



HAL
open science

Evaluation of the RealStar® SARS-CoV-2 RT-PCR kit RUO performances and limit of detection

Benoit Visseaux, Quentin Le Hingrat, Gilles Collin, Valentine Ferré,
Alexandre Storto, Houria Ichou, Donia Bouzid, Nora Poey, Etienne de
Montmollin, Diane Descamps, et al.

► To cite this version:

Benoit Visseaux, Quentin Le Hingrat, Gilles Collin, Valentine Ferré, Alexandre Storto, et al.. Evaluation of the RealStar® SARS-CoV-2 RT-PCR kit RUO performances and limit of detection. *Journal of Clinical Virology*, 2020, 129, pp.104520 -. 10.1016/j.jcv.2020.104520 . hal-03492448

HAL Id: hal-03492448

<https://hal.science/hal-03492448>

Submitted on 18 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Evaluation of the RealStar® SARS-CoV-2 RT-PCR Kit RUO performances and limit of detection

Benoit Visseaux ^{1,2,#}, Quentin Le Hingrat ^{1,2}, Gilles Collin ^{1,2}, Valentine Ferré ^{1,2}, Alexandre Storto ^{1,2}, Houria Ichou ¹, Donia Bouzid ^{2,3}, Nora Poey ⁴, Etienne de Montmollin ⁵, Diane Descamps ^{1,2}, Nadhira Houhou-Fidouh ¹

¹ *Université de Paris, Assistance Publique – Hôpitaux de Paris, Service de virologie, Hôpital Bichat, Paris, France*

² *UMR 1137-IAME, DeSCID: Decision Sciences in Infectious Diseases control and care, INSERM, Université de Paris, Paris, France*

³ *Université de Paris, Assistance Publique – Hôpitaux de Paris, Service d’Accueil des Urgences, Hôpital Bichat, Paris, France*

⁴ *Université de Paris, Assistance Publique – Hôpitaux de Paris, Service des Maladies Infectieuses et Tropicales, Hôpital Bichat, Paris, France*

⁵ *Université de Paris, Assistance Publique – Hôpitaux de Paris, Réanimation Médicale et Infectieuses, Hôpital Bichat, Paris, France*

Abstract word count: 204 words

Main manuscript word count: 1621 words

1 **Introduction**

2 The novel coronavirus identified in China in December 2019 as the etiological agent of the
3 COVID-19 disease has been declared as pandemic by the WHO the 11th of March 2020 [1].
4 The etiological agent of this new infection is the Severe Acute Respiratory Syndrome
5 coronavirus 2 (SARS-CoV-2) [2]. Availability and reliability of robust assays providing sensitive
6 identification of infected individuals are the first steps allowing patient isolation, diagnosis
7 and proper therapeutic measures. Several in-house and commercial RT-PCR assays have
8 been quickly developed [3] but still require to be independently assessed and evaluated. The
9 RealStare[®] SARS-CoV-2 RT-PCR kit (Altona) is a Research Usage Only (RUO) product providing
10 the detection of SARS-CoV-2 and compatible with various real-time PCR platforms. It allows
11 detection and differentiation of lineage B-betacoronavirus (B-βCoV), by targeting the E gene
12 from B-βCoV, and SARS-CoV-2 specific RNA, by targeting the S gene. Evaluating the
13 sensitivity of RT-PCR assays is an essential point as very low viral loads can be identified in
14 patients nasopharyngeal swabs, especially in later disease stages [4,5]. To date, the
15 RealStar[®] SARS-CoV-2 assay has been compared to several other commercial assay [6,7],
16 however thorough limit of detection assessment and performances comparison with the
17 currently WHO recommended assay have not been provided.
18 Here, we report an independent validation of this new commercial PCR assay for SARS-CoV-2
19 detection, determining its limit of detection and comparing its performances on clinical
20 samples with the currently WHO recommended RT-PCR assay [3].

21

22 **Methods**

23 **Analytical sensitivity analysis of the RealStar[®] SARS-CoV-2 assay**

24 A preliminary sensitivity analysis was conducted using serial dilution of a SARS-CoV-2
25 positive nasopharyngeal sample. This sample was serially diluted at 1:10 up to 1/100 000
26 before being serially diluted at 1:5 up to 1/12 500 000. All those dilutions were tested both
27 with RealStar® SARS-CoV-2 and the currently WHO recommended RT-PCR assay (WHO-RT-
28 PCR). The same sample was quantified using a standardised RNA transcript control obtained
29 from the European Virus Archive Program. The limit of detection (LoD) was then determined
30 for the two assays by testing multiple replicates of serial two fold dilutions of the quantified
31 sample around the expected LoD. As recommended by the European Network of GMO
32 Laboratories for LoD definition [8], the LoD was defined as the concentration until obtaining
33 10 out of 10 positive replicates (10/10). All dilutions were done using Virocult transport
34 medium and kept at +4°C before testing within 24h.

35 Briefly, for both assays the viral RNA was extracted from 200 µL of clinical samples with the
36 MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume (Roche Diagnostics) and
37 eluted in 50 µL. The WHO RT-PCR, targeting E and Orf1 genes, was performed as described
38 by Corman *et al* [3] and the RealStar® SARS-CoV-2 was performed according to the
39 manufacturer recommendations. Both PCR assays were performed on an ABI 7500
40 platform (Applied Biosystems®). For both assay, a signal with a cycle threshold (Ct) value
41 above 40 was considered as negative.

42

43 **Evaluation of specificity**

44 To assess the specificity of the RealStar® SARS-CoV-2 assay towards other coronaviruses we
45 used using 2 pools of 3 positive samples for each of them (HKU1, NL63, OC43, 229E)
46 obtained between October and December 2019.

47

48 **Performance comparison using clinical samples**

49 We included 83 nasopharyngeal swabs specimens from patients hospitalized in Bichat
50 Claude Bernard teaching hospital, Paris, France. These specimens were taken from patients
51 suspected of COVID-19, collected in Virocult® viral transport media (Sigma). Samples for the
52 specificity evaluation were obtained for mPCR point of care evaluation approved by the
53 Bichat Claude Bernard ethic committee (N2019-050). All other hospitalized patients,
54 included in this study, were included in the national French-COVID19 cohort and written
55 consent was obtained for clinical and biological sub-studies.

56

57 **Results**

58 **Analytical sensitivity analysis of the RealStar® SARS-CoV-2 assay**

59 All results obtained from initial serial dilutions are depicted in Table 1. The virus was
60 detected for all genes of both methods for all dilutions up to 1:100 000. At 1:500 000
61 dilution, all targets were positive for RealStar® SARS-CoV-2 but only the E gene for the WHO
62 RT-PCR. At 1:2 500 00 dilution, only the two targets of the RealStar® SARS-CoV-2 assay were
63 positive. As we previously observed, the RdRp gene of the WHO RT-PCR assay presented a
64 lower sensitivity than the E gene (Visseaux et al. JCM, in revision).

65 For the LoD assessment, all results obtained with the serial 1:2 dilutions around the
66 expected LoD are depicted in table 2. The detection of 10/10 replicates by the WHO RT-PCR
67 assay was achieved for the dilution containing 1250 copies/mL (*i.e.* for an input of 25
68 copies/PCR in our conditions) whereas it was achieved for the dilution containing 625
69 copies/mL with both E and S genes of the RealStar® SARS-CoV-2 assay (*i.e.* for an input of 13
70 copies/PCR).

71

72 **Evaluation of specificity**

73 No cross-reaction was detected neither for human coronaviruses (229E, OC43, NL63 and
74 HKU1, n=2 pools of three samples for each targets) nor other respiratory viruses (influenza
75 A:H1N1, A:H3N2 and B, respiratory syncytial virus, rhinovirus, parainfluenza virus 1 and 4,
76 and human metapneumovirus; n=1 pool for each).

77

78 **Performance comparison using clinical samples**

79 All results from this comparison are given in Figure 1 and supplementary Table 1. Among the
80 83 clinical samples tested in this study, 45/83 and 36/83 were identified positive and
81 negative with both methods, respectively. Among the two remaining samples: (i) one was
82 identified as positive with the WHO assay (E gene at 37.9 Ct and undetectable RdRp gene)
83 but negative with the RealStar[®] SARS-CoV-2 assay; and (ii) the second one was identified as
84 negative with the WHO assay (E gene above 40 Ct at 40.9 and undetectable RdRp gene) but
85 positive with the RealStar[®] SARS-CoV-2 assay (E gene at 34.4 and S gene at 36.9). Thus, when
86 taking the WHO assay as a gold standard, the RealStar[®] assay demonstrated a sensitivity at
87 97.8% (45/46) and a specificity at 97.3% (36/37).

88

89

90 **Discussion**

91 In this work we assessed the performances and limit-of-detection on clinical samples of the
92 RealStar[®] SARS-CoV-2 RT-PCR Kit RUO in comparison to the currently recommended WHO
93 RT-PCR assay [3]. The LoD obtained for the E gene with the WHO RT-PCR assay, at 50
94 copies/PCR, was slightly higher than previously estimated in the initial description at 5
95 copies/PCR [3]. This may be explained in part by the use of SARS-CoV-2 clinical samples in

96 the current work instead of transcript RNA and the use of different LoD estimation methods.
97 The LoD of the RealStar® SARS-CoV-2 assay was in the same range and even slightly higher
98 than the WHO assay in our work at 25 copies/PCR. The poor sensitivity of the WHO assay for
99 the RdRp gene has been previously evidenced in our previous works [9] and a recent pre-
100 publication comparing the performances of the main reference assays [10]. The sensitivity
101 and specificity of both the RealStar® SARS-CoV-2 RT-PCR RUO assay on clinical samples
102 appears similar with 100% of concordance on 83 clinical nasopharyngeal samples. Thus, the
103 RealStar® SARS-CoV-2 RT-PCR RUO assay provides a promising commercial alternative for
104 SARS-CoV-2 detection with a slightly better sensitivity than the WHO currently
105 recommended assay. It can be used in most laboratories with various extraction and real-
106 time PCR platforms. To date, RT-PCR assays remain the methods of choice for COVID-19
107 diagnosis even if it can lack of sensitivity in swab samples from some patients, mostly
108 presenting late stage diseases and for which chest CT or other respiratory samples can be of
109 valuable help [11–13]. This point also highlights the necessity of having highly sensitive PCR
110 assays and the critical importance of checking and comparing their performances. If the
111 RealStar® SARS-CoV-2 assay will not fulfil all our needs, especially for high-throughput and
112 highly automatized assays, it provides a robust, versatile and sensitive options for all
113 molecular biology laboratories.

114

115 **Acknowledgements**

116 This study has been funded in part by the Assistance Publique – Hôpitaux de Paris, the
117 Université de Paris and the ANRS (Agence Nationale de Recherche sur le SIDA et les
118 hépatites virale) AC43.

119
120
121
122
123

124 **Conflict of interests**

125 BV received travel accommodations and personal fees from Qiagen and Biomérieux.
126 The other authors have no conflict of interest to declare.

127
128
129
130
131
132

133 **Bibliography**

- 134 [1] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F.
135 Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G.F. Gao, W. Tan, China Novel Coronavirus Investigating
136 and Research Team, A Novel Coronavirus from Patients with Pneumonia in China, 2019, *N. Engl.*
137 *J. Med.* 382 (2020) 727–733. <https://doi.org/10.1056/NEJMoa2001017>.
- 138 [2] A.E. Gorbalenya, S.C. Baker, R.S. Baric, R.J. de Groot, C. Drosten, A.A. Gulyaeva, B.L. Haagmans,
139 C. Lauber, A.M. Leontovich, B.W. Neuman, D. Penzar, S. Perlman, L.L.M. Poon, D.V. Samborskiy,
140 I.A. Sidorov, I. Sola, J. Ziebuhr, Coronaviridae Study Group of the International Committee on
141 Taxonomy of Viruses, The species Severe acute respiratory syndrome-related coronavirus:
142 classifying 2019-nCoV and naming it SARS-CoV-2, *Nature Microbiology.* 5 (2020) 536–544.
143 <https://doi.org/10.1038/s41564-020-0695-z>.
- 144 [3] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K. Chu, T. Bleicker, S. Brünink, J.
145 Schneider, M.L. Schmidt, D.G. Mulders, B.L. Haagmans, B. van der Veer, S. van den Brink, L.
146 Wijsman, G. Goderski, J.-L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken,
147 M.P. Koopmans, C. Drosten, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-
148 PCR, *Eurosurveillance.* 25 (2020) 2000045. [https://doi.org/10.2807/1560-](https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045)
149 [7917.ES.2020.25.3.2000045](https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045).
- 150 [4] J. Wu, J. Liu, S. Li, Z. Peng, Z. Xiao, X. Wang, R. Yan, J. Luo, Detection and analysis of nucleic acid
151 in various biological samples of COVID-19 patients, *Travel Medicine and Infectious Disease.*
152 (2020) 101673. <https://doi.org/10.1016/j.tmaid.2020.101673>.
- 153 [5] L. Zou, F. Ruan, M. Huang, L. Liang, H. Huang, Z. Hong, J. Yu, M. Kang, Y. Song, J. Xia, Q. Guo, T.
154 Song, J. He, H.-L. Yen, M. Peiris, J. Wu, SARS-CoV-2 Viral Load in Upper Respiratory Specimens
155 of Infected Patients, (2020). <https://doi.org/10.1056/NEJMc2001737>.
- 156 [6] P.B. van Kasteren, B. van der Veer, S. van den Brink, L. Wijsman, J. de Jonge, A. van den Brandt,
157 R. Molenkamp, C.B.E.M. Reusken, A. Meijer, Comparison of seven commercial RT-PCR

- 158 diagnostic kits for COVID-19, *J. Clin. Virol.* 128 (2020) 104412.
159 <https://doi.org/10.1016/j.jcv.2020.104412>.
- 160 [7] N. Merindol, G. Pépin, C. Marchand, M. Rheault, C. Peterson, A. Poirier, C. Houle, H. Germain,
161 A. Danylo, SARS-CoV-2 detection by direct rRT-PCR without RNA extraction, *J. Clin. Virol.* 128
162 (2020) 104423. <https://doi.org/10.1016/j.jcv.2020.104423>.
- 163 [8] L. HOUGS, F. GATTO, O. GOERLICH, L. GROHMANN, K. LIESKE, M. MAZZARA, F. NARENDJA, J.
164 OVESNA, N. PAPAZOVA, I. SCHOLTENS, J. ZEL, Verification of analytical methods for GMO
165 testing when implementing interlaboratory validated methods: Version 2, EU Science Hub -
166 European Commission. (2018). [https://ec.europa.eu/jrc/en/publication/verification-analytical-](https://ec.europa.eu/jrc/en/publication/verification-analytical-methods-gmo-testing-when-implementing-interlaboratory-validated-methods)
167 [methods-gmo-testing-when-implementing-interlaboratory-validated-methods](https://ec.europa.eu/jrc/en/publication/verification-analytical-methods-gmo-testing-when-implementing-interlaboratory-validated-methods) (accessed
168 February 28, 2020).
- 169 [9] B. Visseaux, Evaluation of the QIAstat-Dx Respiratory SARS-CoV-2 Panel, the first rapid
170 multiplex PCR commercial assay for SARS-CoV-2 detection, *Journal of Clinical Microbiology.*
171 (n.d.).
- 172 [10] C.B.F. Vogels, A.F. Brito, A.L. Wyllie, J.R. Fauver, I.M. Ott, C.C. Kalinich, M.E. Petrone, A.
173 Casanovas-Massana, M.C. Muenker, A.J. Moore, J. Klein, P. Lu, A. Lu-Culligan, X. Jiang, D.J. Kim,
174 E. Kudo, T. Mao, M. Moriyama, J.E. Oh, A. Park, J. Silva, E. Song, T. Takehashi, M. Taura, M.
175 Tokuyama, A. Venkataraman, O.-E. Weizman, P. Wong, Y. Yang, N.R. Cheemarla, E. White, S.
176 Lapidus, R. Earnest, B. Geng, P. Vijayakumar, C. Odio, J. Fournier, S. Bermejo, S. Farhadian, C.
177 Dela Cruz, A. Iwasaki, A.I. Ko, M.-L. Landry, E.F. Foxman, N.D. Grubaugh, Analytical sensitivity
178 and efficiency comparisons of SARS-COV-2 qRT-PCR assays, *Infectious Diseases (except*
179 *HIV/AIDS)*, 2020. <https://doi.org/10.1101/2020.03.30.20048108>.
- 180 [11] C. Long, H. Xu, Q. Shen, X. Zhang, B. Fan, C. Wang, B. Zeng, Z. Li, X. Li, H. Li, Diagnosis of the
181 Coronavirus disease (COVID-19): rRT-PCR or CT?, *Eur J Radiol.* 126 (2020) 108961.
182 <https://doi.org/10.1016/j.ejrad.2020.108961>.
- 183 [12] W. Hao, M. Li, Clinical diagnostic value of CT imaging in COVID-19 with multiple negative RT-
184 PCR testing, *Travel Medicine and Infectious Disease.* (2020) 101627.
185 <https://doi.org/10.1016/j.tmaid.2020.101627>.
- 186 [13] Y.-M. Qu, E.-M. Kang, H.-Y. Cong, Positive result of Sars-Cov-2 in sputum from a cured patient
187 with COVID-19, *Travel Medicine and Infectious Disease.* (2020) 101619.
188 <https://doi.org/10.1016/j.tmaid.2020.101619>.
- 189

Figure 1. Concordance of cycle threshold values obtained for the E gene with RealStar® SARS-CoV-2 assay and the WHO recommended RT-PCR.

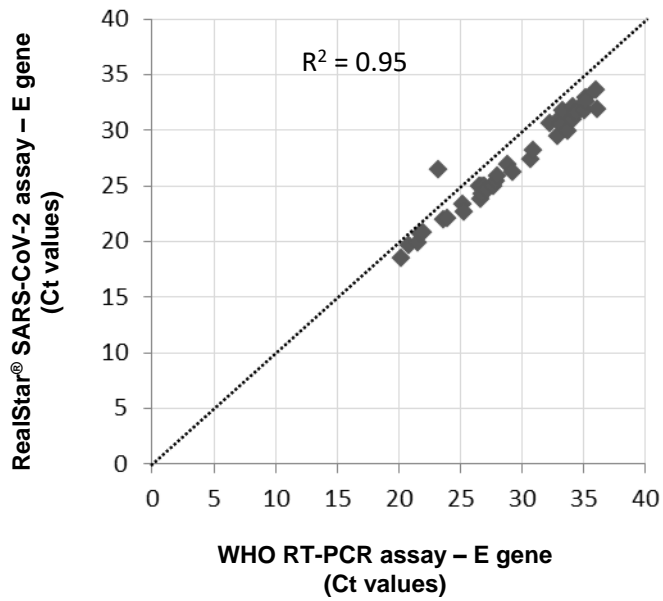


Table 1. Results of the preliminary evaluation of the sensitivity of RealStar® SARS-CoV-2 assay and the currently recommended WHO assay. For the E gene with the WHO assay, used as the gold standard method, the quantification estimated using a standardised RNA transcript control has been calculated. N.D.: Not Detected.

Tested dilution	WHO assay E gene				WHO assay RdRp gene		RealStar SARS-CoV-2 E gene		RealStar SARS-CoV-2 S gene	
	Ct	Interpretation	copies/PCR	copies/mL	Ct	Interpretation	Ct	Interpretation	Ct	Interpretation
1/10	20	Positive	6 254 420	312 721 000	19	Positive	19.6	Positive	19.2	Positive
1/100	24.3	Positive	412 044	20 602 200	24.4	Positive	23.1	Positive	22.7	Positive
1/1000	28.7	Positive	25 648	1 282 400	29.5	Positive	26.6	Positive	26.4	Positive
1/10 000	32.8	Positive	1 877	93 850	33.9	Positive	31	Positive	30.7	Positive
1/100 000	36.4	Positive	198	9 900	40.6	Negative	33	Positive	32.7	Positive
1/500 000	38.9	Positive	41	2 050	N.D.	Negative	36.4	Positive	36	Positive
1/2 500 000	N.D.	Negative	-	-	N.D.	Negative	40.1	Negative	38.6	Positive
1/12 500 000	N.D.	Negative	-	-	N.D.	Negative	N.D.	Negative	N.D.	Negative

Table 2. Limit of detection assessment for RealStar® SARS-CoV-2 and the currently recommended

WHO assay. N.D.: Not Detected.

Dilution	Viral load (copies/mL)	WHO assay	RealStar SARS-CoV-2	
		E gene	E gene	S gene
1/100 000	10 000	36.8	34.5	33
		36.8	34.9	33
		37.7	34.7	33.3
1/200 000	5000	37.3	35.5	33.9
		38.3	36	34.4
		37	36	34.5
1/400 000	2500	38.4	37.4	35.5
		37.9	37.3	35.2
		38.2	36.8	35.1
1/800 000	1250	39.8	39.5	37.8
		39.6	38.2	36.8
		39.4	37.5	35.5
		39	37.9	35.6
		38.9	38.2	37.4
		37.4	39	38.2
		39.4	37.7	37.2
		39.7	39.6	36.4
		38.7	38	39.3
		39.1	38.4	37.1
1/1 600 000	625	39.5	39	36.3
		39.4	39.5	39.2
		39.3	38.6	38.3
		39.4	38.1	36.2
		N.D.	38.6	37.9
		N.D.	38.8	39.2
		N.D.	39	37.4
		N.D.	40.8	38.2
		N.D.	38.1	37.6
		N.D.	38.3	36.4
1/3 200 000	312.5	39.1	40.3	39.1
		N.D.	40.9	37.9
		N.D.	40.9	N.D.
		N.D.	N.D.	N.D.
		N.D.	N.D.	N.D.
		N.D.	N.D.	N.D.
		N.D.	N.D.	N.D.
		N.D.	N.D.	N.D.
		N.D.	N.D.	N.D.
		N.D.	N.D.	N.D.