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

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4 Cyrille Haddar, Paul O. Verhoeven, Thomas Bourlet, Bruno Pozzetto, Sylvie Pillet\*

5 \*Corresponding author:

6 Laboratory of Infectious Agents and Hygiene, University Hospital of Saint-Etienne, 42055, Saint-  
7 Etienne Cedex 02, France. [sylvie.pillet@chu-st-etienne.fr](mailto:sylvie.pillet@chu-st-etienne.fr)

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9 **Affiliations:**

10 CH: BioSpeedia, Institut Pasteur, 75015, Paris, France

11 POV, TB, BP and SP: laboratory of Infectious Agents and Hygiene, University Hospital of Saint-Etienne,  
12 and GIMAP (Groupe Immunité des Muqueuses et Agents Pathogènes) EA-3064, Medicine Faculty of  
13 Saint-Etienne, Campus Santé-Innovations of Saint-Etienne, member of University of Lyon, France

14

15 **Highlights:**

- 16 • In case of virus emergence, laboratory-developed assays (LDA) should be urgently assessed  
17 • Recommended open one-step RT-qPCR reagents are rapidly on tension in case of pandemic  
18 • Five other reagents were shown to be used for SARS-CoV-2 RNA detection by LDA  
19 • This study can help laboratories to assess LDA for detecting emerging RNA viruses

20 **Keywords:**

21 Coronavirus, SARS-CoV-2, RNA virus, emerging virus, laboratory-developed assay, RT-qPCR

22 **Authors contributions:**

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

25

26 **Words counts:**

27 Abstract: 218 words

28 Manuscript: 5361 characters including spaces; 842 words

29 **Abstract**

30 **Background:** Facing the emergence of a new RNA virus, clinical laboratories are often helpless in the face of a  
31 shortage of reagents recommended by Reference Centres.

32 **Objectives:** To compare five open one step RT-qPCR reagents to the SuperScript™ III Platinum™ One-Step  
33 qRT-PCR kit (Invitrogen) considered as the reference one in France at the beginning of the pandemic for  
34 detection of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in respiratory specimens by  
35 using laboratory-developed assay targeting the viral RNA dependant RNA polymerase (RdRp) gene.

36 **Study design:** A total of 51 NUCLISENS easyMAG extracts from respiratory specimens was tested on ABI 7500  
37 thermocycler with TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems), Luna® Universal Probe One-Step  
38 RT-qPCR Kit (New England Biolabs), GoTaq® Probe 1- Step RT-qPCR System (Promega), LightCycler®  
39 Multiplex RNA Virus Master (Roche) and One-step PrimeScript RT-PCR kit (Takara). The CT values obtained  
40 using the 5 challenged reagents were compared to those obtained using the reference assay.

41 **Results:** The percentages of concordance were all above 95%. When comparing the CT values of the 48 extracts  
42 exhibiting CT values < 35 obtained with the reference reagent, the results were similar between the reagents  
43 although the differences of CT values were quite dispersed.

44 **Conclusions:** All five reagents can be considered as alternative reagents to the reference for detecting SARS-  
45 COV-2 RNA.

## 46 **Background**

47 The emergence of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Europe lead the  
48 clinical laboratories to urgently assess a home-brew molecular assay [1]. Between mid-February and mid-March,  
49 a period when no commercial kits were available to cope with the huge amount of molecular tests to be carried  
50 out, all the laboratories equipped for molecular biology testing referred both to the already published techniques  
51 [2] and the National Reference Centres [3,4]. However, they were confronted with a shortage of consumables and  
52 reagents, linked to the use of the same protocol by many laboratories. Mögling et al. recently reported the delayed  
53 response to the pandemic in relation to primers and probe contamination [5]. Another pitfall was the huge delivery  
54 delays of enzymes, especially the SuperScript™ III Platinum™ One-Step qRT-PCR kit (abbreviated as SIII in this  
55 study, Invitrogen, Cergy-Pontoise). Our aim was to compare five open one step RT-qPCR reagents available on  
56 the French market to urgently find an alternative solution for SARS-CoV-2 RNA detection.

## 57 **Study design**

58 A total of 51 respiratory specimens (45 nasopharyngeal e-swabs, 3 tracheal aspirations and 3 sputum specimens)  
59 sampled from patients suspect of SARS-CoV-2 infection and sent at the Laboratory of infectious agents and  
60 hygiene of the University Hospital of Saint-Etienne were processed as described [3,4,6] and using a ABI7500  
61 thermocycler (Thermofisher) after extraction on eMAG (bioMérieux). The assay targeted the viral RNA dependant  
62 RNA polymerase (RdRp) gene. A multiplex assay was set up in our laboratory by adding to the mastermix the  
63 primers (0.2µM) and a Taqman probe (0.1µM) targeting the glyceraldehyde-3-phosphate dehydrogenase  
64 (GAPDH) mRNA [7] to manage the whole analytical process together with the sample cellularity. After testing with  
65 this reference assay, the extract (conserved at 4°C; 5µL for each assay) was tested on the same day using the  
66 same concentrations of primers and probes as those used in the reference assay and one of the five other  
67 mastermixes depicted in the Table. The cycle threshold (CT) values were recorded for each assay and compared  
68 to the reference assay by estimating their agreement with the concordance percentage and the Cohen kappa  
69 coefficient. The positive specimens were drawn on Linear regression and Bland Altman plot.

70

## 71 **Results**

72 The analysis focused for the CT values of the IP4 region of the viral RdR) and those of the GAPDH mRNA. All the  
73 specimens were positive for the GAPDH region, with CT values comprised between 20.1 and 28.7 using the  
74 reference assay; the GAPDH mRNAs were also detected in all tested clinical specimens with all the tested  
75 reagents (data not shown). The analysis of CT values for the IP4 region showed a global good agreement  
76 between the mixes in comparison to the reference assay (Table). Only 3 samples were not detected by at least

77 one of five reagents, but all exhibited extremely low viral loads with the reference assay (CT values between 38.9  
78 and 40.7). After excluding these discordant samples, the correlation of the CT values was analysed on the  
79 remaining 48 specimens (Figure). All CT values were <35 using the reference assay. The mean difference of CT  
80 values was higher (> 0.5) for PrimeScript and GoTaq and lower (<-0.5) for LC MM than SIII. Fast Virus and Luna  
81 exhibited similar CT values than that of SIII (mean difference of CT value comprised between -0.5 and 0.5). The  
82 dispersion of CT values was higher with PrimeScript.

83

## 84 **Discussion**

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

102

## 103 **Declaration of Competing Interest:**

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



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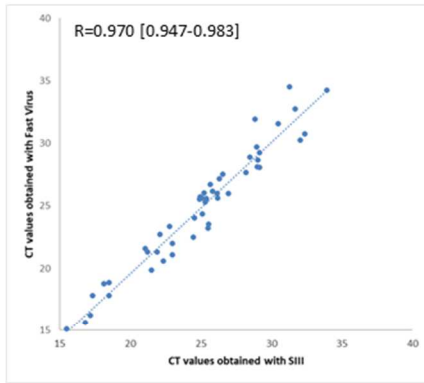
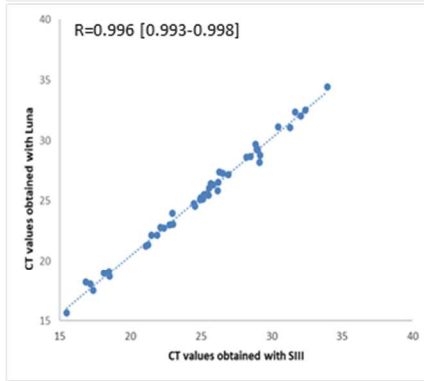
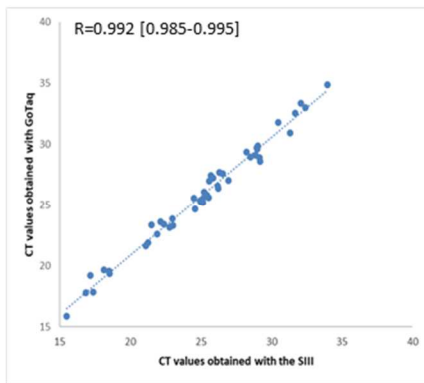
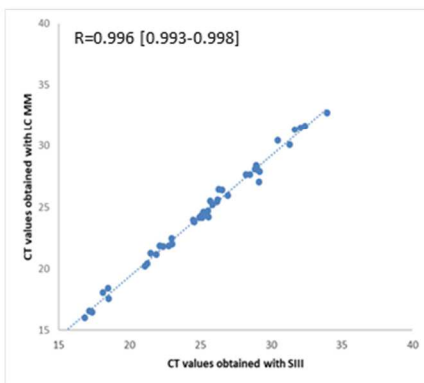
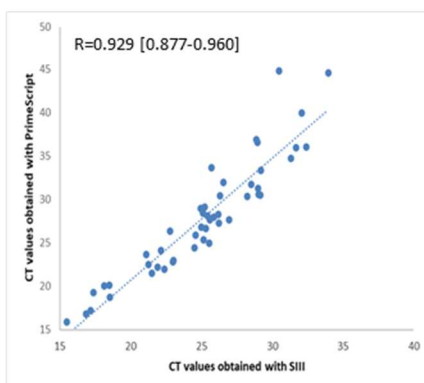
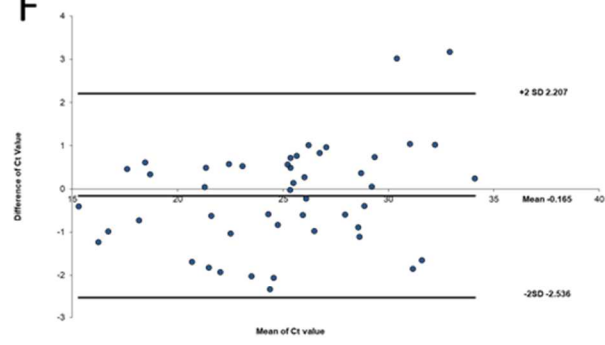
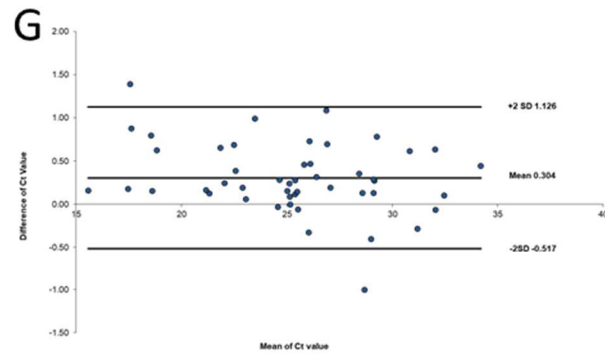
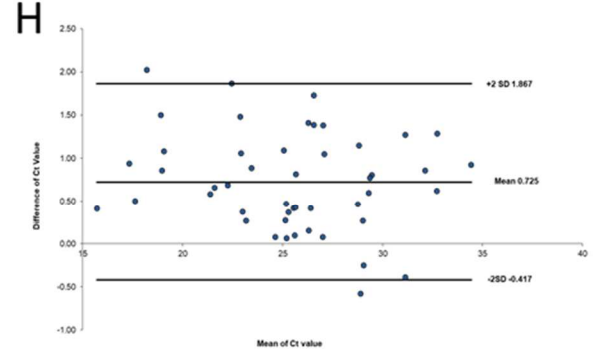
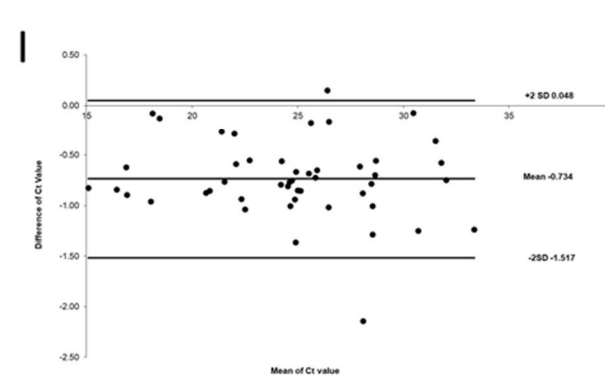
**Table: Overall results comparing the 5 open one step RT-qPCR reagents to the reference assay.**

Complete denomination (commercialization)	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) <sup>a</sup>	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) <sup>b</sup>	Luna	49	3	2	96.30	0.731 (0.474-0.988)
GoTaq® Probe 1- Step RT-qPCR System (Promega France, Charbonnières-les-Bains) <sup>b</sup>	GoTaq	51	3	0	100	1 (0.733-1.267)
LightCycler® Multiplex RNA Virus Master (Roche, Meylan) <sup>b</sup>	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) <sup>a</sup>	PrimeScript	50	3	1	98.15	0.848 (0.584-1.111)

<sup>a</sup>The reverse transcription (RT) step was done during 5 min at 48°C and after inactivation of the enzyme during 20 sec at 95°C, the cycling program was of 5 sec at 95°C and 30sec at 58°C during 50 cycles; <sup>b</sup>: same RT and cycling program than the reference assay.

CI: Confidence interval

**Figure: 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. **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 coefficient with its 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.

**A****B****C****D****E****F****G****H****I****J**