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A comparison study of GeneXpert and In-House N1N2 CDC Real-Time RT-PCR for detection of SARS-CoV-2 infection

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ABSTRACT

COVID-19 is a disease caused by SARS-CoV-2, a new virus from genus β-coronaviruses. This disease has been declared a pandemic by WHO on 11 March 2020 until now. The nucleic acid tests are the most frequently used assays because of their high sensitivity and specificity. One of the tests is the GeneXpert, a real-time reverse transcription polymerase chain reaction (rRT-PCR)-based assay platform. The use of the GeneXpert shows great public health interest because of the rapid (50 min), the minimum number of trained staff, and less infrastructure and equipment. However, there are limited data on the application of the GeneXpert for the detection of SARS-CoV-2. Therefore, we conducted a comparative study between the GeneXpert and in-house N1N2 CDC rRT-PCR assay. Of 86 samples, 17 were rRT-PCR positive while 13 were GeneXpert positive. Of rRT-PCR positive 17 samples, 7 were GeneXpert negative [58.82% (10/17] sensitivity]. We also found that 3 GeneXpert positive samples showed rRT-PCR negative (95.65% [66/69] specificity). It is concluded that negative results by the GeneXpert can not rule out the possibility of SARS-CoV-2 infection, particularly in close-contact individuals and the interpretation of the positive result should be analyzed carefully, particularly amplification with Ct>40.

ABSTRAK

COVID-19 adalah penyakit yang disebabkan oleh SARS-CoV-2, virus baru dari genus β-coronaviruses. Penyakit ini telah dinyatakan sebagai pandemi oleh WHO pada 11 Maret 2020 hingga sekarang. Tes asam nukleat adalah tes yang paling sering digunakan karena sensitivitas dan spesifisitasnya yang tinggi. Salah satu tesnya adalah GeneXpert, platform pengujian berbasis *real-time reverse transcription polymerase chain reaction* (rRT-PCR). Penggunaan GeneXpert menunjukkan minat kesehatan masyarakat yang besar karena kecepatan (50 menit), jumlah staf terlatih yang minimum, dan infrastruktur dan peralatan yang lebih sedikit. Namun, ada keterbatasan data dalam aplikasi GeneXpert untuk deteksi SARS-CoV-2. Oleh karena itu, kami melakukan studi perbandingan antara GeneXpert dan uji rRT-PCR N1N2 CDC *in-house*. Dari 86 sampel, 17 adalah rRT-PCR positif sementara 13 adalah GeneXpert positif. Dari 17 sampel rRT-PCR positif, 7 adalah GeneXpert negatif (sensitivitas 58,82% [10/17]). Kami juga menemukan bahwa 3 sampel positif GeneXpert menunjukkan rRT-PCR negatif (95,65% [66/69] spesifisitas). Disimpulkan bahwa hasil negatif oleh GeneXpert tidak dapat mengesampingkan kemungkinan infeksi SARS-CoV-2, terutama pada individu yang kontak dekat dan interpretasi hasil positif harus dianalisis dengan cermat, terutama amplifikasi dengan Ct>40.

Keywords:
COVID-19;
SARS-CoV-2;
GeneXpert;
PCR;
nucleic acid tests

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from Family *Coronaviridae*.[1,2] Genetic analysis showed that SARS-CoV-2 has similarities with another coronavirus (SARS-CoV) and is grouped within β-coronavirus genus.[2] SARS-CoV-2 is an enveloped virus and has a positive-sense single-stranded RNA genome enclosed in structural nucleocapsid (N) protein.[2,3] Other structural proteins namely E (envelope), M (membrane), and S (spike) proteins form viral envelope.[2,3] Among these structural proteins, S protein facilitates viral entry to the host cell by binding with the ACE receptor.[3]

This is a new virus discovered in 2019 in Wuhan, China, after some patients showed symptoms of flu-like illness.[4] In March 2020, COVID-19 was declared a pandemic by WHO because of the number of cases and countries with cases increase.[5] Globally, the numbers of COVID-19 patients continuously increased, with 500 million confirmed cases and over 6 million deaths have been reported from the beginning of the pandemic until the third week of April 2022.[6] In Indonesia, COVID-19 cases have been over 6 million and more than 155,746 deaths have been reported.[7]

At the beginning of the pandemic, there is an urgent need for a highly specific and sensitive method to detect the virus.[2,4,8] Currently, many testing methods are available for the detection of SARS-CoV-2.[2] The most common genes for nucleic acid detection of SARS-CoV-2 are orf1a/b, RdRp, S, N, and E.[1] Some diagnostic methods used to detect SARS-CoV-2 are serological (antigen and antibody detection) and nucleic acid tests such as standard real-time reverse transcription-polymerase chain reaction (rRT-PCR) and rapid tests like RT-LAMP, and GeneXpert assays.[2,9] The rRT-PCR is a gold standard for detection of SARS-CoV-2 because its sensitivity and specificity.[1,2,8] On the other hand, the GeneXpert using single cartridge-based assay is a rapid method for detection of COVID-19 compared to the standard rRT-PCR assay.[5,8] The rapid turnover of the GeneExpert result makes it as an increasingly popular choice for the detection of SARS-CoV-2. However, to our knowledge there is limited data on the GeneExpert performance particularly on its sensitivity and specificity. Therefore, in this study we compared standard rRT-PCR based on the N1N2 CDC protocol and GeneXpert assay.

MATERIALS AND METHODS

Clinical specimens

Eighty-six nasopharyngeal/oropharyngeal swabs were obtained from suspected COVID-19 individuals in Jakarta from September-December 2020. The swab samples were collected immediately into 1 mL of the viral transport medium (DMEM containing 1% pen-strep and 5% bovine serum albumin) and stored at 2-4°C for not more than 4 h. The viral transport medium was divided for GenXpert and rRT-PCR tests conducted by two separate teams (blind testing). This study was approved by the Ethics Committee, Faculty of Medicine, Universitas Indonesia (KET-395/UN2.F1/ETIK/PPM.00.02/2020).

Viral RNA extraction

The viral RNA genome was extracted by using QIAmp Viral RNA Mini Kit (Qiagen, Germany) in accordance with the manufacturer's instructions. The final elute was stored at -80°C for not more than 4h.

Real-time RT-PCR (rRT-PCR) assay

The primers and probes for N (N1 and N2) and human RNase P (internal control, IC) genes based on the Centers for Disease Control and Prevention (CDC) were used for the detection of SARS-CoV-2.[10] The rRT-PCR was performed with the following composition (20 µl of total volume): 1x SensiFAST™ Probe No-ROX One-Step mix (Bioline, Cat. No: BIO-76005), 1.5 µL each of primer and probe solution (2019-nCoV RUO Kit, IDT Integrated DNA technologies, Cat. no:10006713), 4U of RNase inhibitor, 2 U of reverse transcriptase enzyme, and 7.9 µL of RNA template. The PCR machine, MA-6000 Real-Time PCR System [Molarray, Suzhou, China]), was used under the following conditions: 50°C for 50 min; 95°C for 50 min; 45 cycles of 95°C for 15 sec and 55°C for 30 sec. The rRT-PCR positive was defined if Ct ≤ 40 for both N1 and N2.[10]

Rapid GeneXpert test

The GeneXpert used Xpert® Xpress SARS-CoV-2 kit based on N (N2 CDC) and E genes for detection of SARS-CoV-2.[11] The procedure and the result interpretation were performed according to the manufacturer's instructions.[11] SARS-CoV-2 positivity was defined if either gene (N2 and E) or only N2 were positive. The presumptive SARS-CoV-2 positive was defined if only the E gene was positive (Ct value ≤ 45).

Statistical analysis

The SPSS 16.0 was used for statistical analysis and a fisher test with a 5% (0.05) level of significance was used for hypothesis testing.

RESULTS

The comparison results between real-time RT-PCR (rRT-PCR) and GeneXpert are shown in TABLE 1. Of 86 samples, 17 were rRT-PCR positive while 13 were GeneXpert positive. Of rRT-PCR positive 17 samples, 7 were GeneXpert negative (41.18% [7/17] discrepancy). The GeneXpert negative samples had Ct values above 34 by rRT-PCR (TABLE 2), indicating that GeneXpert failed to detect SARS-CoV-2 with high Ct values above 34.

TABLE 1. Comparison results between real-time RT-PCR (rRT-PCR) and GeneXpert methods (n=86)

Positive		GeneXpert		p*	OR
		Negative			
rRT-PCR	Positive	10	7	< 0.001	31.429
	Negative	3	66		

TABLE 2. Discrepancy results between real-time RT-PCR (rRT-PCR) and GeneXpert for detection of SARS-CoV-2

Sample ID	Real-time RT-PCR			GeneXpert		
	Region target (Ct value)		Result	Region target (Ct value)		Result
	N1	N2		N2	E	
2809-23	34.22	33.93	+	ND	ND	-
2809-31	37.67	38.15	+	ND	ND	-
2809-20	38.34	37.81	+	ND	ND	-
2809-21	35.91	37.72	+	ND	ND	-
2809-14	ND	ND	-	42.4	ND	+
2909-06	34.75	34.49	+	ND	ND	-
2909-13	36.15	38.28	+	ND	ND	-
0510-05	37.56	36.40	+	ND	ND	-
1008-08	ND	ND	-	44.2	39.2	+
1208-38	ND	ND	-	41.3	ND	+

Note: All tests were valid with internal control Ct of < 30. N1 and N2: Regions of N gene. E: Envelope gene. ND: Not detected. Ct: Cycle threshold. +: Positive. -: Negative.

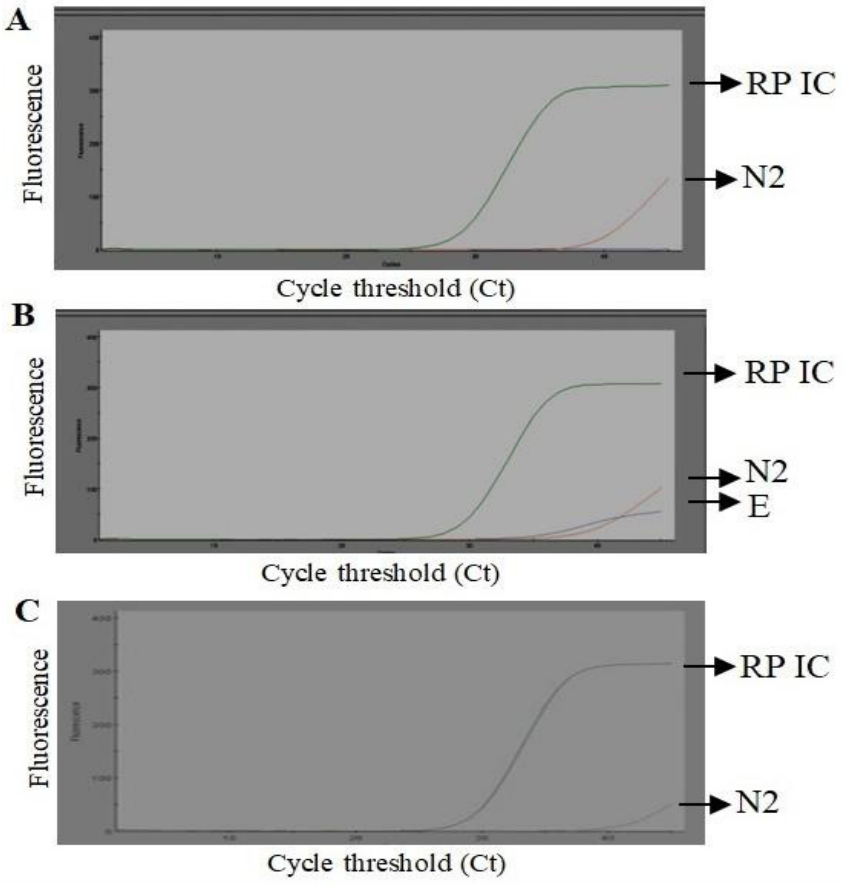

FIGURE 1. Curve Amplification for ID samples 2809-14 (A), 1008-08 (B), and 1208-38 (C). RP IC: Human RNase P gene internal control. N2: Region of N gene. E: E gene.

TABLE 3. Sensitivity, specificity, PPV, and NPV of GeneXpert assay (prior probability of infection 0.05

Test	Value
Sensitivity	0.5882 (58.82 %)
Specificity	0.9565 (95.65 %)
PPV	0.4158
NPV	0.9778

We found that 3 GeneXpert positive samples showed rRT-PCR negative (TABLE 2). Of 3 samples, 2 were detected for N2 (Ct>40) and E (Ct=0), while 1 was detected for N2 (Ct>40) and E (Ct=39.2). Based on manual curve analysis, all samples showed sigmoid curves of RP gene internal controls, while N2 and E curves were not sigmoid (FIGURE 1). Because of the questionable results, another real-time RT-PCR reaction (Detection Kit for 2019-nCoV, Cat. no: #DA-930, Da An Gene Co., Ltd. of Sun Yat Sen University), a kit listed by the WHO Emergency Use for detection of SARS-CoV-2 nucleic acid, was performed for clarification. The results showed that all 3 samples were SARS-CoV-2 negative (Data not shown).

DISCUSSION

The rRT-PCR and GeneXpert compared in this study have the same gene target [nucleocapsid (N)] for SARS-CoV-2; however, the rRT-PCR detects two regions (N1 and N2) of the N gene, while the GeneXpert detects only one region (N2).[10,11] The GeneXpert detects an additional gene, envelope (E) for all coronaviruses.[11] For detection of specific SARS-CoV-2, regions of N gene including N1 and N2 have been reported as rRT-PCR targets with higher sensitivity than other gene targets.[12-14] The high sensitivity might be caused by a high number of subgenomic mRNA of the N gene produced during the replication of coronaviruses.[15] Comparison between N1 and N2 applied for clinical and environmental samples, most of studies reported N1 having higher sensitivity than N2,[12,16-19] and another study reported an otherwise result.[13] Even though N1 was more sensitive than N2, several valid results were N2 positive and N1 negative.[18] Thus, it is suggested that N1 and N2 primer-probe sets should be used for detection of specific SARS-CoV-2.

The GeneXpert failed to detect SARS-CoV-2 in 7 samples that were positive by rRT-PCR (TABLE 2). Procop *et al*. reported that the GeneXpert had afalse-negative rate of 2% compared with N1N2 CDC rRT-PCR.[20] Other studies also reported the false-negative results by the GeneXpert.[21] Based on Ct value, the false-negative occurred in cases with high Ct values above 34 (TABLE 2). The Ct values can be used as surrogate markers for deducing the virus infectivity. For this reason, several studies have reported the association of Ct values with virus infectivity by using cell culture methods. It has been shown that patients with Ct values above 30 or 34 did not excrete infectious viral particles.[22,23]

However, other studies have reported otherwise data.[24-26] Two studies reported that clinical samples with Ct-values above 30 could still be infectious.[24,25] Singanayagam *et al*.[26] reported that 8% of samples with Ct above 35 were still infectious. The different results might be affected by different pre-analytic and post-analytic factors in each laboratory, making Ct values as surrogate markers are unclear and debatable. Thus, Platten

et al.,[27] suggested that the Ct value cut-offs can be defined as acceptable low-risk values; higher Ct values as lower infection risks. Based on the data, it is suggested that SARS-CoV-2 negative by the GeneXpert cannot rule out the possibility of SARS-CoV-2 infection.

On the other side, the GeneXpert showed 3 false-positive results (TABLE 1). Based on Ct values, all 3 samples were detected for N with Ct>40 and only 1 sample was detected for E with Ct= 39.2 (TABLE 2). Moreover, N2 and E amplification curves showed non-sigmoid curves (FIGURE 1). The question results have been clarified by another kit and showed SARS-CoV-2 negative (Data not shown). These GeneXpert false-positive results have been reported by Rakotosamimanana *et al.*,[28] in that they found samples, that were no amplification of E gene (Ct=0) and N2 with Ct>40 by GenXpert, are negative by standard rRT-PCR assay. Other studies also reported the same result patterns.[21,29] Das *et al.* reported 16 (34%) of samples with Ct>35 by GenXpert were only 3 (18.8%) positive by standard rRT-PCR assay.[21] Moreover, Moran *et al.*,[29] reported that the GeneXpert results with E gene (Ct=0) and N2 with Ct>40 were SARS-CoV-2 negative when performing the repeated GeneXpert testing. Therefore, we suggested the repeated GeneXpert testing for clarification when the results were N2 with Ct>40.

CONCLUSION

The negative results by GeneXpert cannot rule out the possibility of SARS-CoV-2 infection, particularly for close-contact individuals. Due to automatic interpretation by the GeneXPert software, the interpretation of the positive result should be analyzed carefully, particularly Ct>40. The sensitivity and specificity of the GeneXpert were 58.82% and 95.65% respectively. However, it is important to know that there is a limitation to this study, namely the small number of the samples used.

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