Austin Journal of Clinical Case Reports

Open Access

Austin Publishing Group

Short Communication

Reliable Sample Preservation Solution: The Critical First Step in Accurate Detection of SARS-CoV-2

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Received: September 08, 2021; **Accepted:** October 06, 2021; **Published:** October 13, 2021

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The unprecedented global pandemic known as COVID-19 caused by SARS-CoV-2 has a profound impact on human life and health, economy and society. Since the outbreak, tremendous effort has been put forth to expand our capacity to diagnose this deadly virus, because accurate detection is essential to effectively combat the epidemic. Numbers of reports have focused on whether the detection methods are sensitive and accurate enough [1]. Little information on the critical first step of detection, sample preservation, is available. At present, there are various of virus Preservation Solutions (PS) on the market, which are used for the preservation of swab samples in nucleic acid detection of SARS-CoV-2. When screening or testing for SARS-CoV-2, pharyngeal swab or nasal swab should be put into PS. Before nucleic acid extraction, the sample may stay in the protective solution for several hours or days. If the sample is not effectively protected in the preservation solution, resulting in the degradation of virus nucleic acid then false negative will occur in the downstream detection process [2]. Therefore, the protection ability to virus RNA of PS is critical important to the reliability of nucleic acid detection results.

In order to investigate whether there are differences in protective ability between different PS, we collected eight kinds of PS products (A, B, C, D, E, F, G, H) for evaluation. The same amount of SARS-CoV-2 pseudovirus Reference Material (RM) or inactivated influenza virus (H9N2) were mixed with these eight PS, and physiological saline (PS, I) and phosphate buffer saline (PBS, J) were used as controls. The protective effect of different PS at 24°C and 37°C for 0h, 6h, 12h, 24h, 48h and 72h were investigated by extracting the nucleic acid of each sample then determining via one step reverse transcribed quantitative PCR (RT-qPCR, Figure).

The results show that the protective effect of PS F on pseudovirus nucleic acid is very poor, which is the worst among the 8 tested products. The Ct value of sample F was significantly higher than that of other PS, even if the test was carried out immediately after the mixing of pseudovirus RNA and PS (defined as 0 hour). And after 24 hours at 24°C and 6 hours at 37°C, no signal was detected in F. Except F, the protective effects of all other guanidine containing PS were satisfactory within 6 hours. From 12 hours, the protective effect of B at 37°C began to become worse, and that of D at 24°C and 37°C began to deteriorate. And as expected, pseudovirus RNA degraded gradually with time in PBS and physiological saline. In comparison, PS A/C/E/G/H have better protection ability to pseudovirus nucleic acid. The consistent results were obtained using inactivated influenza virus samples.

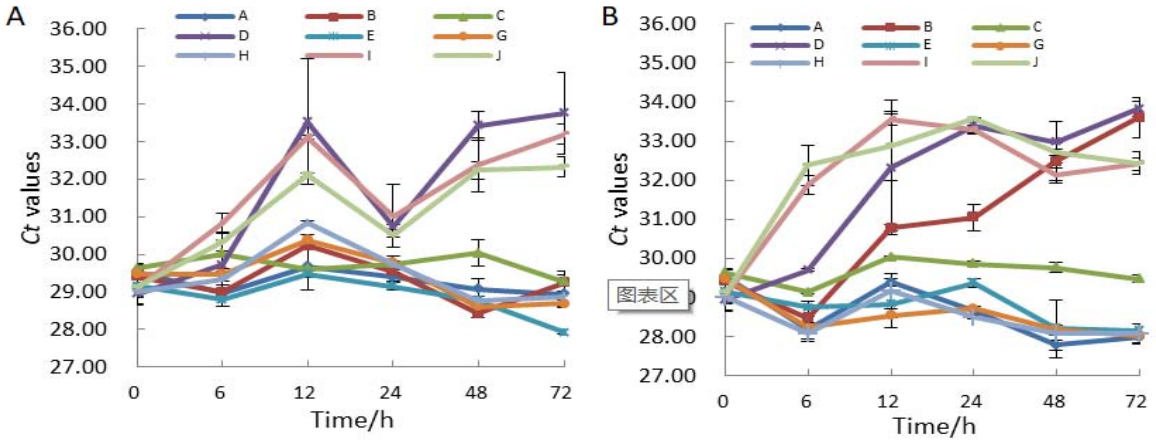

Figure 1: Results of protective effect of different Preservation Solutions (PS) for SARS-CoV-2 pseudovirus RNA reference material. The results were presented by Ct values (Y axis) of one step RT-qPCR detecting RNA extracted from mixtures of pseudovirus RNA and different PS, after same amount of pseudovirus RNA were mixed in 8 kind of PS (A-H), physiological saline (I) and PBS (J) for 0h, 6h, 12h, 24h, 48h and 72h at 24°C (A) and 37°C (B).

Citation: Yang Y, Song Q, Dai X and Dong L. Reliable Sample Preservation Solution: The Critical First Step in Accurate Detection of SARS-CoV-2. Austin J Clin Case Rep. 2021; 8(8): 1228.

Conclusion

As an essential step of nucleic acid detection, the protective ability to virus nucleic acid of PS should be highly valued. According to the results of this experiment, differences in protective effect do exist between different PS, even if these products are all approved by the National Medical Products Administration (NMPA). Among the eight products tested, five PS were reliable within 72h at both 24°C and 37°C, one PS (B) became worse after 12h at 37°C and one (D) become unreliable after 12h at both 24°C and 37°C. The worst PS F didn't even provide any protection for the RNA.

If the products with poor protection ability are widely used and the nucleic acid in collected samples cannot be extracted and detected in a short time, the reliability of the subsequent test results will be very worrying. Since the outbreak of the epidemic, there have been many reports showed that nucleic acid test results were negative for many times even for patients whose clinical indications have been confirmed [3]. Therefore, it is suggested that 1) use guanidine containing PS with stable and reliable quality, 2) before used, the protective effect of each batch of purchased products should be verified with the help of safe pseudovirus reference materials as the quality control sample, 3) after sample collection, the faster subsequent nucleic acid extraction and detection, the better.

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