Inactivation of SARS-CoV-2 Virus by XpressAmp™ Lysis Buffer for Viral RNA Preparation[1]

Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its associated coronavirus disease 2019 (COVID-19) pandemic required rapid in vitro diagnostic assay creation and scale-up. Global testing demand has strained the supply chain of reagents used in the molecular testing workflow for COVID-19. One bottleneck has been the supply of reagents used to extract and purify viral RNA from clinical specimens for subsequent RT-qPCR assays. To help alleviate this, Promega developed the XpressAmp™ Direct Amplification Reagents (Cat.# A8880, A8882)[2] to provide a fast, RNA extraction-free method to prepare viral samples for PCR-based amplification. Preparing an infectious sample requires reagents that safely inactivate viral particles, thereby simplifying the laboratory process. This report describes an evaluation of the ability of the XpressAmp™ Lysis Buffer, used to prepare viral sample for RT-qPCR analysis, to inactivate the SARS-CoV-2 virus.

Methods and Results Discussion

To evaluate the XpressAmp™ Lysis Buffer's ability to inactivate the SARS-CoV-2 virus, a high titer SARS-CoV-2 sample (10^5 infectious units/ml) was mixed with an equal volume of prepared XpressAmp™ Lysis Buffer and then incubated for 10 minutes at room temperature (Figure 1). Since the XpressAmp™ sample preparation method requires the user to prepare the XpressAmp™ Lysis Buffer by adding 1-thioglycerol (1-TG) before use, XpressAmp™ Lysis Buffer was evaluated both with and without the addition of 1% 1-TG. Residual infectious virus was evaluated by adding treated viral samples to the culture media of infection-susceptible CaCo cells, and incubating the cells over a 7-day time course. Cell media samples were taken on days 0, 3 and 7. Viral RNA was purified from the media samples using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330). The presence of SARS-CoV-2 viral particles were detected by RT-qPCR amplification for each of these time points. No culture media change was done between days 0 and 3. On day 3, after sampling, cells were passaged 1:8, which led to a media dilution of the same factor. Cells were grown for an additional 4 days until another culture media sample was taken to assess possible virus replication on day 7.

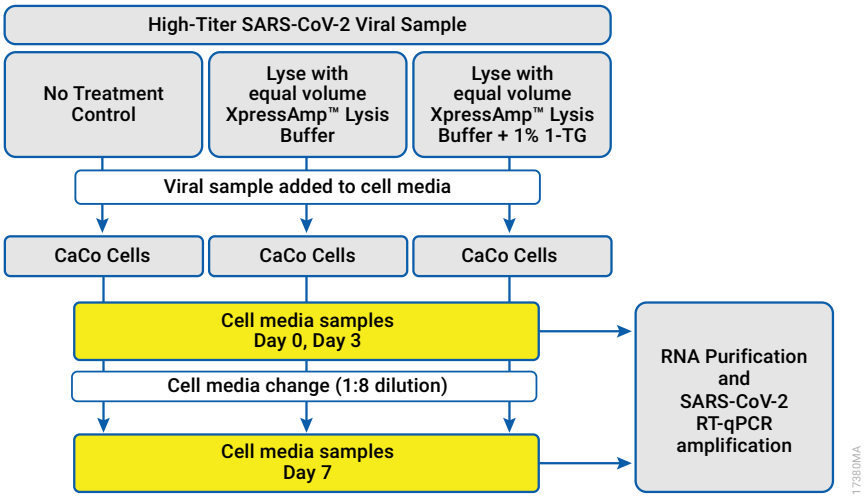

Figure 1. Flow diagram of protocol workflow.

At day 0, the virus input was determined for all the conditions tested and set to "1" (Figure 2). This value was identical for all sample types since both active and lysed viral sample would have the same number of viral RNA copies present in the cell media sample. On days 3 and 7, the untreated control samples show an exponential increase in detectable viral RNA in the supernatant, indicating the infection and production of viral particles in the CaCo cells over the time course (orange line).

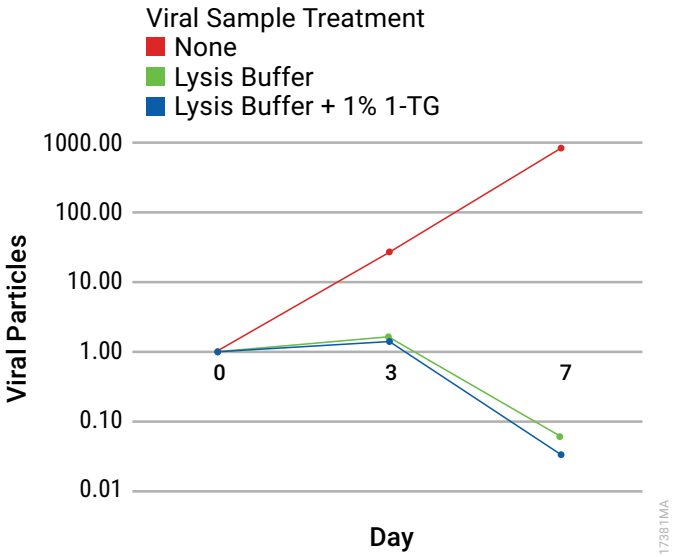

Figure 2. Inactivation of SARS-CoV-2 virus by XpressAmp™ Lysis Buffer.

Comparably, no such increase was observed for samples treated with the XpressAmp™ Lysis Buffer, alone or containing 1% 1-TG (green lines). The unchanged virus RNA levels, detected at the day 3 timepoint compared to day 0, are in line with the reported high particle stability of SARS-CoV-2. The virus decrease seen on day 7 reflects a combination of particle degradation and the dilution in the culture media, with no evidence for any viral amplification. This data indicates a complete inactivation of the SARS-CoV-2 samples after treatment with the XpressAmp™ Lysis Buffer. Furthermore, the virus inactivation is not dependent upon the addition of 1% 1-TG to the XpressAmp™ Lysis Buffer.

To assess the biological safety of treated specimens, treated samples were examined in repeated "blind passages" of cells where they were evaluated for cytopathic changes (CPE). Cell cultures exposed to untreated viral samples developed a strong CPE within 4 days of infection. Comparatively, in cell cultures with added XpressAmp™ treated viral samples, no CPE was observed after 7 days or during 3 weeks of successive passaging. This method reflects a highly sensitive standard cell culture method for detecting residual viable virus. This experiment thus provided additional proof that treatment of the SARS-CoV-2 viral samples with the XpressAmp™ Lysis Buffer inhibits SARS-CoV-2 replication.

Conclusions

Preparing an infectious sample requires reagents to safely inactivate viral particles. These results show that under the conditions tested, the XpressAmp™ Lysis Buffer successfully inactivated the SARS-CoV-2 virus. Furthermore, in the case of user error, where the user forgets to add 1-TG to the XpressAmp™ Lysis Buffer prior to use, viral inactivation is still fully accomplished.

[1]These viral inactivation studies were performed on behalf of Promega Corporation by Dr. Thomas Klimkait from the Molecular Virology Group, Department of Biomedicine, University of Basel, Switzerland. Please address any additional questions regarding this work to your local Promega representative. To learn more about the XpressAmp™ Direct Amplification Reagents (Cat.# A8880, A8882), please visit: **www.promega.com/XpressAmpReagents**

[2]For Laboratory Use