Poxviruses
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# Environmental resistance, disinfection, and sterilization of poxviruses

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## Abstract

The virion of a poxvirus is an enveloped particle that differs significantly from other enveloped viruses. Apart from DNA, proteins and phospholipids, poxvirus virions also contain carbohydrates. They show a high environmental stability and remain contagious over a period of several months in an ambient environment. Poxviruses show an extraordinary high resistance to drying, which is further enhanced by materials in which they are released into the environment (e.g., dermal crusts, serum, blood residues and other excretions). Dried *Vaccinia virus* can be stored at 4°C over a period of more than 35 weeks without any loss of infectivity. Frozen in buffer at –20°C, a titer reduction of only 3 log-steps is observed within 15 years. In general, virus isolated from patients and/or environment is more resistant to environmental conditions than virus deriving from cell cultures. In addition, poxviruses show a high stability towards different pH values. Due to their low lipid content, they are less sensitive to organic solvents/disinfectants compared to other enveloped viruses. This is the reason for the considerably higher resistance of poxviruses to diethylether in comparison to other enveloped viruses. Despite all of these aspects, poxviruses are highly sensitive to all common approved disinfection regimens. Cell-bound poxvirus may show a higher stability than cell-free virus. This phenomenon is not observed if quaternary ammonium compounds are used. Due to the possible renewed importance of smallpox, e.g., in case of abuse in biological warfare, but also because of the impact of poxviruses in veterinary medicine, representatives of the poxvirus family have been chosen to test the efficacy of common disinfectants. The common sterilization procedures – thermal, chemical, an/or radiation – are usually effective against poxviruses.

## Environmental resistance

Poxviruses (*Poxviridae*) are a very diversified family of viruses and still represent a potential danger to health, even for humans [1, 2]. They show a broad occurrence in nature and infect not only vertebrates down to fish, but also insects and even plants. The poxvirus virion is an enveloped particle that differs significantly from all other enveloped viruses. Poxviruses have

only a comparably low content of lipids in their envelope, although there are considerable differences between the different subfamilies and genera of poxviruses. Avipoxviruses, for example, have a higher lipid content than that found in orthopoxviruses. Apart from DNA, proteins and phospholipids, poxviruses also contain small quantities of carbohydrates (about 3%) [3–5].

Poxviruses show an extraordinarily high resistance to drying [6–9]. This property is enhanced by the materials in which the virus is released into the environment, such as dermal crust, serum, blood and other excretions [10–12]. Already in the 18th century it was recognized that material from patients infected with smallpox stays contagious over a period of at least several months [13]. In particular, dust, blankets, bed linen and personal clothes remained contagious for several years [14] and as well as direct human-to-human transmission, transmission *via* personal belongings, clothes and even underwear was presumed to occur [14]. In the past, clothes and linen, especially, possessed a significantly higher commercial value than today. It was, therefore, common practice to pass them on to others even if they originated from severely ill or deceased persons.

A case reported from Galicia in 1912 provides evidence that the virus was, for example, transmitted *via* paper, specifically, by a letter. Its paper seemed to have been contaminated with *Variola virus* (VARV) and this was transported to Mühlacker in Baden (Germany), where an epidemic developed. From there it was reported to have spread to the cities of Pforzheim, Aue and Freiburg in Germany *via* person-to-person contact as well as *via* contaminated textiles [15].

Although the environmental resistance of poxviruses is high at ambient temperatures, it is even greater at lower temperatures. Dried *Vaccinia virus* (VACV) can be stored at 4°C over a period of more than 35 weeks without any decline of infectivity. Frozen in buffer at –20°C a titer reduction of only 3 log-steps was observed after 15 years. Virus isolated from patients and/or the environment is commonly more resistant than virus material derived from cell cultures. Cell-free or purified virus preparations isolated from supernatants of cell cultures are generally less resistant than the corresponding cell-bound virus [12].

Poxviruses show an increased temperature tolerance compared to most other enveloped viruses. A titer reduction of only 2 log-steps was observed for cell-bound virus on heating to 56°C for 15 min. Nevertheless, differences within the temperature stability for the subfamilies and genera seem to exist. For example, avipoxviruses have been reported to be inactivated by heating at 56°C for 60 min, whereas parapoxviruses need inactivation conditions of 2.5 h at 56°C or alternatively 1 h at 80°C. Therefore, a short exposure of even 90°C does not guarantee reliable inactivation of infectivity. Purified virus preparations are considerably easier to inactivate at 56°C for 15 min even in the presence of 2% fetal calf serum (FCS) with a titer reduction of 4 log-steps [16].

## Disinfection

In addition to the high resistance to drying, poxviruses show a high stability across different pH values in the range between pH 4.5 and 10. Due to their low lipid content they are less sensitive to organic solvents compared to other enveloped virus families [17, 18]. This explains their considerably high diethylether resistance in contrast to their sensitivity to chloroform, phenol and ethanol, which has been described, for example, for the *Shope fibroma virus* [19]. Whereas 30–40% ethanol at 0°C for 1 h was sufficient to inactivate this virus, a concentration of 60–70% diethylether was necessary under the same experimental conditions [20].

Poxviruses are highly sensitive to commercial chemical disinfectants, as are all lipid-containing enveloped viruses, although cell-bound poxvirus can exhibit a remarkably high stability [21–23]. If 0.5% formaldehyde is used for a contact time of 5–15 min, a titer reduction of cell-free VACV of 3.5–4 log-steps can easily be achieved. In contrast, only a 1 log-step reduction could be obtained under the same conditions for cell-bound virus. If treated with sodium hydroxide, cell-free VACV can be inactivated in 15 min by a 0.1% solution (4 log-step reduction), whereas cell-bound virus titers could only be reduced by 1 log-step. Comparable results can be observed for peracetic acid: a working concentration of 0.1% (150 ppm active oxygen content) yields a reduction rate of 4–5 log-steps within a 30-min contact time for cell-free virus, but only a 1–2 log-steps of reduction could be obtained under the same conditions for cell-bound virus. If quaternary ammonium compounds (QAC) were used this significant difference was not observed: 0.2% *N*-cetylpiridinium chloride yielded a reduction factor of 4 log-steps for both cell-free and cell-bound virus with a contact time of 15 min (cell-bound virus: reduction factor 3.5–4.0; cell-free virus: 4.0) [24]. The efficacy of some further active ingredients for disinfection is presented in Table 1 [25].

Because of the importance of possible smallpox contamination [26], e.g., due to its exceptional epidemic impact and in terms of a potential abuse in case of biological warfare [27–29], as well as their impact in veterinary medicine, members of the poxvirus family have been chosen for efficacy testing of disinfectants. This has been laid down in several national and/or international guidelines [30–33]. The German Society of Veterinary Medicine (Deutsche Veterinärmedizinische Gesellschaft, DVG) uses samples of VACV both in a suspension for direct tests as well as on pieces of wood (poplar) to simulate carrier contamination [30]. In addition, the German National Health Authorities (Robert-Koch-Institute) together with the German Society for the Control of Virus Diseases (Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten, DVV) employs VACV in their suspension test for the field of human medicine [31]. Orthopoxviruses are prescribed in the French AFNOR guidelines (Association Française de Normalisation) [33] for their suspension test for the human medical field [34]. Correspondingly, broad experiences of the efficacy of commercial chemical disinfectants are

Table 1. Efficacy of common active ingredients of disinfectants against poxviruses tested exemplarily against *Vaccinia virus* as a representative for most other poxviruses [38–49]

| Substance | Concentration/contact time | Test conditions |
|---|---|---|
| Sodium hypochlorite | 200 ppm/10 min | Suspension test WPL |
| $H_2O_2$ | 1%/5–10 min* | Suspension test WPL |
| $KMnO_4$ | 0.02%/5–10 min* | Suspension test WPL |
| Peracetic acid | 0.1%/5–10 min* | Suspension test WPL |
| Formaldehyde | 2%/5 min* | Suspension test WPL |
| Glutaraldehyde | 0.02%/10 min** | Suspension test WPL |
| Phenol | 2%/10 min** | Suspension test WPL |
| o-Phenylphenol | 0.12%/10 min** | Suspension test WPL |
| Ethanol | 40%/10 min** | Suspension test WPL |
| 2-Propanol | 30%/10 min** | Suspension test WPL |
| $HgCl_2$ | 0.02%/10 min** | Suspension test WPL |
| Formic acid | 0.1%/30 min**<br>0.25%/15 min** | Suspension test WPL<br>Suspension test with 0.2% BSA or 10% FCS |
| Propionic acid | 1%/10 min**<br>1%/1 h** | Suspension test WPL<br>Suspension test with 0.2% BSA or 10% FCS |
| Citric acid | 1%/15 min**<br>1%/30 min** | Suspension test WPL<br>Suspension test with 0.2% BSA or 10% FCS |
| Acetic acid | 1%/30 min**<br>2%/15 min** | Suspension test with or WPL<br>Suspension test with or WPL |
| Propionic acid<br>Citric acid<br>Acetic acid | 0.5–2%/7.5–120 min** | Carrier test on wood and cotton (according to DVG) |

BSA, bovine serum albumin; DVG, Deutsche Veterinärmedizinische Gesellschaft (German Society of Veterinary Medicine); FCS, fetal calf serum; WPL, without protein load.
* Reduction factor ≥ 5, ** reduction factor ≥ 4.

available [35], and the results show that poxviruses can be easily controlled by such commercial disinfectants. Table 2 summarizes the efficacy of some marketed disinfectant formulations. As smallpox was eradicated some while ago now, a large number of publications – also on the disinfection issue – derive from the time before the 1970s/1980s.

## Sterilization

Every sterilization procedure used in the medical field is effective against viruses. Although poxviruses have a better tolerance against heat, they do

Table 2. Efficacy of some commercially available disinfectant formulations against pox viruses tested against *Vaccinia virus* according to the RKI (Robert Koch Institute) suspension test

| Disinfectant formula (for 100 g concentrate) | Reduction Factor > 4 log-steps | |
|---|---|---|
| | Application concentration (%) | Contact time (min) |
| 70 g 2-Propanol<br>0.05 g Chlorhexidine digluconate<br>0.45 g $H_2O_2$ (30%) | 90 | 0.5 |
| 46 g Ethanol<br>27 g 2-Propanol<br>1 g Benzyl alcohol | 90 | 1 |
| 10.4 g Ethanol<br>1.67 g $H_2O_2$ (30%)<br>1.5 g Chlorhexidine digluconate | 90 | 1 |
| 38.4 g Ethanol<br>0.35 g Formaldehyde<br>0.066 g Glyoxal<br>0.018 g Glutaraldehyde | 90 | 10 |
| 40 g Ethanol<br>10 g n-Propanol<br>0.018 g Glutaraldehyde<br>0.05 g Benzalconium chloride<br>0.01 g 5-Bromo-5-nitro (1,3)-dioxa-cyclohexane | 90 | 5 |
| 11 g Formaldehyde<br>12 g Glyoxal<br>3.75 g Glutaraldehyde<br>2.7 g Benzalconium chloride<br>1 g Oligo-[di(iminoimidocarbonyl) imidohexamethylene] | 0.5 | 5 |
| 15 g Benzalconium chloride<br>2 g Oligo-[di(iminoimidocarbonyl) iminohexamethylene]<br>2 g 2-Oxydiphenyl | 0.8 | 5 |
| 4.5 g Glutaraldehyde<br>8.8 g Glyoxal | 2 | 5 |
| 8 g Formaldehyde<br>8 g Glyoxal<br>4.5 g Glutaraldehyde | 0.25 | 1 |
| 20 g Sodium perborate<br>15 g Tetraacetylethylene diamine (TAED) | | |
| 6 g Glutaraldehyde<br>5 g Quarternary ammonium compounds (QAV) | 0.5 | 5 |
| 11.1 g Formaldehyde<br>12 g Glyoxal<br>3.75 g Glutaraldehyde<br>2.7 g Benzalconium chloride | 1.5 | 5 |
| 25 g Glucoprotamine | 1 | < 5 |
| 4 g Peracetic acid<br>26 g $H_2O_2$ (30%) | 3 | 2.5 |
| 35 g Sodium hypochloride | 3 | 2.5 |
| 50 g Propylene glycol<br>5 g Potassium hydroxide | 90 | 5 |
| 20 g o-Phenylphenol<br>10 g *p*-Chloro-*m*-cresol | 2 | 5 |

not form an exception to this rule [36]. Dry heat and/or steam sterilization techniques are as effective as chemical sterilization procedures, such as exposure to formaldehyde or ethylene oxide, and radiation [37].

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