SHORT COMMUNICATION

Virucidal Activity of Virkon S on Human Enterovirus 71

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Summary

The efficacy of Virkon S, a commercial disinfectant as a virucidal spray against human enterovirus 71 (HEV71), the causative agent of the fatal form of hand, foot and mouth disease was examined. At least one $\log_{10}$ reduction of HEV71 titer was achieved when one spray of Virkon (1% or 2%) with ten minutes of contact time was applied. The infectivity was completely lost when four sprays of 1% or 2% Virkon were applied, suggesting that at least four sprays of 1% Virkon to the surface bound HEV71 was necessary to completely inactivate the virus. These findings suggest that Virkon S at the proper concentration is suitable to be used as an effective and easy to use disinfectant against HEV71.

Key Words: Enterovirus 71, Disinfection, Virkon S

Enterovirus 71 (HEV71) is a non-enveloped, single stranded positive sense RNA virus belonging to the genus Enterovirus of the *Picornaviridae* family. HEV71 is an important causative agent of hand, foot and mouth disease (HFMD) worldwide affecting millions of young children annually[1]. The pathogenesis of HEV71 infection however, is not well established. It is not known for example why the infection caused many deaths in outbreaks in Malaysia[2] and Taiwan[3] as the virus had caused infections elsewhere but with few deaths if any. Nonetheless, it is known that the virus is transmitted through faecal-oral route and also through direct contact with infected persons secretions[1]. The lack of proper hygienic care and close contacts between children has been attributed as one of the possible means by which the infection may spread. The fear that young children could acquire the infection through direct contact while in close contact with peers had previously resulted in nationwide closure of kindergartens, playgrounds, swimming pools and schools. These had severely disrupted the life of millions and caused severe economic impact on the country[1]. As such effective measures for the immediate containment of the infection and potential future outbreaks are highly desirable. Apart from good sanitation and hygienic practices, effective disinfection strategies are also necessary.

A number of disinfectants have been developed for disinfecting infectious agents including viruses. These disinfectants often are non-specific and in general have broad actions against many microorganisms. Unlike most of the enveloped viruses, which could easily be inactivated with common detergents, enteroviruses are non-enveloped viruses, and in general are completely insensitive to the common disinfectants such as hypochlorite, ether, deoxycholate and other common detergents. Enteroviruses are also relatively resistant to 70% alcohol, 5% Lysol (phenolic agent) and 1% quarternary ammonium compound[4]. Commercial disinfectants for usage against some of the non-enveloped viruses are available but its efficacy for usage against HEV71 in particular under the typical tropical climate such as Malaysian is not known. In the

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present study, the virucidal effects of Virkon S (Antec International, USA), a peroxygen-based commercially prepared disinfectant system was evaluated for its efficacy against HEV71.

HEV71 used in the present study was isolated from the brain of a patient who died of HEV71-associated neurogenic pulmonary edema[2]. The virus was propagated in the laboratory using African green monkey kidney cells (Vero). Virus inoculum was prepared when infected cell cultures manifested ~ 70-80% cytopathic effects. The infected cell cultures were freeze-thawed once and centrifuged to remove cell debris. The supernatant was filtered through 0.20 *mm* porosity filter (Sartorius, Germany) and stored at –70°C until needed. To examine the stability of the virus, initially the virus inoculum was removed from the –70°C freezer, thawed and kept in suspension or plated into a 24 well plate (Costar, USA). The plates or suspension were left to dry for selected intervals under a typical Malaysian classroom environment (temperature between 25-28°C; humidity, 84-88%; evaporation rate 2-6 mm/day). The dried virus inoculum was recovered by adding serum free medium (100 µl) to each well. A ten fold serial dilution was then performed and the inoculum was used to infect Vero cells cultured in 96 well plates (TPP, Switzerland). The end point titer, indicated by the highest dilution that resulted in cytopathic effects, was determined on day seven post-incubation.

Virkon S solution was prepared fresh at 1% and 2% concentrations by diluting Virkon S powder in distilled water. The disinfectant was then dispensed using a plastic spray bottle. To evaluate the efficacy of Virkon S on HEV71, the virus inoculum (100 µl) was added to a 60 mm^2 culture dish (Costar, USA) and allowed to dry for an hour at room temperature in a typical Malaysia classroom environment. Subsequently, Virkon S was sprayed on to the plate at about eight inches away and with the nozzle of the spray bottle perpendicular to the plate held at 45° angle. One spray or four continuous sprays with either 1% or 2% Virkon were applied. The volume of solution delivered was 200 $\pm$ 50 *ml* and 1000 $\pm$ 200 *ml* per spray or per four sprays, respectively. The Virkon mist was left in contact with the virus for exactly ten minutes. Following that serum free medium was added to each dish and allowed to mix well by gentle repeat pipetting of the fluid. Virus infectivity was determined as described earlier.

A drop in HEV71 titer by more than three $\log_{10}$ p.f.u. was recorded when HEV71 was left in a suspension (without any living cells) at room temperature for seven days. When the virus suspension was left to dry at room temperature, the end point titer dropped by 1, 3 and 5 $\log_{10}$ by day 1, 3 and 5 post-treatment, respectively (Figure 1). As expected, no virus was recovered from the mock-treatment using infecting fluid prepared in parallel to the virus inoculum. To examine the efficacy of Virkon S as a potential virucidal disinfectant, HEV71 inoculum was dried over a tissue culture dish surfaces and sprayed with Virkon S solution. A single spray with Virkon S at concentration 1% and 2% resulted only in one $\log_{10}$ reduction of HEV71 titer. No viable HEV71 was recovered when four repeated sprays with 1% and 2% Virkon S were applied and no signs of cells cytotoxicity were seen in Vero cells up to day seven post-inoculation with the recovered samples (Figure 2). Significant reduction (Student's t-test, $P < 0.01$) in HEV71 titer was achieved with four repeated sprays in comparison to no spray or a single spray. On the other hand, undiluted (100%) or at neat concentration, Virkon S was toxic to cells resulting in complete cell lysis.

Findings from the present study established that Virkon S at concentration of at least 1% with ten minutes of contact time was sufficient to completely inactivate HEV71. A minimum of four sprays, however, were required to ensure a complete inactivation of the virus as the virus was shown to be viable up to seven days in suspension and three days in dried form under the typical Malaysia hot and humid room conditions. As Virkon S has previously been reported to be effective for breaking the chain of transmission of the highly contagious foot and mouth disease[5], it could also be the disinfectant of choice as a primary approach for containment of HEV71 transmission among children in the tropic. Furthermore, Virkon S is considered less toxic in comparison to the commonly used disinfectants such as formaldehyde, hypochlorites and glutaraldehyde, effective in the presence of organic matters and across a wide pH spectrum[5], and it is also easily purchased over the counter at most pharmaceutical outlets in the country.

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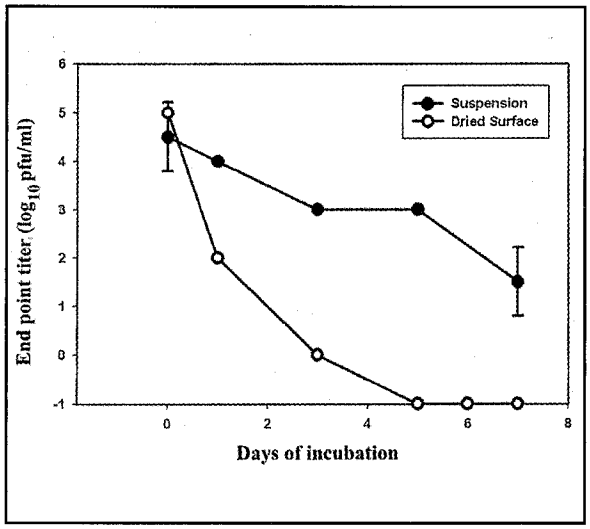

Fig. 1: Survival of human enterovirus 71 in a typical Malaysia classroom environment. Virus inoculum was kept in suspension or dried over a tissue culture plate surfaces and placed in a non-airconditioned classroom up to seven days. At selected intervals, the virus was recovered and cultured in Vero cells. The end point titer was determined based on the highest dilution of the recovered virus that resulted in cytopathic effects.

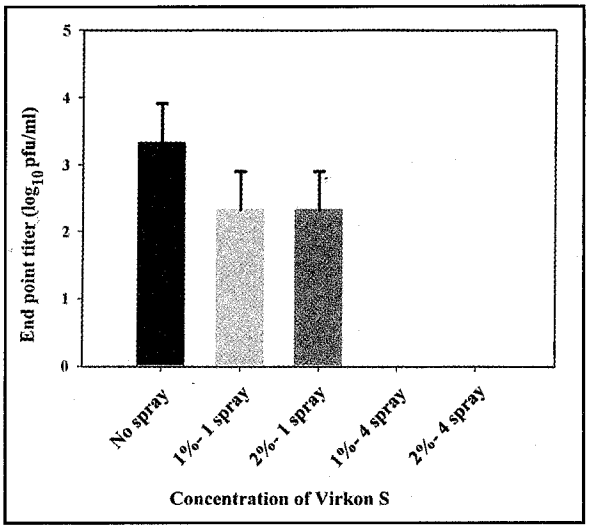

Fig. 2: Efficacy of Virkon S against Enterovirus 71. One spray or four continuous sprays with either 1% or 2% Virkon S were applied to the surface dried HEV71. The Virkon S mist was left in contact with the virus for ten minutes. HEV71 was recovered and the end point titer was determined seven days later.

References

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