Introduction

1. COVID-19 is a serious and potentially deadly disease, and the early diagnosis plays a very important role in stopping the escalation of the disease.
2. One of the biggest challenges about coronavirus containment is asymptomatic transmission of the virus. An early detection is playing a very important role in controlling the spread of this virus.
3. There are different diagnostic tests currently available in the market. Each has its own strengths and downsides and mostly all of them use nasopharyngealthroat swabs to obtain the samples for testing.
4. Currently, different research groups around the world are trying to develop strategies which involve the collection and diagnosis of viral genetic material using saliva as a collection source which is less invasive and more cost-effective diagnostic method.
5. In response to current pandemic, here we proposed various strategies for the standardization of saliva based diagnostic testing. Some of the major bottlenecks are specimen collection, virus inactivation and RNA extraction. Therefore, Saliva is a convenient specimen type that can be self-collected provided easily by all ages of patient, i.e., children, adult and elderly patients.
6. Currently RNA isolation from patient samples is the rate limiting step and purpose of my research is to develop a robust and stable buffer system that can be used to directly perform RT-qPCR on patient samples without going for nucleic acid extraction.
7. This study will assess the diagnostic validity, turn-around time and cost-effectiveness that are associated with the use of saliva-based diagnostics testing.
8. In order to standardize buffer system for the collection and processing of SARS-CoV2 in the absence of BSL-3 facility we simulated the entire procedure using Hepatitis B virus (HBV).

Materials and methods

1) Healthy Saliva samples were bought from Lee Biosolutions, Inc. and were dispensed into 2 ml polypropylene tubes with or without collection buffer i.e. TAE, TE, PBS and water. RNA Later, DNA/RNA Shield. Different additives (Twee20, TritonX-100, NP-40, Glycogen, Proteinase K, BSA, DTT, and PBS-DTT) were also added to the above mix separately. As a results, a total of 39 combinations of buffers and different additives were tested during the present study.
2) HBV particles were harvested from HepG2-BV stable cell line [ATCC® HB-8065™].
3) Saliva samples were spiked with know concentration of HBV suspension.
3) Samples were incubated in a hot water bath at 60°C for 30 min to inactivate HBV.
4) A one step qRT-PCR of two important genes of HBV i.e. X and HBeAg was performed from the above direct samples using one-step SYBR Green qRT-PCR kit. RNA was also isolated from some of the above samples for comparison purposes.

Conclusions

The direct saliva-to-RT-qPCR method described herein, bypasses NP swabs and RNA isolation/purification steps.

Heating of the saliva samples beforehand represents a very simple method to inactivate the virus without opening the collection tubes which also confers added biosafety and thus reduces biohazard risks.

Also, direct qRT-PCR resulted in time-efficient and cost-effective detection of the HBV. We are in the process to test our developed protocol on SARS-CoV-2 and other virus first in BSL3 and then in BSL2.

Large scale SARS-CoV-2 testing will be a powerful weapon in preventing spread of this virus and helping to control the COVID-19 pandemic.

References

1) Ranna D.R.E. et al. Saliva-Based Molecular Testing for SARS-CoV-2 that Bypasses RNA Extraction. doi:10.1101/2020.06.18.159434
3) Azzi L. et al. Saliva is a reliable tool to detect SARS-CoV-2. p. e45-e50

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