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Performance of the QIAprepamp Viral RNA UM Kit assay (Qiagen), an automatable method for RT-qPCR detection of SARS-CoV-2 without RNA extraction

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ABSTRACT
We evaluated the performance of the QIAprepamp Viral RNA UM Kit (Qiagen) for SARS-CoV-2 detection. It displayed specificity and sensitivity required for SARS-CoV-2 RNA detection from swab transport media without RNA extraction. This method identifies accurately patients at risk of transmission while saving time and cost of extraction.

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The severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), responsible for Coronavirus Disease 19 (COVID-19), has emerged in late 2019 and become a dramatic pandemic [1,2]. In order to manage patients appropriately and control virus spread, a rapid diagnostic response is needed [3]. Gold standard diagnostic assays rely on age patients appropriately and control virus spread, a rapid diagnos-

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SARS-CoV-2 RNA was detected with the Alinity m SARS-CoV-2 AMP Kit (Abbott Diagnostics, Chicago, Illinois, USA), according to the manufacturer’s instructions. The positive samples (n = 123) presented a large range of Ct values. Inactivation was performed by mixing 500 μL of transport media with 500 μL of lysis buffer. Sample results with Ct values ≤36 (n = 101) were considered positive, while those with Ct values >36 (n = 22) were considered weakly positive [5].

SARS-CoV-2 negative samples (n = 33) were used to evaluate assay specificity, including samples positive for at least another respiratory virus, as determined by the multiplex PCR assay BioFire® Respiratory Panel 2.1 plus (Biomerieux, Marcy l’Etoile, France) (n = 26, suppl 1).

SARS-CoV-2 RNA detection was performed using QIAprep with specific primers and probes (Qiagen). This assay targets 2 viral genes (N1 and N2), detected with probes labelled with the same dye; the kit includes an internal control (a synthetic RNA) and a 2-target cell control (Beta-2-Microglobulin and RNase P genes), allowing monitoring of both PCR inhibition and sample quality.

After heat inactivation (70°C for 10 minutes), 8 μL of sample transport media were distributed on a microwell PCR plate and 2 μL of lysis buffer were added. After a 2 minutes incubation at room tem-

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In order to be able to manage tens of samples rapidly, we automati
cized the method on the QiaSymphony SP/AS platform (Qiagen). The
RT-qPCR reaction volume was the same but larger volumes of sample
and reagents were needed to allow automated handling. An extra
step was also needed because the platform has 2 modules and the
first module cannot wield small volumes. Briefly, after heat inactiva-
tion, 200 μL of sample were loaded on the SP module: 80 μL were
taken from the sample to a 1st plate, and 20 μL of lysis buffer were
added. After a 2 minutes incubation, 60 μL were transferred into a
2nd plate. The AS module then distributed 10 μL of the beforehand
prepared reaction mix in each PCR wells of a RotorGeneQ plate and
added 10 μL of prepared sample.
QIAprep detected SARS-CoV-2 RNA in all replicates of culture
supernatants up to the 10E-6 dilution. The 10E-7 dilution was the
limit of detection of both assays (Fig. 1A).
SARS-CoV-2 positive clinical samples (fresh or stored frozen,
\( n = 123 \)) were tested either with the manual or with the automated
procedure. We observed no impact of freezing or type of analysis pro-
cedure on assay performance. In 5 of 123 (4%) samples, the cell con-
trol target was not detected. These samples had been collected in a
transport medium that we determined to be not compatible with no-
extraction techniques (either sampled in Cary-Blair medium or in a
viral transport medium containing, among other products, guanidine
isothiocyanate, chelating agent and alcohol). Among the 118 samples
with interpretable results, QIAprep detected 79 of 79 (100%), 17 of 30
(56.7%), and 1 of 9 (11.1%) samples with Alinity m Ct <34, from 34 to
38, and >38, respectively (Table 1; Fig. 1B).
In 3 of 33 (9.1%) negative samples, the cell control target was not
detected due to inappropriate transport medium. In the remaining
30 samples, the specificity of the QIAprep was 100%.
In a context of ongoing pandemic, there is critical need for
diagnostic innovation in order to get results as fast as possible
and to deal with shortages. The QIAprep assay was proven a reli-
able method with excellent specificity and acceptable sensitivity
for SARS-CoV-2 detection from nasopharyngeal swabs. Its perfor-
ance was similar to that of the early developed reference
method of the French NRC for Respiratory Viruses [4]. Compared
to the highly sensitive Alinity m assay [6], the QIAprep reliably
detected samples with Ct<34, a surrogate marker of infectivity:
patients with high Ct values are known to be less contagious [7].
No extraction was required with this method, which makes it
d faster, and to our knowledge, this is the first method to provide
both an internal control and a cell control, allowing the monitor-
ing of both PCR inhibition and sample quality. However, a limit

Fig. 1. (A) Simple linear regression model fitting between culture dilutions and observed QIAprep Ct, with 95% confidence band (CB). The 10E-7 dilution was inconstantly detected (5/9) and was considered as QIAprep detection limit. Statistical analysis and figures were computed on Analyse-it Software V5.65. (B) Qiaprep Results on SARS-CoV-2 positive clinical samples according to Alinity m Ct.
of this absence of nucleic acid purification, with the possible persistence of PCR inhibitors, is the need for validation for each transport medium.

This method can be automatized. In our hands, a round of 71 samples would take 4 hours to a trained technician, and another round can be prepared once amplification starts running for the first 1. Finally, this method requires a small sample volume, even when automatized. This allows retesting, which can be advantageous for low volume samples, such as saliva, that deserves evaluation on this type of procedure.

In conclusion, the QIAprep SARS-CoV-2 assay displays very good diagnostic performances, and is an easy method to set up, relatively quick, even after automatization, and relatively inexpensive for SARS-CoV-2 RNA detection from viral transport media. Depending on the laboratory’s habits, it can be used as a backup in case of saturation or main method breakdown.

Declaration of competing interest

The authors report no conflicts of interest relevant to this article.

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Authors’ contributions

HF, LL and AMRA designed the experiments. MPS and FV performed the experiments. VE provided the culture supernatants. HF, VE and AMRA wrote the paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2022.115700.

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