Journal of Hospital Infection (2005) **61**, 107–111

Available online at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevierhealth.com/journals/jhin

Efficacy of various disinfectants against SARS coronavirus

H.F. Rabenau[a,*], G. Kampf[b,c], J. Cinatl[a], H.W. Doerr[a]

[a]*Institut für Medizinische Virologie, Klinikum der Johann Wolfgang Goethe-Universität Frankfurt, Paul-Ehrlich-Str. 40, 60596 Frankfurt, Germany*
[b]*Bode Chemie GmbH & Co., Scientific Affairs, Melanchthonstr. 27, 22525 Hamburg, Germany*
[c]*Institut für Hygiene und Umweltmedizin, Ernst-Moritz-Arndt Universität Greifswald, Walther-Rathenau-Str. 49a, 17489 Greifswald, Germany*

Received 5 July 2004; accepted 23 December 2004
Available online 31 May 2005

KEYWORDS
SARS-CoV;
Disinfectants;
Virucidal activity;
prEN 14476

Summary The recent severe acute respiratory syndrome (SARS) epidemic in Asia and Northern America led to broad use of various types of disinfectant in order to control the public spread of the highly contagious virus. However, only limited data were available to demonstrate their efficacy against SARS coronavirus (SARS-CoV). We therefore investigated eight disinfectants for their activity against SARS-CoV according to prEN 14476. Four hand rubs were tested at 30 s (Sterillium, based on 45% iso-propanol, 30% n-propanol and 0.2% mecetronium etilsulphate; Sterillium Rub, based on 80% ethanol; Sterillium Gel, based on 85% ethanol; Sterillium Virugard, based on 95% ethanol). Three surface disinfectants were investigated at 0.5% for 30 min and 60 min (Mikrobac forte, based on benzalkonium chloride and laurylamine; Kohrsolin FF, based on benzalkonium chloride, glutaraldehyde and didecyldimonium chloride; Dismozon pur, based on magnesium monoperphthalate), and one instrument disinfectant was investigated at 4% for 15 min, 3% for 30 min and 2% for 60 min [Korsolex basic, based on glutaraldehyde and (ethylenedioxy)dimethanol]. Three types of organic load were used: 0.3% albumin, 10% fetal calf serum, and 0.3% albumin with 0.3% sheep erythrocytes. Virus titres were determined by a quantitative test (endpoint titration) in 96-well microtitre plates. With all tested preparations, SARS-CoV was inactivated to below the limit of detection (reduction factor mostly ≥ 4), regardless of the type of organic load. In summary, SARS-CoV can be inactivated quite easily with many commonly used disinfectants.

* Corresponding author. Tel.: +49 69 6301 5312; fax: +49 69 6301 83061.
E-mail address: rabenau@em.uni-frankfurt.de

0195-6701/$ - see front matter
doi:10.1016/j.jhin.2004.12.023

Introduction

The recent severe acute respiratory syndrome (SARS) epidemic affected over 30 countries,[1] mainly in Asia and Northern America, and involved more than 8000 probable cases and more than 700 deaths worldwide.[2] New cases were reported in 2004.[3] Several hospital outbreaks occurred, affecting both patients and healthcare workers.[4,5] It has been suggested that healthcare workers who are exposed to SARS patients can be infected with SARS coronavirus (SARS-CoV), regardless of the intensity of exposure.[6] This has alerted the global infection control community. Management of infected patients consisted of isolation and strict respiratory and contact precautions.[7] A case-control study among 241 non-infected and 13 infected staff members with documented exposure to 11 index SARS patients suggested that wearing a face mask is the most important infection control tool, followed by appropriate hand hygiene,[8] suggesting that droplets and hands play a major role in transmission of SARS-CoV. Hands may be contaminated by patient excretions or contact with contaminated surfaces. From the inanimate environment, nosocomial pathogens can be transmitted to hands quite easily.[9] SARS-CoV has been described to persist on surfaces for up to 96 h.[10] In another study, dried SARS-CoV retained its infectivity for as long as six days, indicating a relatively strong survival ability. Only after nine days in a dried state did SARS-CoV completely lose its infectivity.[11] Results of a cohort study among visitors of a hotel suggested that environmental contamination should be considered as a possible source of infection.[12] The Robert Koch Institute, Berlin, Germany has recommended the use of disinfectants that have complete virucidal activity including the spectrum of non-enveloped viruses.[13] The World Health Organization (WHO) has suggested that standard disinfectants should be effective against SARS-CoV,[14] but experimental evidence is not available to date. This is why we have investigated the activity of various disinfectants against SARS-CoV.

Materials and methods

Products

Eight commercial products were tested, all manufactured by and obtained from Bode Chemie GmbH & Co., Hamburg, Germany. Four were alcohol-based hand disinfectants: Sterillium, based on 45% iso-propanol, 30% n-propanol and 0.2% mecetronium etilsulphate; Sterillium Rub, based on 80% ethanol; Sterillium Gel, based on 85% ethanol; and Sterillium Virugard, based on 95% ethanol. All alcohol-based hand rubs were tested without dilution. Three products were surface disinfectants: Mikrobac forte, based on benzalkonium chloride and laurylamine; Korsolin FF, based on benzalkonium chloride, glutaraldehyde and didecyldimonium chloride; and Dismozon pur, based on magnesium monoperphthalate. The surface disinfectants were tested at the recommended concentration for the recommended application time at which they have been shown to have bactericidal and yeasticidal activity, as well as sufficient efficacy in tests under practical conditions.[15] The instrument disinfectant Korsolex basic, based on glutaraldehyde and (ethylenedioxy) dimethanol, was included due to the possible transmission of SARS-CoV by flexible bronchoscopes. The product was tested at the recommended concentration and application time that has been shown to have bactericidal and yeasticidal activity, as well as sufficient efficacy in tests under practical conditions.[15]

Test procedure

Viruses and cells

SARS-CoV isolate FFM-1[16] was obtained from the sputum of a patient hospitalized with a diagnosis of probable SARS in the Isolation Unit of Frankfurt University Hospital, Germany. SARS-CoV were grown in Vero cell cultures (African green monkey kidney, ATCC no. CCL-81). The maintenance medium consisted of minimum essential medium (MEM) without fetal calf serum (FCS) and containing 100 IU/mL of penicillin and 100 μg/mL of streptomycin. Virus stock was stored at −80 °C. Infectious virus titres were calculated as described by Kärber[17] and Spearman,[18] and determined as 50% tissue culture infective doses. Different virus stocks were used for the experiments. The initial $\log_{10}$ virus titres were between 8.93 ± 0.25 and 9.30 ± 0.38. In accordance with WHO recommendations, all work involving infectious SARS-CoV was performed under biosafety level (BSL)-3 conditions in a BSL-3 facility.

Susceptibility of SARS-CoV to different chemical disinfectants

The experiments were performed according to prEN 14476.[19] For each of the experiments, eight parts of the compound were adapted to room temperature (RT) and mixed with one part of virus suspension and one part of organic load or MEM. The organic loads used were 0.3% albumin, 10% FCS, and 0.3%

albumin with 0.3% sheep erythrocytes. Immediately after incubation for defined periods of time at RT, the mixture was diluted 1:10 with ice-cold MEM and put into an ice bath to avoid an extension of the effective incubation period. Serial 10-fold dilutions with ice-cold MEM were performed to assess virus titres as described above. For each dilution step, eight wells containing suspended cells were inoculated. After three to four days of incubation at 37 °C in an CO_2 incubator, cells were microscopically examined for virus-specific cytopathogenic effects. All tests were performed in triplicate, and for each experiment, a virus control containing MEM instead of disinfectant was included ('control titration'). Further control experiments included formaldehyde (0.7%) as standard disinfectant, and a 'termination control', which is a 1:10 dilution of the disinfectant. This control demonstrates the first 1:10 dilution step in the above mentioned procedure and should verify if a postincubation effect of the disinfectant exists. Furthermore, cytotoxic effects caused by the compounds at various dilutions were assessed in suspended Vero cells in 96-well plates using the MTT Cell Proliferative Kit I (Roche, Mannheim, Germany) as published previously.[20,21] Tests for cytotoxicity were performed as single assays using 10% FCS and disinfectant but without addition of virus.

Calculation of the reduction factor

The reduction factor (RF) was calculated as the difference in the quotient of the infection titre before ('control titration') and after incubation of the virus with the disinfectant ('remaining virus'). Therefore, the $\log_{10}$ titre and its (double) standard deviation (SD) were calculated as well as the variance of the RF.

Results

The results are shown in Table I. All four alcohol-based hand rubs led to inactivation of SARS-CoV to below the limit of detection (RF $\geq$ 4.3, SD 0.5 to $\geq$ 5.5, SD 0.5), irrespective of the presence and type of organic load, within 30 s (Table I). The three surface disinfectants also inactivated SARS-CoV to below the limit of detection (RF $\geq$ 3.8, SD 0.7 to $\geq$ 6.1, SD 0.4) within 30 min. The same efficacy was seen with the instrument disinfectant at concentrations of 2% (60 min), 3% (30 min) and 4% (15 min), regardless of the type of organic load (Table I). The mean RF with the instrument disinfectant was $\geq$ 3.3, SD 0.5, which is nearly tenfold below the results of the other disinfectants, due to initial virus titre which was tenfold lower. The results of the controls (data not presented) showed that the termination controls had nearly the same titre as the 'control titration'. This means that no post-exposure disinfection effect could be seen. The incubation of SARS-CoV with 0.7% formaldehyde showed an RF $\geq$ 3, and the cytotoxicity controls indicated that the compounds are cytotoxic up to a dilution between 1:10 to 1:100, while 0.7% formaldehyde was cytotoxic up to a 1:10 000 dilution.

Discussion

Data on the efficacy of various types of disinfectants against SARS-CoV are very limited. We were able to show a reproducible activity with all disinfectants at the commonly used concentrations and exposure times, even with different types of organic load. Alcohols have been described to have immediate, very good activity[22] against many different enveloped viruses such as orthopoxvirus,[23,24] influenza A virus,[23,24] herpes simplex virus type 1 and 2,[24] Newcastle disease virus,[25] togavirus,[26] hepatitis B virus[27–29] and human immunodeficiency virus.[24,30,31] Our finding with SARS-CoV is therefore in line with previously reported data against many other enveloped viruses. The use of alcohol-based hand rubs after contamination of the hands with SARS-CoV, e.g. by respiratory secretions during patient contact, should be effective to prevent further transmission of SARS-CoV by healthcare workers' hands.

Patients with SARS may well spread the virus to the inanimate environment, which has been described as a source for SARS infections.[12] SARS-CoV may persist on inanimate surfaces for up to six days,[11] and serve as a source of infection during that time. That is why the disinfection of surfaces provides additional safety to control the spread of SARS-CoV from inanimate surfaces in an outbreak situation. The three tested surface disinfectants were all effective against SARS-CoV at the concentration and exposure time recommended for routine disinfection of surfaces.[15] The recommendation is derived from experimental evidence which includes bactericidal and yeasticidal activity in suspension tests, and which provides sufficient efficacy under practical conditions.[15] This spectrum of activity appears to include SARS-CoV. prEN 14476 allows surface disinfectants to be tested at exposure times of 5 and 15 min, as well as 30 and 60 min. Shorter times are more relevant to the exposure times used in practice for surface

Table I Efficacy of different types of disinfectant at various exposure times against SARS coronavirus, expressed as minimum reduction factors (RFs) of three parallel experiments: 0.3% serum albumin (BSA), 10% fetal calf serum (FCS), and 0.3% BSA with 0.3% sheep erythrocytes

Product	Type of area of application	Concentration	Exposure time	RF (and SD)		
				0.3% BSA	10% FCS	0.3% BSA and 0.3% sheep erythrocytes
Sterillium	Hand rub	Undiluted	30 s	≥4.25 (0.47)	≥4.25 (0.47)	≥4.25 (0.47)
Sterillium Rub	Hand rub	Undiluted	30 s	≥4.25 (0.47)	≥4.25 (0.47)	≥4.25 (0.47)
Sterillium Gel	Hand rub	Undiluted	30 s	≥5.5 (0.54)	≥5.5 (0.54)	≥5.5 (0.54)
Sterillium Virugard	Hand rub	Undiluted	30 s	≥5.5 (0.54)	≥5.5 (0.54)	≥5.5 (0.54)
Mikrobac forte	Surface disinfectant	0.5%	30 min	≥6.13 (0.35)	≥6.13 (0.35)	≥6.13 (0.35)
			60 min	≥6.13 (0.35)	≥6.13 (0.35)	≥6.13 (0.35)
Kohrsolin FF	Surface disinfectant	0.5%	30 min	≥3.75 (0.71)	≥3.75 (0.71)	≥3.75 (0.71)
			60 min	≥3.75 (0.71)	≥3.75 (0.71)	≥3.75 (0.71)
Dismozon pur	Surface disinfectant	0.5%	30 min	≥4.5 (0.54)	≥4.5 (0.54)	≥4.5 (0.54)
			60 min	≥4.5 (0.54)	≥4.5 (0.54)	≥4.5 (0.54)
Korsolex basic	Instrument disinfectant	4%	15 min	≥3.25 (0.47)	≥3.25 (0.47)	≥3.25 (0.47)
		3%	30 min	≥3.25 (0.47)	≥3.25 (0.47)	≥3.25 (0.47)
		2%	60 min	≥3.25 (0.47)	≥3.25 (0.47)	≥3.25 (0.47)

disinfection (i.e. before drying), and are usually achieved with higher concentrations of the surface disinfectant. Especially in critical areas such as paediatric intensive care, a higher concentration of surface disinfectants provides more safety and is preferable.[32]

One instrument disinfectant was tested against SARS-CoV. Patients with SARS may require a bronchoscopy. The flexible endoscope will be processed after use and must not spread the virus to any other patient. Different approaches to achieve optimum results during reprocessing flexible endoscopes are currently debated worldwide,[33–36] but the minimum spectrum of activity for an instrument disinfectant has not yet been defined. We were able to show that a standard instrument disinfectant achieved sufficient activity against SARS-CoV using the recommended concentration and exposure time,[15] indicating that SARS-CoV is quite easily inactivated.

All experiments were carried out with three different types of organic load: 10% FCS, 0.3% albumin, and a combination of 0.3% albumin with 0.3% washed sheep erythrocytes ('dirty conditions'). All disinfectants were found to be active against SARS-CoV regardless of the type of organic load. Against other enveloped viruses, an ethanol-based hand rub was also described to be effective under different types of organic load.[24] Against the feline calicivirus (FCV), however, there was a significant influence of the type of organic load on the efficacy of 70% ethanol and 70% iso-propanol; FCS did not impair the efficacy against FCV but the presence of albumin or sheep erythrocytes significantly reduced the efficacy of the alcohols.[37] Our data indicate that sufficient activity against SARS-CoV can be expected with the tested disinfectants, regardless of the type of organic load.

Acknowledgements

The authors would like to thank Mrs Gaby Bauer for technical assistance. The study was sponsored by a grant from Bode Chemie GmbH & Co., Hamburg, Germany.

References

1. Guan Y, Peiris JS, Zheng B, *et al*. Molecular epidemiology of the novel coronavirus that causes severe acute respiratory syndrome. *Lancet* 2004;**363**:99—104.
2. Berger A, Drosten C, Doerr HW, Sturmer M, Preiser W. Severe acute respiratory syndrome (SARS)—paradigm of an emerging viral infection. *J Clin Virol* 2004;**29**:13—22.
3. Fleck F. SARS virus returns to China as scientists race to find effective vaccine. *Bull WHO* 2004;**82**:152—153.
4. Anonymous. Cluster of severe acute respiratory syndrome cases among protected healthcare workers—Toronto, Canada, April 2003. *MMWR* 2003;**52**:433—436.
5. Li HL, Feng XR, Dai L, Yang Q. Epidemiologic investigation of nosocomial infection of SARS in medical staff of hospital. *Di Yi Jun Yi Da Xue Xue Bao* 2004;**24**:355—358.

6. Ho KY, Singh KS, Habib AG, *et al*. Mild illness associated with severe acute respiratory syndrome coronavirus infections: lessons from a prospective seroepidemiologic study of healthcare workers in a teaching hospital in Singapore. *J Infect Dis* 2004;**189**:642—647.
7. Manocha S, Walley KR, Russell JA. Severe acute respiratory distress syndrome (SARS): a critical care perspective. *Crit Care Med* 2003;**31**:2684—2692.
8. 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). *Lancet* 2003;**361**:1519—1520.
9. Kampf G. Mikrobielle Besiedlung der Hände und ihre epidemiologische Bedeutung. In: Kampf G, editor. *Händehygiene im Gesundheitswesen*. Berlin: Springer; 2003. p. 29—64.
10. Duan SM, Zhao XS, Wen RF, *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—255.
11. 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.
12. Radun D, Niedrig M, Ammon A, Stark K. SARS: retrospective cohort study among German guests of the hotel "M", Hong Kong. *Eurosurveillance* 2003;**8**:228—230.
13. Robert Koch-Institut. *Empfehlung des Robert Koch-Institutes für die Hygienemaβnahmen und Infektionskontrolle bei Patienten mit Schwerem Akutem Respiratorischem Syndrom (SARS)*. Berlin: Robert Koch-Institut; 2003.
14. WHO. *First data on stability and resistance of SARS coronavirus compiled by members of WHO laboratory network*. Geneva: WHO; 2003.
15. DGHM. *Liste der nach den "Richtlinien für die Prüfung chemischer Desinfektionsmittel" geprüften und von der Deutschen Gesellschaft für Hygiene und Mikrobiologie als wirksam befundenen Desinfektionsverfahren*. Wiesbaden: mhp-Verlag; 2002.
16. 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—1976.
17. Kärber G. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Arch Exp Path Pharmak* 1931;**162**: 480—487.
18. Spearman C. The method of 'right or wrong cases' (constant stimuli) without Gauss's formulae. *Br J Psychol* 1908;**2**: 227—242.
19. prEN 14476. *Chemical disinfectants and antiseptics. Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine. Test method and requirements (phase 2, step 1)*. Brussels: Comité Européen de Normalisation; 2002.
20. Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *Lancet* 2003;**361**:2045—2046.
21. Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Treatment of SARS with human interferons. *Lancet* 2003;**362**:293—294.
22. 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—893.
23. Groupe V, Engle CC, Gaffney PE. Virucidal activity of representative antiinfective agents against influenza A and vaccinia virus. *Appl Microbiol* 1955;**3**:333—336.
24. Kampf G, Rudolf M, Labadie J-C, Barrett SP. Spectrum of antimicrobial activity and user acceptability of the hand disinfectant agent Sterillium Gel. *J Hosp Infect* 2002;**52**: 141—147.
25. Cunningham CH. The effect of certain chemical agents on the virus of Newcastle disease of chicken. *Am J Vet Res* 1948; **9**:195—197.
26. Bucca MA. The effect of various chemical agents on eastern equine encephalomyelitis virus. *J Bacteriol* 1956;**71**: 491—492.
27. Bond WXV, Favero MS, Petersen NJ. Inactivation of hepatitis B virus by intermediate to high-level disinfectant chemicals. *J Clin Microbiol* 1983;**18**:535—538.
28. Kobayashi H, Tsuzuki M, Koshimizu K. Susceptibility of hepatitis B virus to disinfectants or heat. *J Clin Microbiol* 1984;**20**:214—216.
29. Payan C, Pivert A, Kampf G, Ramon C, Cottin J, Lemarie C. Assessment of new chemical disinfectants for HBV virucidal activity in a cell culture model. *J Hosp Infect* 2004;**56**: S58—S63.
30. Martin LS, Meoougal JS, Loskoski SL. Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy associated virus. *J Infect Dis* 1985; **152**:400—403.
31. Spire B, Barre-Sinoussi F, Montagnier L. Inactivation of lymphadenopathy associated virus by chemical disinfectants. *Lancet* 1984;**2**:899—901.
32. Reiss I, Borkhardt A, Fussle R, Sziegoleit A, Gortner L. Disinfectant contaminated with Klebsiella oxytoca as a source of sepsis in babies. *Lancet* 2000;**356**:310—311.
33. Rutala WA, Weber DJ. Reprocessing endoscopes: United States perspective. *J Hosp Infect* 2004;**56**:S27—S39.
34. Martiny H, Floss H, Zühlsdorf B. The importance of cleaning for the overall results of processing endoscopes. *J Hosp Infect* 2004;**56**:S16—S22.
35. Heeg P. Reprocessing endoscopes: national recommendations with a special emphasis on cleaning—the German perspective. *J Hosp Infect* 2004;**56**:S23—S26.
36. Darbord JC. Importance of cleaning for reprocessing endoscopes and thermolabile sterile medical devices: French use and regulations. *J Hosp Infect* 2004;**56**:S40—S43.
37. Kampf G, Grotheer D, Steinmann J. Efficacy of three ethanol-based hand rubs against feline calicivirus (FCV). *J Hosp Infect* 2005 [in press].