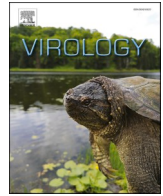




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Analytical and clinical comparison of Viasure (CerTest Biotec) and 2019-nCoV CDC (IDT) RT-qPCR kits for SARS-CoV2 diagnosis.

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ABSTRACT

Background: Several RT-qPCR kits are available for SARS-CoV-2 diagnosis, some of them with Emergency Use Authorization (EUA) by FDA, but most of them lacking of proper evaluation studies due to covid19 emergency.

Objective: We evaluated Viasure RT-qPCR kit (CerTest Biotec, Spain) for SARS-CoV-2 diagnosis using FDA EUA 2019-nCoV CDC kit (IDT, USA) as a gold standard.

Results: Although we found the lack of RNA quality control probe as the main limitation for the Viasure kit, the sensitivity was 91.9% and the specificity was 100%. The limit of detection (LOD) was 2000 copies/mL and 1000 copies/mL for Viasure and IDT kits, respectively.

Conclusions: Viasure RT-qPCR kit is a reliable tool for SARS-CoV-2 diagnosis but improvement of an alternative RT-qPCR reaction for RNA extraction quality control as RNaseP is recommended.

1. Introduction

The COVID19 pandemic has challenged public health systems worldwide, not only for patient care or surveillance and control, but also to guarantee the quality of SARS-CoV-2 related diagnosis tools. For instance, multiple in vitro RT-qPCR diagnosis kits are available on the market for the detection of SARS-CoV-2. Some of them have received emergency use authorization (EUA) from the U.S. Food & Drug Administration (FDA), while for others only limited validation reports made by manufacturers are available. The CDC designed FDA EUA 2019-nCoV CDC kit (IDT, USA) is based on N1 and N2 probes to detect SARS-CoV-2 that have received positive evaluation on recent reports (Lu et al., 2020; Interim Guidelines for Co, 2019; Rhoads et al., 2020; Nallaa et al., 2020), and RNase P as an RNA extraction quality control. Among the commercial kits available in the market, Viasure SARS-CoV-2 RT-qPCR kit (CerTest Biotec; Spain) includes “ORF1ab” and “N” probes for SARS-CoV-2 detection. However, no probe for RNA extraction quality control is included but an “internal positive control” to guarantee that PCR reaction performs well. Viasure SARS-CoV-2 kit is made in Spain, one of the countries leading COVID19 cases and deaths worldwide, where it has been used for SARS-CoV-2 diagnosis. Also, it was recently authorized for SARS-CoV2 diagnosis in Ecuador. However, it is not included on the list of FDA EUA kits (<https://www.fda.gov/medic>, 2020) and only an evaluation study for Viasure SARS-CoV-2 RT-qPCR kit has been reported comparing to an automatized system like de Cobas 6800, besides the limited validation provided by manufacturer’s manual (Matzkies et al., 2020; <https://www.certest.es/wp, 2020>).

We herein present a comparison of the analytical and clinical performance of Viasure and 2019-nCoV CDC kits for SARS-CoV-2 RT-qPCR diagnosis from nasopharyngeal swab samples.

2. Materials and methods

Study design. 156 clinical specimens (nasopharyngeal swabs collected on 0.5 mL TE pH 8 buffer) were included in this study, coming from individuals selected for SARS-CoV-2 surveillance. Also, seven negative controls (TE pH 8 buffer) were included as control for carryover contamination, one for each set of RNA extractions.

RNA Extraction and RT-qPCR for SARS-CoV-2 diagnosis using 2019-nCoV CDC kit. All the samples included on the study were tested following an adapted version of the CDC protocol (Lu et al., 2020): using PureLink Viral RNA/DNA Mini Kit (Invitrogen, USA) for RNA extraction method (Interim Guidelines for Co, 2019); using CFX96 BioRad instrument (Freire-Paspuel et al., 2020a, 2020b, 2020c).

RT-qPCR for SARS-CoV-2 diagnosis using Viasure kit. Same RNA

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extractions from all the samples included on the study were tested using the Viasure SARS-CoV2, following manufacturer’s manual (<https://www.certest.es/wp>, 2020).

Analytical Sensitivity. Limit of detection (LoD) was performed using commercial positive controls provided by both manufacturers. For Viasure SARS-CoV2, a positive control is included on the kit; although the concentration is not detailed, the manufacturer reported that the positive control is provided at 10.000 genome equivalents/mL upon request. For 2019-nCoV CDC kit, the 2019-nCoV N positive control (IDT, USA) was used, provided at 200.000 genome equivalents/mL.

Ethics statement. All samples have been submitted for routine patient care and diagnostics. Ethics approval was not sought because the study involves laboratory validation of test methods and the secondary use of anonymous pathological specimens that falls under the category ‘exempted’ by Comité de Ética para Investigación en Seres Humanos" from "Universidad de Las Américas".

3. Results

Clinical performance of “Viasure SARS-CoV-2 RT-qPCR kit” compared to the CDC gold standard protocol.

156 samples were tested for SARS-CoV-2 following both protocols described on the methods. For the 2019-nCoV CDC EUA kit, 86 samples tested positive and 70 samples tested negative (Table 1 and Supplementary Tables 1 and 2). All the 70 samples tested negative for 2019-nCoV CDC kit were also SARS-CoV-2 negative for Viasure SARS-CoV-2 kit, so the specificity obtained in our study was 100% (see Table 2).

Considering only true positive samples for Viasure SARS-CoV-2 kit (amplification for both N and Orf1ab gene targets according to manufacturer’s manual), 74 samples tested positive and 82 samples were negative; but if we consider as positive not only true positives but also “presumptive positive” samples (only amplification of N gene target), 79 samples tested positive and 77 were negative. In summary, sensitivity for Viasure SARS-CoV-2 kit compared to 2019-nCoV CDC EUA was 86.04% if we considered only true positives, but up to 91.9% if we considered also presumptive positives as positive samples for SARS-CoV-2 (Table 1 and Supplementary Tables 1 and 2).

Analytical sensitivity: calculation of the limit of detection (LoD) of “Viasure SARS-CoV-2 kit”.

The viral loads (copies/uL) detailed on Supplementary Table 1 were calculated running a calibration curve with 2019-nCoV N positive control (IDT, USA). The LoD for the CDC protocol was set at 1000 viral RNA copy per mL of sample (or 5 RNA copies/uL of RNA extraction solution) on previous studies (Lu et al., 2020; Freire-Paspuel et al., 2020a, 2020b, 2020c). For Viasure SARS-CoV2 kit, the positive control included on the kit at a concentration of 10.000 genome equivalents/mL was used on serial dilutions to calculate de LoD for both viral probes. As the LoD is defined as the lowest viral load in which all replicates are detected (100% sensitivity), our data indicates that the LoD for “ORF1ab” probe was 1000 viral RNA copies/mL of sample (5 RNA copies/uL of RNA extraction solution) and for “N” probe 2000 viral RNA copies/mL of sample (10 RNA copies/uL of RNA extraction solution). As both “N” and

Table 1

Performance of Viasure SARS-CoV-2 compared to 2019-nCoV CDC EUA for RT-qPCR SARS-CoV-2 diagnosis (% values: sensitivity and specificity). "Presumptive positive" samples only amplified for N probe with Viasure SARS-CoV-2 kit.

	Viasure SARS-CoV-2 true positive only	Viasure SARS-CoV-2 true and "presumptive positive"	Viasure SARS-CoV-2 negative
2019-nCoV CDC positive	74 (86,04%)	79 (91,86%)	12 (7)
2019-nCoV CDC negative	0	0	70 (100%)

Table 2

Analytical sensitivity of Viasure SARS-CoV-2. A) limit of detection for ORF1ab probe. B) limit of detection for N probe (* : LoD for each probe).

Panel A.		
Viral load	replicates	sensitivity
10,000 copies/mL	5/5	100%
5000 copies/mL	5/5	100%
2000 copies/mL	5/5	100%
1000 copies/mL*	5/5	100%
Panel B.		
Viral load	replicates	sensitivity
10,000 copies/mL	5/5	100%
5000 copies/mL	5/5	100%
2000 copies/mL*	5/5	100%
1000 copies/mL	4/5	80%

“ORF1ab” gene targets must yield a positive result for a sample to be considered positive, the LoD for Viasure SARS-CoV-2 is set at 2000 viral RNA copies/mL of sample.

4. Discussion

Although the main limitation of our study is the sample size (156 specimens), our results support that Viasure SARS-CoV-2 RT-qPCR kit had a good performance in terms on sensitivity and specificity compared to 2019-nCoV CDC EUA, with values of 86,04% and 100%, respectively. Moreover, according to Viasure SARS-CoV-2 kit manufacturer’s manual, when a sample only yields amplification for N gene target but Orf1ab gene target is negative, the result should be SARS-CoV-2 negative and a possible infection by other coronavirus must be considered (<https://www.certest.es/wp>, 2020). However, on our hands, all the Viasure SARS-CoV-2 N gene target positive but Orf1ab gene target negative were confirmed as SARS-CoV-2 positive by CDC protocol (Lu et al., 2020; Interim Guidelines for Co, 2019), and thus we referred to this samples as "presumptive positive". So, if we calculate the sensitivity of Viasure SARS-CoV-2 kit including both true positives and presumptive positives samples, the value rise up to 91.9%.

As we have described on the results, we could calculate de LoD of Viasure SARS-CoV-2 kit on 2000 viral RNA copies/mL of sample, that according to our experimental procedure is equivalent to 10 viral RNA copies/uL of RNA extraction solution, confirming the LoD indicated at manufacturer’s manual (<https://www.certest.es/wp>, 2020). Actually, with the only exception of sample 448 (viral load of 4.300 copies/uL), all the presumptive positive and negative samples for Viasure SARS-CoV-2 kit yielded viral loads below 10 copies/uL (see Supplementary Table 1).

It came to our attention a recent study about the clinical performance of Viasure SARS-CoV-2 compared to Cobas 6800 system using a total number of 95 samples (Matzkies et al., 2020). The authors reported a lost of sensitivity of 30,9% for Viasure SARS-CoV-2 kit, with only 47 out of 68 positive samples detected. However, all the samples that failed to be positive for Viasure SARS-CoV-2 kit had viral loads over 2000 copies/mL of sample (Matzkies et al., 2020). Considering the viral loads frequency distribution for SARS-CoV-2 (Lavezzo et al., 2020; KleiboekerScott et al., 2020), this study would have a bias toward ultra low viral loads below 2000 copies/mL as less than 10% of SARS-CoV-2 positive patients are expected to have those loads (Lavezzo et al., 2020; KleiboekerScott et al., 2020).

The lack of any probe for RNA extraction quality control like RNaseP is a limitation to be considered when using Viasure SARS-CoV-2 kit. Although in our experience, samples failing to yield any RNA after extraction are below 1%, an extra RT-qPCR reaction for a RNA extraction quality control probe would be recommended for laboratories starting SARS-CoV-2 diagnosis.

On the other hand, Viasure SARS-CoV-2 kit is provided on precast

format of 8 tubes strips containing a mix of enzymes, primers, buffer and nucleotides in stabilized format, so only rehydration buffer and RNA samples have to be added. This format is a great advantage in terms of time saving and reduction on pipetting mistakes. Also, the manufacturer provided us upon request the concentration of the positive control included on Viasure SARS-CoV-2 kit (10.000 copies/uL). That means viral loads can be easily calculated without need of purchase any extra positive control, despite the manufacturer's manual does not include instruction for viral load calculation (<https://www.certest.es/wp,2020>).

Considering the worldwide high demand of reagents for SARS-CoV RT-qPCR diagnosis, supplies shortage is a fact, actually affecting harder to developing countries like Ecuador. Under this scenario, validation studies are helpful to guarantee the quality of the supplies in the market for every country in the world, as not necessary all the SARS-CoV-2 RT-qPCR kits show the performance indicated by manufacturer as it was the case for Viasure SARS-CoV-2 kit.

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Authorship contribution statement

All authors contributed to study conceptualization, experimental procedures and revision and approval of final version of the manuscript. Byron Freire-Paspuel and Miguel Angel García Bereguiain analyzed the data and wrote the manuscript.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.virol.2020.10.010>.

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