, a disposable gown, and a cotton gown.

Inoculation, $TCID_{50}$/mL	Time taken to inactivate SARS-CoV, by surface		
	Paper	Disposable gown	Cotton gown
10^6	24 h	2 days	24 h
10^5	3 h	24 h	1 h
10^4	<5 min	1 h	5 min

CoV. After incubation with various disinfectants, a reduction in the virus load of >3 log was taken to indicate inactivation (table 2). All disinfectants reduced the virus load by >3 log within 5 min after incubation. Cell toxicity was observed in wells inoculated with a virus/disinfectant mixture at a 1:10 dilution. Thus, when trying to calculate the residual $TCID_{50}$, results from wells with dilutions starting from 1:100 were used.

DISCUSSION

A recent study showed evidence that SARS-CoV has contaminated a variety of environmental surfaces in some hospital settings [8]. The presence of SARS-CoV on surfaces is always a concern, although few studies in which live virus has been successfully isolated from an environmental surface have been reported. Surfaces were usually contaminated with patient's droplets or by indirect transfer of virus from gloves that were contaminated with excreted virus. The increase in the isolation rate of methicillin-resistant *Staphylococcus aureus* at the intensive care unit of a Hong Kong hospital during the SARS outbreak suggested that increased cross-contamination could occur if gloves and gowns were worn all of the time [9]. It is important to gather evidence of the survival of SARS-CoV on surfaces so that appropriate infection-control measures can be taken.

The present study demonstrates that SARS-CoV can survive in respiratory samples for 5 days at room temperature and for up to 3 weeks at 4°C. Although normal fecal material seems to have a deleterious effect on its survival, the present study shows that the virus could have a prolonged survival when present in diarrheal stool. The virus can survive for 4 days at room temperature after being spiked in diarrheal stool with an alkaline pH. This observation lends evidence that fecal droplets containing SARS-CoV remain infectious for a period of time. This may explain the Amoy Gardens outbreaks, in which the drainage and sewage system was implicated in facilitating the spread of SARS, as was pointed out in the SARS Expert Committee study [10] and in a simulation study by Yu et al. [11].

On the basis of quantitative data obtained from our own study [5], stool samples contain a much higher viral load than do NPA samples. The mean virus concentration may reach 10^5 $TCID_{50}$/mL at 2 weeks after onset of disease in stool samples, compared with $10^{2.2}$ $TCID_{50}$/mL for NPA samples. Our present data show that, at a high concentration of virus (10^6 $TCID_{50}$/mL), SARS-CoV can survive for 4–5 days at room temperature in both respiratory and diarrheal stool samples. From the point of view of infection control of SARS, it is important to know that excreta from patients with SARS (especially those who have diarrhea) may remain highly infectious for a considerably long period, and appropriate precautions must be taken to prevent formation of aerosols, because of probable airborne transmission of SARS.

During the SARS outbreak in 2003, contamination of paper documents was a concern for health care workers, who frequently had to handle such documents in their daily work. The present study simulates a situation in which large respiratory droplets (volume, 5 μL; radius, ~1 mm) that contain the virus fall onto paper. Even with a higher concentration of virus (10^4 $TCID_{50}$/mL) than would normally occur in NPA samples ($10^{2.2}$ $TCID_{50}$/mL), no virus infectivity remained after the paper was dried. Paper contaminated with a higher concentration of virus (equivalent to that of fecal excreta [i.e., 10^5 $TCID_{50}$/mL]) was not infectious after 3 h, and no viral infectivity was shown after 24 h, even with a concentration of 10^6 $TCID_{50}$/mL. Our study shows that the risk of infection through contact with a droplet-contaminated paper is small. Standard infection-control measures, such as hand washing after touching any potential infectious material, are effective against nosocomial transmission of SARS [12].

Table 2. Effect of disinfectants on severe acute respiratory syndrome coronavirus.

Duration of exposure, min	Disinfectant (dilution), residual $TCID_{50}$/mL					
	Hypochlorite (1:50)	Hypochlorite (1:100)	Detergent (1:50)	Detergent (1:100)	1% Virkon S[a]	VTM
5	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	10^5
10	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$10^{5.5}$
20	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$10^{5.5}$
30	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$10^{5.75}$

NOTE. VTM, viral transport medium.

[a] Manufactured by Antec International.

A previous study reported that coronavirus 229E and OC43 can survive for a few hours after drying on 3 different surfaces (aluminium, cotton gauze sponges, and latex gloves) [13]. In the present study, we compared the survival of SARS-CoV on 2 types of gowns: disposable gowns and cotton gowns. Our results showed that, even with a high concentration of virus (10^5 $TCID_{50}$/mL), the droplets will lose all infectivity after 1 h on cloth, compared with 24 h needed for the disposable gown. Apparently, droplets will be absorbed more quickly on cotton material than on fluid-repellent material. The present data show that an ordinary cotton gown offers reasonable protection against small droplets containing SARS-CoV. Our study also raises the possibility that any droplets that hang on a nonabsorbent disposable gown may pose a risk of contaminating the environment when health care workers wear the gown all of the time or when they try to remove the gown. A similar conclusion may also be drawn for gloves, although gloves were not tested in the present study. A specially designed disposable garment with a fluid-repellent lamination that has an outer fluid-absorbing sheet may offer better protection for the personnel.

Finally, our study shows that 3 common liquid detergents/disinfectants are equally effective against the SARS-CoV. All demonstrated a minimum 10^3-fold reduction in the initial virus titer within 5 min after incubation in solution [14]. The household detergents tested in this study were shown to be effective against the SARS-CoV with a lipid envelope and could be used for cleaning common items and surfaces that are not grossly contaminated with secretions or excreta.

Although we did not perform specific neutralizing steps for the 3 detergents/disinfectants, the fact that the wells that we examined to calculate the residual virus $TCID_{50}$ were free of cell toxicity highly suggests that nonneutralized disinfectants also have no effect on the virus during the 4 days of incubation.

The SARS-CoV is a newly discovered virus. Thus far, there have only been a few reports of its survival characteristics [15]. Here, we demonstrate that this deadly virus can remain infectious for a long period in stool specimens. The samples that we spiked with SARS-CoV were incubated in closed containers during the entire period of incubation, simulating the conditions in a sewage drainage pipe. Thus, our results showed that, in this situation, droplets may be a concern with regard to disease transmission, as occurred in the Amoy Gardens outbreaks. This has significant implications for sewage treatment in both domestic and hospital environments. Fortunately, this virus is also susceptible to drying. We showed that, when virus-containing droplets were dried, the virus was inactivated rapidly on paper and cotton cloth. Transmission through droplet-contaminated paper and cotton gowns is unlikely, and common household detergents can be effective decontaminating agents for use in the laboratory and hospital.

Acknowledgments

Potential conflicts of interest. All authors: no conflicts.

References

1. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med **2003**; 348:1967–76.
2. Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. SARS study group. Lancet **2003**; 361:1319–25.
3. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. SARS Working Group. N Engl J Med **2003**; 348:1953–66.
4. World Health Organization. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. **2005**. Available at: http://www.who.int/csr/sars/country/table2004_04_21/en/. Accessed 12 August 2005.
5. Cheng PK, Wong DA, Tong LK, et al. Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. Lancet **2004**; 363:1699–700.
6. World Health Organization. First data on stability and resistance of SARS coronavirus compiled by members of WHO laboratory network. Available at: http://www.who.int/csr/sars/survival_2003_05-04/en/. Accessed 22 December 2004.
7. Schmidt NJ, Emmons RW, eds. Diagnostic procedures for viral, rickettsial and chlamydial infections. 6th ed. Washington, DC: American Public Health Association, **1989**.
8. Dowell SF, Simmerman JM, Erdman DD, et al. Severe acute respiratory syndrome coronavirus on hospital surfaces. Clin Infect Dis **2004**; 39: 652–7.
9. Yap FHY, Gomersall CD, Fung KSC, et al. Increase in methicillin-resistant *Staphylococcus aureus* acquisition rate and change in pathogen pattern associated with an outbreak of severe acute respiratory syndrome. Clin Infect Dis **2004**; 39:511–6.
10. SARS Expert Committee. SARS in Hong Kong: from experience to action. Report of the SARS Expert Committee. Hong Kong: Health, Welfare, and Food Bureau, Hong Kong Government, **2003**.
11. Yu ITS, Li Y, Wong TW, et al. Evidence of airborne transmission of the severe acute respiratory syndrome virus. N Engl J Med **2004**; 350: 1731–9.
12. Seto WH, Tsang D, Yung RWH, et al. Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (SARS). Advisors of Expert SARS group of Hospital Authority. Lancet **2003**; 361:1519–20.
13. Sizun J, Yu MWN, Talbot PJ. Survival of human coronavirus 229E and OC43 in suspension and after drying on surfaces: a possible source of hospital-acquired infections. J Hosp Infect **2000**; 46:55–60.
14. Russell AD, Hugo WB, Ayliffe GAJ. Principles and practice of disinfection, preservation and sterilization. 2nd ed. Oxford, UK: Blackwell Scientific Publications, **1992**.
15. Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, Doerr HW. Stability and inactivation of SARS coronavirus. Med Microbiol Immunol (Berl) **2005**; 194:1–6.