Is a Real Time-Polymerase chain Reaction a Reliable Confirmatory Test for COVID-19?
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ABSTRACT
Importance: Corona virus Diseases 2019 (COVID-19) is an unprecedented global health pandemic that occurs in the twentieth century, caused by a virus genre called SARS-CoV-2 which infects the lower respiratory system. Started in Wuhan city in Chine, then disease has spread worldwide until the WHO declared as pandemic disease.
Recent View: The current pandemic SARS-CoV-2. Confuses the scientific and the medical community about which accurate diagnostic tool should be relied on to diagnosis COVID-19. RT-PCR is considered as a gold stander technique for COVID-19, however, there are a few viewpoints doubting underestimating its sensitivity and specificity. Here in the current narrative review, we have shed light on the both opinions and we have added our perspectives, as well.
Critical concept: In spite of a little concern about the reliability of RT-PCR, up to date RT-PCR has been accredited as a fundamental diagnostic technique. Nevertheless, to eliminate this concern regarding the sensitivity and specificity of RT-PCR; there are efforts from scientists to develop more molecular diagnostic technique; such as multiplex PCR.
Future prospect: To reduce the variation on the RT-PCR result report, it would be better; if there is a universal guideline or protocol to be applied in this critical situation.
Keywords: COVID-19; Real Time-PCR; SARS-CoV-2; Sensitivity; Specificity

INTRODUCTION
Since December 2019, Corona Virus which is later known as COVID-19 has occupied top news all around the world. In the beginning, Corona virus started in Wuhan city in China as local outbreak. But later the disease has spread out all around the world until the WHO declared COVID-19 as a pandemic disease. It has not only represented a critical issue for health authorities, but also caused concerns for political leaders, because of its direct impact on economic situation. Whereas, most of the businesses over the world are lockdown [1]. This is an unprecedented global health pandemic at least in the twentieth century. COVID-19 is one of the viruses that infects lower respiratory system and causes severe acute respiratory syndrome (SARS). The virus which caused COVID-19 is called SARS-CoV2 [2,3]. In the beginning, the COVID-19 genome was unknown, it has been sequenced and became available online on the first of January 2020 [4]. Then after knowing the SARS-CoV2 genome sequence, another controversial issue has started in the medical community, regarding which analytical tool could be used as a reliable mechanism for diagnosis of COVID-19. Actually, up to date, the diagnosis of COVID-19 is basically based on RT-PCR, in addition to CT, and recently serological antibody detection is also used. However, from all these techniques, RT-PCR is considered as a gold stander for diagnosis of COVID-19 as established in the previous reports. Up to date the RT-PCR is accredited globally as laboratory technique for identification of SARS-CoV2 [5]. Nevertheless, there are some opposing views around the sensitivity of RT-PCR in the detection of SARS-CoV-2.
Here, this narrative review has focused on a degree of reliability of RT-PCR and attempted to evaluate and clarify the literature
regarding the accuracy of RT-PCR and makes recommendations based on this literature.

Molecular diagnosis of COVID-19

RT-PCR is a molecular diagnostic technique used to detect presence of specific genetic material in any pathogen, including a virus. The principle of this technique based on conversion of RNA to a complementary DNA (cDNA) by a process called reverse transcription. Then, the PCR amplifies the cDNA exponentially which is detected by real-time using fluorescence probes [6]. At the current pandemic situation, RT-PCR is used to transcribe and amplify the specific sequence(s) of SARS-CoV-2 from target specimen (nasal or nasopharyngeal swabs) [7].

Pros of RT-PCR

Some of the published data showed that the sensitivity of the RT-PCR reached up to 95% for diagnosis of COVID-19. That means enabling early detection of low viral load [8]. In spite of that, there are different laboratory methods to detect viruses such as Cell Culture, Immunofluorescence (IF) Assay, Immunoblotting, Flow Cytometry (FCM) and Transmission Electron Microscopy (TEM), but among them, the RT-PCR is preferred to be used for detection of COVID-19, because it may be able to provide results within hours or in maximum within 2 days [9-11]. The cost of RT-PCR detection test is cheaper if compared with other molecular diagnostic techniques [12].

Cons of RT-PCR

There are some perspectives that should be taken into consideration around the sensitivity of the RT-PCR test in the detection of COVID-19. Firstly, the similarity of genome between SARS-CoV-2 and SARS-CoV, whereas, about 82% of nucleotide identity with some of the primer-probes of SARS-CoV and other SARS-related viruses can lead to have a cross-reaction [13,14]. Secondly, critical concern of low sensitivity as reported for RT-PCR assays, especially when patients are in the early stages [15,16]. Thirdly, the RT-PCR results revealed a fluctuating trend. Theses may happen by inadequate viral material in the specimen, laboratory error during sampling collection or transportation [17].

In addition to that, the RT-PCR machine requires tremendous knowledge for its technical operation, whereas the operator who works on the machine should be well trained [18]. RT-PCR could not be used to detect past infections (which may help to understand the development and spread of the virus) [19]. So, to reduce the potential risk of false-negative PCR, some studies have recommended to examine chest CT for clinically suspected patients with negative initial RT-PCR [20].

DISCUSSION

False-negative and false-positive results of the RT-PCR still are considered as one of the critical issues in the diagnosis of COVID-19. Especially, when patients with a negative result, couldn’t exclude the possibility of COVID-19 infection, and should not take the only RT-PCR result as a criterion for patient management decisions [21]. As recommended by experts, it’s important to run another confirmatory test such as a CT or antibodies test to emphasize the RT-PCR result [22].

Pre analytical errors

Due to pre analytical errors, perhaps miss-diagnosis occurs in patient infected by COVID-19. These errors could be divided into many categories [23] see Figure 1.

Modifications of RT-PCR

One of the significant drawbacks of the diagnosis of severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) is sensitivity. To minimize the risk of false-negative results, there was a need to develop a convenient RT-PCR to more advanced RT-PCR, therefore, appears a multiplex rRT-PCR. Whereas, in multiplex PCR, occurs an amplification of more than one target gene which leads to the prevention of amplification of less abundant targets genes and subsequently increases the sensitive detection of target genes. This mechanism confirms the WHO recommendation regarding detecting at least two different targets gene on the COVID-19 virus genome [24,25].Another modification of molecular diagnostic of COVID-19 is COVID-19-nsp2, whereas did not amplify other human-pathogenic corona viruses and respiratory viruses that means only amplify the Coronavirus-2 target genes, to some extent it could be highly sensitive and specific [26].

AUTHOR’S PERSPECTIVE

According to the published data at this point, the degree of reliability of RT-PCR specificity and sensitivity for the diagnosis of COVID-19 is extremely acceptable, especially after the modifications which have been entered by using multiplex rRT-PCR.
Nevertheless, there is a little concern about the biological validity of quantitative data of RT-PCR results. In our perspective view, we think that, if a global agreement to determine the quality is implemented, and quantity of RNA control, and guidelines about basic issues such as how to write unified result report for COVID-19 all over the world, all these amendments may be helpful to reduce the variations between RT-PCR results. Finally, the authors of this review support and recommended that, as long as RT-PCR is considered as gold stander and reliable technique for diagnosis of COVID-19, it would be better if the future efforts focus on resolving the limited negative drawback of RT-PCR (as mentioned above). They could benefit from misconception which accompanied the current pandemic COVID-19 result (either due to false negative or false positive result), and through correction of this misconception by scientific explanation, they could reach to a satisfactory result. Moreover, by figuring out the accurate diagnostic tool for the current pandemic COVID-19, the world will be ready if the second wave of SARS-CoV-2 is to come.

CONCLUSION
To sum up, a rapid changing of new research findings represents major Challenges to laboratory diagnosis of the unprecedented Pandemic COVID-19. However, RT-PCR assay is still a fundamental method to be applied for the detection of SARS-CoV-2 to date.

ACKNOWLEDGEMENTS
Our great Thanks are to Mr: Yassir Aboalbashar Younis and the Sudanese Medical Laboratory Technologists in Oman (SMLT) for their logistic support.

AUTHOR CONTRIBUTIONS
Abdelmonem M Ali, Ahmed O Abuelhassan, Ebtehal M fawzi, Nagia S Ahmed, Shieima A Elbasheer have contributed equally in conceptualizing and writing the original draft; Alfatih Abo Albashar Younis has edited and reviewed it the final draft.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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