Viral Inactivation Using Norgen's Total Nucleic Acid Preservative and Norgen's Saliva RNA Preservative

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INTRODUCTION

Herein, Norgen Biotek Corp. proposes two novel COVID-19 sample collection and preservation kits. The first is Norgen's Swab Collection and Total Nucleic Acid Preservation System, comprised of swab collection device (oropharyngeal and/or nasopharyngeal swab) and tube with a preservative. The second kit is Norgen's Saliva RNA Collection and Preservation Devices, comprised of a collection tube with a funnel and an ampoule filled with a 2 mL preservative that is added to 2 mL of saliva immediately after collection. The used preservative functions dually as an RNA preservative solution and lysing agent. This COVID-19 specimen collection kit will be provided to end-users—such as medical personnel in hospitals, clinics, or long-term care facilities—who may collect clinical specimens from patients (oropharyngeal and/or nasopharyngeal cell brushings as well as saliva), place them in the transport/lysis media tube, and send them to core laboratory facilities for reliable molecular detection of SARS-CoV-2 RNA. In effect, this preservative solution will: (1) inactivate and lyse the virus within less than 2 min, thus preventing COVID-19 transmission and enabling safe shipping and handling to core laboratory facilities; and (2) preserve SARS-CoV-2 RNA for up to two months, enabling reliable and accurate COVID-19 detection. This claim is supported by data obtained from an experiment conducted on Ad-GFP (recombinant adenovirus, green fluorescent protein), a highly stable virus. The preservative/lysis solution was able to successfully inactivate recombinant adenovirus-GFP (Ad-GFP) at 1X and 0.5X concentrations.

MATERIALS AND METHODS

Cell Line

Human Embryonic Kidney 293 cell line (HEK 293) (Graham et ai, 1977; ATCC CRL-1573) was used in this experiment. Cells were maintained as monolayer cultures. The medium used was autoclavable MEM (Invitrogen Corp., Gibco) supplemented with 3% (v/v) sodium bicarbonate (Invitrogen Corp., Gibco), 1 % L-glutamine (Invitrogen Corp., Gibco), 10% (v/v) FBS (PAA Laboratories Inc.) and 1% (v/v) Antibiotic-Antimycotic (10,000 units/mL penicillin G sodium, 25 mg/mL amphotericin B, and 10,000 units/mL streptomycin sulphate, Invitrogen Corp., Gibco). Growing cells were incubated in a water-jacketed incubator (Fisher Scientific, Pittsburgh PA) at 37X with 96% humidity and 5% CO2.

Virus and Viral Infection

A recombinant adenovirus type 5 with green fluorescence protein (GFP) gene inserted in place of the AdE1 region (Ad-GFP) was used. Infection of HEK 293 cells was carried out in 6-well plates by using a viral concentration of 10e7 viral particle/mL. This viral volume was mixed with PBS++ (0.01% CaCh.2H20 and 0.01 MgCh.6H20 dissolved in PBS) in a total volume of 100 µL/well and then added to the cell monolayer (after aspirating the medium). The 6-well plate was then incubated for 1 hour at 37°C with 96% relative humidity and 5% CO2, with swirling the plate every 15 minutes. After that, 2 mL of the culture medium was added to each well and the plate was returned to the incubator.

Treatment of Viruses with Preservative

Ad-GFP was added to 1X concentration of Norgen's Total Nucleic Acid Preservative or 0.5X concentration of Norgen's Saliva RNA Preservative (to mimic 1:1 dilution upon collection), in a ratio of 1:9, where 20 µL of Ad-GFP stock (10^{10} viral particle/mL) was mixed with 180 µL of preservative. The resulting viral-preservative mixture (109 viral particle/mL) was incubated for 10 minutes at room temperature. The mixture was immediately diluted 100-fold (to avoid cellular toxicity by the preservative) using PBS++ to yield a viral concentration of 107 viral particles/mL and use to infect HEK 293 cells. Positive control viral infection was performed with the same multiplicity of infection (MOI) used in the preservative treatment condition, by diluting the Ad-GFP stock to 107 viral particle/mL in PBS++. Negative control cells were included where only PBS++ was added to the cells during the infection procedures. Cells were incubated for 36 hours post-infection and then images were acquired by Zeizz Axio Observer Microscope (Brock University, St. Catharines, ON) using Hamamatsu Camera at an excitation wavelength of 488 nm and emission wavelength of 509 nm and exposure time of 1 s.

RESULTS AND DISCUSSION

The effect of Norgen's Total Nucleic Acid Preservative as well as Norgen's Saliva RNA Preservative on viral activity was investigated in the present study using a replication deficient recombinant adenovirus 5 with GFP gene (Ad-GFP) together with HEK 293 cell line that allows for the replication of the virus. Viral stock was mixed with the preservative

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in a ratio of 1:9 (virus:preservative) in order to have a minimum change in the molarity of the preservative components and to generate minimum dilution conditions that are similar to the use of a swab applicator added directly to the concentrated preservative or saliva collected and mixed (1:1) with the saliva RNA Preservative. Viral-preservative mixture was then incubated for 0, 2, 5 and 10 minutes before proceeding with further dilutions and infection of the cell line. Positive and negative controls were included as described earlier.

We monitored the cells and observed that both the negative control cells and cells infected with preservative-treated virus remained healthy and intact, while the positive control cells that were infected with the untreated-adenovirus started to develop a cytopathic effect (CPE) that was obvious at 18 hours post-infection. At 36 hours post-infection, the negative control cells and cells infected with the preservative-treated virus were still growing and remained healthy and intact. The induced CPE in the positive control cells was more pronounced. Florescence microscope was then used to monitor expression of the GFP as an indicator of active viral infection and expression of viral proteins and to further confirm the loss of infectivity of the preservative-treated virus. Only the positive control showed active GFP expression while the negative control and cells infected with the preservative-treated virus did not show any signs of GFP expression (Figures 1 and 2). This indicates that viral inactivation can be achieved by the preservative within 0 or 2 minutes. The mechanism of viral infectivity inactivation is due to the chemical lysis of the viral capsid by the preservative. The exposed viral nucleic acid will then be preserved by the preservative as indicated by the stability of nucleic acid (Nucleic acid preservative) or RNA (Saliva RNA Preservative) over time (2 years for DNA and 2 months for RNA in nucleic acid preservative and 2 months for RNA in Saliva RNA preservative, at room temperature).

CONCLUSION

From the data presented in this report, we concluded that Norgen's Total Nucleic Acid Preservative as well as Norgen's Saliva RNA Preservative are efficient chemical reagents that inactivates viral infectivity in less than 2 min to generate a safe storage and transport system with extended nucleic acid stability.

▶ ACKNOWLEDGEMENT

The authors would like to thank Dr. Jeff Stuart and Christopher Moffatt (Department of Biological Sciences, Brock University) for providing the facility and help to acquire the fluorescent images.

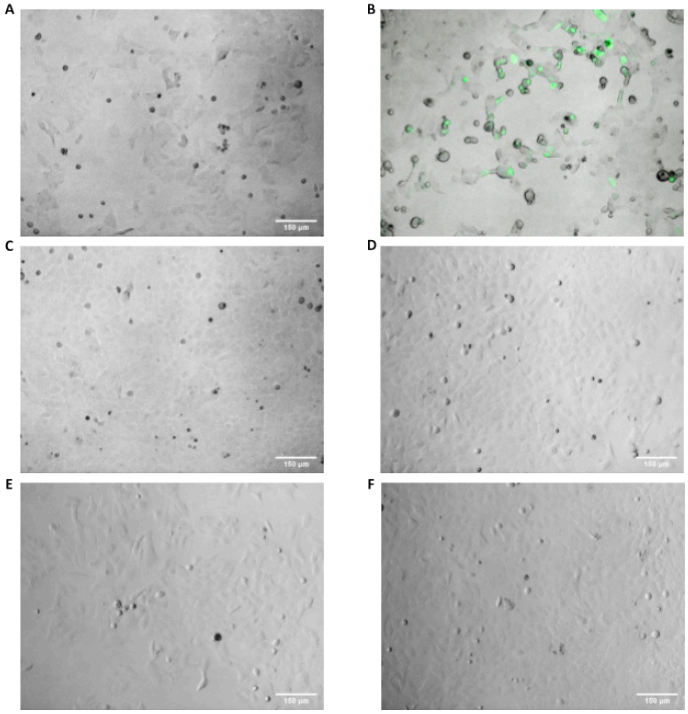

Figure 1. Images of HEK 293 cells acquired by confocal microscope. (A) control cells with no viral infection. (B) cells infected with Ad-GFP and (C to F) cells infected with Ad-GFP at the same MOI as in panel B, after treatment with Norgen's Total Nucleic Acid Preservative. Panels C, D, E and F represent preservative treatment time of 0, 2, 5 and 10 minutes, respectively. Ad-GFP that was treated with preservative/lysis solution did not infect the cells as can be seen in the total absence of CPE and GFP expression in Panels C to F. On the other hand, cells infected with Ad-GFP that is not treated with a preservative (panel B) show clear CPE with GFP expression. Images were developed using Zeizz Axio Observer Microscope with Hamamatsu Camera.

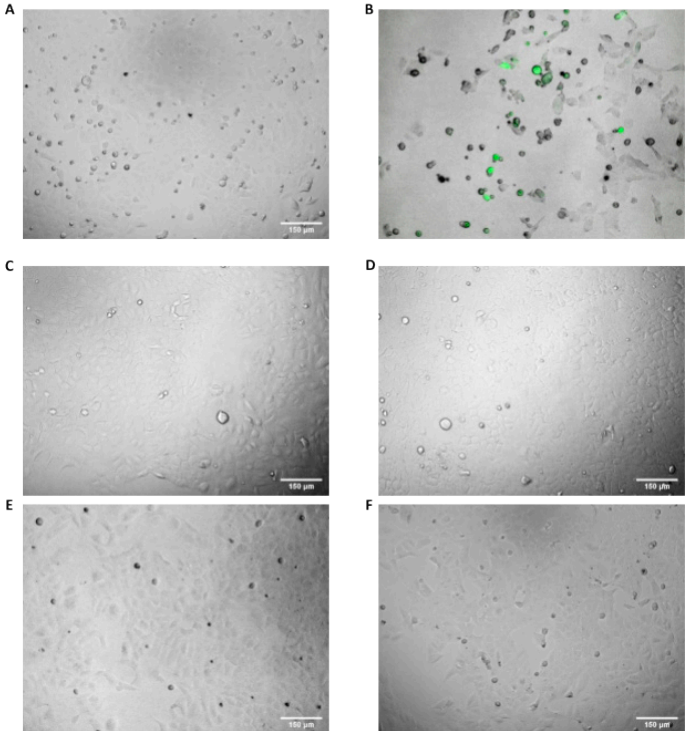

Figure 2. Images of HEK 293 cells acquired by confocal microscope. (A) control cells with no viral infection. (B) cells infected with Ad-GFP and (C to F) cells infected with Ad-GFP at the same MOI as in panel B, after treatment with 0.5X of Norgen's Saliva RNA Preservative. Panels C, D, E and F represent preservative treatment time of 0, 2, 5 and 10 minutes, respectively. Ad-GFP that was treated with preservative/lysis solution did not infect the cells as can be seen in the total absence of CPE and GFP expression in Panels C to F. On the other hand, cells infected with Ad-GFP that is not treated with a preservative (panel B) show clear CPE with GFP expression.

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