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Reply to: Letter to the editor of heliyon re: "Diagnostic evaluation of nCoV-QS, nCoV-QM-N, and nCoV-OM detection kits based on rRT-PCR for detection of SARS-CoV-2 in Ecuador"

Dear Editor,

First of all, we are grateful for Dr. Garcia-Bereguiain's interest in our article. Here is the answer to every comment.

Regarding Dr. Garcia-Bereguiain's initial comment. We agree with the statement that ARCSA did not carry out any evaluations of commercial SARS-CoV-2 kits. For this reason, we reviewed on the FDA web page [1] each of the 35 COVID-19 diagnostic kits that were in use in Ecuador and we found that just 10 of them got the FDA's authorization for use in an emergency. The FDA served as a reference organization throughout the pandemic, validating the scientific data of each kit and approving its use for the diagnosis of COVID-19 in the context of a medical emergency.

We read the article of Dr. Garcia-Bereguiain [2], his team evaluated the nCoV-QS kit against an adapted version of 2019-nCoV CDC EUA, by finding an overall positive percentage agreement (PPA) of 66.7% (n = 54 samples), they concluded that nCoV-QS had a significant lower performance. They used a CFX-96 instrument to analyze the nCoV-QS kit, however according to the instruction manual nCoV-QS kit is designed to load the sample onto a special PCR plate (LabChip) and specifically run with the Veri-Q PCR 316-QD-P100 device [3]. Following that, they indicated that only 29 samples followed the instruction manual recommendation. Consequently, the use of an unvalidated instrument for the nCoV-QS kit could be the reason for the different results reported by the Dr. Garcia-Bereguiain group. Additionally, the sample size is a limitation of their results, as they described.

In our study, we used the Veri-Q PCR 316-QD-P100 device and loaded the samples onto a LabChip in accordance with the nCoV-QS and nCoV-QM-N kits' instruction manuals.

Second, the general argument of our paper supports the necessity to assess the COVID-19 diagnostic kits that have arrived in our country in order to evaluated their performance. We clearly mentioned that most of the kits Ecuador had, were not FDA approved. By doing this kind of research, the academy aimed to confirm the performance of the kits that were already in use and may have been utilized in Ecuador.

Third, we use the LightMix SarbecoV *E*-gene plus *EAV* control and LightMix Modular SARS-CoV-2 (COVID-19) *RdRp* kits as a references kit in our research; as such, our statistical analyses consist of calculating the PPA and negative percentage agreement (NPA) rather than sensitivity and specificity, which would be determined with the use of a gold standard diagnosis kit. Therefore, we made the cautious decision to adhere to the FDA guidelines, which advise doing so when a non-reference standard is used [4]. Consequently, verifying the ability of the nCoV-OM, nCoV-QS, and nCoV-QM-N to agree with the comparison method LightMix *E/RdRp*, reflecting that the estimates are not of accuracy but of agreement. At this point, it is important to emphasize that the LightMix SarbecoV *E*-gene plus *EAV* control and LightMix Modular SARS-CoV-2 (COVID-19) *RdRp* kits (FDA approved) are for emergency use only, whose assessments have shown to be the most reliable for identifying COVID-19, without any evidence of cross-reactivity with common human respiratory viruses, as noted in the IFUs cited in our paper [5,6].

Our analyses showed a Cohen's Kappa value of more than 90% for all of the kits, which statistically shows a strong level of agreement between the outcomes of the analyzed kits. However, the discussion section addressed aspects such sample size, RNA eluate volume, infection phase of each patient, and limit of detection (LoD) of each kit in order to handle the discrepancy rate in NPA that was approximately 8% for nCoV-OM.

Fourth, the One-Step RT-PCR Polymerase Mix Lyophilized enzyme reports analytical sensitivities of 5.2 copies per reaction with the E gene and 10.6 copies per reaction with the RdRp gene when used with the LightMix *E/RdRp* kits. Studies comparing different reagents, such as the One-Step RT-PCR Polymerase Mix Lyophilized enzyme and the SuperScript III One-Step RT-PCR System enzyme used in the Corman protocol, showed the same amplification fidelity [7]. Regarding the mismatched nucleotides in the *RdRp* gene primers reported in 2020 [8]. Corman, in response to these comments, detailed that mismatched base pairs do not reduce the sensitivity of RT-PCR [9].

Fifth, 49% of the positive cases found with the *RdRp* primer are due to LoD whose cut-off is at Ct < 40, while for the *E* gene (47%) it

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is at Ct < 36 as described in table 1 in Ref. [10]. Furthermore, as we mentioned in the materials and methods section, for all kits, the samples were diagnosed as positive for SARS-CoV-2 when at least one viral gene had a valid Ct value according to the recommendations by WHO [11]. Lastly, Corman points out that the high Ct values of the *RdRp* primer in comparison to those of the *E* primer are not the result of mismatched nucleotides, but rather due to the general oligonucleotide design, such as the predicted lower melting temperature of the reverse primer compared to the other oligonucleotides [9], it explains why the cut-off of the *RdRp* in the LightMix *E/RdRp* kit is 4 Ct higher than the *E*.

Finally, the lack of a three-way comparison utilizing a reference standard kit to explain the inconsistencies in the results was also noted in the discussion section. Thus, the main findings of our research just express the comparable SARS-CoV-2 diagnostic performance between the kits.

Author contribution statement

Marco Salinas, Diana Aguirre, Lucy Baldeón, Jorge Pérez-Galarza: Wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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