OMNIgene™•GUT

Inactivation of viral pathogens in the OMNIgene™•GUT collection device

Introduction

Biological samples can contain viruses, some with the potential to cause disease in humans. The presence of live pathogens in biological samples can pose a significant risk to laboratory personnel responsible for handling and processing of these samples. Viral human pathogens exist as either "naked" non-enveloped particles (e.g., human papillomavirus, poliovirus, norovirus) or as enveloped particles surrounded by a lipid membrane (e.g., influenza, SARS-CoV-2, herpes simplex, dengue).[1] The former are known to be more resistant to inactivation due to the lack of a detergent-sensitive lipid bilayer.[2] Here, we conducted a research study to evaluate the ability of the stabilization solution contained in the DNA Genotek OMNIgene™•GUT collection device to inactivate common viral pathogens. We specifically assessed the inactivation of lentivirus, an enveloped RNA virus, and adenovirus, a non-enveloped DNA virus. According to the U.S. Centers for Disease Control and Prevention (CDC) guidelines[3] and the American Society for the Testing of Materials (ASTM) standards on viral inactivation[4], an inactivating agent should render viral pathogens non-infectious in permissive cell culture assays to be considered virucidal. Thus, in inactivation assays, infectious viruses are first incubated with the inactivating agent (i.e., the OMNIgene™•GUT collection device stabilization solution) before being added to the culture medium of permissive cells to measure remaining infectivity (Figure 1). Dilution of the inactivation mixture in culture medium is often necessary to avoid cytotoxicity caused by the inactivating agent. In some cases, concentration and/or buffer exchange are advantageous approaches to circumvent cytotoxicity as opposed to dilutions, since the latter can impact the sensitivity and the limit of detection (LoD) of the assay (Figure 1).[3,4]

Results

This research study demonstrated that the OMNIgene™•GUT collection device stabilization solution inactivates lentivirus (an enveloped RNA virus) and adenovirus (a non-enveloped DNA virus) by a minimum reduction of 5-$\log_{10}$, equivalent to 99.999%. Figure 1 shows an overview of the inactivation procedure developed in-house.

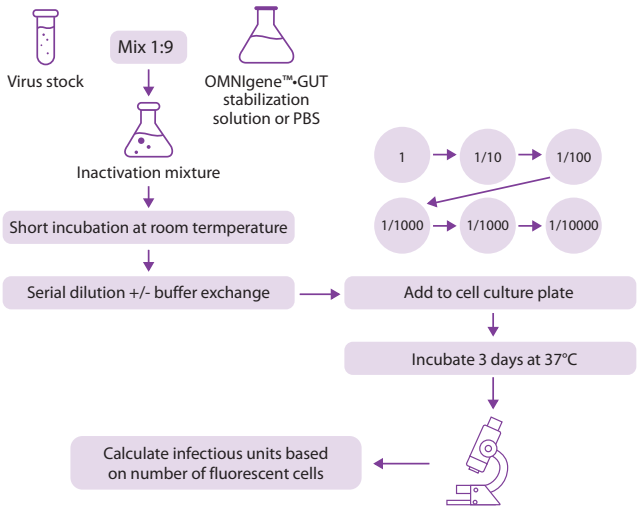

Figure 1. Diagram of the protocol developed in-house and used to determine the viral inactivation potential of the OMNIgene™•GUT collection device stabilization solution. *Viral stocks (lentivirus-mCherry or adenovirus-GFP) were mixed with the stabilization solution at a 1:9 ratio and incubated for 5-30 minutes before being diluted and added to permissive-cell cultures. The cells were incubated for 3 days, and live viral titers were determined by fluorescence microscopy.*

Briefly, live recombinant lentivirus and adenovirus stocks containing a fluorescent reporter protein (mCherry and GFP, respectively) were incubated with the OMNIgene™•GUT collection device stabilization solution. The inactivation mixture (virus + stabilization solution) was then diluted before being added to permissive-cultured cells. Live viral titers were determined by fluorescence microscopy after 3 days. As observed in Figure 2A and 2D, no infectious event was recorded when chemistry-treated samples were added to permissive cells, while the control phosphate-buffered saline (PBS) samples showed significant number of fluorescent cells, indicative of high titers of infectious virus (> 10^8 infectious units [IFUs]/mL).

Cytotoxicity was assessed by brightfield microscopy for each dilution to ensure that the decrease in infectivity was not due to chemistry-driven cytotoxicity (Figure 2A, 2D). Results showed that the OMNIgene™•GUT collection device stabilization solution was toxic to cultured cells at lower dilutions, therefore, viral titers could only be determined from wells showing no signs of cytotoxicity (i.e., generally higher dilutions). Our data demonstrate that the OMNIgene™•GUT collection device stabilization solution inactivated both lentivirus and adenovirus to the limit of detection (LoD) of their respective assays (Figure 2B, 2E). The LoD corresponds to the viral titer that would be measured should a single infection event (i.e., 1 fluorescent cell) be detected in the lowest dilution showing no evidence of chemistry-driven cytotoxicity. In addition, the lentivirus and adenovirus viral genomes were quantified by qPCR and RT-qPCR in the inactivation mixtures as well as PBS controls. The data show similar number of viral genome copies in both conditions (Figure 2C, 2F), further demonstrating that the loss of infectivity is driven by inactivation of the viral particles following exposure to the OMNIgene™•GUT collection device stabilization solution.

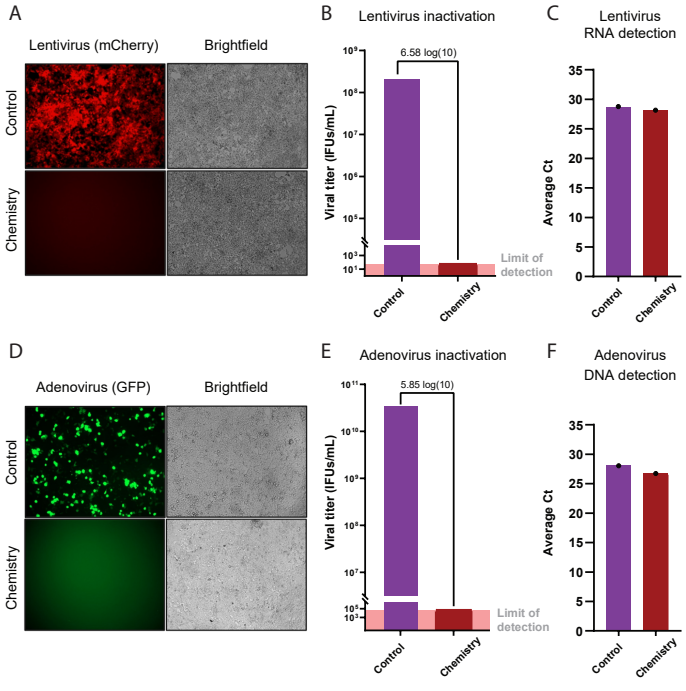

Figure 2. The OMNIgene™•GUT collection device stabilization solution reduces live titers of lentivirus and adenovirus to the limit of detection of the assay. *A live lentivirus carrying an mCherry reporter gene or an adenovirus carrying a GFP reporter gene was mixed with the chemistry, or PBS as a control, at a ratio of 1:9 (virus:chemistry). The mixtures were incubated for 5-30 minutes respectively at room temperature. (A),(D) Fluorescence microscopy images of HEK293T cells grown in cell media treated with the inactivation mixture or PBS-treated virus (control) 3 days post infection. Brightfield images were used to assess chemistry-driven cytotoxicity and to determine which dilution should be used for measuring inactivation and LoD. (B),(E) Measured live titers of lentivirus in the control (PBS) and chemistry-treated conditions. Since no fluorescent events were detected when cells were incubated with the chemistry-treated virus, the titer was deduced to be lower than the limit of detection (5.75E + 01 units/mL for lentivirus, and 5.00E + 04 units/mL for adenovirus). (C),(F) The lentivirus RNA and adenovirus DNA genomes are detectable by RT-qPCR and qPCR, respectively, in the inactivation mixture to the same levels as the PBS control.*

Conclusions

The OMNIgene™•GUT collection device stabilization solution broadly inactivates common viral pathogens such as non-enveloped viruses (i.e., adenovirus) and enveloped viruses (i.e., lentivirus) by > 5-$\log_{10}$ (99.999%). We demonstrated inactivation of adenovirus and lentivirus to the LoD of our assays, indicating that the stabilization solution has potent virucidal activity. Overall, this research study highlights reduced risk of pathogen exposure when working with samples collected in the OMNIgene™•GUT collection device, providing an extra layer of safety during downstream handling by laboratory personnel.

Materials and methods

Inactivation assay protocol

Viruses used for this inactivation testing study were an mCherry-expressing lentivirus (University of Ottawa Genome Editing and Molecular Biology core facility) and a GFP-expressing adenovirus (Vector Biolabs, Cat. No. 1060). For lentivirus, viral stock was mixed with the OMNIgene™•GUT collection device stabilization solution (inactivation mixture) or PBS (control) at a ratio of 1:9 (virus:chemistry) and the resulting reactions were incubated at room temperature for 5 minutes. The reactions were diluted 100-fold in PBS with 0.5% BSA and added to a pre-treated Amicon Ultra-15 100 KDa filter and centrifuged at 4,000 x *g* until ~200 µL remained. The contents of two filter tubes of the same condition were combined, and 9.6 mL of fresh PBS with 0.5% BSA was added to the Amicon filter, which was centrifuged again at 4,000 x *g* until ~200 µL remained. The concentrated sample was transferred to 2 mL of full-culture media (DMEM + 10% BCS) and serially diluted 10-fold before being added

to HEK293T cells grown in 6-well plates. For adenovirus, viral stocks were mixed with the OMNIgene™•GUT collection device stabilization solution (inactivation mixture) or PBS (control) at a ratio of 1:9 (virus:chemistry) and the reactions were incubated at room temperature for 30 minutes. Both conditions were serially diluted 10-fold to full cell media (DMEM + 10% BCS) before being added to HEK293T cells grown in a 6-well plate. The cells were incubated for 72 hours at 37°C. After a 72-hour incubation, the number of infected cells at each dilution was determined by fluorescence microscopy (mCherry or GFP). Titers were determined by counting the number of fluorescent colonies in 5 representative fields of view per well and multiplying the average of those counts by the number of fields of view per well and by the dilution factor of the well. The LOD of each assay was determined as the titer that would be measured if a single event were to be detected in the first well that shows no sign of chemistry-driven cytotoxicity.

qPCR assay protocol

Viral stocks (mCherry-expressing lentivirus or GFP-expressing adenovirus) were mixed at a 1:9 (virus: chemistry) ratio and incubated at room temperature for 5-30 minutes, respectively. The nucleic acids were then purified using the QIAamp MinElute Virus Spin Kit (QIAGEN, Cat. No. 57704) following the manufacturer's protocol. The eluates were diluted 1:500 and used as a template for RT-qPCR (lentivirus) or qPCR (adenovirus) using the GoTaq Probe 1-Step RT-qPCR (Promega, Cat. No. A6120) and GoTaq Probe-qPCR systems (Promega, Cat. No. A6101), respectively. Primer and probe sets (IDT) were designed to target mCherry for the lentivirus RNA and GFP for the adenovirus DNA.

References:

1 Ryu WS. (2016). Molecular virology of human pathogenic viruses. *Academic Press.*

2 Zhou SS. (2022). Variability and relative order of susceptibility of non-enveloped viruses to chemical inactivation. *Disinfection of viruses.* 1-28.

3 U.S. Centers for Disease Control and Prevention, Division of Select Agents and Toxins. (2018). Guidance on the inactivation or removal of select agents and toxins for future use. https://www.selectagents.gov/compliance/guidance/inactivation/index.htm. [webpage].

4 ASTM International. (2020). E1052 – 20 Standard Practice to Assess the Activity of Microbicides against Viruses in Suspension. https://www.astm.org/e1052-20.html. [webpage].

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