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Review

Efficacy of biocidal agents and disinfectants against the monkeypox virus and other orthopoxviruses

G. Kampf*

University Medicine Greifswald, Greifswald, Germany

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SUMMARY

The number of human monkeypox virus infections is increasing in many countries. The typical mode of transmission is by direct contact. As orthopoxviruses may stay infectious on inanimate surfaces under laboratory conditions for up to 42 days, disinfection may be relevant in the surroundings of confirmed cases. The aim of this review was to evaluate published data on the antiviral efficacy of biocidal agents and disinfectants against the monkeypox virus and other orthopoxviruses. A Medline search was carried out on 5th June 2022. The terms 'monkeypox virus', 'poxvirus' and 'orthopoxvirus' were used in combination with 'disinfection'. Publications were included and results were extracted where they provided original data on any orthopoxvirus regarding its inactivation by disinfectants. Vaccinia viruses could be inactivated by at least 4 $\log_{10}$ in suspension tests and on artificially contaminated surfaces by 70% ethanol (≤1 min), 0.2% peracetic acid (≤10 min) and 1–10% of a probiotic cleaner (1 h), mostly shown with different types of organic load. Hydrogen peroxide (14.4%) and iodine (0.04–1%) were effective in suspension tests, sodium hypochlorite (0.25–2.5%; 1 min), 2% glutaraldehyde (10 min) and 0.55% orthophthalaldehyde (5 min) were effective on artificially contaminated surfaces. Copper (99.9%) was equally effective against vaccinia virus and monkeypox virus in 3 min. Disinfectants with efficacy data obtained in suspension tests and under practical conditions with different types of organic load resembling compounds of the blood, the respiratory tract and skin lesions are preferred for the inactivation of the monkeypox virus.

Introduction

Monkeypox is regarded as a typical zoonotic infectious disease that can occasionally cause infections in humans, typically transmitted from animals [1]. Overall case numbers have been low in the UK in the past years with three cases in 2018, one case in 2019 and three cases in 2021, most of them associated with travel from Nigeria [2]. The number of new monkeypox virus infections in humans, however, is currently increasing in many countries worldwide [3]. An important insight is that each of the sequenced viral genomes collected from people with monkeypox in Belgium, France, Germany, Portugal and the USA closely resembles that of a monkeypox strain found in western Africa [4] which is less lethal with a death rate below 1% in poor, rural populations. The typical strains detected in central Africa, however, have a death rate up to 10% [5]. The World Health Organization (WHO) reported until 15th June 2022 a total of 2103 confirmed cases of monkeypox from 42 member states, including one death; 81% of the cases were reported from Europe ($N = 1773$) with most of them found in the UK ($N = 524$), Spain ($N = 313$) and Germany ($N = 263$) [6].

* Address for correspondence: University Medicine Greifswald, Ferdinand-Sauerbruch-Strasse, 17475 Greifswald, Germany.
E-mail address: guenter.kampf@uni-greifswald.de.

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The DNA of monkeypox is typically detected in ulcerated lesions, in the upper respiratory tract, in blood and in urine [7]. Nosocomial and household transmissions have been described [7]. Viral transmission occurs by direct contact with infected body fluids or lesions, via contaminated fomites, or through respiratory secretions that typically require prolonged interaction [8]. A pooled estimate from a systematic review suggested a secondary attack rate of approximately 8% (range 0–11%) among household contacts who were unvaccinated against smallpox [9].

Results from an *in vitro* study showed that cultured orthopoxviruses may remain infectious at room temperature on galvanized steel and glass under laboratory conditions for 3 days (89–100% relative humidity) or up to 42 days (1–10% relative humidity) in the absence of organic load [10]. Variola virus in crusts obtained from a single smallpox patient with an initial viral load of approximately 2.2×10^8 could remain infectious in a sterile bottle at room temperature for up to 8 weeks at 85–90% relative air humidity or for up to 12 weeks in a desiccator [11]. Disinfection may therefore be relevant in the surroundings of confirmed cases to reduce the potential for viral spread via contaminated surfaces. Although it is unlikely that monkeypox will become a global health emergency [3], it is important to know which disinfectants and biocidal agents are effective against the monkeypox virus and other orthopoxviruses. The aim of this review was therefore to compile and evaluate published data on the antiviral efficacy of biocidal agents and disinfectants against the monkeypox virus and other orthopoxviruses.

Methods

A Medline search was performed on 5th June 2022. The terms 'monkeypox virus', 'poxvirus' and 'orthopoxvirus' were used in combination with 'disinfection' and resulted in three, 127 and 106 hits, respectively. Research articles from all available years and in all languages were included. Results were extracted given they provided original data on any orthopoxvirus regarding its inactivation by single biocidal agents used for disinfection or by formulated products based on a single or multiple biocidal agents (e.g., suspension tests or carrier tests). Reviews were not included, but were screened for any information within the scope of this review.

Results

Alcohols

Ethanol was effective in suspension tests against the vaccinia virus strain Elstree and the modified vaccinia virus Ankara (MVA) in concentrations between 50% and 95% within 1 min, even with different types of organic load. Ethanol at 45% supplemented with phosphoric acid was also effective in 30 s. At concentrations of 40% or less, ethanol revealed only poor efficacy against vaccinia viruses (Table I). Isopropanol was effective against both viruses in concentrations between 40% and 75% within 1 min, mostly shown in the presence of 10% foetal calf serum (FCS), whereas a concentration of 30% revealed only a partial efficacy within 1 min (Table I). Formulations based on two types of alcohol with a total alcohol concentration between 75% and 77.8% were also very effective in 15 s (Table I).

A sufficient efficacy of 70% ethanol against vaccinia viruses on artificially contaminated surfaces was shown on stainless-steel carriers with different types of organic load at exposure times of 1 min, 10 min and 1 h (Table II).

Aldehydes

Glutaraldehyde has been described to be effective in suspension tests against the vaccinia virus strain Elstree and MVA at concentrations between 0.05% and 0.5% within 5 min, mostly in the presence of 10% FCS. At shorter contact times of 30 s or 2 min, however, sufficient efficacy against vaccinia viruses was not consistently described (Table III).

A solution and formulation of 2% glutaraldehyde were effective against vaccinia virus on artificially contaminated stainless-steel carriers under dirty test conditions within 10 min. Orthophthaldehyde at 0.55% revealed a comparable efficacy in 5 min against the vaccinia virus under dirty conditions (Table II).

Peroxides

Hydrogen peroxide was effective in suspension tests against vaccinia virus at 14.4% in 30 s. Peracetic acid was also proved to quickly inactivate vaccinia viruses at concentrations between 0.005% and 0.2% within 1 min and with 10% FCS as organic load. Monopercitric acid inactivated vaccinia virus at 0.05% (30 s), 0.025% (2 min) and 0.01% (15 min). Ozone was effective against vaccinia virus at 0.12% in 1 h. The data obtained with monopercitric acid and ozone indicate that the efficacy is impaired in the presence of organic load (Table IV).

The efficacy against vaccinia viruses on artificially contaminated stainless-steel carriers was sufficient under dirty test conditions when a solution of 7.5% hydrogen peroxide was applied for 10 min or when a solution or formulation of 0.2% peracetic acid were applied for 5 min (Table II).

Halogens

Chlorine was effective in suspension tests against vaccinia viruses at 0.64% (active chlorine) in 1 min and at 0.525% (sodium hypochlorite) in 3 min with a low organic load. Lower concentrations required longer exposure times or were insufficiently effective. A higher concentration of albumin as an organic load reduced the virucidal efficacy (Table V). Iodine was also effective against vaccinia viruses in concentrations between 0.045% and 1% within 1 min under clean and dirty test conditions (Table V).

Sodium hypochlorite (0.25% and 2.5%) was in addition effective against vaccinia virus under dirty test conditions on artificially contaminated stainless-steel carriers in 1 min (Table II).

Benzalkonium chloride

Solutions based on benzalkonium chloride (BAC) at 0.05% and 0.13% were not sufficiently effective in suspension tests against vaccinia viruses within 10 min. Products based on 0.0125% BAC (30 min) and 0.025% BAC (5 min), however, were effective but were tested only in the absence of organic load. A product based on either a 'quaternary ammonium compound' at 0.1% (30 min) or BAC at 0.015% (1 min) reduced vaccinia virus sufficiently. Products based on BAC with additional chlorhex

Table I
Efficacy of solutions and formulations primarily based on ethanol, isopropanol and n-propanol against vaccinia viruses in suspension tests

Biocidal agent(s)	Concentration	Test strain	Exposure time	Type of organic soil	Temperature	Log_{10}	Reference
Ethanol	95% #	Strain Elstree	15 s	None	20°C	≥4.8	[12]
			15 s	10% FCS	20°C	≥4.5	[12]
			15 s	0.2% BSA	20°C	≥4.1	[12]
			15 s	Clean conditions	20°C	≥4.5	[12]
			15 s	Dirty conditions	20°C	≥5.5	[12]
			30 s	Not described	20°C	4.1	[13]
	85% #	Strain Elstree	15 s	None	Not described	>4.6	[14]
			15 s	10% FCS	Not described	>5.3	[14]
			15 s	Dirty conditions	Not described	>5.0	[14]
	80% #	Strain Elstree	15 s	None	20°C	≥5.0	[12]
			15 s	10% FCS	20°C	≥5.3	[12]
			15 s	0.2% BSA	20°C	≥5.3	[12]
			15 s	Clean conditions	20°C	≥5.0	[12]
			15 s	Dirty conditions	20°C	≥5.4	[12]
		MVA	30 s	0.3% BSA	Not described	5.0	[15]
	70% ##	Vaccination strain	1 min	None	RT	≥4.0	[16]
		Vaccination strain	10 min	None	25°C	6.8	[17]
	60% ##	ATCC CRL-1549	1 min	10% FCS	20°C	≥4.4	[18]
		MVA	1 min	10% FCS	20°C	≥3.6 *	[18]
	55% #, **	Strain Elstree	30 s	Not described	20°C	5.1	[13]
	50% ##	ATCC CRL-1549	1 min	10% FCS	20°C	≥4.4	[18]
		MVA	1 min	10% FCS	20°C	≥3.6 *	[18]
	45% #, **	Strain Elstree	30 s	Not described	20°C	5.1	[13]
	40% ##	ATCC CRL-1549	1 min	10% FCS	20°C	2.3–3.9	[18]
		MVA	1 min	10% FCS	20°C	1.7–5.3	[18]
	30% ##	ATCC CRL-1549	1 min	10% FCS	20°C	≤0.1	[18]
		MVA	1 min	10% FCS	20°C	0.0	[18]
Isopropanol	99% ##	Vaccination strain	10 min	None	26°C	≥6.7	[17]
	75% #	MVA	30 s	0.3% BSA	Not described	5.0	[15]
	60% ##	ATCC CRL-1549	1 min	10% FCS	20°C	≥4.4	[18]
		MVA	1 min	10% FCS	20°C	≥3.6 *	[18]
	50% ##	ATCC CRL-1549	1 min	10% FCS	20°C	≥4.4	[18]
		MVA	1 min	10% FCS	20°C	≥3.6 *	[18]
	48.5% ##	Vaccination strain	10 min	None	26°C	6.5	[17]
	40% ##	ATCC CRL-1549	1 min	10% FCS	20°C	≥4.4	[18]
		MVA	1 min	10% FCS	20°C	≥3.6 *	[18]
	30% ##	Vaccination strain	10 min	None	RT	≥4.0	[16]
		ATCC CRL-1549	1 min	10% FCS	20°C	0.3–1.9	[18]
		MVA	1 min	10% FCS	20°C	0.5–3.7	[18]
Isopropanol, n-propanol, mecetronium etilsulphate	45%, 30%, 0.2% #	Strain Elstree	15 s	None	20°C	≥6.3	[12]
			15 s	10% FCS	20°C	≥5.3	[12]
			15 s	0.2% BSA	20°C	≥5.6	[12]
			15 s	Clean conditions	20°C	≥5.7	[12]
			15 s	Dirty conditions	20°C	≥6.4	[12]
Isopropanol, ethanol, povidone iodine	38.9%, 38.9%, 3.24% #	MVA	15 s	Clean conditions	20°C	≥5.7	[19]
			15 s	Dirty conditions	20°C	≥5.7	[19]

BSA, bovine serum albumin; clean conditions = 0.03% bovine albumin; dirty conditions = 0.3% bovine albumin and 0.3% sheep erythrocytes; FCS, foetal calf serum; MVA, modified vaccinia virus Ankara; RT, room temperature.

Formulated product.

Solution of biocidal agent

* Limit of detection <4.0 log_{10} in some experiments.

** Contains phosphoric acid as an auxiliary agent.

Table II
Efficacy of solutions or products based on various biocidal agents against vaccinia viruses on artificially contaminated surfaces

Biocidal agent(s)	Concentration	Applied volume	Test strain	Type of carrier	Type of organic soil	Temperature	Exposure time	Log_{10}	Reference
Ethanol	70% #	50 μL	Laboratory strain	Stainless steel	Dirty conditions	20°C	1 min	4.5	[32]
							10 min	>5.0	[32]
	70% #	100 μL	MVA	Stainless steel	Clean conditions	RT	1 h	5.8	[24]
Glutaraldehyde	2% #	50 μL	Laboratory strain	Stainless steel	Dirty conditions	20°C	10 min	>5.0	[32]
	2% ##						10 min	>5.0	[32]
Orthophthalaldehyde	0.55% #	50 μL	Laboratory strain	Stainless steel	Dirty conditions	20°C	5 min	>5.0	[32]
	0.55% ##						5 min	>5.0	[32]
Hydrogen peroxide	7.5% #	50 μL	Laboratory strain	Stainless steel	Dirty conditions	20°C	10 min	4.9	[32]
							20 min	>5.0	
Peracetic acid	0.2% #	50 μL	Laboratory strain	Stainless steel	Dirty conditions	20°C	10 min	>5.0	[32]
	0.2% ##						10 min	>5.0	[32]
Sodium hypochlorite	2.5% #	50 μL	Laboratory strain	Stainless steel	Dirty conditions	20°C	1 min	>4.6	[32]
	0.25% #						1 min	>4.6	[32]
Benzyldimethyltetradecylammonium chloride	0.05% #	50 μL	Laboratory strain	Stainless steel	Dirty conditions	20°C	1 min	2.8	[32]
							10 min	3.2	[32]
Glucoprotamin	0.065% ##	100 μL	Strain Elstree	Stainless steel	Clean conditions	RT	5 min	1.4	[31]
							15 min	1.6	
							30 min	2.3	
		100 μL	Strain Elstree	Glass	Clean conditions	RT	5 min	1.2	[31]
							15 min	1.5	
							30 min	1.7	
		100 μL	Strain Elstree	PVC	Clean conditions	RT	5 min	1.1	[31]
							15 min	1.3	
							30 min	2.1	
Glucoprotamin	0.13% ##	100 μL	Strain Elstree	Stainless steel	Clean conditions	RT	5 min	1.8	[31]
							15 min	1.7	
							30 min	2.3	
		100 μL	Strain Elstree	Glass	Clean conditions	RT	5 min	1.6	[31]
							15 min	1.5	
							30 min	2.2	
		100 μL	Strain Elstree	PVC	Clean conditions	RT	5 min	1.6	[31]
							15 min	1.6	
							30 min	2.4	
Glucoprotamin	0.26% ##	100 μL	Strain Elstree	Stainless steel	Clean conditions	RT	5 min	2.0	[31]
							15 min	2.1	
							30 min	2.3	
		100 μL	Strain Elstree	Glass	Clean conditions	RT	5 min	1.9	[31]
							15 min	2.3	
							30 min	2.3	

	100 µL	Strain Elstree	PVC	Clean Conditions	RT	5 min	2.1	[31]
						15 min	2.1	
						30 min	2.7	
Probiotic cleaner	10% ##	MVA	Stainless steel	Clean conditions	RT	1 h	5.8	[24]
	2% ##					1 h	5.8	
	1% ##					1 h	2.8	
	1% ##					2h	5.8	
Alkaline cleaner	0.9% ##	50 µL	Laboratory strain	Stainless steel	Dirty conditions	20°C	10 min	>5.0	[32]

MVA, modified vaccinia virus Ankara; RT, room temperature.
dirty conditions = 0.3% sheep erythrocytes and 0.3% bovine serum albumin; clean conditions = 0.03% bovine serum albumin.
Solution of biocidal agent.
Formulated product.

idine digluconate or glutaraldehyde were also effective in 1 min in the absence of organic load during testing (Table VI).

The potentially low effect of quaternary ammonium compounds against vaccinia virus was also shown on artificially contaminated stainless-steel carriers under dirty test conditions. A solution of 0.05% benzyldimethyltetradecylammonium chloride reduced the test virus by up to 3.2 $\log_{10}$ in 10 min (Table II).

Glucoprotamin

A formulated product based on glucoprotamin was tested under clean conditions against the vaccinia virus with a 5-min exposure time. Diluted product reduced the test virus by at least 2.7 $\log_{10}$ (0.13% and 0.065% glucoprotamin) or at least 1.7 $\log_{10}$ (0.26% glucoprotamin). The limit of detection did not allow measurement of a higher reduction (Table VI).

The same product was insufficiently effective in a carrier test under clean conditions with an incomplete inactivation of dried vaccinia virus on stainless steel (2.3 $\log_{10}$), polyvinyl chloride (2.7 $\log_{10}$) or glass carrier (2.3 $\log_{10}$) using 0.26% glucoprotamin for 30 min (Table II).

Chlorhexidine digluconate

A commercially available antimicrobial soap based on 4% chlorhexidine digluconate reduced the vaccinia virus strain Elstree in suspension tests in 30 s by 1.0 $\log_{10}$ and was not sufficiently effective [13].

Copper

The vaccinia virus strain Elstree and the virulent monkeypox virus strain Copenhagen were both tested on surfaces with 99.9% copper and stainless steel at room temperature. The initial viral titre of both viruses (approximately 10^6 pfu) was reduced by $\geq$4 $\log_{10}$ within 3 min on copper whereas the decline was less than 2 $\log_{10}$ on stainless steel within 5 min and remained small after 20 min [23].

Probiotic cleaner

A formulated probiotic detergent product containing 10^7 cfu/mL spores of *B. subtilis*, *B. pumilus* and *B. megaterium* was tested against MVA (ATCC VR-1508) in suspension tests at dilutions of 10%, 2% and 1% with exposure times of up to 24 h. Sufficient efficacy of at least 4 $\log_{10}$ was found at concentrations of 10% and 2% within 1 h whereas a solution of 1% was sufficiently effective within 2 h [24].

Similar results were found according to EN 16777:2019 on artificially contaminated surfaces under clean conditions. The same concentrations of 10%, 2% and 1% were able to inactivate MVA when applied after the artificial contamination of surfaces (Table II). In addition, the formulation was effective when applied prior to a viral contamination of the surface. The antiviral effect of treated surfaces (2-h exposure time) against a subsequent viral contamination remained with all concentrations for at least 24 h [24].

Alkaline cleaner

An alkaline cleaner at 0.9% was found to inactivate vaccinia virus on artificially contaminated stainless-steel carriers under dirty conditions by more than 5 $\log_{10}$ in 10 min (Table II).

Table III
Efficacy of solutions based on glutaraldehyde against vaccinia viruses in suspension tests

Biocidal agent	Concentration	Test strain	Exposure time	Type of organic soil	Temperature	Log_{10}	Reference
Glutaraldehyde	0.5%	ATCC CRL-1549	30 s	10% FCS	20°C	≥2.8 *	[18]
			2 min	10% FCS	20°C	≥2.8 *	[18]
			5 min	10% FCS	20°C	≥2.8 *	[18]
		MVA	30 s	10% FCS	20°C	≥3.1 *	[18]
			2 min	10% FCS	20°C	≥3.1 *	[18]
			5 min	10% FCS	20°C	≥3.1 *	[18]
	0.1%	ATCC CRL-1549	30 s	10% FCS	20°C	2.1 to ≥3.9 *	[18]
			2 min	10% FCS	20°C	≥3.8 *	[18]
			5 min	10% FCS	20°C	≥3.8 *	[18]
		MVA	30 s	10% FCS	20°C	0.7 to ≥3.1 *	[18]
			2 min	10% FCS	20°C	≥3.1 *	[18]
			5 min	10% FCS	20°C	≥3.1 *	[18]
	0.05%	ATCC CRL-1549	30 s	10% FCS	20°C	0.5 to ≥3.9 *	[18]
			2 min	10% FCS	20°C	3.9 to ≥4.4 *	[18]
			5 min	10% FCS	20°C	≥4.6	[18]
		MVA	30 s	10% FCS	20°C	0.2—1.9	[18]
			2 min	10% FCS	20°C	2.1 to ≥3.9 *	[18]
			5 min	10% FCS	20°C	≥3.1 *	[18]
	0.02%	Vaccination strain	10 min	None	RT	≥4.0	[16]

FCS, foetal calf serum; MVA, modified vaccinia virus Ankara; RT, room temperature.
* Limit of detection <4.0 log_{10} in some experiments.

Ultraviolet light

UVC light (254 nm) has been described to inactive aerosolized vaccinia virus strain WR in a benchtop one-pass aerosol chamber in 7.6 s by 0.02—2.3 log_{10}. A lower relative air humidity increased the susceptibility of the vaccinia virus to UVC [25]. Similar results were found with the vaccinia virus Western reserve strain exposed for 10 min in aerosol to UVC light (254 nm). Under steady-state conditions a reduction between 0.9 and 2.4 log_{10} was found with higher values in winter [26].

Discussion

Only very few data were found to describe the efficacy of biocidal agents or disinfectants against the monkeypox virus. Most studies were carried out with different strains of vaccinia virus which could be inactivated by at least 4 log_{10} in suspension tests and on artificially contaminated surfaces by 70% ethanol (≤1 min), 0.2% peracetic acid (≤10 min) and 1—10% of a probiotic cleaner (1 h), mostly shown with different types of organic load. Hydrogen peroxide (14.4%) and iodine (0.04—1%) were effective in suspension tests, sodium hypochlorite (0.25—2.5%), 2% glutaraldehyde and 0.55% orthophthalaldehyde were effective on artificially contaminated surfaces. Glucoprotamin at 0.07%, 0.13% and 0.26% showed an insufficient efficacy on artificially contaminated surfaces and some effect in suspension tests where the limit of detection did not allow measurement of a 4 log_{10} reduction. Benzalkoniumchloride was partly effective depending on its concentration and the exposure time; chlorhexidine digluconate at 4% (30 s) was not sufficiently effective. A surface of 99.9% copper was also very effective against vaccinia virus and monkeypox virus.

Only one study was found with a direct comparison of the susceptibilities of a vaccinia virus and a monkeypox virus to copper. A similar susceptibility of both viruses was found towards the biocidal agent [23]. In addition, a comparable susceptibility of the vaccina virus strain Elstree (e.g., ATCC VR-1549, used for decades in disinfectant efficacy testing) and MVA (recently established in disinfectant efficacy testing) to four commercially available disinfectants used in veterinary medicine was shown by Hartnack *et al.* [33]. Finally, early experiments showed that the variola virus could be effectively inactivated by 50—70% ethanol in 1 min, 40—50% isopropanol in 1 min, 0.1—2% sodium hypochlorite in 1 min and 0.5% benzalkonium chloride in 5 min [34], which corresponds to the results obtained with vaccinia viruses described previously. Based on these data, it is reasonable to assume that the different orthopoxviruses have a similar susceptibility to disinfectants.

A relevant limitation may be the type of organic load used in virucidal efficacy testing. Crusts are an obstacle to determine real-life virucidal activity, especially when suspension tests reveal favourable results [35]. In addition, it has been shown that vaccinia virus embedded in rabbit dermal scabs are more difficult to inactivate. Under these experimental conditions, complete viral inactivation, which is not required by a disinfection procedure, was achieved by 2% glutaraldehyde in 1 h, 80% ethanol, 70% isopropanol and 60% n-propanol in 3 h, whereas a quaternary ammonium compound did not achieve complete viral inactivation in 18 h [36]. The type and amount of organic load has been established to have a major impact on the efficacy of disinfectants against vaccinia viruses [22,37]. Taking into account that the DNA of the monkeypox virus is typically detected in ulcerated lesions, in the upper respiratory tract, in blood and in urine [7], some of the organic loads used in suspension tests have certainly clinical relevance such as FCS (skin lesions) as well as the combination of sheep erythroctes and albumin (blood). The ASTM tripartite organic load containing mucin, bovine serum albumin and tryptone may be the most suitable soil for a virus detected in the upper respiratory tract [38] but is currently not described in the European norms.

Table IV
Efficacy of solutions and formulations based on hydrogen peroxide, peracetic acid, monopercitric acid and ozone against vaccinia viruses in suspension tests

Biocidal agent(s)	Concentration	Test strain	Exposure time	Type of organic soil	Temperature	Log_{10}	Reference
Hydrogen peroxide	14.4% #	ATCC CRL-1549	30 s	None	20°C	≥4.3	[20]
			30 s	FCS *	20°C	≥4.2	[20]
Peracetic acid	0.2% #	Strain Elstree	30 s	None	Not described	>4.0	[21]
			30 s	10% FCS	Not described	>4.0	[21]
	0.1% ##	ATCC CRL-1549	1 min	10% FCS	20°C	≥3.9 **	[18]
		MVA	1 min	10% FCS	20°C	≥3.9 **	[18]
	0.05% ##	ATCC CRL-1549	1 min	10% FCS	20°C	≥3.5 **	[18]
		MVA	1 min	10% FCS	20°C	≥4.1	[18]
	0.01% ##	ATCC CRL-1549	1 min	10% FCS	20°C	≥3.5 **	[18]
		MVA	1 min	10% FCS	20°C	≥3.6 **	[18]
	0.005% ##	ATCC CRL-1549	1 min	10% FCS	20°C	≥4.5	[18]
		MVA	1 min	10% FCS	20°C	≥5.7	[18]
	0.0025% ##	ATCC CRL-1549	1 min	10% FCS	20°C	3.4	[18]
		MVA	1 min	10% FCS	20°C	4.8	[18]
	0.001% ##	ATCC CRL-1549	1 min	10% FCS	20°C	0.6–0.9	[18]
		MVA	1 min	10% FCS	20°C	0.2–1.1	[18]
Monopercitric acid	0.05% ##	Strain Elstree	30 s	None	Not described	>4.0	[21]
			30 s	10% FCS	Not described	>4.0	[21]
	0.025% ##		1.5 min	None	Not described	3.1	[21]
			1.5 min	10% FCS	Not described	2.0	[21]
			2 min	None	Not described	>4.0	[21]
			2 min	10% FCS	Not described	>4.0	[21]
	0.01% ##		5 min	None	Not described	1.3	[21]
			5 min	10% FCS	Not described	1.0	[21]
			15 min	None	Not described	>4.0	[21]
			15 min	10% FCS	Not described	>4.0	[21]
Ozone	0.12% ##	Strain Elstree	30 min	None	Not described	5.5	[22]
			30 min	10% FCS	Not described	2.9	[22]
			45 min	10% FCS	Not described	4.2	[22]
			60 min	10% FCS	Not described	5.5	[22]
			30 min	50% FCS	Not described	1.0	[22]
			45 min	50% FCS	Not described	2.1	[22]
			60 min	50% FCS	Not described	5.5	[22]
			60 min	80% FCS	Not described	2.1	[22]

FCS, foetal calf serum; MVA, modified vaccinia virus Ankara.
Formulated product.
Solution of biocidal agent.
* Concentration not described.
** Limit of detection <4.0 log_{10} in some experiments.

Overall, it may be useful to establish an additional organic load for disinfectant efficacy testing against pathogens typically isolated from the respiratory tract, especially for biocidal substances such as sodium hypochlorite or ozone which have an impaired activity against vaccinia viruses when tested with increasing amounts of organic load [22,28]. Alternatively, it may be helpful to provide evidence that the standard organic loads used in the European norms also cover the typical organic soil found in respiratory tract secretions.

Another limitation is the lack of efficacy data under practical conditions for many disinfectants, e.g., on artificially contaminated surfaces according to EN 16777 without mechanical action or according to EN 16615 with mechanical wiping. For surface disinfection, one study with a glucoprotamin-based disinfectant described that data from suspension tests do not correlate with data from carrier tests, indicating that the efficacy in real life may be substantially lower than in suspension tests [31]. Conversely, it was described with a probiotic cleaner that data from suspension tests do correlate well with data from tests under practical conditions [24]. Although the results from suspension tests are relevant to determine the spectrum of antiviral activity it seems desirable to have additional results from tests under practical conditions to have more confidence in the disinfectants efficacy in real life.

The efficacy of a biocidal agent may also depend on the formulation itself including its auxiliary substances. Data obtained with carrier tests under dirty conditions revealed that 2% glutaraldehyde, 0.55% orthophthalaldehyde and 0.2%

Table V
Efficacy of solutions and formulations based on chlorine as sodium hypochlorite and iodine as iodophor against vaccinia viruses in suspension tests

Biocidal agent(s)	Concentration	Test strain	Exposure time	Type of organic soil	Temperature	Log_{10}	Reference
Chlorine as sodium hypochlorite	0.636% *, #	Strain Guarani (VACV-GP2)	1 min	None	RT	5.7	[27]
	0.525% #	Strain Copenhagen	3 min	1% BSA	RT	≥4.4	[28]
			3 min	7% BSA	RT	3.8	[28]
	0.0525% #	Strain Copenhagen	3 min	1% BSA	RT	1.8	[28]
			3 min	7% BSA	RT	0.2	[28]
	0.02% ##	Vaccination strain	10 min	None	RT	≥4.0	[16]
	0.00525% #	Strain Copenhagen	3 min	1% BSA	RT	0.3	[28]
			3 min	7% BSA	RT	0.2	[28]
Iodine as iodophor	1% **, #	MVA	15 s	Clean conditions	20°C	≥4.0	[19]
			15 s	Dirty conditions	20°C	≥4.2	[19]
	0.75–0.81% **, #	Strain Elstree	30 s	Not described	20°C	≥3.7 ***	[13]
	0.75% **, #	MVA	15 s	Clean conditions	20°C	≥4.0	[29]
			15 s	Dirty conditions	20°C	≥4.2	[29]
	0.4% **, #	MVA	15 s	Clean conditions	20°C	≥4.2	[29]
			15 s	Dirty conditions	20°C	≥4.0	[29]
	0.1% **, #	MVA	15 s	Clean conditions	20°C	6.5	[29]
			15 s	Dirty conditions	20°C	6.5	[29]
	0.1% **, #	MVA	15 s	Clean conditions	20°C	≥5.7	[19]
			15 s	Dirty conditions	20°C	≥5.5	[19]
	0.075% **, #	MVA	15 s	Clean conditions	20°C	≥5.5	[29]
			15 s	Dirty conditions	20°C	≥5.7	[29]
	0.04% **, #	MVA	15 s	Clean conditions	20°C	4.5	[29]
			15 s	Dirty conditions	20°C	4.3	[29]
	0.01% **, #	MVA	15 s	Clean conditions	20°C	4.3	[19]
			15 s	Dirty conditions	20°C	2.8	[19]
			30 s	Dirty conditions	20°C	3.5	[19]
			60 s	Dirty conditions	20°C	3.5	[19]
	0.01% **, #	MVA	15 s	Clean conditions	20°C	4.8	[29]
			15 s	Dirty conditions	20°C	3.5	[29]
			30 s	Dirty conditions	20°C	4.0	[29]
	0.0075% **, #	MVA	30 s	Clean conditions	20°C	4.2	[19]
			60 s	Clean conditions	20°C	4.5	[19]
			30 s	Dirty conditions	20°C	1.7	[19]
			60 s	Dirty conditions	20°C	1.8	[19]
	0.045% #	Strain Guarani (VACV-GP2)	1 min	None	RT	5.5	[27]
	0.004% **, #	MVA	60 s	Clean conditions	20°C	3.7	[29]
			60 s	Dirty conditions	20°C	1.0	[29]
	0.001% **, #	MVA	60 s	Clean conditions	20°C	0.7	[29]
			60 s	Dirty conditions	20°C	1.0	[29]
	0.0009% **, #	Strain Guarani (VACV-GP2)	1 min	None	RT	0.9	[27]
			5 min	None	RT	1.8	[27]
			30 min	None	RT	2.8	[27]
	0.0005% **, #	Strain Guarani (VACV-GP2)	1 min	None	RT	0.1	[27]
			5 min	None	RT	1.1	[27]
			30 min	None	RT	2.1	[27]

BSA, bovine serum albumin; clean conditions = 0.03% bovine albumin; dirty conditions = 0.3% bovine albumin and 0.3% sheep erythrocytes; MVA, modified vaccinia virus Ankara; RT, room temperature.

* Active chlorine.

** Available iodine.

*** Limit of detection <4.0 log_{10} in all experiments.

\# Formulated product.

\#\# Solution of biocidal agent.

Table VI
Efficacy of solutions or products primarily based on surface-active biocidal agents such as benzalkonium chloride or glucoprotamin against vaccinia viruses in suspension tests

Biocidal agent(s)	Concentration	Test strain	Exposure time	Type of organic soil	Temperature	Log_{10}	Reference
Benzalkoniumchloride	0.13% #	Strain IHD	10 min	None	30°C	2.8	[30]
	0.05% #	Strain IHD	10 min	None	30°C	1.8	[30]
	0.025% ##	Strain Guarani (VACV-GP2)	1 min	None	RT	3.4	[27]
			5 min	None	RT	≥6.0	[27]
			30 min	None	RT	≥6.0	[27]
	0.0125% ##	Strain Guarani (VACV-GP2)	1 min	None	RT	2.1	[27]
			5 min	None	RT	2.4	[27]
			30 min	None	RT	≥6.0	[27]
'Quaternary ammonium compound'	0.015% ##	Strain Guarani (VACV-GP2)	1 min	None	RT	≥6.0	[27]
			5 min	None	RT	≥6.0	[27]
			30 min	None	RT	≥6.0	[27]
	0.01% ##	Strain Guarani (VACV-GP2)	1 min	None	RT	1.1	[27]
			5 min	None	RT	1.7	[27]
			30 min	None	RT	≥6.0	[27]
Benzalkonium chloride, chlorhexidine digluconate	0.025%, 0.01% ##	Strain Guarani (VACV-GP2)	1 min	None	RT	≥6.0	[27]
			5 min	None	RT	≥6.0	[27]
			30 min	None	RT	≥6.0	[27]
Benzalkonium chloride, glutaraldehyde	0.01%, 0.007% ##	Strain Guarani (VACV-GP2)	1 min	None	RT	≥5.7	[27]
			5 min	None	RT	≥5.7	[27]
			30 min	None	RT	≥5.7	[27]
Glucoprotamin	0.26% ##	Strain Elstree	5 min	Clean conditions	RT	≥1.7 *	[31]
	0.13% ##		5 min	Clean conditions	RT	≥2.7 *	[31]
	0.065% ##		5 min	Clean conditions	RT	≥2.7 *	[31]

RT, room temperature.
Solution of biocidal agent.
Formulated product.
* Limit of detection <4.0 log_{10} in all experiments.

peracetic acid were equally effective against vaccinia virus as a simple solution and as a formulated product [32]. With ethanol as a biocidal agent, it was shown that the efficacy against poliovirus can be enhanced in the presence of 0.7% phosphoric acid [39]. This finding may also explain the sufficient efficacy of a formulation based on 45% ethanol plus phosphoric acid in 30 s [13] while ethanol alone at 40% had mostly insufficient efficacy against vaccinia virus in 1 min [18]. It is therefore essential to have efficacy data for disinfectant products and formulations used in healthcare or in community settings.

In healthcare, it is important that a disinfectant exerts its virucidal effect in a short contact time, especially when volatile biocidal agents such as alcohols are used. The data obtained with vaccinia viruses indicate that some biocidal agents are sufficiently effective within 1 min depending on their concentration such as alcohols, glutaraldehyde, peracetic acid, hydrogen peroxide, monocitric acid, sodium hypochlorite, iodine and some formulations with two different biocidal agents. Ozone, a probiotic cleaning agent and benzalkonium chloride mostly required a longer exposure time of up to 1 h which may be too long in the patients' surroundings.

In conclusion, data from suspension tests and carrier tests show that most biocidal agents and disinfectants have sufficient activity against vaccinia viruses with different types of organic load. Susceptibility data of the monkeypox virus and the vaccinia virus to copper indicate that disinfectants with sufficient activity against vaccinia virus should also be effective against the monkeypox virus. Disinfectants with efficacy data obtained in suspension tests and under practical conditions with different types of organic load resembling compounds of the blood, the respiratory tract and skin lesions should be preferred for the inactivation of the monkeypox virus.

Conflict of interest statement
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