

Hygiene Protocols for the Prevention
and Control of Diseases
(Particularly Beak and Feather Disease)
in Australian Birds

The Effectiveness of Disinfectants Used on
Viruses
Closely Related to BFDV



Australian Government

Department of the Environment and Heritage

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The Effectiveness of Disinfectants used on Viruses Closely Related to BFDV

Introduction

A disinfectant is a chemical agent that destroys pathogenic bacteria, but not necessarily spores and not all viruses. A disinfectant is usually applied to an inanimate object (such as a bench) to destroy infective agents that might be present on the object. A dirt, sand or gravel floor cannot be disinfected as easily.

Although many disinfectants have a noticeable odour, they should never be used as a deodoriser. The source of any objectionable odours should be eliminated and not masked by a more acceptable odour.

For a disinfectant to be effective:

1. it must be applied to a surface that has been cleaned of debris and macroscopic organic matter, usually by thorough scrubbing and washing with soap or detergent solutions. If left on the surface, such debris and organic matter will reduce the effectiveness of the disinfectant; and
2. it must be applied to the surface for the recommended minimum period, usually 5-10 minutes, but up to 30 minutes for some disinfectants.

Direct contact between the disinfectant and microorganism is required for disinfection. Microorganisms may be protected from the disinfectant solution by dirt, blood, bubbles, grease and so on. Soaps and detergents are an important facet of disinfection because they are used to remove organic material, dirt or grease from surfaces to be disinfected, and can even kill lipid-enveloped viruses. Many common disinfectants have detergent combinations that enhance their actions.

Microorganisms vary in their resistances to physical and chemical agents that might be applied to kill them. Disinfectants that destroy bacteria may not destroy fungi or viruses. Bacterial spores are more resistant than vegetative forms, non-enveloped non-lipid-containing viruses are more resistant than lipid-enveloped viruses. Microorganism resistance may be summarised (in order of decreasing resistance) in Figure 1 as follows:

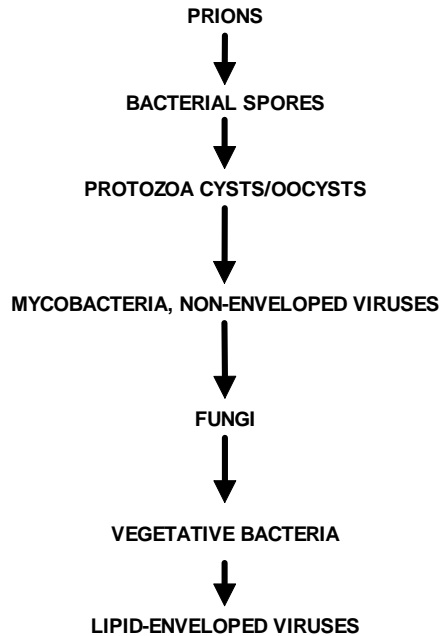


Figure 1: Descending Order of Resistance to Disinfectants

Types of Disinfectants

The types of disinfectants which might be used in a bird facility will be described as follows: aldehydes (formaldehyde and glutaraldehyde), peroxyacid compounds, hypochlorites, iodophors, phenol, quaternary ammonium compounds and alcohols.

i. Aldehydes

Formaldehyde

This may be purchased as either a powder (paraformaldehyde) or a liquid (40% solution with water [37% by weight], called formalin. Because of its carcinogenicity, volatility, caustic action, and danger to humans, it should never be used near birds. It has previously been used extensively as a fumigant in hatcheries in the poultry industry.

Glutaraldehyde

Glutaraldehyde at an acid pH will act only at a bacterial spore's surface, whereas at an alkaline pH it penetrates the spore. Thus it is usual to add an alkalinising agent such as a quaternary ammonium compound (QAC) to glutaraldehyde products. Glutaraldehyde is chemically related to formaldehyde with similar efficiency but without the handling disadvantages of formalin. However, glutaraldehyde usage is associated with occupational health and safety issues - some people develop extreme sensitivity to it, and staff refuse to handle it. It is contraindicated for use in surgical equipment because of the need to rinse the equipment thoroughly with sterile water to avoid the irritation it causes to tissues.

ii. Peroxygen Compounds

Peroxyacetic acid or peracetic acid is made by mixing acetic acid with hydrogen peroxide. Most preparations contain peracetic acid (~5%) and hydrogen peroxide (~20%). On degradation, peroxyacetic acid breaks down to acetic acid (vinegar), water and oxygen. The destruction of spores is greatly increased with both higher temperatures and stronger concentrations. One of the

most striking characteristics of peracetic acid compared to other disinfectants is the low concentration needed to achieve the desired antimicrobial efficacy.

Virkon S is comprised of potassium peroxymonosulfate (50%), sodium chloride, sulphamic acid (5%), malic acid, sodium hexametaphosphate (a buffer), sodium dodecyl benzene sulphonate (a detergent - 15%), amaranth colour (an indicator) and lemon peel extract to provide a characteristic odour. Virkon S is sold as a pink powder that is mixed with water to form a 1% or 2% solution (i.e. 10g or 20g per litre). As the solution ages it becomes pale, indicating that it needs to be replaced. The solution is stable for 7 days. It should not be used as a hand-wash. A 1% solution has a pH of 2.6 and is non-irritant to skin and eyes. It is corrosive to metals over time if it is not thoroughly rinsed off.

iii. Hypochlorites

Chlorine disinfectants are effective against enveloped viruses, vegetative bacteria and fungi.

Hypochlorites contain about 70% of available chlorine. Hypochlorites are available either as:

- a powder containing calcium hypochlorite and sodium hypochlorite (NaOCl), combined with hydrated trisodium phosphate; or
- a liquid containing NaOCl in concentrations 1-15% (Dychdala, 1991).

Scott (1980) found in test conditions that 0.175 percent sodium hypochlorite was the most effective and practical broad-spectrum disinfectant of 22 products tested against a range of different viruses.

Hypochlorites are rapidly inactivated by organic matter, light and heat. Increasing the pH decreases the biocidal activity of chlorine, while decreasing the pH increases the activity. Hypochlorites function best at pH 5-7 (optimum 6), the range at which hypochlorous acid is formed, the chemical that exerts the disinfectant activity. Free chlorine is destructive to clothing and metal, including stainless steel, and is very irritating to the skin, eyes and respiratory system. Hypochlorites must be used carefully around birds because of their odour and residual effect.

iv. Iodophors

An iodophor is a combination of iodine complexed with carriers such as polyvinylpyrrolidone or non-ionic surfactants. The carrier allows a sustained release of iodine, and the iodine remains bound to the carrier until the free iodine in solution falls below equilibrium, when additional iodine is released into solution. When used correctly, they are supposed to enhance the bactericidal activity of iodine (Gottardi, 1991). They are less toxic and irritating than aqueous or alcoholic iodine solutions.

When an iodophor becomes colourless, it is no longer effective. It is effective in cold or hot water, soft or hard water, and can be applied to all surfaces, especially in hatcheries and nurseries.

Iodophors must be applied at the manufacturer's recommended concentration - for example a full-strength (10%) solution of povidone iodine releases only 10% of the free available iodine that is released from a 1% solution.

v. **Phenol (carbolic acid)**

Phenol is a colourless crystalline solid with a characteristic lysol-like odour. It is the chemical that is used to compare other disinfectants for determining the “phenol coefficient”, their ability to kill test microorganisms to that of phenol (O’Connor and Rubino, 1991). Phenol disinfectants are unsuitable for use in a bird environment, because of their odour and persistence in the environment.

vi. **Quaternary ammonium compounds**

One product, F10, consists of polihexanide 4g/L, benzalkonium chloride 54g/L, non-toxic ampholytics and sequesterants. Polihexanide is a chemical used as a biocide for control of micro-organisms and algae in swimming pools, spas, disinfectant in veterinary products and as a sanitiser for milk handling equipment. Its efficacy claims are:

- bacteria (1:500 for 2 minutes)
- Fungi, yeasts and moulds (1:500 15 minutes)
- fungal spores (1:250 30 minutes)
- enveloped viruses (1:500, 10-30 minutes)
- non-enveloped viruses - infectious bursal disease virus and parvovirus (1:125, 30 minutes), and
- bacterial spores (1:125 for 30 minutes).

The product has been used safely as an aerosol to treat respiratory infections, including fungi and yeasts (Verwoerd, 2001, Chitty, 2002). No claims are made for circoviruses.

There is no evidence at present that F10 inactivates BFDV.

QACs are classified as follows:

- **First Generation:** An example is benzalkonium chloride. These have minimal biocidal activity and are commonly used as preservatives;
- **Second Generation:** These are substituted benzalkonium chlorides, an example of which is alkyl dimethyl benzyl ammonium chloride. These have high biocidal activity.
- **Third Generation:** These are also called "dual QACs" (for example: one contains equal parts of alkyl dimethyl benzyl ammonium chloride and alkyl dimethyl ethylbenzyl ammonium chloride. These QACs have increased biocidal activity, stronger detergency, and increased safety to the user (lower toxicity).
- **Fourth Generation:** These are also called "Twin or Dual Chain QACs". Examples are didecyl dimethyl ammonium chloride and dioctyl dimethyl ammonium chloride. They are superior to other QACs in germicidal performance, lower foaming, and have an increased tolerance to protein loads and hard water.
- **Fifth Generation:** These are mixtures of fourth generation and second-generation QACs (e.g., didecyl dimethyl ammonium chloride + alkyl dimethyl benzyl ammonium chloride). They have excellent germicidal performances, are active under more hostile conditions and are safer to use.

vii. **Alcohols**

Alcohols denature bacterial proteins. In the absence of water, proteins are not readily denatured by alcohol, so a 70-85% solution is generally recommended. Ethyl alcohol and isopropyl alcohol

kill most vegetative bacteria and enveloped viruses but are ineffective against bacterial spores, mycobacteria, protozoa and non-enveloped viruses.

Resistance of Related Viruses to Physical and Chemical Agents

BFDV, chicken anaemia virus (CAV) and porcine circovirus (PCV) are classified as *Circoviridae*. Thus it would be expected that BFDV would have similar environmental sensitivities to those of CAV and PCV. As BFDV's ability was unaffected by incubation to 80°C for 30 min (Raidal and Cross, 1994 - see Figure 2), BFDV appears to have a similar heat sensitivity to that of CAV and PCV. At higher incubation temperatures the titre declined. No change in HA titre occurred following treatment with chloroform. CAV and PCV can be propagated in tissue culture (BFDV cannot) so it is possible to investigate their physical and chemical stabilities. Table 1 shows some physical and chemical characteristics of these viruses.

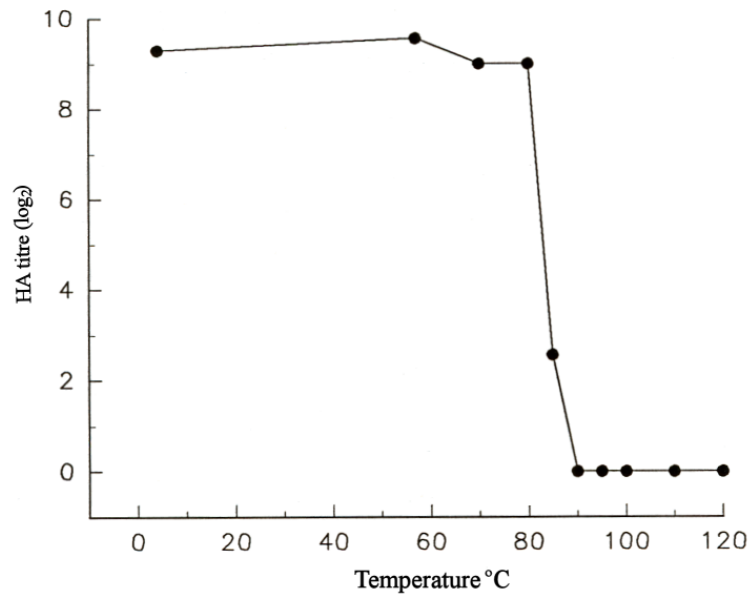


Figure 2: Effects of incubating BFDV for 30 minutes at temperature (Raidal, 1994).

Table 1 Physical and Chemical Characteristics of CAV and PCV

Treatment	CAV	PCV
Ethyl ether	resistant ¹	resistant ^{1,7}
Chloroform	resistant ¹	resistant ^{1,7}
pH 3.0 for 3 hours	stable ¹	stable ^{1,7}
70% ethyl alcohol	resistant ¹	resistant ^{1,7}
90% acetone for 24 hours	resistant ²	-
50% phenol in liver suspension	inactivated ³	-
5% phenol @ 37°C for 2 hours	resistant ³	-
0.1N NaOH 2h @ 37°C; 24 h @ 15°C	resistant ⁴	-
1% glutaraldehyde for 10 min @ RT	inactivated ⁴	-
0.4% β-propiolactone @ 5°C for 24 h	inactivated ⁴	-
5% formaldehyde @ RT for 24h	inactivated ⁴	-
10% iodine @ 37°C for 2 h	inactivated ⁴	-
10% hypochlorite @ 37°C for 2 h	inactivated ⁴	-
Formaldehyde fumigation for 24 h	resistant ⁴	-
Chlorhexidine 0.78%	-	resistant ⁷
Formaldehyde 0.78%	-	resistant ⁷
Iodine 0.47%	-	resistant ⁷
Ethylene oxide fumigation for 24 h	resistant ⁴	-
70°C 1 hr, @ 80°C for 15 min	resistant ⁵	resistant ¹
95°C 35 min; @ 100°C for 10 min	inactivated ⁶	-
70°C for 15 min	resistant ⁵	resistant ¹
5-10% NaOH	-	resistant ¹ reduction ⁷
1:32 hypochlorite	-	resistant ¹
8% formaldehyde	-	resistant ¹
Potassium perogymonosulohate, sodium chloride (Virkon S) 1-2%	inactivated ⁸	inactivated ⁷
Quaternary ammonium 0.5-2.34%	-	reduction ⁷
Phenol 0.4%	-	reduction ⁷
Hypochlorite 4.68%	-	reduction ⁷

1. Allan *et al.*, 1994
2. Taylor, 1992
3. Yuasa *et al.*, 1979
4. Yuasa, 1992

5. Goryo *et al.*, 1985
6. Urlings *et al.*, 1993
7. Royer *et al.*, 2001
8. Wyeth, 1990

Table 2: Properties of some Disinfectants

Disinfectant group	Example	Use Dilution	Contact Time Mins 20°C	BACTERIA			VIRUSES		Fungi	Comments
				Vegetative	Spore	Mycobacteria	Non-enveloped	Enveloped		
Organic acid surfactant Peroxygen compound	Virkon S	0.5-1%	10	Yes	Yes	Yes	Yes	Yes	Yes	OK in presence of organic matter Mild skin irritant - Respiratory irritant (dust)
Glutaraldehyde	Glutacide Key 200 Microcide disinfectant Terminator broad spectrum disinfectant	1-2%	10	Yes	Yes	Yes	Yes	Yes	Yes	OK in presence of organic matter Toxic to birds Skin, eye, respiratory irritant No evidence that it is carcinogenic
Hyochlorites, chlorine	Bleach	100-10,000 ppm	10-60	Yes	Yes*	Yes*	Yes*	Yes	Yes	Rapidly inactivated by organic matter Work best at pH 5-7 Lose activity >80°C Toxic to birds, ruins clothing Produces carcinogenic by-products Skin, eye, respiratory irritant Corrosive to metals
Iodophors	Betadine (Povidone iodine)	25-1600 ppm	20-30	Yes	Variable*	No	No	Variable*	Yes	Rapidly inactivated by organic matter Work best at pH 5-7. Can be corrosive Lose activity >80°C Skin, eye irritant
Phenol	1-stroke environ	1:256	10	Yes	No	No	No	Variable*	Yes	Rapidly inactivated by organic matter Toxic to birds Skin, eye, respiratory irritant
Quaternary ammonium compounds QACs	ViraClean (F10)	1000-20000 ppm	30	Yes	No	No	No	Variable*	Variable	May support growth of Gram negative bacteria Work best at pH >7 Inhibited by hard water Inactivated by proteins, soaps and detergents
Polihexanide	F10	1000-10,000ppm	30	Yes	Yes*	No	only IBD virus Parvovirus	Yes*	Yes	APVMA review on possible carcinogenicity July 2005
Acohols	Ethyl alcohol Isopropyl alcohol	70-85%	15-20	Yes	No	No	No	Variable	Yes	Eye irritant Requires long contact time Evaporates, so items must be soaked in alcohol

* At higher concentrations. Protozoa - killed by glutaraldehyde or peroxygen compound. IBD - infectious bursal disease

Table 1 demonstrates that both CAV and PCV are very resistant viruses. They are inactivated by recommended dilutions of glutaraldehyde and Virkon S applied for the recommended times, and it may be inferred that BFDV would also be inactivated by these disinfectants.

Table 2 shows examples of disinfectants, recommended dilutions, recommended contact times, and efficacy against vegetative bacteria, bacterial spores, mycobacteria, non-enveloped and enveloped viruses and fungi. The table shows that Virkon S and glutaraldehyde are effective across a broad range of pathogens.

Figure 3 shows inferred efficacy from Table 2.

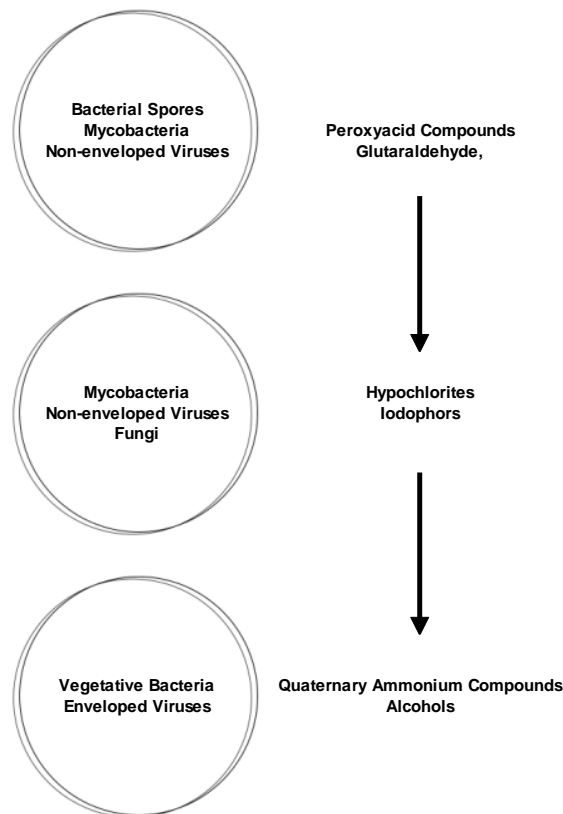


Figure 3: Descending efficacy of disinfectants

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