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Automation of the RT-PCR to detect SARS-CoV-2 approved in Mexico by using the BD Max open system

Abstract

The exponential increase in the number of cases of COVID-19 and the technical difficulties to obtain an RT-PCR result for SARS-CoV-2, according to Berlin protocol, enabled the migration of the manual PCR assay, validated by Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), to the BD Max system utilizing the use of reagents from BD Max open system. A total of 48 clinical samples were taken from patients who went to the emergency room with COVID clinical features; 46 % (22/48) were positive for SARS-CoV-2. The sensitivity of the automated BD MAX assay was 92% (20/22), compared with the manual PCR. The mean Ct was lower (27,1±5,5) with the automatized method for the RdRp gene, compared with the manual procedure (27,9±6,1), there was no significant difference (p=0.39). The use of the BD MAX open system allows SARS COV detection because it has a high performance and diminishes the time to obtain the results.

Keywords: RT-PCR, SARS-CoV-2, primers, automated system

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Introduction

In December 2019, the cases of pneumonia —caused by the new coronavirus SARS-CoV-2— spread in Wuhan, Hubei (China). Since then, the confirmed COVID-19 cases have increased rapidly. On December 30, 2019, the World Health Organization (WHO) announced that such a fact was a Public Health Emergency of International Concern (PHEIC). On February 28, 2020, WHO escalated COVID-19 risk assessment to "very high" across the world because of the transmission all over China and 26 more countries.[1–3] By April 4, WHO's report informed that the total number of confirmed COVID-19 cases around the world had overcome a million; on April 7, there were 1,279,722 confirmed cases, 26% occurred in the United States of America as well as 13% of all deaths around the world.[3] As the pandemic of active cases and deaths arrive in the Americas, especially to Mexico who was still in phase two of the pandemic, approaching the 3,000 confirmed cases and almost 200 deaths from SARS-CoV-2, the cause of COVID-19 disease, since the first case reported in Mexico diagnosed on February 28 of the same year.[4]

Based on the situation in the USA, Europe, Western Pacific and Mexico going through an accelerated increase in suspicious cases and the high demand of accurate diagnosis in patients attending the Instituto Nacional de Enfermedades Respiratorias (INER), seeking care at the national center of reference in respiratory diseases, the laboratory based in the same institute and the first in the country to be authorized by the National Network of Public Health Laboratories recognized by the Instituto de Diagnóstico y Referencia Epidemiológica (InDRE) to perform diagnostic tests for SARS-CoV-2, we saw necessary the research for alternatives with diagnostic tools to detonate actions of epidemiological surveillance for the care, prevention, and control of the transmission of the disease in our country.[4]

Since there are no specific therapeutic or vaccines for the new 2019 coronavirus disease (COVID-19), it is essential to identify it in the early stages and to isolate the infected person from the healthy population immediately.[6] By January 2020, the sequence of the virus was already traced, which enabled the development of several real-time reverse transcription-polymerase chain reaction assays (RT-PCR).

RT-PCR has also been broadly used to detect SARS-CoV-2[7] and is used as a marker to isolate, discharge, or transfer patients diagnosed with COVID-19. Nevertheless, to carry out this assay, numerous manual steps are necessary, but they are long-lasting and error-prone. They include preparing the solutions, extraction, purification of nucleic acids along with the master mixture to the amplifying device. All that requires specialized equipment, and from 6 to 8 hours to obtain the result.[8] These technical inconveniences may be avoided using automated PCR platforms.

The BD MAX (Becton Dickinson, Diagnostic Systems, Sparks MD, EE. UU.) is a fully-integrated open automated molecular platform that combines the sample processing with real-time PCR. Besides a series of assays approved by the FDA, BD MAX also offers generic extraction kits and PCR reagents ready to be used in an open platform, which allows the users to create their assays using a specific set of primers and probes.[9,10] The main objective of this trial is to migrate the reference RT-PCR assay approved by the Instituto de Diagnóstico y Referencia Epidemiológica (InDRE) according to Berlin protocol suggested by WHO to the BD MAX platform.

Material and methods

Clinical Microbiology Laboratory carried out a prospective trial. The pharyngeal and nasopharyngeal swabs, as well as the bronchial

aspirate samples included in the study, were taken from patients who attend to medical consultation because of their symptomatology compatible with COVID-19; an RT-PCR was necessary to detect SARS-CoV-2. The pharyngeal and nasopharyngeal samples were taken with nylon swabs and placed in a universal transport medium for viruses. Samples were processed simultaneously for the reference RT-PCR and by the automated open BD MAX system.

Primers and Probes: T4-OLIGO synthesized and provided all the oligonucleotides. The primer sequences (5'-3') approved by InDRE and utilized to detect target genes for SARS-CoV-2 are summarized in Table 1:

RNA Extraction: 200µl taken from the universal viral transport were used to extract genetic material. The extraction process was performed using the QIAamp RNA Viral Mini (Qiagen) kit, and the genetic material was eluted in a final elution buffer volume of 140 µl.

Reference RT-PCR: The reference RT-PCR was performed by a method standardized by InDRE using primers and probes synthesized by T4-OLIGO. The SuperScript™ One-Step RT-PCR System with Platinum™ Taq DNA Polymerase (Invitrogen) kit was used, it includes a 2X concentration reaction buffer and a mixture of enzymes Retrotranscriptase and *Taq* polymerase. To detect the *E* and *RNaseP* genes, the PCR mixture was carried out with 1 µL of forward and reverse primers (10mM), 0.5 µL of probe (10mM), 0.5 µL of enzyme, and 12.5 of µL 2X reaction buffer. The detection of the *RNaseP* gene was used as a sample quality control trial. To detect the *RdRp* gene (confirmatory assay), the PCR mixture was carried out with 1.5 µL of forward primer (10mM), 2 µL of reverse primer (10mM), 0.5 µL of probe (10mM), 0.5 µL of enzyme, and 12.5 µL of 2X of reaction buffer. Both reaction mixtures led to a final volume of 20 µL with molecular grade water or RNAse free water. The RT-PCR was performed in a 7500 Fast Real-Time PCR (Applied Biosystems™) thermocycler under the following amplifying conditions: RT: 15 minutes/55°C; Initial denaturing: 2 minutes/95 °C; 45 cycles of denaturing: 15 seconds/95°C and alignment 30 seconds/55°C. Fluorescence detection was carried out during the alignment.

BD max system: The RT-PCR by the BD MAX system was carried out at from 150 µL of the universal transport medium, which was inoculated and mixed inside an SBT tube (sample buffer tube) included in the BD MAX ExK TNA-3 kit, here the lysis of the sample was performed. The reaction mixture of E and *RdRp* genes was carried out in the same conditions and using the same reagents, probes, and primers as in the reference RT-PCR (previously described). A reaction strip (BD MAX TNA Reagent Strip) from the BD MAX ExK TNA-3 kit was used for each sample, where the RNA extraction and amplification of *E* and *RdRp* genes were taken. The reaction strip was loaded as follows, the SBT tube containing the sample, the TNA extraction tube placed in position 1; 12.5 µL of *E* gene reaction mix in position 2; 25 µL of molecular grade water in position 3; and 12.5 µL of *RdRp* gene reaction mix in position 4. After the extraction process, the BD MAX equipment defaulted 12.5 µL of eluted nucleic acids into each of both reaction mixes tubes to have a final volume of 25 µL per reaction. The detection of *E* (Sarbecovirus) and *RdRP* (SARS-CoV-2) genes was carried out into the same reaction strip by sample (dual RT-PCR). The amplifying conditions for amplification performance were the same as the reference RT-PCR (previously described). The amplification was carried out using the BD MAX PCR cartridges. *RNasaP* detection was not performed by this method.

Ethics

The trial was performed in strict adherence to ethical standards established for the use of the patients' data, and it was approved by the INER's ethics committee.

Results

Forty-eight samples were included in the clinical trial; 83% (40/48) of them were nasopharyngeal swabs, seven were pharyngeal, and one was a bronchial aspirate. The reference RT-PCR method indicated that 22 (46%) samples, out of the 48, were positive; 26 (54%) were negative for SARS-CoV-2. The BD MAX system showed 20 positive samples (42%) for SARS-CoV-2 and 28 negative samples (58%).

The BD MAX system has a sensitivity of 92% to detect SARS-CoV-2 and a 100% specificity (Table 2).

The sensitivity of the automated RT-PCR for samples taken from the nasopharynx was 88%, and the specificity was 100%, compared with the manual RT-PCR. The assay performed using BD MAX was highly sensible for the pharyngeal samples (Table 3).

The automated system showed a lower Ct mean, compared with the manual method, and there was no statistically significant difference (Table 4). There was an excellent correlation using the assays (Figure 1).

Table 1 Primer sequences (5'-3') approved by InDRE and utilized to detect target genes for SARS-CoV-2

Primer/Probename	Direction	Target	Sequence (5'- 3')
E_Sarbeco_F1	Primer Forward	SARS-CoV-2 *E* gene	ACAGGTACGTTAATAGTTAATAGCGT
E_Sarbeco_R2	Primer Reverse		ATATTGCAGCAGTACGCACACA
E_Sarbeco_P1	Probe		FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ
RdRP_SARSr-R1	Primer Forward	SARS-CoV-2 *RdRp* gene	CARATGTTAAASACACTATTAGCATA
RdRP_SARSr-F2	Primer Reverse		GTGARATGGTCATGTGTGGCGG
MAPGD_19_RdRP_SARSr	Probe		CAGGT(G)(G)AACC[BHQ1-Dt]CATCA(G)(G)AGATGC
Rnase P for	Primer Forward	Human *RNasa P* gene	AGATTTGGACCTGCGAGCG
Rnase P Rev	Primer Reverse		GAGCGGCTGTCTCCACAAGT
Rnase P	Probe		FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ1

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Table 2 Comparison between the performance of RT-PCR (reference method) and RT-PCR by BD MAX

Real-time result of the PCR, BD MAX	Real-time result of the manual PCR (reference method)		Sensitivity (%)	Specificity (%)	VPP (%)	VPN (%)
	Positive	Negative				
Positive	22	0	92	100	100	93
Negative	2	26				

Table 3 Comparison between the performance of RT-PCR (reference method) and RT-PCR by BD MAX

Real-time result of the PCR, BD MAX	Real-time result of the manual PCR (reference method)		Sensitivity (%)	Specificity (%)	VPP (%)	VPN (%)
Nasopharyngeal Swabs	**Positive**	**Negative**				
Positive	14	0	88	100	100	92
Negative	2	24				
Pharyngeal Swabs						
Positive	5	0	100	100	100	100
Negative	0	2				

Table 4 Ct mean of the real-time PCR

	Real-time PCR (reference)	Real-time PCR (BD MAX)	
	CtMV± SD	VM Ct± SD	p Value
RdRp gene	27,9 ± 6,1	27,1 ± 5,5	0,39
E gene	25,3 ± 5,24	23,6 ± 5,2	0,74

CtMV, Ct median value; SD, standard deviation

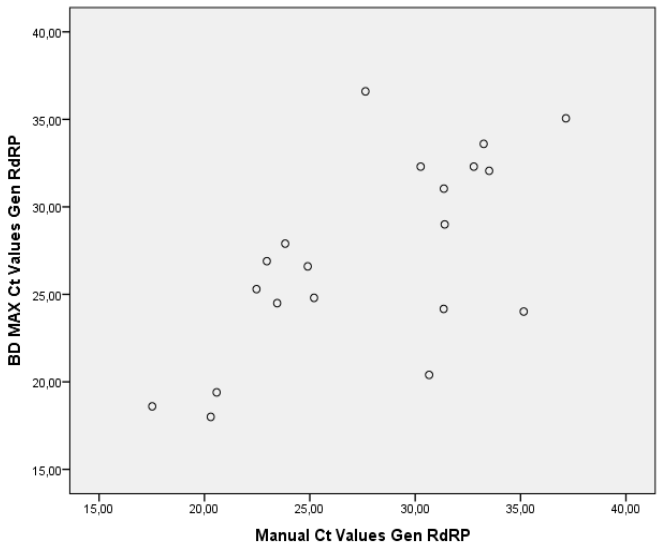

Figure 1 Comparison between real-time PCR with BD MAX and the manual PCR assay. The sample distribution of Ct of RdRp genes (confirmatory test) shows an excellent correlation (r = 0.45).

Discussion

With 3,000 confirmed cases and almost 200 deaths in Mexico of SARS-CoV-2, early detection in clinical specimens is essential for national and global control efforts. Although manual PCR is the reference method for diagnosis of COVID-19 in Mexico, fast molecular methods are now preferred due to their speed and ability to decrease sample handling. The well-recognized difficulties in reproducing manual assays from the extraction of genetic material to PCR due to variation in the performance of thermocyclers, the efficiency of different DNA polymerases, and personnel, they can hinder implementation in laboratories.[11]

We succeeded in migrating to a system of the RT-PCR reference protocol for the detection of SARS-CoV-2. Although it is not the first time that a comparison study has been performed between a reference method and the BD MAX system, during the assay, it was observed that migrating towards the automated platform was easy, the sensitivity and specificity obtained were high, the workflow was optimal, and the time to get the result decreased 50% because of the ease to perform the sample processing.[12]

The automation of the sample to detect SARS-CoV-2 enables the increase in the diagnostic capability. That is to say, the automated method may process above 200 samples per day. Reducing the time to

obtain a result, helps the physicians to take the proper isolation actions and to diminish the potential nosocomial spreading of the emerging virus.

The results will facilitate the increase of the number of samples that may be performed in our country because it is no longer necessary to have specific areas and equipment for carrying out the processing and amplification separately. Laboratories lacking those facilities may perform the tests to detect this emerging virus using the platform, and they will obtain a reliable result because of the ease to use it and because of a decrease in the error rates during the sample processing.

Some essential differences observed between the two assays are; the reduction volume of the PCR and the sample used in the BD Max system. The reduction in the volume reaction mixture didn't compromise the quality of the results. Other studies have determined that PCR reaction volumes can be reduced[14]. The strategy allows the laboratory to reduce the consumption of samples and reagents without affecting sensitivity. Other differences observed were a reduction of the mistakes of the analysts by the less contact with de samples during the process as the cross-contamination. The mean Ct was lower with the BD MAX system, a fact that may indicate a better performance of the BD MAX and explain why numerous steps may be eliminated to extract and purify RNA using the extraction kit in the automated system.

Conclusion

The results obtained with BD MAX confirm it is an accurate assay, whose sensitivity is higher and whose amplification has shown good efficiency. We suggest the assay herein described could be a tool to identify the SARS-CoV-2 virus, especially in laboratories, with the growing number of tests to be performed. Additional to this, it is essential to mention that this technology could be adapted for the diagnosis of other emerging viral diseases.

The afore mentioned, does not rule out the need for areas and processing equipment, as well as the need for amplifying separately, but implies less practical time for the technician and multiplies the capability of processing more samples per day, which improves laboratory efficiency.

Acknowledgments

None.

Conflicts of interest

The authors declare no Conflicts of interest.

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