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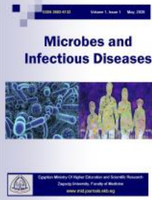

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Letter to the Editor

Do we need a quality standard to use primers and probes from literature?

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To the Editor

It is common practice for many research groups to use the primers and probes from the literature to conduct different kinds of studies in the field of medical microbiology. Our laboratory indicates that many of these primers and probes give questionable results [1]. One important example is the primers and probes recommended by World Health Organization for detection of SARS CoV-2 as these oligos were published from a German and Dutch research group [2]. These lead to questionable results. Many groups have confirmed that the questionable results are given through these primers and probes [3].

Not only this, but there are many research groups, which have published. The literature is full of such questionable oligos. Here are a few examples: The following primers are used to detect the Rift Valley virus [4].

TCTGTCCGTCTCCTATAGACACAAAGACCGG TGCAACTTCA-3

The in-silico results show that these primers are not in a position to detect this virus. Such PCR detection system also raises questions about the correctness of the results of such German publication.

In another publication from Germany, there are primers and probes for the detection of Chikungunya virus, but the results in our laboratory show that these primers and probe are not in the position to detect these viruses properly; therefore, we developed our own primers and probes for our Chikungunya virus kit. The in-silico results showed that these primers and probes are not good enough to give the best results. The forward primer and the probe are not in the position to detect all strains circulating worldwide as shown below:

CTCCTATAGACACAAAGACCGGTGCAACTT CA-3. These oligos are outdated, as they were published in 2008. They have been used in recent publications [5].

Here is another questionable backwards primer to detect La crosse virus as shown below:

GCC TTC CTC TCT GGC TTA-3 [6].

In a recent publication from Bangladesh, the primers were used to detect *Pseudomonas* genus, but the in-silico results showed that these oligos are not in a position to do so. In this publication, there are primers for *E. coli*, which are likely to give all *E. coli* strains; hence the results of this research can be questioned [7].

Influenza A covers a broader range of influenza strains ranging from H1 to H18 and N1 to N11. There are many primers and probes available in the literature, but many of them are outdated because they come from older publications. They should be avoided or updated. There is a recent publication that

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claims that they have developed a universal test for the detection of Influenza A, but the careful analysis of this work shows that the primers and probe provided in this work detects most of H1N1, H3N2, H3N6 and some H5N1 strains. Even the abstract and the publication itself accept these facts [8]. Therefore, scientists using such primers and probe must know the limitations of such publications, and their published results must point out this weakness. Moreover, such publications may not be able to provide highly accurate results. It is very difficult to develop a universal influenza kit, but it is not impossible. There are a number of publications, which are using these oligos, as the title of this publication is doing a questionable claim i.e., development of universal primers and probes for influenza A [8].

A few days ago, analysis from a publication is from Microbes and Infectious Diseases showed that the primers used to detect the antibiotics' genes were not in the position to detect them properly as they were very outdated from very old publications. In silico studies confirm these results [9].

Another example from Microbes and Infectious Diseases website was the detection of *Klebsiella pneumoniae*, where forward and back primers were not designed properly as they may react with other organisms also. These need to be validated for cross reaction panel for their specificity along with sensitivity. Moreover, the band length should be 534 or 535 bp instead 516 [10]. Each kit, which we manufactures go through strongest validation process.

Similarly, the publication about the detection of *Entamoeba moshkovskii* indicated that the primers used should not give correct results, as in-silico studies show this fact [11]. Furthermore, a research group from Africa has published the molecular detection of different pathogenic *Trypanosoma*, where primers were used from Holland and Belgium group from year 2007. It seems that these tests should be able to detect other related pathogens as well [12].

In 2016, the author made a presentation at a conference in Puerto Rico, USA, where he compared double check PCR test primer and probes with the primer and probes from a publication from CDC, USA, to show that double check real time PCR kit covers a wider range of Zika virus strains.

These are only few examples, but the author encounters so many publications where primers and probes from literature were used, but these should not give the correct results; hence results of many hard-working scientists are questionable, and they cannot build the basis of developing new therapies, vaccines, and monitoring measurements. This leads to wastage of taxpayer money and financial resources.

There may be many reasons for these questionable PCR tests from the literature as there may be mistakes during the publication process. These may be outdated as they might have functioned properly at the time of their development many years ago, but new information about the pathogens is being published daily making these oligos outdated. There are many thousands of publications in microbiology, which may face such problem. There are also publications where the primers and probes still would give good results; therefore, scientists must carefully choose the primers and probes before conducting the assays.

There are companies offering designing of PCR oligos for microbiology. They use software, which is not sufficient to design primers and probes. One must have deep knowledge of virology or microbiology and may work in many cases for a very long time to design and develop good tests. Similarly, the ready to use kits on the market are developed on the basis of primers and probes from literature as there is very less knowledge of virology in many cases. This fact can be proved through the failure of diagnostics for many pathogens like Influenza A, H5N1, Zika, Dengue genotyping, HCV, Norovirus and respiratory syncytia viruses as examples. These kits may be approved through European regulatory authorities, WHO and FDA. The scientists must still know the drawbacks and they should ask their governments to establish their own reference laboratories in each country to compare the commercial kits available on the market. One should also do the comparisons between the approved kits and research use kits in order to show that approved kits are better, but there will be many surprises. This practice can help finding the best kits, which leads to less burden on health system.

Therefore, my laboratory is going to suggest quality standards for how to cite these primers and probes during the publication process to make sure that the primers and probe provide accurate results. During the peer review process, the journals must ensure that PCR oligos used are updated.

Such development of quality standards will help to remove outdated oligos from scientific research process. Once we will do this, we are very sure that we are going to have accurate results to

develop excellent therapies, vaccines and monitoring measurements. There will be a publication in future.

In this letter, we want to request the hard-working scientists in the world to check these primers and probes along with writing in this publication that the primers and probes used are thoroughly checked for their correctness to be used in current research.

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