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Cristina García-Salguero[1]
Luis Vallejo[1]
Mercerdes Martínez-Rodríguez[1]
Alberto Delgado-Iribarren[1-2]
Esther Culebras[1-2]

Role of Hologic® Panther Aptima™ SARS-CoV-2 assay in the detection of SARS-CoV-2: screening or diagnostic technique?

[1]Department of Clinical Microbiology, IML and IdISSC, Hospital Clínico San Carlos, Madrid, Spain.
[2]Department of Medicine, Facultad de Medicina, Universidad Complutense, Madrid, Spain.

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ABSTRACT

During the multiple waves of COVID-19 suffered all over the world, having a rapid and sensitive diagnostic test has become a priority for microbiology laboratories. The AptimaTM SARS-CoV-2 transcription-mediated amplification (TMA) assay running on the Panther system (Hologic) was presented as a very good option to cover this need. To evaluate this system, 570 respiratory samples were included in the study and were processed both by the Panther (Hologic) system and by qRT-PCR (Thermo Fisher Science, Waltham, USA), current assay for the diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A high number of false positives (n=76) was obtained with Panther system (Hologic), but the number of false positives decreases as the relative light units (RLU) value increases. These results show that this technique can be a good option for sample screening but checking for positive results should be mandatory, especially those with low RLU values.

Keywords: SARS-CoV-2; COVID-19; RT-qPCR; Panther; TMA.

Papel del ensayo Hologic® Panther Aptima™ SARS-CoV-2 en la detección del SARS-CoV-2: ¿técnica de cribado o de diagnóstico?

RESUMEN

Durante las múltiples oleadas de COVID-19 sufridas en todo el mundo, disponer de una prueba diagnóstica rápida y sensible se ha convertido en una prioridad para los laboratorios de microbiología. El ensayo de amplificación mediada por transcripción (TMA) AptimaTM SARS-CoV-2 que se ejecuta en el sistema Panther (Hologic) se presentó como una muy buena opción para cubrir esta necesidad. Para evaluar este sistema, se incluyeron en el estudio 570 muestras respiratorias y se procesaron tanto por el sistema Panther (Hologic) como por qRT-PCR (Thermo Fisher Science, Waltham, EE. UU.), técnica utilizada actualmente para el diagnóstico del síndrome respiratorio agudo severo por coronavirus 2 (SARS-CoV-2). Se obtuvo un alto número de falsos positivos (n=76) con el sistema Panther (Hologic), pero el número de falsos positivos disminuye a medida que aumenta el valor de las unidades relativas de luz (RLU). Estos resultados muestran que esta técnica puede ser una buena opción como técnica de *screening*, pero la verificación de resultados positivos debería ser obligatoria, especialmente aquellos con valores bajos de RLU.

Palabras clave: SARS-CoV-2; COVID-19; RT-qPCR; Panther; TMA

INTRODUCTION

The multiple waves of the covid pandemic have highlighted the need for an automatic, fast and reliable technique for positive detection [1].

Current tests for the diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) relies on real-time polymerase chain reaction (qRT-PCR) diagnostic assays [2, 3]. Although this technique has many advantages, it does not allow for continuous automated random-access testing or the possibility to perform on-demand testing avoiding run series. In this sense, the Hologic Aptima transcription-mediated amplification (TMA) assay running on the Panther system (Hologic) was presented as a very good option since, in addition to having this characteristic of continuous loading of reagents and specimens during the process, it is also easy to use and fast, being able to perform a high number of determinations in one day (up to 60 per hour), which is a limiting factor for other diagnostic techniques [4].

Correspondence:
Cristina García Salguero.
Postal address: Microbiology Department.
Hospital Clínico San Carlos.
Calle Profesor Martín Lagos, s/n
28040, Madrid. Spain
E-mail: cgsalguero@salud.madrid.org

However, TMA tests have the disadvantage of not providing any type of semiquantitative result since there is no evidence of any relationship between the viral load of the samples and the units of measurement of the system (RLU). As healthcare workers often require an estimate of viral load to decide on patient management, samples with a positive result by this system (TMA) were retested by qRT-PCR in order to obtain Ct value and use it for clinical decision making. Some negative ones were also retested by qRT-PCR.

The results obtained were analyzed to determine the sensitivity of the Panther device and to check if any type of correlation could be established between the Ct obtained by qRT-PCR and the units of measure used by the Panther (RLU).

MATERIAL AND METHODS

Five hundred and seventy respiratory samples collected in a virus transport medium (VTM) were included in the study. Five hundred positive and 70 negative samples by Panther (Hologic) were re-analyzed by the TaqPath COVID-19 RT-PCR kit (Thermo Fisher Science, Waltham, USA) in order to determine the Ct. In addition, some of these samples with discordant result, were repeated by both techniques and 50 positive (10%) and 5 negative (7%) were verified by Cepheid Xpert Xpress SARS-CoV-2.

Both assays were performed following the manufacturer's instructions.

Samples were always handled in a biosafety hood and taking extreme precautions to avoid contamination and filter tips were used throughout the process.

Positive samples were selected from those with negative samples around them to ensure there was no cross contamination.

RESULTS

TaqPath COVID-19 RT-PCR confirmed the negative results in all samples detected as negative by Panther Hologic (n= 70). The results obtained with the 500 positive samples are shown in Table 1.

Of the total positive samples, four hundred twenty-four (84,8%) confirmed the result by qPCR. The remaining 76 samples (15.2%) were clearly negative by qPCR. Some of the non-concordant results (16 of 76) were repeated again by both techniques obtaining in all cases the same result as initially, and all samples retested by Cepheid Xpert Xpress SARS-CoV-2, showed 100% agreement with the qRT-PCR.

When the positive samples were divided according to the Panther RLUs, it can be seen that the percentage of false positives decreased as RLUs increased as well as the positive percent agreement (PPA) increase. The percentage of false positives goes from 80,55%, in the range >500- <1000 RLUs, to only 8,85% when the RLU value is greater than 1200.

The sensitivity was very high, 100% in all RLU ranges, but the global specificity was very low, 47,9%. However, since the number of negative samples included is much lower than the number of positives, this last data may be biased.

Although there was not a clear correlation between the RLU and Ct values, since there were high Ct values (Ct >40) in all RLU ranges, it can be seen that the lowest RLU values were associated with higher Ct ranges.

DISCUSSION

This study evaluated the usefulness of the Aptima assay for the diagnosis of SARS-CoV-2 compared to the TaqPath COVID-19 RT-PCR kit as the reference gold standard [2, 3].

Several studies have previously evaluated Hologic Panther Aptima assay ability for SARS-CoV-2 detection in comparison to different qPCR commercial kits [5, 6, 7, 8, 9]. The efficacy of the technique has already been demonstrated, although it is true that a previous study by our group [10] showed that the sensitivity of Hologic Panther Aptima assay is lower than TaqPath COVID-19 RT-PCR.

However, in all these previous studies samples were selected by qPCR results and later on analysed by Aptima assay. The approach of the present study was just the opposite and the selection of specimens' was made based on the results of Aptima to later perform the analysis by qPCR.

The results showed and excellent sensitivity and negative

Table 1 Relationship between results obtained by qRT-PCR y TMA in panther positive samples

	N	Positive qRT-PCR	Negative qRT-PCR	Ct range	False positives (%)	PPA
Panther RLU >500	500	424	76	10->40	15.2%	84.8%
Panther RLU >500- <1000	36	7	29	34->40	80.55%	19.45%
Panther RLU >1000-<1200	12	5	7	25->40	58.33%	41.67%
Panther RLU >1200	452	412	40	10->40	8.85%	91.15%

Ct (cycle threshold); PPA (positive percent agreement); RLU (relative light units)

percent agreement (100%), an acceptable positive percent agreement (84,8%), but a very low specificity (47,9%) when all samples were considered. However, the specificity improved when samples with the lowest RLU values were discarded.

Other comparator studies showed similar values of sensitivity but higher PPA values [7, 8, 9]. The differences are probably due to the dissimilar way of sample selection. The inclusion in the comparative studies of only positive samples by qPCR does not allow the detection of false positives by Hologic since all specimens are true positives.

Some of the false positives corresponded to samples from patients with previous positives who had recently become negative. So, there is a possibility that what the Hologic system detected is some viral residue with no clinical value. Nevertheless, since this was not the case in all samples, there must be other factors responsible for the false positives detected.

In conclusion, analytical validation of this study shows that the Aptima assay can be a very good tool for screening samples. This system is fast, easy to use and get ability to continuously load reagents and samples during the process; it is also able to discriminate true negative samples. However it would be recommendable to check positive results by a different technique, especially those with RLU values below 1000.

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None to declare.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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