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ABSTRACT

Background: With the persistent COVID-19 pandemic, there is an urgent need to use rapid and reliable diagnostic tools for highly urgent cases. Antigen tests are disappointing with their lack of sensitivity. Among molecular tools allowing a diagnosis in less than an hour, only one, the Cepheid Xpert Xpress SARS-CoV-2 assay, has exhibited a good sensitivity. However, we are also facing a global shortage of reagents and kits. Thus, it is imperative to evaluate other point-of-care molecular tests.

Methods: We evaluated the VitaPCR™ RT-PCR assay, whose sample analysis time is of approximately 20 minutes, in nasopharyngeal secretions from 534 patients presenting to our Institute, for the diagnosis of COVID-19, and compared it to our routine RT-PCR assay. We also compared the two assays with tenfold dilutions of a SARS-CoV-2 strain.

Results: Compared to our routine RT-PCR and the previous diagnosis of COVID-19, the sensitivity, specificity, positive and negative predictive values of VitaPCR™ can be evaluated to be 99.3% (155/156), 94.7% (358/378), 88.6% (155/175) and 99.7% (358/359), respectively. Tenfold dilutions of a SARS-CoV-2 strain show that the VitaPCR™ was more sensitive that our routine RT-PCR assay.

Conclusion: The VitaPCR™ SARS-CoV-2 is an accurate rapid test, suitable for clinical practice that can be performed as part of a point-of-care testing, for the rapid diagnosis of COVID-19.

INTRODUCTION

The onset of the coronavirus disease 2019 (COVID-19) pandemic in March 2020 initiated a race to develop rapid detection tools for the causative virus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in order to optimize the management and triage of patients [1-4]. The congestion of emergency departments also required that we could offer an accurate point-of-service test that could be performed directly there. Antigen tests are easy to perform and can provide rapid diagnosis, but lack sensitivity, which makes them unreliable for the diagnosis of COVID-19 [1]. Therefore, RT-PCR assays remain the gold standard for the diagnosis of COVID-19. Several molecular tools allowing a diagnosis in less than an hour have been evaluated. Of these, the fastest two (the Abbott ID NOW and the Mesa Accula) with less than 30 minutes of delay between sampling and answer accumulated evidence of poorer diagnostic performance with a lack of sensitivity [5, 6]. Only the Cepheid Xpert Xpress SARS-CoV-2 assay has shown to be a valuable tool with a run-time of 45-50 minutes with hands on time limited to 2-3 minutes [7]. However, due to the pandemic, we are also facing a global shortage of reagents and kits and uncertainty over the availability of Xpert Xpress cartridges [5]. It is therefore imperative to evaluate other point-of-care molecular tests for emergency diagnosis.

In this context, we evaluated the VitaPCR^{TM} RT-PCR assay (Credo Diagnostics Biomedical, Singapore), whose sample analysis time is of approximately 20 minutes.

MATERIAL AND METHODS

From September 28th to October 1st, 2020, 534 patients presenting to the Mediterranee Infection Institute (Marseille, France), for the diagnosis of COVID-19, were included in the study. Each patient benefited from two naso-pharyngeal swab samplings, one per nostril. VitaPCR^{TM} SARS-CoV-2 was systematically compared to our routine in-house real-time RT-PCR as reference method [8, 9].

The VitaPCR™ assay includes three detection systems: (1) one targeting the human β-globin gene, to check the quality of DNA extracts; (2) a second targeting a specific sequence on the nucleocapside N-encoding gene; (3) a third targeting a conserved sequence common to SARS-CoV-2, SARS-CoV, and SARS-like bat coronavirus, also located on the N-encoding gene. We strictly followed the manufacturer's instructions for VitaPCR™ SARS-CoV-2 assay (Credo Diagnostics Biomedical, Singapore). For virus lysis and inactivation, the swab was discharged in the kit-provided collection buffer by stirring it 15 times. We allowed the lysis buffer to act for 5 minutes. Thirty µL of lysate were transferred to the tube containing the lyophilized PCR reagents. They were mixed well by pipetting. We avoided bubbles during all the process. The tube was then introduced into the apparatus in order to perform the analysis by RT-PCR, and then the latter returned the results in 20 minutes.

For our routine assay, automated nucleic acid extraction was performed using a KingFisher™ Flex system (Thermo Fisher Scientific), following the manufacturer's instructions. Our routine SARS-CoV-2 RT-PCR assay, that targets the envelope protein E-encoding gene, was performed as previously reported [8]. Besides, PCR targeting the human β-actin gene was performed to check the quality of DNA extracts [9]. In routine, the cycle threshold (Ct) to conclude that an analysis is positive using our RT-PCR is less than or equal to 35 Ct. In parallel, we also assessed the impact of delayed testing on Ct values using VitaPCR™ assay for twelve positive samples tested directly and 3 hours later.

Besides, we also determined the level of detection of the two molecular assays by analyzing tenfold dilutions of a suspension of Vero E6 cell-cultured SARS-CoV-2 IHUMI-3 strain [10]. This strain was obtained from a nasopharyngeal swab of an RT-PCR positive patient, as previously reported [10].

RESULTS

By analyzing tenfold dilutions of IHUMI-3 strain, from 780 x 10^6 copies/ml at a dilution of 10^{-1} to 1,484 copies/ml at a dilution of 10^{-6}, the Ct values were 16 and 34 for the highest (10^{-1}) and lowest (10^{-6}) using the VitaPCR™ and 20 and 36, respectively, using our routine PCR assay (Figure 1).

Among the 534 analyzed samples, 119 were positive and 358 negative using both assays (Supplementary Figure). One from recent diagnosis of COVID-19 was positive only with our routine RT-PCR. Fifty-six were positive only with the VitaPCR™. Among them, nine were negative for β-actin PCR showing thus the poor quality of the DNA extracts and the impossibility of interpreting the SARS-CoV-2 results obtained by routine RT-PCR; in contrast, β-globin was correctly detected from the naso-pharyngeal swabs from these patients interpreted using VitaPCR™. Eighteen exhibited a cycle threshold (Ct) value from 35 to 38 using our routine RT-PCR (including 6 from patients with a recent diagnosis of COVID-19). In our laboratory, a threshold of Ct 35 was selected in order to prioritize diagnoses of putatively contaminant patients. Nine were also from recent diagnosis of COVID-19, including 6 with a Ct value greater than 31 with VitaPCR™. Overall, these data support false negative results from our routine RT-PCR due to a biased threshold or at least a lower sensitivity. Finally, among the other twenty patients, two were asymptomatic whereas eighteen exhibited clinical and biological data highly evocative of COVID-19, such as fever, cough, anosmia, ageusia, and eosinopenia (Table 1) [11]. Compared to our routine RT-PCR with a Ct less than or equal to 38 and the previous diagnosis of COVID-19, the sensitivity, specificity, positive, and negative predictive values of VitaPCR™ can be evaluated to be 99.3% (155/156), 94.7% (358/378), 88.6% (155/175) and 99.7% (358/359), respectively.

Finally, a 3-hour delayed testing using VitaPCR™ assay has an impact on Ct values with an increase in these (Table 2).

DISCUSSION

Our study shows that the VitaPCR™ assay exhibits a high sensitivity for SARS-CoV-2 detection in nasopharyngeal samples. Moreover, the apparent lack of specificity must be heavily weighted with the patient data which suggests a potential lack of sensitivity of our routine RT-PCR. Of note, the manufacturer has reported no cross-reactivity with human coronavirus 229E, human adenovirus 1, influenza A virus (H1N1, H2N3), influenza B virus, and respiratory syncytial virus A. The assay is not only fast but also easy to handle. After the nasopharyngeal sampling, the swab is discharged into a specific lysis buffer and tested directly using ready-for-use reagents, stored at room temperature. The results are automatically interpreted, limiting human interpretation bias. The training required for operators is simple and does not last long. It required us an hour to train technicians, medical students and pharmacy students from collecting the sample to analyzing it. The device is not bulky and can therefore be installed in a delocalized laboratory, close to patients to be tested. Finally, the system is secure as the virus is inactivated by the kit-provided collection buffer.

Potential limits are that only one sample is processed by apparatus at a time (3 tests per hour) but several devices can be used concomitantly by a single person. Besides, extracted viral RNAs in lysis buffer is rapidly degraded, which prevents delayed testing.

Overall, the VitaPCR™ is a highly sensitive test that enables to deliver results in less than half an hour and to decentralize testing for SARS-CoV-2 in acute-care hospital or emergency departments where rapid triage decisions are required for the establishment of specific isolation for contagious patients, the use of adequate personal protective elements for the healthcare workers, and for the management of the patient. The VitaPCR™ can therefore be included in point-of-care tests.

Transparency declaration

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The authors declare no conflict of interest in relation to this research.

The patients gave an informed consent for this study.

Authors' contributions

PEF and FF conceptualized and supervised the study. CZ, EP, LN, MM, HTD, and PC performed the investigations and experiments. PEF and FF wrote the initial draft of the manuscript. All authors agreed to the publication of this version of the manuscript.

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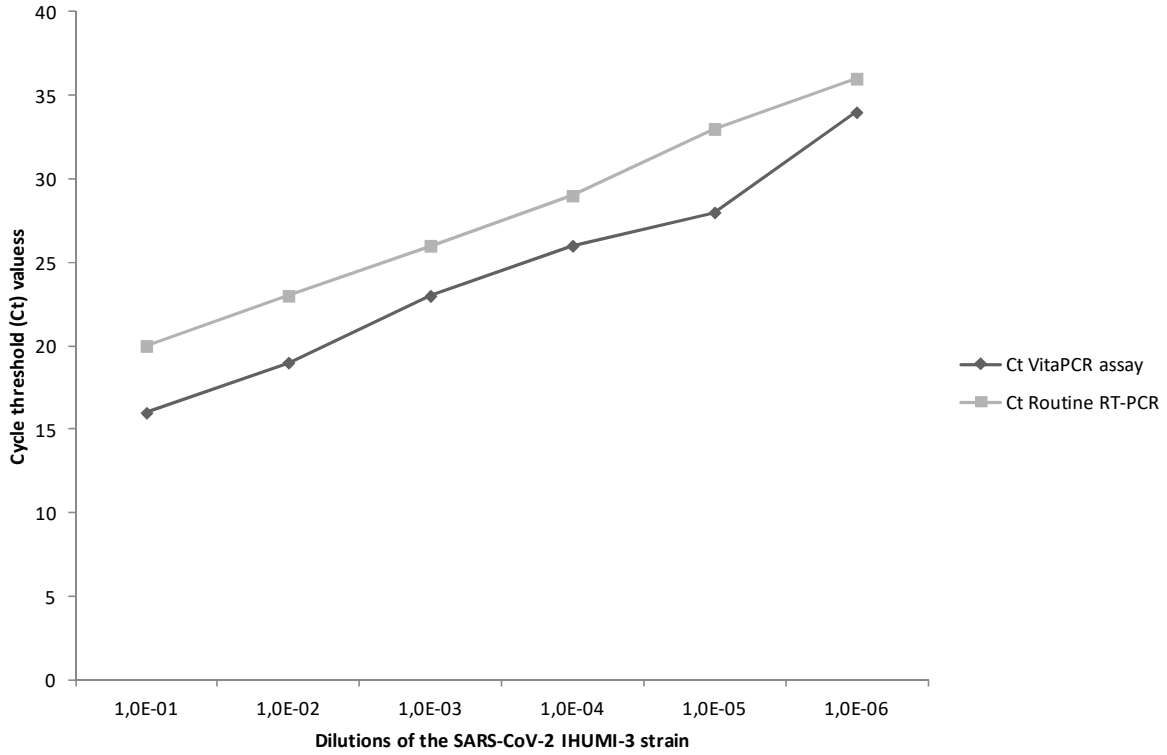
Legend Figure 1. Evaluation of the *in vitro* sensitivity of VitaPCR™ SARS-CoV-2 assay by comparison with our routine RT-PCR using tenfold dilutions of a suspension of Vero E6 cell-cultured SARS-CoV-2, IHUMI-3 strain.

Figure 1. Evaluation of the *in vitro* sensitivity of VitaPCR™ SARS-CoV-2 assay by comparison with our routine RT-PCR using tenfold dilutions of a suspension of Vero E6 cell-cultured SARS-CoV-2, IHUMI-3 strain.



216 **Table 1.** Clinical and biological data for the twenty patients positive only with the VitaPCR™ and without previous diagnosis of COVID-19..

Patients	Sex, age	Date of sample	Date of symptoms onset	Clinical and biological data
1	F, 20 y	24 sept	None	Asymptomatic (another sample collected on 09/28 was negative by both techniques)
2	M, 83 y	25 sept	None	Asymptomatic (another sample collected on 09/30 was negative by both techniques)
3	M, 31 y	25 sept	23 sept	Cough, aches, asthenia, leucopenia (3.6 Giga/l), eosinopenia (0.03 Giga/l), lymphopenia (0.87 Giga/l)
4	F, 47 y	29 sept	20 sept	Fever, cough, anosmia, ageusia, thoracic pain, rhinitis, diarrhea, eosinopenia (0 Giga/l), elevated CRP (42.1 mg/l), elevated ferritin (660 µg/l), elevated γGT (41 UI/l), elevated transaminases (ALT [45 UI/l] and AST [43 UI/l]), elevated LDH (272 UI/l), elevated fibrinogen (5.4 g/l)
5	F, 40 y	29 sept	28 sept	Cough, headache, leucopenia (3.8 Giga/l), lymphopenia (0.63 Giga/l)
6	M, 40 y	28 sept	19 sept	Fever, cough, anosmia, ageusia, diarrhea, headache, eosinopenia (0.02 Giga/l), elevated ferritin (943 µg/l), elevated CRP (21.8 mg/l), elevated transaminases (ALT [72 UI/l] and AST [63 UI/l]), elevated LDH (301 UI/l)
7	F, 39 y	29 sept	25 sept	Fever, anosmia, ageusia, headache, leucopenia (3.6 Giga/l), eosinopenia (0.08 Giga/l), thrombocytopenia (134 Giga/l), elevated fibrinogen (4.15 g/l), elevated d-dimers (3 µg/ml)
8	M, 24 y	25 sept	18 sept	Anosmia, ageusia, leucopenia (3.9 Giga/l), neutropenia (1.9 Giga/l), eosinopenia (0.02 Giga/l), elevated CRP (22.2 mg/l), elevated LDH (223 UI/l), elevated ferritin (10.8 µg/l)
9	M, 62 y	01 oct	26 sept	Fever, diarrhea, aches, abdominal pain; leucopenia (2,9 Giga/L), neutropenia (1.7 Giga/l), eosinopenia (0.03 Giga/l), lymphopenia (0.79 Giga/l), thrombocytopenia (129 giga/l), elevated CRP (16.3 mg/l), elevated ferritin (1400 µg/l), elevated fibrinogen (5.15 g/l), elevated LDH (242 UI/l)
10	M, 43 y	28 sept	14 sept	Fever, cough, headache, leucocytosis (23 Giga/l), neutrophilic leucocytosis (20 Giga/l), eosinopenia (0.04 Giga/l), elevated CRP (33 mg/l), elevated γGT (90 UI/l)
11	M, 35 y	29 sept	26 sept	Ageusia, headache, asthenia, aches, eosinopenia (0.02 Giga/l), elevated CRP (13.5 mg/l), elevated fibrinogen (4.6 g/l)
12	M, 19 y	28 sept	21 sept	Cough, ageusia, headache, asthenia, no biological abnormalities reported
13	M, 23 y	28 sept	21 sept	Rhinorrhea, headache, asthenia, eosinopenia (0.05 Giga/l)
14	F, 43 y	28 sept	22 sept	Cough, anosmia, rhinitis, aches, diarrhea, eosinopenia (0.01 Giga/l), elevated ferritin (212 µg/l)
15	F, 41 y	29 sept	22 sept	Fever, anosmia, ageusia, headache, diarrhea, rhinitis, chest pain, aches, eosinopenia (0.02 Giga/l), elevated LDH (222 UI/l)
16	M, 43 y	30 sept	20 sept	Anosmia, ageusia, eosinopenia (0.08 Giga/L), elevated ALAT (54 UI/l), elevated γGT (173 UI/l)

17	F, 16 y	25 sept	18 sept	Cough, anosmia, ageusia, headache, rhinitis, thoracic pain, aches, elevated transaminases (ALT [59 UI/l] and AST [46 UI/l])
18	F, 67 y	28 sept	20 sept	Fever, diarrhea, breathlessness, aches, asthenia, eosinopenia (0.00 Giga/l), elevated ferritin (264 µg/l), elevated transaminases (ALT [53 UI/l] and AST [43 UI/l]), elevated LDH (276 UI/l), elevated fibrinogen (5.5 g/l), elevated d-dimers (0.67 µg/ml)
19	F, 34 y	29 sept	15 sept	Cough, anosmia, ageusia, eosinopenia (0.08 Giga/l)
20	F, 73 y	29 sept	14 sept	Cough, leucocytosis (11 Giga/l), neutrophilic leucocytosis (7.9 Giga/l), eosinopenia (0.08 Giga/L)

CRP (C-reactive protein); γGT (Gamma glutamyl transpeptidase); ALT (alanine transaminase); AST (aspartate transaminase); *LDH (lactate dehydrogenase*)

Table 2. Cycle threshold values obtained using VitaPCR™ assay when analyses were performed directly after the sampling and 3 hours later.

	VitaPCR™ cycle threshold values	
Patients	**Directly performed**	**Performed 3 hours later**
1	22	22
2	30	32
3	19	20
4	17	25
5	16	27
6	28	30
7	25	31
8	19	20
9	28	30
10	20	22
11	27	30
12	22	26