Duck hepatitis B virus: a model for assessing the efficacy of disinfectants against human hepatitis B virus infection

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One of the most important aspects of infection control is the interruption of transmission of infectious organisms to and from patients within the hospital environment. Of particular concern are the blood-borne viruses HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV). Disinfectants play an important role in infection control, but their virucidal efficacy is difficult to measure *in vitro* because of the high susceptibility of tissue culture systems to damage by chemical agents and the relatively low titres which are achieved in growing many important viruses. Additionally, HBV is almost uncultivable *in vitro* and fails to infect more common laboratory animals. Therefore, the duck model of HBV infection has been used for testing disinfectant action against HBV.

Human HBV is the prototype hepadnavirus and chronically infects over 350 million people. Approximately 20% of these will succumb to cirrhosis and hepatocellular carcinoma. HBV is highly contagious and is spread both vertically, from mother to infant, and horizontally through body secretions such as blood, semen and saliva. The very high viral titres (10^{10} virions/ml) present in the blood create a risk of hospital-acquired infections (HAI) via microscopic contamination of instruments and other fomites with blood or body fluids.

For disinfectants to be registered by the Therapeutic Goods Administration (TGA) for sale in Australia, manufacturers have

to present data relating to the microorganisms they claim their disinfectant inactivates obtained by the testing procedures set out in Therapeutic Goods Order (TGO)#54. Instrument-grade disinfectants claiming to be virucidal must show activity against Adenovirus (medium non-enveloped), herpes simplex virus (enveloped) and either poliovirus or parvovirus (small non-enveloped)[1]. Disinfectants are tested against virus dried onto an inanimate surface (carrier method) and are conducted in the presence of inorganic soil to represent hard water and a minimum of 5% organic soil. The disinfectants must show complete viral inactivation with a minimum 4-log reduction in viral titre.

If the manufacturer claims their disinfectant is active against a specific virus such as HBV then separate data for that virus must be provided. These additional tests are preferably conducted using a carrier method but may, under certain circumstances, be conducted against virus in suspension. For blood-borne viruses the organic soil is a minimum of 50% whole blood, reflecting their origin clinically. Limited disinfectant efficacy studies have been conducted using the chimpanzee model of HBV infection[2,3]. However, the endangered status of the chimpanzee and costs involved prohibit routine use of this. For viruses that cannot be readily cultured and fail to infect laboratory animals, both the TGA and the USA FDA accept surrogate viruses. Duck hepatitis B virus (DHBV) is the accepted surrogate virus for HBV[1].

The family *Hepadnaviridae* include other primate viruses such as the woolly monkey hepatitis B virus, rodent viruses such as the woodchuck and ground squirrel hepatitis B viruses, as well as numerous hepatitis B viruses that infect a variety of birds such as DHBV. All are hepatotrophic and can lead to chronic infection in their representative hosts. They have similar biology, being small, enveloped, partially double-stranded, with circular DNA of roughly 3.2kb. They have a unique replication strategy utilising overlapping open reading frames (ORF) and a DNA polymerase that involves a reverse transcription of a RNA intermediate.

Based on this similarity in biology and replication strategy, we developed the duck model of HBV infection for efficacy testing of disinfectants both in suspension and dried onto filter paper as a carrier method[4]. The advantage of suspension tests is that they are easy to perform and reflect the situation encountered in blood spills or when decontaminating heat-sensitive instruments such as endoscopes. Carrier tests reflect the situation when blood is dried onto surfaces or surgical instruments.

Virus and test disinfectant were mixed together *in vitro*, allowed to react for the specified time, the disinfectant inactivated and residual infectivity determined by inoculation into groups of day-old ducklings. The combination of high titre inoculate obtained in natural infection and ease at which ducklings are infected by very small doses of DHBV make this model very sensitive and ideal for showing large log reductions in viral loads. Additionally, the low cost and ease of handling of ducklings facilitates inoculation of multiple ducks for each concentration used, allowing for meaningful statistical analysis.

Using filter paper as a carrier we found that there was some difficulty in standardising the amount of recoverable virus[4].

Table 1. Comparison of *in vitro* and *in use* disinfectant efficacy testing.

			Time (min)	% transmission
***In vitro* testing**				
		untreated control		100
		2% glutaralderhyde	2.5	0
		2% glutaralderhyde	5	0
***In use* testing**				
	Uncleaned laparoscope	untreated control		100
		2% glutaralderhyde	2.5	0
		2% glutaralderhyde	5	0
	Uncleaned angioscope	untreated control		100
		2% glutaralderhyde	5	90
		2% glutaralderhyde	10	70
		2% glutaralderhyde	20	6
		ethylene oxide		6
	Cleaned angioscope	untreated control		100
		2% glutaralderhyde	5	0
		2% glutaralderhyde	10	0
		2% glutaralderhyde	20	0
		ethylene oxide		0

However, by substituting the filter paper with glass fibre mats this problem was rectified and used to test sterilising systems where liquid culture was inappropriate[5].

There have been limited studies comparing HBV and DHBV inactivation[3]. Prince *et al.* compared the ability of two quaternary ammonium disinfectants and a phenolic disinfectant to inactivate HBV and DHBV[3]. Following disinfectant treatment, residual viral infectivity was assayed using chimpanzee inoculation and visual inspection for morphological alteration for HBV and by inoculation of primary duck hepatocyte culture for DHBV. The three disinfectants inactivated both HBV and DHBV ≥99.9%. This paper highlights the difficulty of using a primate model of HBV in that only one time point was measured and only one chimpanzee per disinfectant test was used, preventing statistical analysis.

Using a different disinfectant, Tsiquaye and Barnard[6] compared sodium hypochlorite inactivation of DNA polymerase (DNAP) in HBV and DHBV positive serum. They found that HBV and DHBV DNAP inactivation correlated with viral inactivation by injecting treated serum into ducklings. Prince and Tsiquaye's papers suggest that HBV and DHBV have similar sensitivity to disinfectants.

Pugh's laboratory was instrumental in promoting the *in vitro* duck hepatocyte culture model for disinfectant testing[7]. Like HBV, DHBV is not directly cytopathic, so indirect methods of detecting viral growth in culture are required. Various techniques have been used by different authors ranging from indirect immunofluorescence, direct immunofluorescence, *in situ* hybridisation, PCR and quantitative PCR. Generally hepatocytes are harvested from one-week-old ducklings but Wang *et al.*[8,9] found that hepatocytes purified from duck embryos produced higher titres of DHBV compared with hepatocytes purified from the liver of ducklings.

The *in vitro* primary duck hepatocyte culture model has the advantage of lower costs and in association with PCR is very sensitive. However, while *in vitro* studies are important and can fulfil regulatory purposes, *in use* factors including the amount of biological soil, the shape of instruments and presence of shielded access may contribute to decontamination breakdown. The *in vivo* model not only allows *in vitro* testing of disinfectants but allows simulation of patient to patient transmission of infection and can be used to assess *in use* factors. We have used laparoscopes on infected ducks, processed the laparoscope and then used the instrument to perform laparoscopic investigation on DHBV-negative day-old ducks[10]. Similarly, we tested patient to patient transmission of HBV during angioscopic investigation[11]. The duck model has also been used to test the validity of decontaminating single use medical items in an effort to decrease spiralling health costs[12].

The above studies were interesting in that *in vitro* testing of 2% glutaralderhyde in a tube had resulted in complete inactivation (Table 1). Similarly treating solid instruments (laparoscope) with 2% glutaralderhyde resulted in complete viral inactivation with five minutes' contact time, despite the presence of visible blood contaminating the instrument. However, when hollow instruments were inadequately cleaned, DHBV was not inactivated even with 20 minutes' contact time or ethylene oxide sterilisation. Correct instrument cleaning prior to disinfection resulted in complete inactivation. These studies emphasise the importance of instrument cleaning prior to disinfection and also highlights how *in use* factors affect disinfection efficacy. The TGA requires is simulated *in use* testing for sterilants and high-level disinfectants prior to registration.

In conclusion, the duck has proved to be a versatile laboratory model for testing the efficacy of both commercial formulations and new chemical agents against hepadnaviruses. The *in vivo* model has a special role in simulating *in use* situations.

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Biography

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