Short communication

Comparison of the GeneFinder^{TM} COVID-19 Plus RealAmp Kit on the sample-to-result Platform ELITe InGenius to the national reference method: An added value of N gene target detection?

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A B S T R A C T

Background: Due to the emergence of the coronavirus disease 2019 (COVID-19) pandemic there is an urgent need for rapid and accurate testing on the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
Objectives: The aim of this study was to assess the diagnostic performance of the GeneFinder^{TM}COVID-19 Plus RealAmp Kit on the ELITe InGenius sample-to-result platform, which is a commercial nucleic acid amplification test (NAT) targeting genes of SARS-CoV-2.
Study design: Patients were eligible between March 18 and May 27, 2020, when they had respiratory symptoms that were suspected for COVID-19. The InGenius platform was compared to routine in-house NAT that was validated according to the national reference.
Results: Of 128 randomly selected patients, 58 (45 %) tested positive and 55 (43 %) tested negative in both platforms. Sensitivity of the InGenius platform was 100 % (95 % confidence interval 94–100). In the remaining 15 (12 %) cases E and RdRp genes were not detected in both platforms but the nucleoprotein (N) gene was tested positive by the InGenius platform. All solitary N gene positive cases were confirmed by a N-gene specific in-house validated NAT, and most of these patients could also be considered positive based on other recently available COVID-19 positive respiratory samples or highly suspected radiological findings.
Conclusion: The InGenius platform for SARS-CoV-2 detection has excellent sensitivity, is easy to use and provides fast results. The inclusion of the N gene as a third gene target may further increase sensitivity for the diagnosis of COVID-19 in comparison to the national reference method.

1. Introduction

In December 2019 the coronavirus disease 2019 (COVID-19) outbreak started in Wuhan (China) [1], but COVID-19 rapidly spread to other countries as well [2,3]. In the fight against this pandemic, accurate and rapid diagnostics of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are important. Nucleic acid amplification tests (NATs) are generally characterised by high specificity, but their sensitivity depends on the timing of disease presentation, quality and location of sampling, and severity of illness [4]. Following the first validated national and international NATs, numerous commercial NAT platforms have been introduced into the market, of which many can produce faster results and do not require advanced molecular diagnostic skills to perform these tests.

This study aimed to assess the diagnostic performance of the Gene-Finder™COVID-19 Plus RealAmp Kit on the sample-to-result InGenius® platform in comparison to the national reference standard in the Netherlands, and to determine the added value of nucleoprotein (N) gene detection to establish the diagnosis of COVID-19.

2. Methods

Between March 18 and May 27, 2020, patients presenting to a teaching hospital, healthcare workers working in the same hospital, patients from nursing homes and outpatients were eligible for COVID-19 testing if they had respiratory symptoms that were suspected for

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respiratory tract infection. The Institutional Review Board (IRB) waived the need for informed consent as tests were performed on samples which had been acquired for routine clinical care (IRB protocol number 2020−071).

Patients were sampled from the oral cavity and subsequently from the nasal cavity using the same nasopharyngeal swab, which was tested by a validated in-house NAT assay on the presence of COVID-19 envelope protein (E) gene and RNA dependent RNA polymerase (RdRp) gene according to a reference method that was established after international collaboration [5]. RNA extraction was performed from clinical samples using the DNA and Viral NA Large Volume kit on the MagNA Pure 96 system (Roche, Penzberg, Germany) and subsequently real-time reverse-transcription polymerase chain reaction using the Fast Viral Master mix (Life Technologies) on the LightCycler 480 system (Roche, Penzberg, Germany). Samples were considered to be positive in case both RdRp and E genes or only RdRp gene were detected. The sample was retested when only the E gene was detected and the result was interpreted as positive in case the E gene was detected again.

In total, 58 SARS-CoV-2 positive and 70 negative samples, as determined by the national reference method, were randomly selected from a larger available collection of samples obtained during routine clinical care. Samples were stored at 4 °C before analysis in accordance with manufacturer instructions using the CE-IVD kit GeneFinder™ COVID-19 Plus RealAmp Kit on the sample-to-result platform ELITe InGenius® (Elitech, Puteaux, France). This platform used the same E and RdRp targets but additionally included the SARS-CoV-2 N gene as a third gene target. Samples that were tested negative for E and RdRp genes but tested positive for N gene were send for further analysis by another real-time polymerase chain reaction based on the Centers for Disease Control and Prevention (CDC) N gene (N2) assay [6].

Before the implementation for routine use, the in-house NAT, InGenius® platform and the specific N gene NAT were all internally validated by testing a validation panel provided by the National Institute for Public Health and the Environment, and all performed well.

All analyses were performed using SAS 9.2 (Cary, North Carolina). We compared groups using non-parametric tests for continuous variables. P-values <0.05 were considered to be statistically significant.

3. Results

A total of 128 samples from 128 unique patients were selected, of which 58 were tested COVID-19 positive according to the reference method: 54 both E gene and RdRp gene positive, 3 only RdRp gene positive, and 1 only E gene positive (Table 1). In these samples, the InGenius® platform detected all three genes in 47 (84 %) samples, E gene and N gene only in 1 (2%), RdRp and N gene only in 2 (3%), and N gene only in 8 (14 %). The sensitivity of the InGenius® platform was 100 % (95 % confidence interval (CI) 94–100).

In the 70 COVID-19 negative patients according to the reference method, 55 were tested negative for all three genes by the InGenius® platform and 15 patients were positive for the N gene only, which resulted in a specificity of 79 % (95 % CI 67–87). However, all 15 solitary N-gene positive cases were confirmed positive by the CDC N2 gene

NAT. Furthermore, in this subgroup 7 patients were tested positive in other recently available respiratory samples, another 5 patients were regarded by attending physicians as COVID-19 positive based on highly suspected radiological findings, and insufficient information was available for the remaining 3 patients. When these 12 patients were recategorized as COVID-19 positive, specificity increased to 95 % (95 % CI 86–99).

At the moment of testing, solitary N-gene positive patients had a median duration of symptoms of 14 (interquartile range 11–21) days as compared to 7 (interquartile range 4–10) in patients with positive E or RdRp gene (p < 0.01).

4. Discussion

This study shows that the sensitivity of the InGenius® platform was excellent as all positive samples according to the reference method were also identified as positive by this platform. Our data strongly suggest that the inclusion of the N gene as additional gene target may improve sensitivity of SARS-CoV-2 detection, because most solitary N gene positive cases that were tested negative by the reference method were considered positive based on other recently available COVID-19 positive respiratory samples or COVID-19 matching radiological findings. Moreover, solitary N gene positive cases were associated with longer time between onset of symptoms and timing of NAT, which suggested an increased detection window for N gene in comparison to other target genes. Theoretically, the N gene should be the most sensitive target for SARS-CoV-2 detection as a result of higher abundance of subgenomic N gene messenger RNAs in comparison to other targets [7].

In comparison to the in-house NAT, advantages of the InGenius® platform include the lower turn-around-time of three hours (in contrast to five hours), reduced hands-on-time and easy-to-use making it suitable for a broad group of laboratory technicians not limited to molecular technicians. The platform has integrated automated nucleic acid extraction, real-time polymerase chain reaction amplification and result analysis.

To the best of our knowledge only one other study has assessed the GeneFinder™ assay in 41 nasopharyngeal samples [8], in which high agreement was found between this assay and another commercial assay. Of note, in our study we primarily assessed nasopharyngeal swabs and many sample-to-result CE-IVD assays have been validated for nasopharyngeal samples only [9,10]. The InGenius® platform has been registered for nucleic acid extraction and purification of all types of respiratory samples.

There are some study limitations to consider. This study included only one commercial sample-to-result NAT, but currently many other easy-to-use tests are available on the market, including platforms that can detect the N gene. Second, it remains unknown whether solitary N positive cases are clinically relevant in terms of infectiousness and consequences for clinical management and infection control.

In conclusion, the InGenius® platform as a fully automated device has excellent sensitivity and could be a valuable asset in the molecular diagnostic testing arsenal of microbiological laboratories. Further validation is needed to determine whether including the N gene as a third gene target for establishing the diagnosis of COVID-19 can also improve clinical sensitivity, particularly in those patients with a longer time interval between onset of symptoms and testing.

Authors contribution

DSYO and NV contributed to the conception and design of the study. DSYO, SB and NV acquired the data. DSYO and NV analysed the data.

Table 1
NAT gene characteristics in 58 COVID-19 positive samples.

	InGenius	In-house NAT
E gene, number positive	48 (83)	55 (95)
E gene, Ct-value	25 (21–29)	33 (28–36)
RdRp gene, number positive	49 (84)	57 (98)
RdRp gene, Ct-value	26 (23–30)	32 (28–36)
N gene, number positive	58 (100)	N.A.
N gene, Ct-value	27 (23–30)	N.A.

Data are presented in median (interquartile range) or in absolute number (percentage).
N.A. = not applicable.

DSYO, EC, SB and NV contributed to the interpretation of the data. DSYO drafted the first manuscript and EC, SB and NV revised it critically for important intellectual content. All authors approved this manuscript version to be submitted.

Declaration of Competing Interest

The authors report no declarations of interest.

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