Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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A brief performance evaluation and literature review of Abbott ID Now COVID-19 rapid molecular-based test

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ABSTRACT

The qualitative ID Now COVID-19 assay combines claimed performance and ease of use that seem to position it as a reliable test for urgent patient management. However, the declared limit of detection (LOD) of 125 genome equivalents/mL is not confirmed by the published studies, which observed a range of LOD varying from 276 to 20.000 copies/mL. We decided to establish the LOD value on more robust basis using serial dilutions of a SARS-CoV-2 culture supernatant sample of defined concentration. Afterwards, we tested the analytical performances of the assay with 23 QCMD external quality control measurements. Hence, taking into consideration the additional dilution in the sample receiver cup, we found a lower 95 % LOD of 64 copies/mL. For its intended use and with the new established LOD, ID Now COVID-19 proved to be a suitable test for the diagnosis of COVID-19 in contagious patients, as proposed by the latest Belgian recommendations.

1. Introduction

In late 2019, an outbreak of pneumonia cases with flu-like symptoms of unknown origin began in Wuhan, China. Soon, several studies confirmed a human to human transmission and showed that some cases of this new respiratory disease could cause acute respiratory distress syndrome (ARDS), multiple organ failure (MOF) and death (Zhu et al., 2020; Wu and McGoogan, 2020; Chen et al., 2020).

Early January 2020, a new virus was identified by genome sequencing (Lu et al., 2020) as a betacoronavirus family-related virus, named "Severe Acute Respiratory Syndrome Coronavirus 2" (SARS-CoV-2) by the International Committee on Taxonomy of Viruses. Rapidly, the new virus spread all over the world causing over 85.000 confirmed cases and nearly 3.000 deaths by the end of February 2020 (WHO, 2021a). On March 11, WHO officially declared this new disease, called Coronavirus 2019 (COVID-19), as a global pandemic.

SARS-CoV-2 is the seventh coronavirus with human-to-human transmission (especially through small droplets from upper respiratory tract). It can cause a wide panel of symptoms, from mild forms (cough, temperature, flu-like symptoms) to severe ones, particularly in elderly subjects. The molecular characteristics of SARS-CoV-2 explaining its wide and rapid spread and the existence of healthy carriers and pre-symptomatic contagiousness cause a challenge for diagnostic strategy. Therefore, reliable and easy performing tests are needed. The first validated test was Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using nasal, nasopharyngeal and oropharyngeal swabs (WHO, 2021b). Various countries developed their own assays based on National Reference Center RT-PCRs (Anon, 2021a). Nowadays, RT-PCRs targeting multiple genes, such as nucleocapsid protein, RNA-polymerase and envelop protein genes, remain the most reliable diagnostic test for COVID-19 (Corman et al., 2020) and various platforms and commercial assays are largely used.

In our laboratory, we use the following diagnostic strategy: antigen-based tests for outpatients with symptoms (confirmed with RT-PCR in case of negative result), Cepheid Xpert Xpress SARS-CoV2 RT-PCR (on Cepheid GeneXpert, Cepheid, Sunnyvale, California, US) for patients from emergency department before hospitalization, and finally, we send non-urgent sample to a bigger laboratory which uses several RT-PCR platforms. Like most labs, we are regularly confronted with a limited supply of Cepheid reagents. To meet the increasing demand for diagnostic tests and to offset shortages and delays in delivery of reagents, we needed a rapid and reliable molecular biology test in addition to our diagnostic strategy.

We here evaluate the Abbott ID Now COVID-19 assay, recently available in Belgium only since January 2021, which uses nicking enzyme amplification reaction (NEAR) technology, targeting RdRp

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gene. This test was claimed with a LOD of 125 genome equivalents/mL. In fact, the objective of our study was to assess this expected performance. We used a reference viral material from the Belgian National Reference Center for Respiratory Pathogens (Katholieke Universiteit Leuven, Brussels, Belgium) and the external controls from all three challenges of 2020 and the first 2021 challenge of Quality Control for Molecular Diagnostics (QCMD, Glasgow, Scotland) assessment programs (Matheeussen et al., 2020). We also reviewed the published literature on the subject. Finally, we interpret our results in the light of the recent recommendations of the Belgian Scientific Public Health Institute.

2. Materials and method

2.1. SARS-CoV-2 standards

The standards used in this study have two different origins:

(a) A sample of heat-inactivated SARS-CoV-2 supernatant, with a known viral load of 1.1×10^9 copies/mL, supplied by the Belgian National Reference Center for Respiratory Pathogens. We carried out serial dilutions in isotonic solution of sodium chloride so that we could dispose of concentrations between 1.1×10^2 and 1.1×10^8 copies/mL.
(b) A total of 23 samples from 2020/2021 QCMD, an independent company of external quality controls, were used. Two samples do not contain viral material, 17 samples show SARS-CoV-2 concentrations ranging between 2.0×10^2 and 2.0×10^5 copies/mL and 4 samples contain other human coronaviruses (229E, NL63 and OC43).

2.2. Abbott ID Now COVID-19 assay

This assay is a rapid molecular test based on the nicking enzyme amplification reaction (NEAR) technology, allowing to give a qualitative result (positive, negative, uninterpretable) for the detection of the RNA-dependent RNA polymerase (RdRp) gene segment of SARS-CoV-2 in 13 min reaction time. It also includes an internal control. After an isothermal nucleic acid amplification, this assay specifically identifies the amplified RNA targets with fluorescently labeled beacons. All testing was performed according to the manufacturer's instructions except the type of sample used. Indeed, the use of direct nasopharyngeal swabs is recommended by the test procedure without mentioning the possible use of liquid transport medium testing. However, based on the protocol of published studies (Zhen et al., 2020) we transferred 200 µL of each viral medium directly to the sample receiver cup in order to perform the assay. Therefore, instead of depositing a swab in the sample receiver cup, that contains already 2.5 mL of elution buffer, we deposited 200 µL of viral medium in that 2.5 mL volume of elution buffer, thus adding into the procedure of this assay an additional 13.5-fold-dilution.

2.3. Cepheid Xpert Xpress SARS-CoV-2 assay

This is a molecular test based on real-time reverse transcription PCR (RT-PCR) amplification technology and allowing to give a semi-quantitative result for the combined detection of the nucleocapsid gene (N2) and the envelope gene (E) of SARS-CoV-2. The sample is deposited into single-use cartridges containing all reagents and controls, and housing the extraction and the RT-PCR process once the cartridge is placed in the GeneXpert instrument system. All testing was performed according to the manufacturer's instructions, i.e. direct analysis of 300 µL of viral transport media without additional dilution step in the reaction cup.

2.4. Study design

Serial dilutions of the reference material and all 23 QCMD samples were analyzed with Abbott ID Now COVID-19 assay and with Cepheid Xpert Xpress SARS-CoV-2 assay.

In order to assess the analytical sensitivity, serial dilutions were analyzed, starting from a concentration of 1.1×10^8 copies/mL up to 110 copies/mL. Near the published limits of detection, starting from 22,000 copies/mL, 8 replicates of each dilution were tested. When we reached the LOD, we ran tests in triplicates for the subsequent dilutions. All tests were performed by the same operator in a standardized manner in which each sample was vortexed 40 s prior to analysis. The 95 % LOD was then determined using Probit regression, as recommended by CLSI guidelines (document EP17-A2) with MedCalc Software Version 20.009.

3. Results and literature review

3.1. Analytical sensitivity

The results allowing analytical sensitivity assessment are presented in Table 1. Additional to the declared viral load, the actual measured viral load, corrected with the 13.5-fold dilution in the reaction cup of ID Now, is presented for each dilution.

A total of 8/8 replicates gave positive results with all serial dilutions up to 81 copies/mL. The next dilution, i.e. 41 copies/mL, gave 0/8 positive results. Using Probit regression with MedCalc® software, the 95 % LOD for the final concentration, considering the 13.5-fold dilution, was calculated at 64 copies/mL. Both Probit graphs, the one with the declared concentrations and the one with the calculated concentrations, are shown on Fig. 1.

3.2. Analytical performances of ID Now COVID-19

The results of the QCMD quality controls are presented in Table 2. It is to be noted that contrary to Cepheid Xpert Xpress SARS-CoV-2 assay which directly measures the declared viral load, ID Now COVID-19 assay presents always a dilution when liquid samples are used. Consequently, we also present here the calculated concentration of SARS-CoV-2 viral load inside the ID Now receiver cup considering this additional 13.5-fold dilution. Seventeen QCMD samples were positive for SARS-CoV-2 with declared RNA concentration varying from 100 to 200.000 copies/mL. ID Now correctly detected 12 out of 17 resulting with a PPA of 70.6 %. All 6 negative samples were negative on ID Now (NPA of 100 %). Four of these samples contained other coronaviruses (coronavirus 229E, coronavirus NL63 and coronavirus OC43). The overall agreement was calculated as 78.3 %. All positive and negative QCMD samples were correctly assessed by Cepheid Xpert Xpress SARS-CoV-2 assay.

Table 1
Serial dilutions of heat-inactivated SARS-CoV-2 supernatant measured by ID Now COVID-19.

Dilution	SARS-CoV-2 RNA declared concentration (copies/mL)	SARS-CoV-2 RNA calculated concentration in sample receiver (copies/mL)	No. replicates	No. positive results
10^{-1}	1.1×10^8	8,148,148	1	1
10^{-2}	1.1×10^7	814,815	1	1
10^{-3}	1.1×10^6	81,481	1	1
10^{-4}	110,000	8,148	1	1
1:5 of 10^{-4}	22,000	1,630	8	8
3:20 of 10^{-4}	16,500	1,222	8	8
10^{-5}	11,000	815	8	8
10^{-6}	1,100	81	8	8
1:2 of 10^{-6}	550	41	8	0
10^{-7}	110	8	3	0
NA	Negative[a]	Negative[a]	1	0

[a] Free RNA physiological serum (NaCl 0.9 %).

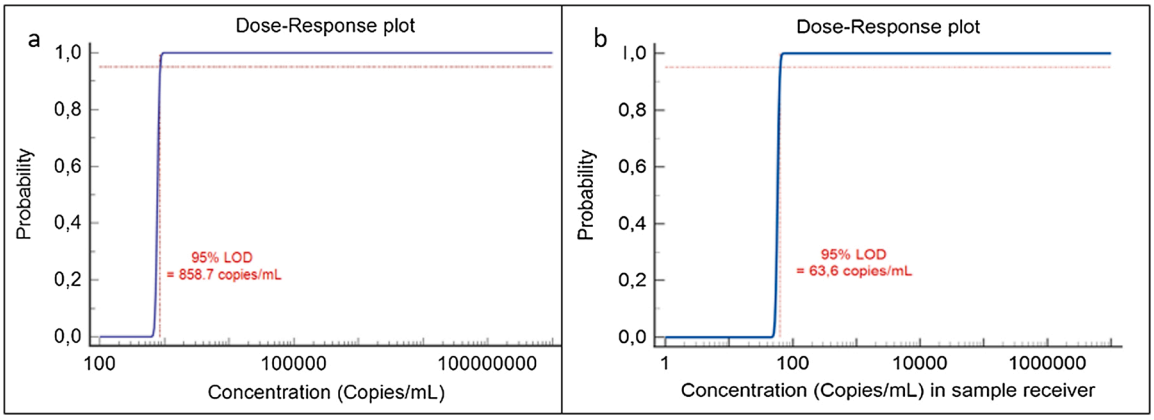

Fig. 1. Probit graph showing concentration of SARS-CoV2 RNA in copies/mL as "Dose variable" and the fraction of positive replicates as "Response variable". **a. Probit graph** showing final declared concentration of SARS-CoV2 RNA. **b. Probit graph** showing final calculated concentration of SARS-CoV2 RNA in the sample receiver. Horizontal reference line shows 95 % probability of positive agreement, the 95 LOD. Vertical reference line shows the concentration corresponding to the 95 % LOD.

Table 2
Agreement for detection of SARS-CoV-2 by Xpert Xpress and ID Now with QCMD external quality controls.

Program	Sample ID	Sample Viral load[a] (copies/mL)	ID Now Receiver cup Viral Load (copies/mL)	Xpert Xpress		ID Now
				E gene (Ct)	N gene (Ct)	
QCMD	SCV2 101C1-01	Coronavirus 229E (8,511)	Coronavirus 229E (630)	0.0	0.0	Not detected
QCMD	SCV2 101C1-02	13.183	976	28.1	30.2	Positive
QCMD	SCV2 101C1-03	1.413	105	31.3	33.9	Positive
QCMD	SCV2 101C1-04	661	49	32.1	34.8	Positive
QCMD	SCV2 101C1-05	661	49	32.3	35.3	Not detected
QCMD	SCV2 101C2-01	Coronavirus OC43 (10,000)	Coronavirus OC43 (741)	0	0	Not detected
QCMD	SCV2 101C2-02	1.862	138	30.7	33.6	Positive
QCMD	SCV2 101C2-03	302	22	34.9	37.7	Not detected
QCMD	SCV2 101C2-04	302	22	34.2	37.1	Not detected
QCMD	SCV2 101C2-05	Negative	Negative	0	0	Not detected
QCMD	CVOP2OS-01	19.953	1.478	28.3	30.2	Positive
QCMD	CVOP2OS-02	Coronavirus NL63 (43.652)	Coronavirus NL63 (3.233)	0	0	Not detected
QCMD	CVOP2OS-03	1.995	148	31.2	33.4	Positive
QCMD	CVOP2OS-04	Coronavirus OC43 (10,715)	Coronavirus OC43 (794)	0	0	Not detected
QCMD	CVOP2OS-05	Negative	Negative	0	0	Not detected
QCMD	CVOP2OS-06	19.953	1.478	28.3	30.3	Positive
QCMD	CVOP2OS-07	199.526	14.780	24.6	26.8	Positive
QCMD	CVOP2OS-08	200	15	33.7	36.5	Positive
QCMD	SCV2_21C1B01	13.490	999	28.6	31.0	Positive
QCMD	SCV2_21C1B02	324	24	32.3	35.4	Positive
QCMD	SCV2_21C1B03	100	7	35.9	38.3	Not detected
QCMD	SCV2_21C1B04	871	64	32.8	35.1	Positive
QCMD	SCV2_21C1B05	1.413	105	33.0	35.3	Not detected

Abbreviations: Ct, cycle threshold.

[a] If not specified, viral load corresponds to RNA concentration for SARS-CoV-2. Values were initially given in log10 copies/mL. We converted those in copies/mL and rounded to the nearest whole number. When a control contained another coronavirus, we specified the viral load between parenthesis after the virus scientific name.

3.3. Current available literature on the performance evaluation of ID Now COVID-19 assay

Several studies have already compared ID Now COVID-19 assay with a reference method (RT-PCR) showing PPA value from 48 to 94 % (Moore et al., 2020; Mitchell and George, 2020; Basu et al., 2020; Smithgall et al., 2020; Rhoads et al., 2020; Jin et al., 2020; Harrington et al., 2020; Thwe and Ren, 2020; Lephart et al., 2021; Cradic et al., 2020; Merens, 2021; Serei et al., 2021) and a LOD widely ranging from 262 copies/mL (Lephart et al., 2021) to 20,000 copies/mL and even higher (Bruno and Escuret, 2021), data presented in Tables 3 and in 4 . Literature data on test comparisons, number of specimens included and ID Now performances are presented in Table 3. Summary of published data of the analytical sensitivity of the assay are presented in Table 4. These were obtained with serial dilutions of positive samples or external quality controls and are expressed in copies/mL when available. In both tables, an additional column informs if the dilution in the sample receiver cup was considered or not for ID Now analytical performances and sensitivity establishment.

4. Discussion

The qualitative ID Now COVID-19 test became available in Belgium at the same time as the recommendations of the Belgian authorities demanding to each laboratory to calibrate its RT-PCRs in order to provide and report results interpretation in terms of viral load (Anon, 2021b). It was therefore essential to know the precise LOD of the ID Now assay before integrating it into our diagnostic strategy. However, we were intrigued by the fact that ID Now was declared by the manufacturer with a LOD of 125 genome equivalents/mL which is even lower than the LOD of RT-PCR, e.g. the declared LOD for Cepheid Xpert Xpress SARS-CoV-2 is of 250 copies/mL. Moreover, the literature shows LOD

Table 3
Summary of current literature on the performance evaluation of ID Now COVID-19 assay.

Tests compared	Number of specimens[1]	Dilution in sample cup considered[2]	Conclusion for ID Now performance	Source
ID Now COVID19[a] RealTime[b] RT-PCR Laboratory-developed RT-PCR	200 NP swabs in VTM	No	PPA: 80.3 % (vs laboratory developed RT-PCR) and 75.2 % (vs Real Time); NPA: 100 %; Overall agreement: 83.5 %	Moore et al. (2020)
ID Now COVID19 CDC or New York EUA RT-PCR	61 NP swabs in VTM	No	Sensitivity: 71.7 %; Specificity: 100 %; Overall agreement: 78.7 %	Mitchell and George (2020)
ID Now COVID19 Xpert Xpress[c]	101 dry nasal swabs paired with NP swabs in VTM	Yes (both specimen types were used on ID Now, as separate comparisons)	PPA: 54.8 %; NPA: 98.6 % ; overall agreement: 85.1% PPV: 94.4 % and NPV: 83.1 % (results for dry nasal swabs on ID Now).	Basu et al. (2020)
ID Now COVID19 Xpert Xpress Cobas SARS-CoV-2[d]	113 NP swabs in VTM or UTM	No	PPA: 73.9 % and NPA:100 % for ID NOW compared to cobas	Smithgall et al. (2020)
Xpert Xpress ID Now COVID19 GenMark ePlex[e]	108 NP swabs in UTM	No	PPA: 87.7 % for ID Now and limit of detection: 20 000 copies/mL	Zhen et al. (2020)
ID Now COVID19 Simplexa[f] CDC FDA EUA RT-PCR	96 (11 nasal swabs in normal saline and 85 NP swabs in UTM)	No	PPA: 94 % for ID Now	Rhoads et al. (2020)
ID Now COVID19 Cobas SARS-CoV-2 NxTAG CoV[g]	124 NP or swabs in UTM and 56 dry NP swabs	Yes, partially (Dry NP swabs used after update of manufacturer's instructions)	ID Now had the lower analytical sensitivity. PPA: ~83 % compared to cobas	Jin et al. (2020)
ID Now COVID19 RealTime RT-PCR	524 paired dry nasal swabs and NP swabs in VTM	Yes (Dry nasal swabs for ID Now, NP swabs in VTM for the other assay)	PPA: 75 % and NPA: 99 % between assays	Harrington et al. (2020)
ID Now COVID19 Xpert-Xpress Simplexa Panther Fusion RealTime RT-PCR	182 paired dry NP swabs and NP swabs in VTM	Yes (Dry NP swabs for ID Now, NP swabs in VTM for the other assay)	PPA: 53.3 % (7 false negative out of 15 positive), NPA: 100 % and overall agreement of 96.2 %	Thwe and Ren (2020)
ID Now COVID19 Xpert-Xpress Simplexa Panther Fusion[h] RealTime RT-PCR	88 paired NP swabs in VTM and dry nasal swabs.	Yes (dry nasal swabs for ID Now, NP swabs in VTM for the other assays)	PPA: 48 % for nasal, 64 % for NP swabs and NPA: 100 % compared to the composite reference standard. Increase in performance when limiting data to an acute patient population: PPA: 69 %.	Lephart et al. (2021)
ID Now COVID19 Simplexa Roche Cobas	184 NP swabs in UVT	No	PPA: 91 %; NPA: 100 % for ID Now compared to consensus standard. Analytical LOD 10–100 times higher for ID Now	Cradic et al. (2020)
ID Now COVID 19 Pasteur National Reference Center RT-PCR[i]		No	PPA: 82.8 %; NPA: 100 %; Increase in performance when excluding samples with negligible viral load (<1000copies/mL): PPA: 94.1 %.	Merens (2021)
ID Now COVID 19 Xpert-Xpress	105 nasal swabs in VTM paired with dry nasal swabs	Yes (dry nasal swabs for ID Now, NP swabs in VTM for the other assays)	PPA: 60 %. All false negatives were positive for N2 gene on Xpert Xpress	Serei et al. (2021)

Abbreviations: NP, nasopharyngeal; VTM, viral transport medium; PPA, positive percent agreement; NPA, negative percent agreement; CDC, Centers for Disease Control and Prevention; EUA, Emergency Use Authorization; PPV, positive predictive value; NPV, negative predictive value; FDA, Food and Drug Administration.

[1] Paired samples means that several specimen types were taken for each patient.

[2] In the studies cited, for samples in VTM, 200 µL of liquid sample were added to the 2.5 mL of elution buffer. We give here the information whether the authors used dry swabs for ID Now assay instead of swabs in VTM/UTM, in order to avoid a 13.5-fold additional dilution.

[a] ID NOW, ID NOW COVID-19 (Abbott Molecular Inc, Des Plaines, Illinois).

[b] RealTime SARS-CoV-2 (Abbott Molecular Inc, Des Plaines, Illinois).

[c] Xpert Xpress SARS-CoV-2 (Cepheid, Sunnyvale, California).

[d] cobas SARS-CoV-2 Test (Roche Molecular Systems, Inc, Pleasanton, California).

[e] ePlex SARS-CoV-2 Test (GenMark Diagnostics, Inc, Carlsbad, California).

[f] Simplexa COVID-19 Direct Kit (Diasorin Molecular LLC, Cypress, California).

[g] NxTAG CoV Extended Panel (Luminex Molecular Diagnostics, Inc, Toronto, Canada).

[h] Panther Fusion SARS-CoV-2 (Hologic, Inc, Marlborough, Massachusetts).

[i] RT-qPCR on SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (ThermoFisher, Waltham, Massachusetts) with Kit Extraction NucleoSpin Dx Virus.

values inconsistent with the manufacturer declared LOD, and in variable proportions ranging from two times up to 300 times greater than the claimed value. An additional question for us was to know what challenge we were facing with respect to the national recommendations by introducing a qualitative test probably less sensitive for the detection of SARS-CoV-2 in respiratory specimens than RT-PCR. Therefore, we decided to define ourselves the analytical sensitivity of ID Now COVID-19 using reference viral materials, CLSI guidelines for 95 % LOD determination, and considering the 13.5-fold additional dilution in the reagent cup. Furthermore, our 95 % LOD was challenged with precisely quantified external controls from QCMD assessment programs.

Our study found a 95 % LOD of 64 copies/mL, which is even lower than the manufacturer product insert (Fig. 1 and Table 1). This surprising finding was further sustained by the results of QCMD external controls (Table 2). Indeed, ID Now COVID-19 missed a total of five positive QCMD samples out of 17 which is explained for at least four of them by the 95 % LOD of the assay considering the receiver cup additional dilution. The false negative samples presented indeed with receiver cup viral load lower than 64 copies/mL. The result of only one false negative sample (SCV2_21C1B05), with receiver cup viral load of

Table 4
Current literature on analytical sensitivity of ID Now COVID-19 assay using limit of detection with serial dilutions.

Specimen	Dilution medium	Dilution in sample cup considered	LOD in copies/mL	Source
ZeptoMetrix inactivated SARS-CoV-2 virus[a]	VTM	Yes	262	Lephart et al. (2021)
Exact Diagnostics synthetic RNA quantified control[b]	RNA Storage solution[c]	No	20.000	Zhen et al. (2020)
Supernatant from BGM cells infected with SARS-CoV-2	Not precised	No	~15.000	Bruno and Escuret (2021) [1]
Positive clinical sample	Negative NP swab medium	No	~37.000	
10 Positive NP swabs	UVT	No	Not mentioned: 10x higher than Simplexa[d] 100x higher than Cobas SARS-CoV-2[e]	Cradic et al. (2020)

Abbreviations: VTM, viral transport medium; BGM cells; Buffalo Green Monkey cells; NP, nasopharyngeal; UVT, Universal Viral Transport.

[1] The results from this study were given in cycle threshold (respectively Ct30.1 for supernatant and Ct29 for the clinical sample) aligned with those of the Pasteur Institute (Anon, 2021c). We converted those results in copies/mL using data source from the reference method, the RT-PCR on SuperScript™ III Platinum® One-Step Quantitative RT-PCR System, with Kit Extraction NucleoSpin Dx Virus performed in Pasteur National Reference Center, Paris, France.

[a] Zeptometrix Corporation, Buffalo, New York.

[b] Exact Diagnostic, Fort Worth, Texas.

[c] ThermoFisher, Waltham, Massachusetts.

[d] Simplexa COVID-19 Direct Kit (Diasorin Molecular LLC, Cypress, California).

[e] cobas SARS-CoV-2 Test (Roche Molecular Systems, Inc, Pleasanton, California).

105 copies/mL, remains although unexplained.

These findings further confirm that comparing results of different quantitative PCR platforms should always consider an eventual pre-analytical dilution which is namely the case of ID Now COVID-19. Unfortunately, many published studies did not take into consideration this systematic pre-analytical bias, which partially explains the heterogeneity in the analytical performance evaluations (Tables 3 and 4). Moreover, regarding the literature specifically on the analytical sensitivity, the 13.5-fold dilution factor was not considered in any of the study protocols using liquid samples, except by the one by Lephart et al. (Table 4). In that study, the determination of the LOD of ID Now COVID-19 assay implied different dilution factors in order to reach within the sample receiver cup the concentrations to which the other PCR platforms were subjected (Table 4). The LOD value determined by Lephart et al. is comparable to the one declared by the manufacturer and to the one determined in this study, despite the differences within the study protocols and the definition of LOD. Indeed, both studies considered, although in different ways, the existence of this dilution factor in the sample receiver cup when defining the analytical sensitivity of ID Now COVID-19 assay. However, if the 13.5-fold dilution factor is omitted, 95 % LOD would be determined as 859 copies/mL instead of 64 copies/mL (Fig. 1a and b), which is much closer to the published data.

Regarding QCMD external controls' expected results and compared to Cepheid Xpert Xpress assay, our determination of PPA of ID Now COVID-19 of 70.6 % is comparable to the one of the various published studies (Table 3). In order to further assess the clinical significance of the discordant results, we followed the recommendations of the Belgian Risk Assessment Group (Sciensano, Scientific Public Health Institute, Brussel, Belgium) (Anon, 2021b) which stated that samples can be considered "very strongly positive" when they present viral load higher or equal to 10^7 copies/mL, "strongly positive" higher or equal to 10^5 copies/mL and "positive" higher or equal to 10^3 copies/mL. Below the threshold of 10^3 copies/mL, samples are reported as "weakly positive" and patients may be considered no longer infectious if the clinical and serologic evidence supports an old infection or the absence of infection. Therefore, in a second analysis, we divided the positive samples into two groups: higher than 10^3 copies/mL receiver cup viral load and lower. All 3 highly charged samples were correctly identified. A total of 14 positive QCMD samples presented a receiver cup viral load below 10^3 copies/mL and 9 out of them were correctly detected by ID Now COVID-19 assay. These findings further underline the ample analytical performances of ID Now.

All of these data were sufficiently satisfactory in order to introduce the ID Now platform in our COVID-19 screening strategy, alongside with the GeneXpert platform. As acknowledged, ID Now being a qualitative test, does not allow evaluation of the viral load of samples positive for SARS-CoV-2, as recommended by the Belgian Public Health Institute (ref). However, the analytical sensitivity calculated in our study demonstrates that this assay can detect all ranges of viral loads reported by the 4 categories, including the one with the lowest concentrations ($<10^3$ RNA copies /mL).

In conclusion, we here defined the analytical sensitivity of ID Now COVID-19 assay using reference viral materials, CLSI guidelines for 95 % LOD determination, and considering the additional dilution in the sample receiver. Surprisingly, ID Now COVID-19 assay presents molecular grade performance characteristics comparable to more complex and time-consuming RT-PCR assays. Its analytical performances, combined with the very short 13 min reactional time and the friendly device-guided handling procedure, constitute an additional advantage of ID Now COVID-19 for setting up a rapid diagnosis within the clinical laboratories or in relocated forms of laboratories.

Disclosure summary

The authors have nothing to disclose.

Author contributions

All the authors have approved the entire content of the submitted manuscript and any subsequent revised version and have accepted responsibility for the entire work.

Data availability

The data that supports the findings of this study are available in the main manuscript of this article.

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CRediT authorship contribution statement

Antoine Aupaix: Methodology, Validation, Formal analysis, Investigation, Writing - original draft. **Elena Lazarova:** Methodology, Writing - review & editing, Supervision. **Monia Chemais:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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