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Letter to the Editor

Pitfalls in the performance of real time PCR tests for SARS CoV-2 and time to improve these tests

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To the Editor

In your recent issue, there is a publication about SARS CoV-2 diagnostics, but this review is not touching the real issues related to real time PCR tests being used through the world to detect SARS CoV-2 [1]. In January, 2020, there was a publication from Holland and Germany about three different tests targeting different regions of this virus to be used for the detection of SARS CoB-2 (Wuhan strain) [2]. World Health Organization (WHO) has recommended these tests to be used to detect this pandemic virus [3]. At the same time, my laboratory in Germany developed a kit (FR475) January 2020, which is targeting one conservative part of the virus and till today, none of the mutations is able to affect the performance of this test. This test is highly sensitive, accurate and specific, along with giving a robust performance. (Publication underway).

The whole industry and institutes take the primers and probes from WHO recommendation and use in their institutes to detect the virus in clinical samples. At the same time, biotech industry from different countries used these WHO tests as basis to develop their own commercial kits. These kits were approved and brought on the market with FDA/CE/WHO approval on the market through many commercial companies including biggest in the world. One biggest pharmaceutic company from Switzerland also provided these questionable primers and probes to many reference laboratories through out the world and these laboratories used them. Many approved kits have been modified or withdrawn because of mutations as they lack the correct performance. Moreover, these tests are multiplex, hence they are more prone to give questionable results.

In September 2021, we were conducting the tests with our kit and WHO primer and probes. We found that these primers and probes gave false positive results against our Kit (FR475). These results are published under the title Pitfalls found in SARS CoV-2 specific test performance during the comparison between WHO recommended method and a commercial test [4].

In the literature, there are many groups, which were also reporting that these WHO primers and probes are giving questionable results. In Germany, a research group found similar results like our laboratory, but this German group was thinking that the positive control is creating these false positive results [5]. In reality, these were primer and probes producing the false positive results [4]. Another group from Italy found that the commercial tests are questionable results too. The commercial

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tests were distributed from biggest biotech companies around the world [6]. Analysis from this Italian group shows that they have similar problem as mentioned above. There are other laboratories reporting such type of pitfalls [7,8].

The biggest concern is that diagnostic laboratories worldwide were unable to notice this issue. Therefore, it is highly recommended that the laboratories must run some negative samples along with their own validations. In our publication, we have written full recommendations [4].

Therefore, an urgent need that there must be laboratories in each country, which should compare the performance of the commercial approved tests as well as tests recommended from any institutes like WHO/CDC/FDA in order to avoid such pitfalls. Through comparing the performance of various tests, one is going to know the exact performance features of each test and it will lead to removal or reduction of use of questionable tests.

These false positive results have created a lot of financial burden along with psychology fear among the tested innocent persons. This may be the cause that so many symptomless persons are PCR positive. Such issues must be avoided [4].

This is not one example. The author of this letter has pointed out this problem with an interview in Bloomberg in 2016 that many of these commercial companies do not have sufficient knowledge of virology, but they develop the kits and get approvals. This issue must be investigated because we need accurate tests to control the pandemic viral outbreak. Because of these questionable tests, one will not be able to stop SARS CoV-2 spread and its fate will be like Influenza H1N1, it means that it will mutate and cause outbreaks in future.

My humble request, if there will be pandemic outbreak of Influenza H5N1, it will be many times stronger than what we are seeing with SARS CoV-2 outbreak. This is time to improve the detecting system!

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