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Short communication

Brief comparative evaluation of six open one-step RT-qPCR mastermixes for the detection of SARS-CoV-2 RNA using a Taqman probe

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ABSTRACT

Background: Facing the emergence of a new RNA virus, clinical laboratories are often helpless in the case of a shortage of reagents recommended by Reference Centres.
Objectives: To compare five open one step RT-qPCR reagents to the SuperScript™ III Platinum™ One-Step qRT-PCR kit (Invitrogen) considered as the reference one in France at the beginning of the pandemic for detection of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in respiratory specimens by using a laboratory-developed assay targeting the viral RNA dependant RNA polymerase (RdRp) gene.
Study design: A total of 51 NUCLISENS easyMAG extracts from respiratory specimens was tested on ABI 7500 thermocycler with TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems), Luna® Universal Probe One-Step RT-qPCR Kit (New England Biolabs), GoTaq® Probe 1- Step RT-qPCR System (Promega), LightCycler® Multiplex RNA Virus Master (Roche) and One-step PrimeScript RT-PCR kit (Takara). The CT values obtained using the 5 challenged reagents were compared to those obtained using the reference assay.
Results: The percentages of concordance were all above 95 %. When comparing the CT values of the 48 extracts exhibiting CT values < 35 obtained with the reference reagent, the results were similar between the reagents although the differences of CT values were quite dispersed.
Conclusions: All five reagents can be considered as alternative reagents to the reference for detecting SARS-CoV-2 RNA.

1. Background

The emergence of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Europe lead the clinical laboratories to urgently assess a home-brew molecular assay [1]. Between mid-February and mid-March, a period when no commercial kits were available to cope with the huge amount of molecular tests to be carried out, all the laboratories equipped for molecular biology testing referred both to the already published techniques [2] and the National Reference Centres [3,4]. However, they were confronted with a shortage of consumables and reagents, linked to the use of the same protocol by many laboratories. Mögling et al. recently reported the delayed response to the pandemic in relation to primers and probe contamination [5]. Another pitfall was the huge delivery delays of enzymes, especially the SuperScript™ III Platinum™ One-Step qRT-PCR kit (abbreviated as SIII in this study, Invitrogen, Cergy-Pontoise). Our aim was to compare five open one step RT-qPCR reagents available on the French market to urgently find an alternative solution for SARS-CoV-2 RNA detection.

2. Study design

A total of 51 respiratory specimens (45 nasopharyngeal e-swabs, 3 tracheal aspirations and 3 sputum specimens) sampled from patients suspect of SARS-CoV-2 infection and sent at the Laboratory of infectious agents and hygiene of the University Hospital of Saint-Etienne were processed as described [3,4,6] and using a ABI7500 thermocycler

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Table 1
Overall results comparing the 5 open one step RT-qPCR reagents to the reference assay.

Complete denomination (commercialisation)	Abbreviation used in this study	True positive samples (n)	True negative samples (n)	False negative samples (n)	Concordance (%)	Kappa coefficient (CI 95 %)
SuperScript™ III Platinum™ One-Step qRT-PCR kit (Invitrogen, Cergy-Pontoise)	SIII (reference assay)	51	3	0	–	–
TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems)[a]	Fast Virus	50	3	1	98.15	0.848 (0.584−1.111)
Luna® Universal Probe One-Step RT-qPCR Kit (New England Biolabs, Evry)[b]	Luna	49	3	2	96.30	0.731 (0.474−0.988)
GoTaq® Probe 1- Step RT-qPCR System (Promega France, Charbonnières-les-Bains)[b]	GoTaq	51	3	0	100	1 (0.733−1.267)
LightCycler® Multiplex RNA Virus Master (Roche, Meylan)[b]	LC MM	49	3	2	96.30	0.731 (0.474−0.988)
One-step PrimeScript RT-PCR kit (Takara, Ozyme, Saint-Cyr-L'École)[a]	PrimeScript	50	3	1	98.15	0.848 (0.584−1.111)

CI: Confidence interval.
[a] The reverse transcription (RT) step was done during 5 min at 48 °C and after inactivation of the enzyme during 20 s at 95 °C, the cycling program was of 5 s at 95 °C and 30 s at 58 °C during 50 cycles.
[b] Same RT and cycling programs than the reference assay.

(Thermofisher) after extraction on eMAG (bioMérieux). The assay targeted the viral RNA dependant RNA polymerase (RdRp) gene. A multiplex assay was set up in our laboratory by adding to the mastermix the primers (0.2μM) and a Taqman probe (0.1μM) targeting the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA [7] to manage the whole analytical process together with the sample cellularity. After testing with this reference assay, the extract (stored at 4 °C; 5 μL for each assay) was tested on the same day using the same concentrations of primers and probes as those used in the reference assay and one of the five other mastermixes depicted in the Table 1. The cycle threshold (CT) values were recorded for each assay and compared to the reference assay by estimating their agreement with the concordance percentage and the Cohen kappa coefficient. The positive specimens were drawn on Linear regression and Bland Altman plot.

3. Results

The analysis focused for the CT values of the IP4 region of the viral RdRp and those of the GAPDH mRNA. All the specimens were positive for the GAPDH region, with CT values comprised between 20.1 and 28.7 using the reference assay; the GAPDH mRNAs were also detected in all tested clinical specimens with all the tested reagents (data not shown). The analysis of CT values for the IP4 region showed a global good agreement between the mixes in comparison to the reference assay (Table 1). Only 3 samples were not detected by at least one of five reagents, but all exhibited extremely low viral loads with the reference assay (CT values between 38.9 and 40.7). After excluding these discordant samples, the correlation of the CT values was analysed on the remaining 48 specimens (Fig. 1). All CT values were <35 using the reference assay. The mean difference of CT values was higher (> 0.5) for PrimeScript and GoTaq and lower (<-0.5) for LC MM than SIII. Fast Virus and Luna exhibited similar CT values than that of SIII (mean difference of CT value comprised between -0.5 and 0.5). The dispersion of CT values was higher with PrimeScript.

4. Discussion

The SuperScript™ III Platinum™ One-Step qRT-PCR kit, both exhibiting high analytical performances and being easy to use, is often recommended for the development of techniques for emerging viruses detection such as influenza viruses or MERS-CoV; it was also proposed for SARS-CoV-2 RNA detection in the early reports [2–4]. However, due to its own success, its widespread use resulted in disruption of deliveries for high throughput testing. This study showed that several other reagents could successfully be used as an alternative. Although our study included a limited number of samples, no replicates were analysed, and only one thermocycler was used, it provides an overview of the results that can be obtained with these alternative solutions. Discrepancies were mainly observed for high CT values that corresponds to extremely low viral loads in respiratory samples. CT values are not necessarily comparable between techniques and the observed differences do not predict the sensitivity of the assay. The limit of detection should therefore be determined by each laboratory under its own conditions and the primers and probe sets chosen [4]. The sensitivity of the molecular assay for SARS-CoV-2 RNA detection by using laboratory-developed assays is currently not the goal to achieve for respiratory specimens testing as CT values > 35 are observed at late stage of the COVID 19 and even correspond to non-infectious viral genomes [8–10]; consequently, sensitivity of the assays was not the aim of this comparison. Several commercial solutions are now available for SARS-CoV-2 RNA detection [11,12]; manufacturers and clinical laboratories are now prepared to meet strong demands of SARS-CoV-2 detection by using RT-qPCR assays. However, we consider that our study may be useful in the event of a future RNA virus emergence.

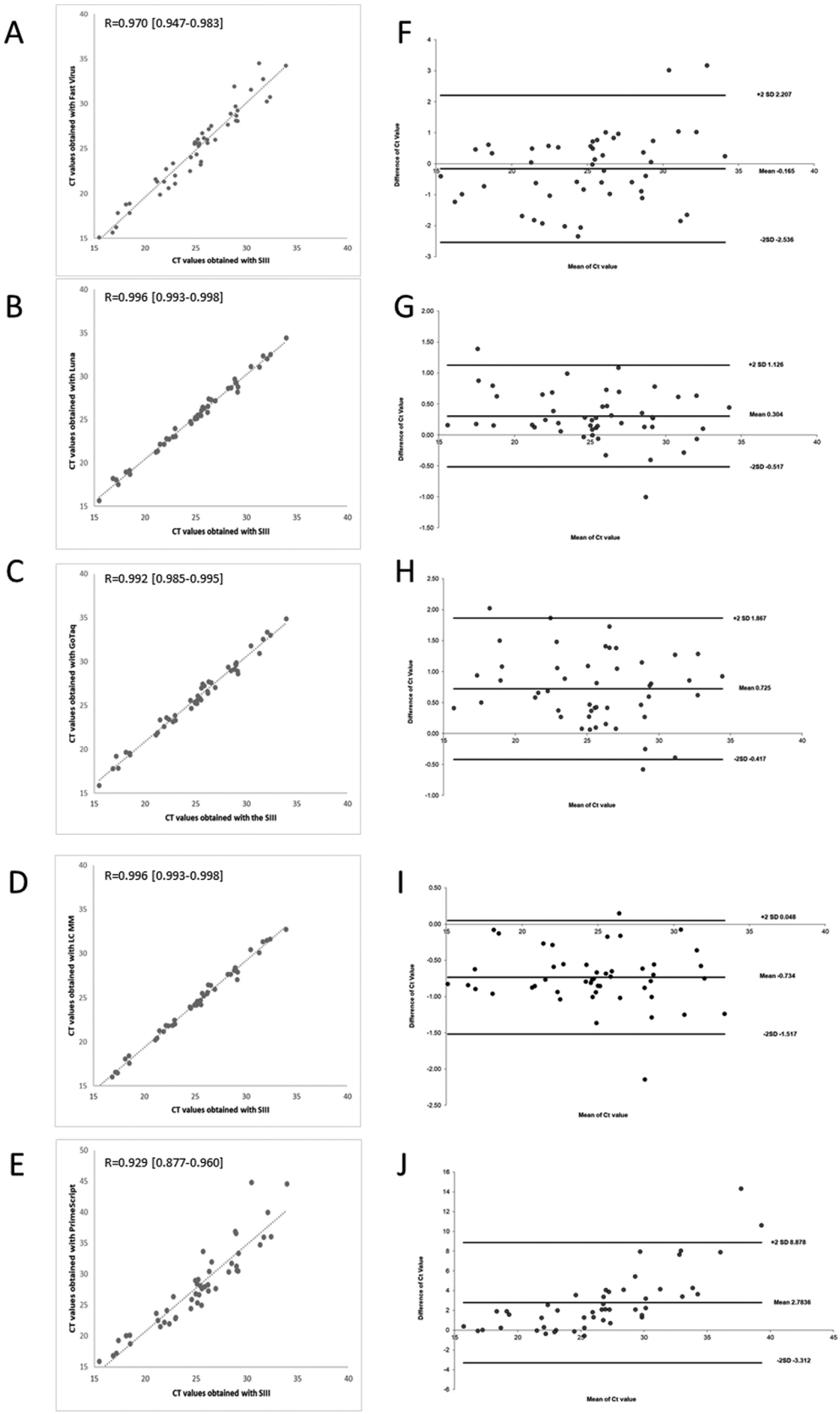

Fig. 1. Scatter plots of correlation between the CT values of the IP4 region of the viral RNA dependant RNA polymerase gene determined using 5 alternative open one step RT-qPCR reagents by analysis with the 48 extracts exhibiting a CT value <35 with the reference assay (SuperScript™ III Platinum™ One-Step qRT-PCR kit Invitrogen). The abbreviations of all reagents tested are listed in the Table 1. **A to E-** Logistic regression. Each point represents the CT value measured by the tested reagent in comparison to that of the reference assay (SIII) of the same extract. The Pearson coefficients with their 95 % Confidence interval are drawn on each figure. **F to J-** Bland-Altman plot. Each point represents the difference observed between the results of the two methods against their mean. **A and F-** Comparison of the Fast Virus to the SIII. **B and G-** Comparison of the Luna to the SIII. **C and H-** Comparison of the LC MM to the SIII. **D and I-** Comparison of the GoTaq to the SIII. **E and J-** Comparison of the PrimeScript to the SIII.

Authors contributions

POV and SP designed the study. CH performed the assays. All the authors contributed to the redaction of the manuscript and approved the final version of the manuscript.

Declaration of Competing Interest

None. Despite the affiliation of CH to the BioSpeedia company (a spin-off of the Pasteur Institute, Paris), BioSpeedia did not interfere to the design of the study, the production of the results, nor to the redaction of the manuscript.

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