# Rapid Detection of SARS-CoV-2 Virus Via Novel Direct Amplification Methods

**Dana Brecklin[1], Jennifer Mook[1], Brad Hook[1], PhD, Curtis Knox[1], Terri McDonnell[1], Rod Pennington[1], PhD**

**[1]Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711**

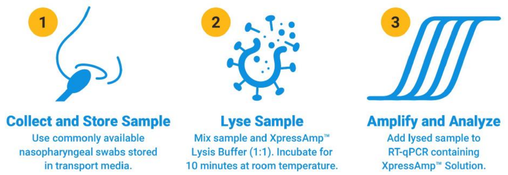
## 1. Introduction

The COVID-19 pandemic has challenged clinical labs to explore ways to increase testing capacity in response to unprecedented demand. This has created a global shortage of viral RNA extraction kits, necessitating the search for alternative methods to meet testing needs. Here, we describe a novel set of reagents and protocols to support rapid viral testing via direct amplification without the need for viral RNA extraction.

## 2. Direct Amplification Protocol

The protocol for direct amplification using nasopharyngeal swab samples in Viral or Universal Transport Medium is simple and straightforward, requiring approximately 15 minutes to go from sample to RT-qPCR-ready lysates. A portion of sample VTM is subjected to a 10-minute room temperature incubation with a prepared lysis buffer (1:1 sample:buffer). The resulting lysate is then added to an amplification reaction containing a new amplification enhancer solution (XpressAmp™ Solution), GoTaq® Probe 1-Step RT-qPCR System* reagents (Promega), and appropriate primers/probes.

**Fig. 1: Direct amplification workflow**




## 3. Detection of Inactivated Viral Targets

Initial protocols were developed using inactivated viral targets to optimize lysis conditions and confirm reproducibility. Influenza B and RSV samples showed viral RNA detection at levels as low as 10 copies/µl.

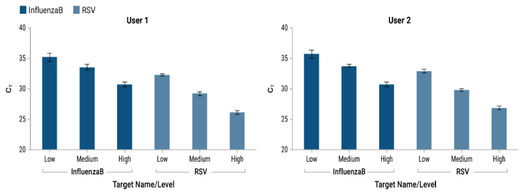



**Fig. 2:** Viral transport media (VTM) was inoculated with a nasopharyngeal swab and spiked with RSV A and Influenza B (Hong Kong) virus reconstituted from Helix Elite™ Inactivated Standard, Inactivated Influenza A/B and Respiratory Syncytial Virus. This high-level virus sample (1 ×$10^3$ copies/µl) was diluted 1:10 and 1:100 in VTM to create the medium-and low-level virus samples. In parallel, two users created sample lysates from the spiked VTM samples using the XpressAmp™ Direct Amplification Reagents, Custom*. Both users then detected the presence of RSV A and Influenza B by RT-qPCR using GoTaq® 1-Step Probe RT-qPCR System* (Cat.# A6121) supplemented with the XpressAmp™ Solution. N=6 amplification replicates.

*All Products Discussed - For Laboratory Use

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## 4. Direct Amplification of SARS-CoV-2 RNA

Testing of SARS-CoV-2 RNA targets were performed using synthetic RNA controls and the original CDC Emergency Use Authorization SARS-CoV-2 assay[3].

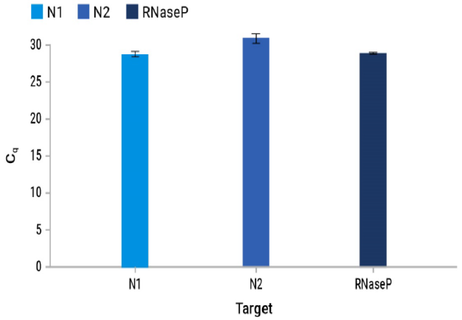


**Fig. 3:** Viral transport media (VTM) was inoculated with a nasopharyngeal swab and spiked with Synthetic SARS-CoV-2 RNA Control 2 (Twist Biosciences, Cat.# 102024, final concentration in VTM 1 ×$10^4$ copies/µl). Spiked VTM samples were then lysed by combining 5µl of sample with 5µl of prepared XpressAmp™ Lysis Buffer and incubated at room temperature for 10 minutes. Following incubation, 5µl of sample lysate was then added to a monoplex GoTaq®Probe 1-Step RT-qPCR (25µl) containing XpressAmp™ Solution and amplified using the 2019 nCoV RUO kit (IDT, Cat.# 10006713) and thermal cycled according to the CDC protocol. N=8 amplification replicates.

## 5. Viral Inactivation†

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 temperate. Since the XpressAmp™ sample preparation method instructs the user to prepare the XpressAmp™ Lysis Buffer before use by adding 1-thioglycerol (1-TG), XpressAmp™ Lysis Buffer was evaluated both with and without the addition of 1% 1-TG. To evaluate the rescue of any residual infectious virus, treated viral samples were then added to the culture media of infection-susceptible CaCo cells, and incubated 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, and the presence of SARS-CoV-2 virus viral particles were measured 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.

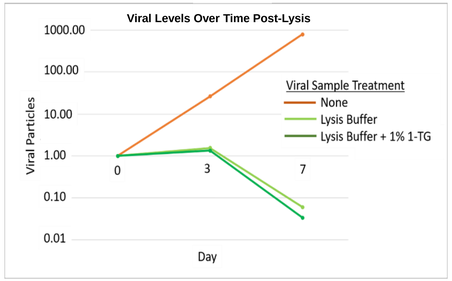


**Fig. 4:** The recovered yields of SARS-CoV-2, after a 7-day incubation period of susceptible cells as described in section 5.

At day 0, the virus input was determined for all the conditions tested and set to "1". 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). 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 after the addition of 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**

- Under the conditions tested, the XpressAmp™ Lysis Buffer successfully inactivated the SARS-CoV-2 virus.
- In case of user error, where the user forgets to add 1-TG to the XpressAmp™ Lysis Buffer before use, viral inactivation is still fully accomplished.

†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.

## 6. Direct Amplification from Saliva Samples

Saliva samples have recently become a frequently used sample type for SARS-CoV-2 testing, notably as by the Yale SalivaDirect™ protocol. We modified our swab/VTM protocol to be compatible with saliva and compared it to the published Yale method[1,2], both with Promega's GoTaq® 1-Step Probe RT-qPCR System and the Luna® Universal Probe One-Step RT-qPCR Kit. Using an MS2 bacteriophage model, we saw earlier Cq values using XpressAmp™, with both RT-qPCR reagents, compared to SalivaDirect™. With an inactivated SARS-CoV-2 virus model we observed similar and more reproducible Cq values for the XpressAmp™ Direct Amplification methods despite an expected 1 cycle delay due to the 1:1 sample dilution in the XpressAmp™ protocol.

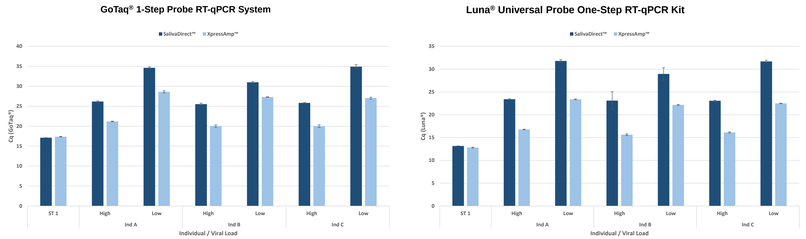


**Fig. 5: Direct amplification of MS2 bacteriophage RNA from saliva.** Saliva from 3 individuals was spiked with MS2 bacteriophage at a high (5 x $10^5$ PFU/µl saliva) or low (5 x $10^3$ PFU/µl saliva) viral load. 50µl of each saliva sample was combined with 50µl prepared XpressAmp™ Lysis Buffer and 125µg Proteinase K. Prepared samples were incubated at room temperature for 10 minutes followed by 95°C for 10 minutes. Saliva samples were also processed according to the SalivaDirect™ protocol[1,2]. 25µl (XpressAmp™) and 20µl (SalivaDirect™) RT-qPCR reactions were prepared using the GoTaq® 1-Step Probe RT-qPCR System or the Luna® Universal Probe One-Step RT-qPCR Kit, MS2 specific primers/probe, and 5µl of saliva lysate. XpressAmp™ lysates were amplified in the presence of XpressAmp™ Solution. Recommended cycling conditions were used for each set of amplification reagents[2,3]. The same analysis threshold was applied to each set of amplifications, the first standard from the standard curve for each set is included for reference. Average Cq and standard deviation of duplicate lysates amplified in duplicate is shown. Standard curve efficiency and $R^2$ values were as follows: GoTaq® (SalivaDirect™ – 86.5%, 0.999; XpressAmp™ – 91.0%, 1); Luna® (SalivaDirect™ – 100.3%, 0.999; XpressAmp™ – 98.6%, 1).

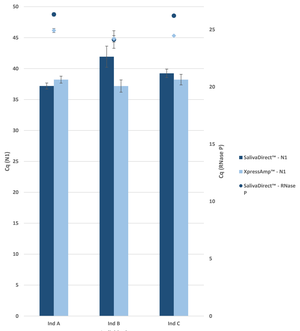


**Fig. 6: Direct amplification of SARS-CoV-2 RNA and Human RNase P from saliva.** Saliva from 3 individuals was spiked with SARS-CoV-2 virus reconstituted from Helix Elite™ Inactivated SARS-CoV-2 Whole Virus (Microbiologics, Cat.# HE0065) at approximately 66 copies/µl. 50µl of each saliva sample was combined with 50µl prepared XpressAmp™ Lysis Buffer and 125µg Proteinase K. Prepared samples were incubated at room temperature for 10 minutes followed by 95°C for 10 minutes. Saliva samples were also processed according to the SalivaDirect™ protocol[1,2]. 25µl (XpressAmp™) or 20µl (SalivaDirect™) duplex RT-qPCR reactions were prepared using the GoTaq® 1-Step Probe RT-qPCR system with N1 and RNase P specific primers and probes[1,2] and 5µl of saliva lysate. XpressAmp™ lysates were amplified in the presence of XpressAmp™ Solution. Reactions were subjected to thermal cycling according to the CDC protocol[3]. The same analysis threshold was applied to each set of amplifications. Average Cq and standard deviation of duplicate lysates amplified in duplicate is shown. Standard curve efficiency and $R^2$ values were as follows: SalivaDirect™ (87.1%, 0.988); XpressAmp™ (90.9%, 0.998).

**Citations:**

1. Vogels, *et al.*, (2020) SalivaDirect: Simple and sensitive molecular diagnostic test for SARS-CoV-2 surveillance. *medR,iv.* **Aug 2020**.

2. Chantal Vogels, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne Wyllie 2020. SalivaDirect: RNA extraction-free SARS-CoV-2 diagnostics. protocols.io https://dx.doi.org/10.17504/protocols.io.bgwekn9e

3. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel EUA. CDC-006- 00019, Revision: 05, Effective 07/13/2020.

## 7. Conclusions

- Effective lysis of viral RNA targets, including SARS-CoV-2, can be achieved in as little as 10 minutes, with the resulting lysates compatible with direct amplification in commercial RT-qPCR master mixes.
- Detection of viral RNA from saliva lysates is possible, with results demonstrating greater reproducibility when using XpressAmp™ Direct Amplification Reagents compared to the Yale SalivaDirect™ protocol.
- The XpressAmp™ lysis step inactivates SARS-CoV-2 virus, reducing contamination risk within the laboratory.
- The XpressAmp™ protocol is designed to be automation-friendly, and we are able to provide support for those wishing to use liquid handlers for the process.

Corresponding author: curtis.knox@Promega.com
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