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1 Multicenter comparison of the Cobas 6800 system with the RealStar RT-PCR kit for the
2 detection of SARS-CoV-2

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25 **Running title:** RT-PCR kit for the detection of SARS-CoV-2

26 **Keywords:** COVID-19; SARS-CoV-2; RT-PCR

27

28 **ABSTRACT**

29

30 **Background**

31 RT-PCR testing is crucial in the diagnostic of SARS-CoV-2 infection. The use of reliable and
32 comparable PCR assays is a cornerstone to allow use of different PCR assays depending on
33 the local equipment. In this work, we provide a comparison of the Cobas® (Roche) and the
34 RealStar® assay (Altona).

35 **Methods**

36 Assessment of the two assays was performed prospectively in three reference Parisians
37 hospitals, using 170 clinical samples. They were tested with the Cobas® assay, selected to
38 obtain a distribution of cycle threshold (Ct) as large as possible, and tested with the RealStar
39 assay with three largely available extraction platforms: QIAasymphony (Qiagen), MagNAPure
40 (Roche) and NucliSENS-easyMag (BioMérieux).

41 **Results**

42 Overall, the agreement (positive for at least one gene) was 76%. This rate differed
43 considerably depending on the Cobas Ct values for gene E: below 35 (n = 91), the
44 concordance was 99%. Regarding the positive Ct values, linear regression analysis showed a
45 determination correlation (R^2) of 0.88 and the Deming regression line revealed a strong
46 correlation with a slope of 1.023 and an intercept of -3.9. Bland-Altman analysis showed that
47 the mean difference (Cobas® minus RealStar®) was + 3.3 Ct, with a SD of + 2.3 Ct.

48 **Conclusions**

49 In this comparison, both RealStar® and Cobas® assays provided comparable qualitative
50 results and a high correlation when both tests were positive. Discrepancies exist after 35 Ct
51 and varied depending on the extraction system used for the RealStar® assay, probably due to
52 a low viral load close to the detection limit of both assays.

53 INTRODUCTION

54 The SARS-CoV-2 is the new coronavirus, a member of the subgenus Sarbecovirus (beta-
55 Coronavirus lineage B), responsible for the ongoing pandemic of infectious respiratory
56 disease called COVID-19 [1,2]. This epidemic, declared a Public Health Emergency of
57 International Concern on 30 January 2020 by the World Health Organization (WHO), has
58 spread rapidly around the world and has caused many infections and deaths worldwide [3]. To
59 control the outbreaks, many countries have implemented confinement instructions and have
60 closed places of groupings with heavy economic consequences. As recommended by WHO,
61 diagnostic tests by reverse transcription-polymerase chain reaction (RT-PCR) via respiratory
62 samples, should be performed widely to detect infected patients, to follow the pandemic
63 evolution and to help stopping the spread of the clusters [4]. RT-PCR testing is also a
64 cornerstone to allow a gradual deconfinement in good sanitary conditions and early detect any
65 viral resurgence. To meet the high demand for these tests and to face the supply difficulties
66 worldwide, the laboratories had to adapt using the different systems available depending on
67 the PCR and nucleic acid extraction equipment already present in their establishment [5–10].
68 Manufacturer-independent evaluation data are still scarce. These tests can be a single-use
69 cartridge, reagent kits for batch testing used with different instruments for the extraction and
70 amplification stages, or fully automated molecular testing platforms. These are real-time RT-
71 PCR which target two or three different regions of the SARS-CoV-2 genome and provide a
72 cycle threshold (Ct) value inversely proportional to the amount of virus. Pre-analytical
73 processing of respiratory samples can be also used to neutralize the virus before testing, and
74 the sample input volume used varies depending on the test performed. All these differences in
75 the pre-analytical and analytical process can have an impact on the sensitivity of the test and
76 the concordance of their results has to be evaluated.

77

78 In this study, we compared two different widely used tests in three major Parisian university
79 hospital laboratories. These are the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 (Altona
80 diagnostics, France) which can be associated to different extraction and amplification devices,
81 and the Cobas® SARS-CoV-2 kit used on the Cobas® 6800 system (Cobas 6800; Roche
82 Diagnostics, Mannheim, Germany).

83

84 **METHODS**

85 **Samples**

86 In April 2020, 140 patients were included in this prospective study performed in 3 virological
87 laboratories located in Paris (Saint Louis hospital (n=45), Bichat hospital (n=49) and La Pitié-
88 Salpêtrière hospital (n=46)). Then, each laboratory selected 45 to 49 samples firstly detected
89 using the Cobas 6800 with a stratification according to the Ct of the E gene Cobas results,
90 allowing to cover the whole linear range of the assays. Thus, three categories were retained:
91 $Ct < 25$, Ct between 25 and 34 and with a $Ct \geq 35$. Rapidly, in the same day or within 48
92 hours, the leftover samples stored at $+4^{\circ}\text{C}$ were tested with the RealStar assay. Thirty
93 nasopharyngeal swab samples collected in 2019 (in the pre-epidemic Covid 19 period) were
94 also tested with both techniques (10 in each laboratory).

95

96 **Cobas 6800 SARS-CoV-2 test**

97 The Cobas[®] SARS-CoV-2 test is a single-well dual target assay, which targets the non-
98 structural ORF1a/b region specific of SARS-CoV-2 and the structural protein envelope E
99 gene for pan-sarbecovirus detection. The test used RNA internal control for sample extraction
100 and PCR amplification process control. To take into account the available sample volume and
101 the security conditions required for this virus before loading on the Cobas 6800 system, the
102 pre-analytical protocol has been adapted as recommended by the manufacturer as follows:
103 400 μl of each sample were transferred at room temperature into barcoded secondary tubes
104 containing 400 μl of Cobas lysis buffer for the SARS-CoV-2 neutralization. Then, the tube
105 was loaded on the Cobas 6800 where 400 μl from those 800 μl were used for RNA extraction,
106 and eluted in 50 μl of which 27 μl were used for the RT-PCR. The test was performed in
107 batches, including one negative and positive control each. According to the manufacturer's
108 instructions, a tested sample was considered SARS-CoV-2 positive if Cobas 6800 showed

109 positive results either for both ORF1a/b and E genes or for the ORF1a/b gene only. In the
110 case of single E gene positivity, the result should be reported as SARS-CoV-2 presumptive
111 positive and repeated, but were considered as positive for this study.

112

113 **RealStar SARS-CoV-2 RT-PCR**

114 The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 assay targets the E gene specific for
115 sarbecoviruses, and the S gene specific for SARS-CoV-2. It includes a heterologous
116 amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm
117 the integrity of the kit reagents. This kit contains only reagents for the SARS-CoV-2 real-time
118 RT-PCR step, extraction and amplification can be performed with various equipment listed in
119 the kit insert. In this study, RNA extraction was performed with MagNA Pure LC 2.0 System
120 (Roche) (Bichat hospital), QIASymphony (Qiagen) (Saint Louis hospital) and NucliSENS®
121 EasyMag® (bioMérieux) (Saint Louis hospital and Pitié Salpêtrière hospital) according to
122 manufacturer's protocol. In each cases, 200 µl of nasopharyngeal samples were diluted in 2 ml
123 of lysis buffer and eluted in 50 µl. Ten µl of extracted RNA was used to perform the real-time
124 RT-PCR with the LightCycler® 480 (Roche) in Pitié Salpêtrière hospital or the ABI Prism®
125 7500 SDS (Applied Biosystems) in the two other laboratories. All these instruments are listed
126 into the RealStar assay instructions. The sample was considered as positive if at least one of
127 both targets was detected.

128

129 **Statistical analysis**

130 Statistical analyses were performed on GraphPad Prism version 6.0. The negative results
131 obtained with the RealStar test were excluded from the analyses. All tests were two-sided,
132 with p values of <0.05 denoting statistical significance. The Ct values obtained with both
133 assays were compared in Wilcoxon tests, and we presented correlation curves with the

134 coefficient of determination, R^2 . Bland-Altman analysis was used to represent the degree of
135 agreement between the Cobas 6800 System and the RealStar[®] SARS-CoV-2 RT-PCR based
136 on the mean difference and standard deviation (SD) of the positive results. The comparison
137 between the EasyMag and QIA Symphony extraction was done with a paired-samples Student
138 test.

139 **RESULTS**

140 **Comparison of the Cobas® 6800 System with the RealStar® kit**

141 A total of 170 patient samples were included in this study: 30 collected in 2019, before the
142 French epidemic period, and 140 with a positive detection for SARS-CoV-2 with the Cobas
143 6800. All the 30 samples collected in 2019 before the epidemic period were negative with
144 both Cobas 6800 and RealStar assays. The qualitative results of the 140 selected samples are
145 summarized in Table 1. Overall, the agreement (positive with the two tests regardless of the
146 gene detected) was 76%. Of note, 3 samples positive in gene E with the COBAS 6800 were
147 negative in gene E but positive in gene S with the RealStar assay. However, this rate differed
148 considerably depending on the Cobas 6800 E Ct: below 35 (n = 91), the concordance was
149 99%. Only one sample with a Cobas 6800 E Ct at 34.3 was negative in RealStar assay with an
150 EasyMag extraction. For samples with a Cobas 6800 E Ct \geq 35 (n = 49) only 14/49 was
151 positive in both techniques with a concordance of 29%.

152 For the gene E Ct < 35 obtained with Cobas® 6800, the median of the value obtained with
153 RealStar® assay was 23.5, 23.4 and 18.6 with EasyMag, QIASymphony and MagNA Pure,
154 respectively. Moreover, for samples with a Ct \geq 35 with Cobas 6800 (n=49), the detection rate
155 observed with the RealStar assay differed depending on the extraction system, 1/13 with
156 EasyMag, 1/15 with the QIASymphony and 13/21 with MagNA Pure.

157 Regarding the positive Ct values of gene E (n=104), linear regression analysis revealed a R²
158 of 0.88 and the Deming regression line revealed a strong correlation with a slope of 1.023 and
159 an intercept of -3.9 (Fig. 1a). The Bland Altman plot showed higher Ct values for the Cobas
160 6800 with a homogeneous distribution up to Ct 35 with a mean difference (Cobas 6800 minus
161 RealStar) of + 3.3 Ct and a SD of + 2.3 Ct (Fig. 1b).

162

163 **Comparison of the RealStar results after extraction with EasyMag and QIASymphony**
164 **systems**

165 In Saint Louis hospital, 45 samples previously detected with the Cobas 6800 (15 with a Ct <
166 25, 15 with a Ct between 25 and 34 and 15 with a Ct \geq 35) were extracted both with the
167 EasyMag kit and the QIASymphony kit before RealStar testing. All the samples with a Ct <
168 35 (n=30) were positive regardless of the extraction system. Among the 15 samples with a
169 Cobas 6800 Ct \geq 35, all were negative after EasyMag extraction while 2 samples were
170 positive after QIASymphony extraction (Ct: 34.9 and Ct: 38.8). We found a R² of 0.99 and the
171 Deming regression revealed a strong correlation with a slope of 0.99 and an intercept of -0.81
172 (Fig. 2a). Bland-Altman analysis showed that the mean difference (QIASymphony minus
173 EasyMag) was + 1.1 Ct, with a SD of - 0.70 Ct (Fig. 2b). Two differences exceeded 5 Ct
174 corresponding to the 2 samples positive using QIASymphony and negative with EasyMag.
175 Although there was no significant difference in Ct values for the gene E (p=0.21), we have a
176 significant difference in Ct values for the gene S (p<0.0001, mean Ct gene S=1.19, 95% CI:
177 1.95 to 1.63) in favor of EasyMag.

178

179 **Comparison of the detection of gene E and ORF for Cobas 6800**

180 Amplification of gene E (139/140, 99.3%) was more frequently positive compared to
181 amplification of gene ORF (115/140, 82.1%). A R² of 0.84 and the Deming regression line
182 revealed a good correlation with a slope of 1.22 and an intercept of -6.3.

183

184 **Comparison of the detection of gene E and S for RealStar® RT-PCR**

185 Amplification of gene E (104/140, 74.3%) and amplification of gene S (115/140, 82.1%)
186 seem to be equivalent. A R² of 0.93 and the Deming regression line revealed a strong
187 correlation with a slope of 1.01 and an intercept of -0.4.

188 **DISCUSSION**

189 In this study, two very different assays were compared: the RealStar assay used with various
190 extraction equipment, allowing a use in a wide range of PCR laboratories, and the Cobas 6800
191 kit used with the fully automated Cobas 6800 platform, allowing more intensive workflows.
192 As all other SARS-CoV-2 PCR diagnostic tests, both assays are qualitative but yield a Ct
193 value inversely proportional to the amount of virus. In our work, below a Cobas 6800 Ct
194 value of 35, the qualitative results are highly concordant and the Ct values have a high
195 correlation even though the values of RealStar are lower than those of the Cobas 6800. Above
196 a Cobas 6800 value Ct of 35, the RealStar failed to detect about one third of the SARS-CoV-2
197 genes while COBAS 6800 detected at least one of both targets. However, this observation is
198 impacted by the extraction method in use, as demonstrated by the slightly lower Ct values and
199 higher positivity rate observed with the MagNA Pure system among samples showing E gene
200 Ct ≥ 35 with the COBAS 6800. This suggests a better extraction process with the MagNA
201 Pure system. The comparison, from same samples, between EasyMag and QIASymphony
202 systems showed a slight improvement for the SARS-CoV-2 detection with QIASymphony.
203 Among samples with Ct values above 35, the E target is mostly the only gene detected with
204 the Cobas 6800 assay. This is in accordance with the Cobas 6800 insert information reporting
205 a higher sensitivity for the E gene detection than for the ORF1/a, and also a drop in the
206 positivity rate above 35 Ct for the E target. This may explain why the RealStar test yielded
207 many negative results in such cases as both tests probably reached their detection limits. This
208 is a limitation of our study as we did not assessed comparatively the limit of detection of the
209 two methods but the reliability of their Ct values among COBAS 6800 positive samples,
210 excluding those that could be negative with COBAS 6800 and positive with RealStar in this
211 range of low viral loads. Our work highlights the impact of the extraction system on the
212 sensitivity of the RealStar assay.

213

214 Overall, we demonstrated the good performances and concordance between the two assays, at
215 least for viral loads above the detection limit of both assays. This concordance allows to
216 reliably compare Ct values obtained from both methods. However, the variations observed
217 between the Ct values of the two assays, evaluated here as about 3.5 additional Ct with the
218 Cobas 6800 assay, has to be taken into account for Ct values follow-up done for the most
219 severe patients in case of successive use of the two methods, depending of reagent and
220 analyser availability.

221

222

223 **Legends:**

224 **Figure 1.** Correlation Curve (a) and Bland-Altman plot (b) for the Ct values of gene E with
225 Cobas 6800 versus RealStar, when both assays are positive (n =104). For the correlation curve
226 the samples extracted by MagnaPure are represented by squares, those with QIASymphony by
227 triangles and those by EasyMag by dots.

228

229 **Figure 2.** Correlation Curve (a) and Bland Altman plot (b) for the Ct values of gene E
230 RealStar for QIASymphony versus EasyMag extraction, when both assays are positive (n
231 =30).

232

233

234 **Conflict of interest**

235 The authors declare no conflict of interest.

236

237 **Acknowledgments**

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239 hospital virology department and La Pitié-Salpêtrière hospital virology department.

240

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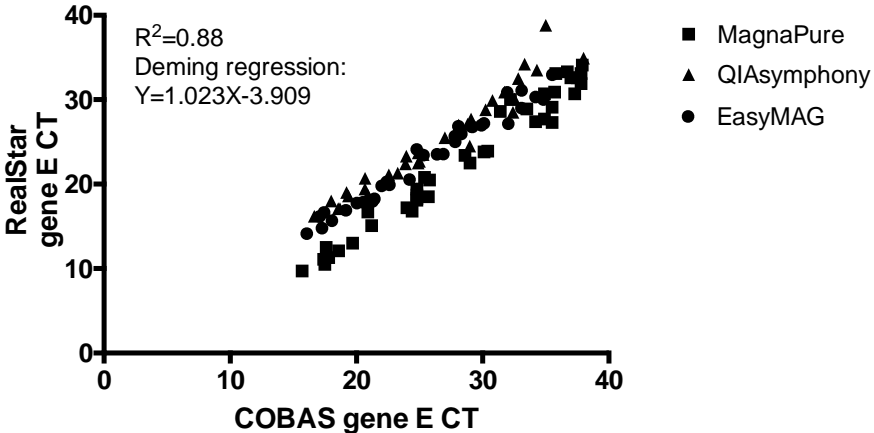
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286

Figure 1.

a.



b.

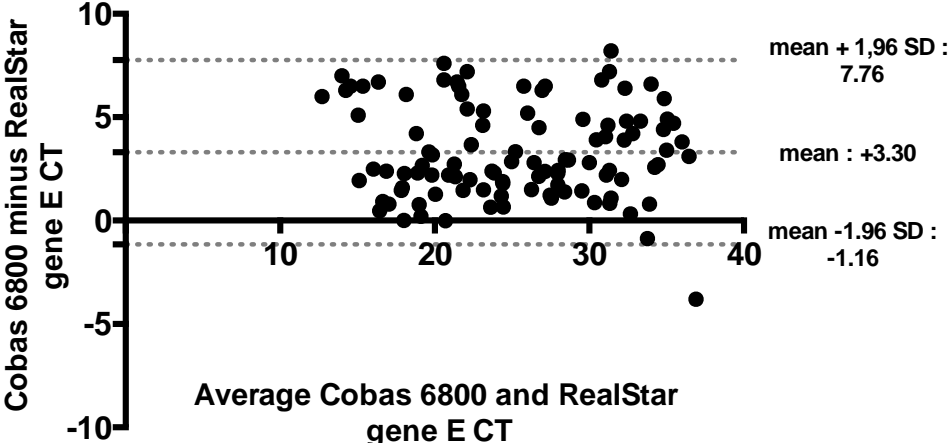
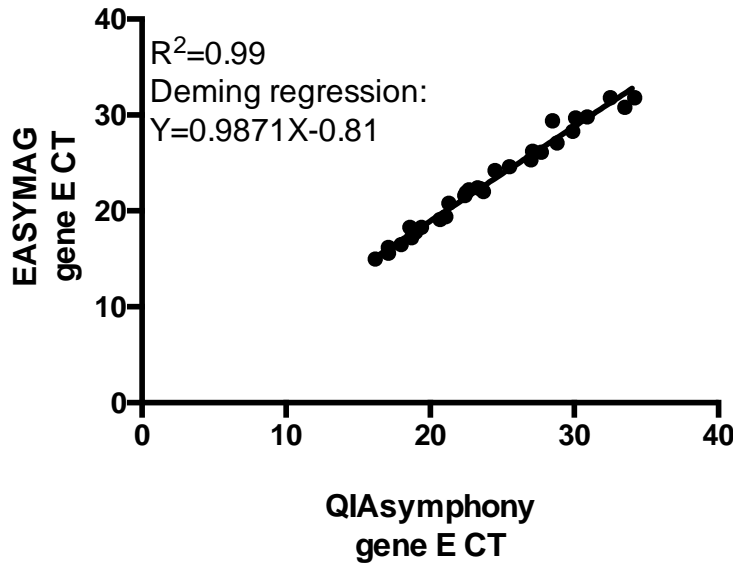
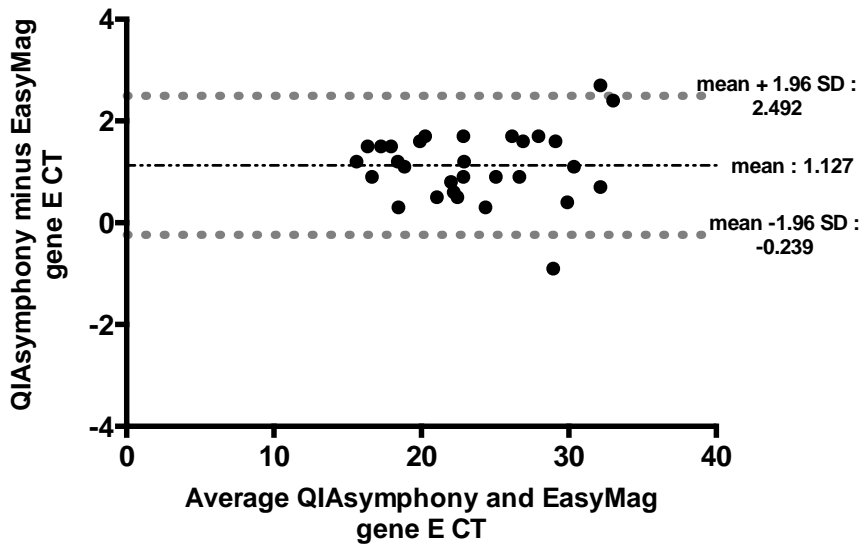


Figure 2.

a.



b.



	gene	Cobas 6800		total
		E+ ORF-1+	E+ ORF-1-	
RealStar®	E+ S+	95 (67.9%)	5 (3.6%)	100 (71.4%)
	E+ S-	2 (1.4%)	2 (1.4%)	4 (2.9%)
	E- S+	2 (1.4%)	1 (1.4%)	3 (2.1%)
	E- S-	16 (11.4%)	17 (12.1%)	33 (23.6%)
total		114 (81.4%)	25 (18.5%)	140 (100%)

Table 1: Agreement between the Cobas 6800 SARS Cov-2 and the RealStar® SARS Cov-2 results according to each gene. E: envelope, S: spike, ORF: open reading frame