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Disinfectant Investigation, March/April 2009

Protocol:

Feline herpesvirus (FHV), feline calicivirus (FCV) and canine parvovirus (CPV) were propagated in cell culture to achieve significant viral titer. The virus-infected cultures were harvested, subjected to a freeze-thaw cycle, and the homogenous mixture was divided into 5 ml portions and frozen at -80C. An aliquot of the frozen virus was thawed and titrated according to standard procedure.

Titrated virus stock was diluted 1:100 in PBS to reduce the concentration of FBS (from 5% to .05%) thereby avoiding disinfectant inhibition. Ten ml of the diluted stock was mixed with an equal volume of 2x disinfectant (final pH after dissolution in water was 9.4) in a polypropylene tube (this would achieve a 1:200 final dilution of the virus stock, and 1X dilution or working concentration of the disinfectant). Contact was done at room temperature for 10 minutes. The solution was then dialyzed using 10,000 MWCO dialysis tubing and Hank's balanced salt solution, through five buffer changes over a 48 hour period at 4C, to remove the disinfectant. The remaining solution (virus free of disinfectant; approximately 10ml total volume) was removed from the tubing and a portion was used to make 10-fold serial dilutions in DMEM. For FHV and FCV, each dilution was applied to CRFK cells in 96-well plates in five replicates. Comparing to original viral stock concentration, viral dilutions of $2x10^{-2}$ through 10^{-8} were titrated post disinfectant treatment. For CPV, viral propagation using dilutions of dialyzed virus were done in six-well plates in two replicates given that splitting (1:2) of cell cultures was required 24 hrs post-inoculation to enhance viral growth. In addition, titers of 10^{-3} through 10^{-7} and one uninoculated well were made for each replicate. Cell cultures were monitored for 5 days total (with day 1 being the day of inoculation). Resultant titers were determined based on cytopathic effects (FHV and FCV) or antigen detection by immunofluorescence (CPV).

Negative control consisted of water (FCV, FHV) or PBS (CPV) only (no disinfectant), while positive control utilized a 3% sodium hypochlorite solution.

	A - Titer of Stock	B - Post Wysiwash	C- Viral Titer Post Water/PBS	D- Post Clorox
FHV	10^6	No viral growth in lowest dilution tested (to $2x10^{-2}$ – 1:200 dilution of original virus stock)	$10^{4.5}$	No viral growth
FCV	10^7	No viral growth in lowest dilution tested (to $2x10^{-2}$ – 1:200 dilution of original virus stock)	10^6	No viral growth
CPV	10^7 (minimum)	No viral growth in lowest dilution tested (to 10^{-3} – 1:1000 dilution of original virus stock)	10^7	No viral growth

From this investigation, this disinfectant was able to inactivate all viruses at least 4 logs. Compared to negative control, inactivation of 2.5-4 logs was achieved. Comparison can only be made to the negative control in order to account for loss of virus in processing.

As 1:200 or 1:1000 dilutions of virus stock were made both in preparation of, and by mixing with the disinfectant solution, complete inactivation of all virus present could not be assessed; i.e. direct mixing of concentrated virus stock with working solution of disinfectant was not made. This was necessitated by the protocol in order to remove inhibitory organic matter present in the cell-culture propagated stock. The conclusion from this experiment is that significant reduction in viral titer was achieved with the Wysiwash solution, and that it was comparable to Clorox in terms of efficacy.

All viruses consist of a protein shell surrounding the nucleic acid. Some viruses also have a lipid membrane surrounding this shell, in which proteins used for cellular attachment are embedded. Inactivation of viruses methods differ for enveloped vs nonenveloped viruses. For the former, removal of the lipid membrane is sufficient to render the virus noninfectious because the attachment proteins are lost. Thus, any solution with detergent activity is efficacious against these viruses, examples of which include influenza and HIV. For the nonenveloped viruses, inactivation generally requires an agent with oxidizing activity to alter the protein shell such that cellular attachment and infection cannot occur. Examples of these viruses include canine parvovirus, feline calicivirus, hepatitis A virus and norovirus. Disinfectants containing sodium hypochlorite have historically been used to inactivate these agents; this solution has drawbacks including its corrosive nature. A disinfectant with efficacy against the nonenveloped

viruses without the disadvantages of sodium hypochlorite would be tremendously useful in many settings, including veterinary clinics and animal shelters.

To evaluate the efficacy of new disinfectants, we tested their effectiveness against viruses of three levels of hardiness: feline herpesvirus (a relatively labile enveloped virus), feline calicivirus (a hardy nonenveloped virus), and canine parvovirus (ostensibly the most environmentally stable virus). Working solution (final dilution when mixed 1:1 with virus stock) of the disinfectant mixed with each virus was incubated per the manufacturer's recommendation, followed by dialysis to remove the disinfectant. The remaining solution was serially diluted and applied to cell culture to evaluate disinfectant efficacy. The Wysiwash solution was able to reduce the virus titer of all viruses at least 4 fold, and performed comparably to sodium hypochlorite.