Inactivation of Coronavirus in Samples using Norgen's Saliva RNA Preservative

Application Note 94

Keywords

+ Virus
+ Viral
+ RNA
+ Inactivation
+ Buffer
+ Media
+ Coronavirus
+ Pathogen
+ Storage
+ Method
+ Protocol

INTRODUCTION

In December of 2019, an outbreak of a respiratory illness started in Wuhan City of the Hubei Province, China and has now spread to well over 200 countries and territories around the world. It was soon determined that this respiratory disease was caused by a novel coronavirus and in February of 2020, the World Health Organization (WHO) announced that the official name of the disease as COVID-19. The official name of causative agent of COVID-19 is SARS-CoV-2. While coronaviruses have been known to infect animals and humans for some time, the sudden emergence, spread and global impact of this novel strain has led to a drastic expansion of diagnostic testing efforts for the disease. Clinical samples can now be collected from patients using oropharyngeal and/or nasopharyngeal cell brushings as well as saliva. Norgen's Saliva RNA preservative (also known as Norgen's Total Nucleic Acid Preservative) functions as an RNA preservative solution and lysing agent. The reagent is used in both Norgen's Total Nucleic Acid Preservation Tubes (Cat. #69200) and Saliva RNA Collection and Preservation Devices (Cat. #RU53800). It is of the utmost importance for preservative agents to inactivate coronavirus thus preventing COVID-19 transmission and to enable safe shipping and handling to core laboratory facilities. The purpose of this experiment is to determine whether Norgen's Saliva RNA Preservative can effectively inactivate human coronaviruses.

MATERIALS AND METHODS

Inactivation of human coronavirus strain OC-43 and SARS-CoV-2 were tested using Norgen's Saliva RNA preservative (0.5X and 1.0X concentrations). These studies were carried out by ViraSource Labs (Durham, North Caroline, USA) and ImPaKT Centre (London, Ontario, Canada), respectively.

Condition Number	Virus	Preservative Conentration	Time (min)
1	Yes	No Preservative	0
2	Yes	0.5x	1
3	Yes	0.5x	5
4	Yes	1x	1
5	Yes	1x	5
6	No	0.5x alone	0
7	No	1.0x alone	0
8	No	No Preservative	0

Table 1. Experimental conditions used in virus inactivation assay. Each treatment was initiated after a 24 hour incubation period.

Human coronavirus strain OC-43

Vero African green monkey kidney cells were used in the virus inactivation assay using the Tissue Culture Infectious Dose-50 (TCID-50) method. Cells were dispensed at a density of 10,000 cells/well on each well of a 96-well plate. After 24 hours, the experimental conditions outlined in Table 1 were used.

As demonstrated in Table 3, Ten microlitres of human coronavirus OC43 virus at 2e7 PFU/mL was combined with 90 µL of Norgen's Saliva RNA Preservative (0.5x or 1x concentrations) for either 1 or 5 minutes (for Conditions 2-5). The volume was then increased to 1 mL using 900 µL of media (total of 100-fold dilution). One hundred microliter of preservative was mixed with 900 µL of media for the preservative only conditions (Conditions 6 and 7). Twenty microliters of each of the previous mixtures prepared for Conditions 2-7 was mixed with 180 µL of media and added to each well in the first row (1:1000 in row 1, 8 wells total), and then serially diluted 1:10 in 5 more rows (total of 6 rows per condition). For the virus with no preservative condition (Condition 1), 20 µL of the virus was mixed directly with 180 µL of media and added to each of the 6 rows as described earlier. In the negative control (i.e. Condition 8) only media was used. After applying the various conditions, cells were monitored for cytopathic effects (CPE) and were fixed with 1% formaldehyde for 30 minutes, stained with 1% crystal violet for 2 minutes and then rinsed with water.

SARS-CoV-2

The Norgen Saliva RNA Preservative was provided as 2 Solutions (A = 1X Norgen's Saliva RNA Preservative; B = 0.5X Norgen's Saliva RNA Preservative). It should be noted that Norgen's Saliva RNA Preservative is the same as Norgen's Total Nucleic Acid Preservative. In preparation for testing, 500 µL of each solution was prepared in 1.5 mL tubes and a control aliquot of Dulbecoo's Modified Eagle's Medium (DMEM) was prepared for the control condition.

The SARS-CoV-2 virus stock used was at a titer of $10^{5.8}$ infectious units (IU)/mL. A volume of 20 µL of the viral stock was added to each 1.5 mL tube containing a Norgen solution. Each tube containing the virus and Norgen solution was inverted for 5 min at 22°C. As a positive control, the virus was added at the same concentration to a 1.5 mL tube containing 500 µl DMEM. After the 1 min mixing period, the virus/Norgen preservative tubes were diluted at 1:100 and frozen at -80°C for further testing. Collected solutions were 100-fold diluted serially from 1:100 to 1:100,000,000 and then added to 20,000 Vero E6 cells in 96-well flat bottom plates. The 1:100 dilution of the virus stock infecting 20,000 cells represents an MOI of 0.5. Infection of the Vero E6 cells was monitored by viral cytotoxicity.

RESULTS AND DISCUSSION

Human coronavirus strain OC-43

Please refer to figures 1 through 4 for the following results.

No CPE was observed with viral conditions treated with the 0.5x or 1x preservative, at both 1 and 5 minutes incubation (Conditions 2-5). As seen in

Condition ID	CPE (TCID-50 measurement)	Cytotoxitity
1	Detected CPE down to 4-fold dilution (TCID at 1e3 PFU/mL)	No cytotoxicity
2	No CPE detected	In first row (1:100 fold preservative dilution)
3	No CPE detected	In first row (1:100 fold preservative dilution)
4	No CPE detected	In first row (1:100 fold preservative dilution)
5	No CPE detected	In first row (1:100 fold preservative dilution)
6	No CPE detected	In first row (1:100 fold preservative dilution)
7	No CPE detected	In first row (1:100 fold preservative dilution)
8	No CPE detected	No Preservative

Table 2. Summary of TCID-50 measurement and cytotoxicity observations for each experimental condition.

Solution ID	Solution Name	Viral Titer	Reduction Factor (Log^{10}) v. Control	% Viral Reduction
Control	N/A	$10^{5.5}$	N/A	N/A
A	1X Norgen's Saliva RNA Preservative	0	>6	99.99%
B	0.5X Norgen's Saliva RNA Preservative	0	>6	99.99%

Table 3. SARS-CoV-2 Viral Titer Reduction following incubation in Norgen's Saliva RNA Preservative (also known as, Norgen's Total Nucleic Acid Preservative).

Condition 7, the cytotoxic effect associated with a high preservative concentration (1e-2x) is not evident in conditions with a lower preservative concentration. The first row in each of the preservative conditions (Conditions 2-7) showed cytotoxicity due to the relatively high preservative concentration (0.5e-2x and 1e-2 for the 0.5x and 1x preservative concentrations, respectively). The cytotoxic effect was more pronounced at 1x preservative concentration (Conditions 4, 5, 7) relative to the 0.5x preservative concentrations (Conditions 2, 3, 6). Signs of cytotoxicity can be seen in the first row of each condition with the highest preservative concentration (i.e. 1e-2X). The active virus condition (Condition 1), showed CPE effect with viral concentrations from 2e6 to 2e3, where CPE was observed in 5 out of the 8 used wells. This indicates a TCID-50 of around 1e3. The non-treated condition (Condition 8) did not show any signs of CPE or cytotoxicity.

SARS-CoV-2

Solutions (diluted 1:100) of untreated virus (no Norgen preservative) applied to Vero E6 cells for 1 hour resulted in no significant decrease in infectious titer relative to the original virus stock (104.5 IU/mL). As seen in Table 3, infectious SARS-CoV-2 was reduced by 6 Logs corresponding to a 99.99% reduction in infectious virus when treatment was performed for 5 minute with Solutions A or B.

CONCLUSIONS

Norgen's Saliva RNA Preservative has been formulated to inactivate viruses including SARS-CoV-2, seasonal influenza, HIV and HPV. The present study demonstrates this viral inactivation capacity at two preservative concentrations (0.5x and 1x) using human coronavirus OC43 and SARS-CoV-2. The use of samples collected in Norgen's Saliva RNA Preservative allows researchers and medical lab technicians the ability to safely handle otherwise potentially pathogenic samples for high throughput disease screening programs.

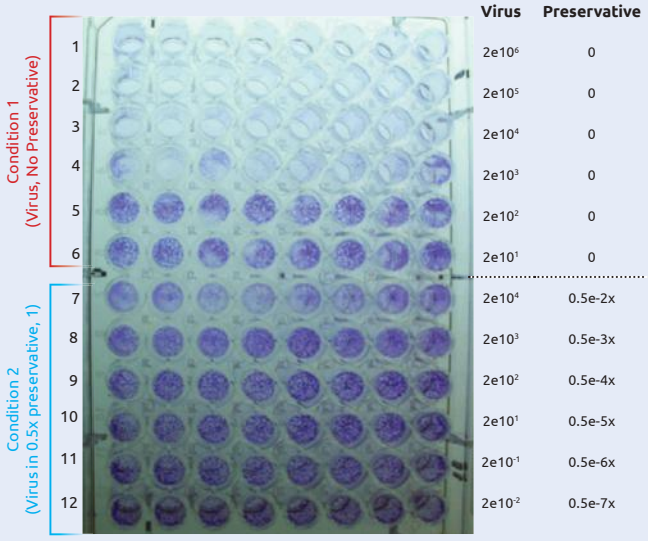

Figure 1. Plaque assay of saliva preservative-treated SARS-CoV-2: Conditions 1 and 2. In Condition 1 (i.e. rows 1-6) Vero cells were infected with the virus at the indicated titers in the absence of any preservative. Complete CPE is observed down to 2e3 PFU/well. In Condition 2 (i.e. rows 7-12) Vero cells were infected with the virus, pre-treated with 0.5x preservative for 1 minute, at the indicated titers. Total inactivation of the virus is observed, as indicated by the viable cells stained in blue.

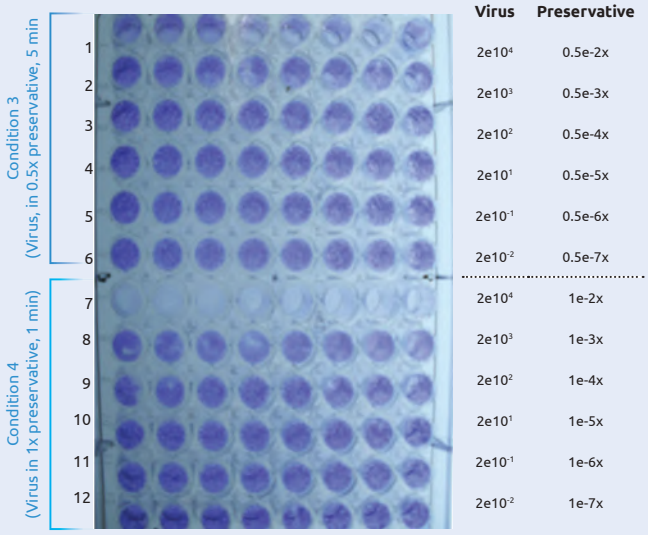

Figure 2. Plaque assay of saliva preservative-treated SARS-CoV-2: Conditions 3 and 4. In Condition 3 (i.e. rows 1-6) Vero cells were infected with virus incubated in 0.5x preservative for 5 minutes at the indicated titers. In Condition 4 (i.e rows 7-12) Vero cells were infected with virus incubated in 1x preservative for 1 minute. In both conditions, total inactivation of the virus is observed, as indicated by the viable cells stained in blue.

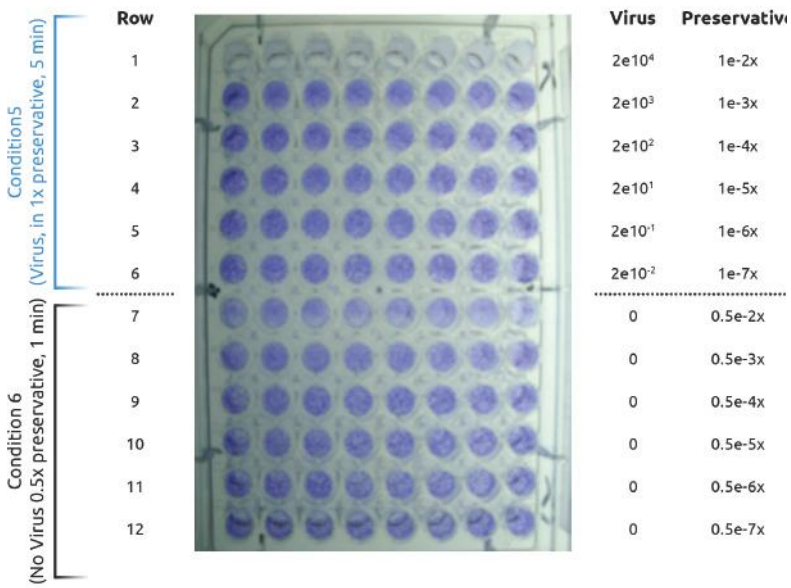

Figure 3: Plaque assay of saliva preservative-treated SARS-CoV-2: Conditions 5 and 6. In Condition 5 (i.e. rows 1-6) Vero cells were infected with virus incubated in 1x preservative for 5 minutes. Total inactivation of the virus is observed, as indicated by the viable cells stained in blue. In Condition 6 (i.e. rows 7-12) 0.5x preservative was added to the Vero cells to compare the cytotoxic effect of the preservative on cells in the absence of viral cells. Only row 7 (0.5e-2x preservative) showed signs of relatively mild cytotoxicity. All remaining rows (8-12), showed no toxic effect on cells.

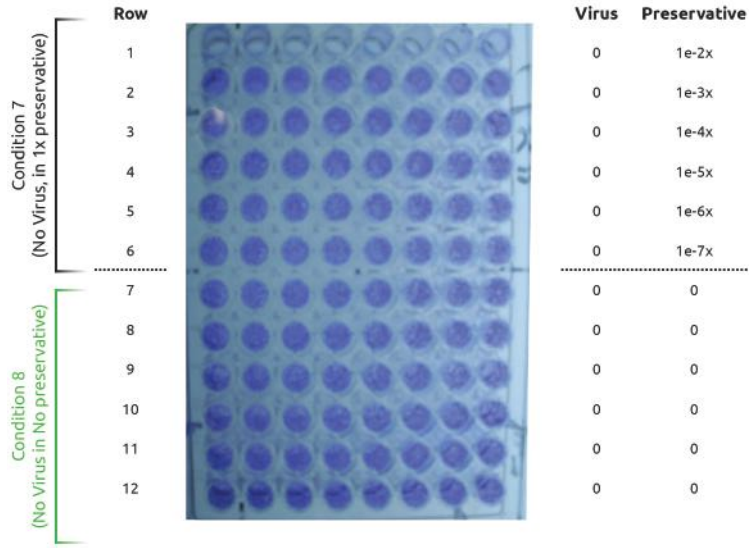

Figure 4: Plaque assay of saliva preservative-treated SARS-CoV-2 Conditions 7 and 8. In Condition 7 (i.e. rows 1-6) 1x preservative was added to compare at the cytotoxic effect of the preservative on Vero cells, at the indicated preservative concentrations, with no viral infection. Only row 1 (1e-2x preservative) showed signs of cytotoxicity due to a high concentration of the preservative. All remaining rows (2-6), shows no toxic effect on cells, as indicated by the viable cells stained in blue. Condition 8 consisted of non-infected cells with no preservative added (negative control), no CPE or cytotoxicity was detected in any of the wells (rows 7-12).

Related Products	Reasearch Use	CE Marked CE
Total Nucleic Acid Preservation Tubes	69200	Dx69200
Saliva RNA Collection and Preservation Devices Dx	RU5300	53800
Saliva/Swab RNA Purification Kit Dx	69100	Dx69100
Saliva/Swab RNA Purification 96-well Kit Dx	69300	Dx69300
COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx	TM67200	DxTM67200
2019-nCoV TaqMan RT-PCR Kit	TM67100	DxTM67100

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