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Abstract

Complete Virus Inactivation Using a Combined Heat and Chemical Treatment †

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Abstract: Validated inactivation protocols are required for the safe handling and disposal of virus samples. This is particularly important for hazard group 3 agents and above. Various methods are employed for virus inactivation, some of which include the use of heat or chemical chaotropic agents such as guanidine hydrochloride. It is generally accepted that these processes are sufficient to denature all viruses. While inactivation of certain viruses with such methods have been reported, validation of their activity against a wide of range of viruses is required. Here, we examined the inactivation of a panel of hazard group 2 viruses (*Rhabdoviridae*, *Togaviridae*, *Peribunyaviridae*, and *Flaviviridae*) using a combination of heat, guanidine hydrochloride-containing buffer, and ethanol. Viruses were treated with proteinase K and guanidine hydrochloride-containing buffer, and heated at 56 °C for 30 min. This was followed by additional treatment with absolute ethanol. Resulting virus–buffer–ethanol mixtures were column-purified to remove residual ethanol and other toxic reagents, before being introduced to cells. Column purification was confirmed to be insufficient for virus removal. Cultures were incubated at 37 °C for 1 h, after which media supplemented with 2% foetal bovine serum was added. Cultures were then observed daily for cytopathic effects. Samples that showed no evidence of cytopathic effects were passaged thrice to confirm absence of cytopathic effects and complete inactivation of viruses. Cultures infected with control viruses that had not been treated with buffer, heat, and ethanol but column-purified developed cytopathic effects, while cultures infected with treated viruses showed no cytopathic effects even after three passages, thus confirming complete virus inactivation. Results from this study provide evidence of the use of a combination of heat, guanidine hydrochloride-containing buffer, and ethanol for the complete inactivation of all members of the four families investigated.

Keywords: inactivation; guanidine hydrochloride; ethanol; heat; *Rhabdoviridae*; *Togaviridae*; *Peribunyaviridae*; *Flaviviridae*