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Short communication

A two minute liquid based sample preparation for rapid SARS-CoV2 real-time PCR screening: A multicentre evaluation

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ABSTRACT

Background: Apart from major health concerns associated to the SARS-coronavirus-2 (SARS-CoV-2) pandemic, also the diagnostic workflow encountered serious problems. Limited availability of kit components, buffers and even plastics has resulted in suboptimal testing procedures worldwide. Alternative workflows have been implemented to overcome these difficulties. Recently a liquid based sample prep has been launched as solution to overcome limitations in relation to nucleic acid extraction.

Objective: Multicenter evaluation of the QIAprep& Viral RNA UM kit (QIA P&A) for rapid sample preparation and real-time PCR detection of SARS-CoV-2 in comparison to standardized laboratory testing methods.

Study design: Selected samples of the routine diagnostic workflow at Clinical Microbiology Laboratories of four Dutch hospitals have been subjected to the rapid QIA P&A protocol and the results have been compared to routine diagnostic data.

Results: Combining results of manual and automated procedures, a total of 377 clinical samples of which 202 had been tested positive with a wide range of C_T values, showed almost complete concordance in the QIA P&A assay for samples up to C_T values of 33 with one exception of C_T 31. Prospectively 60 samples were tested and also showed 100 % concordance with 5 positives. The method has been automated by two centres.

Conclusions: Despite an input of only 8 μL of clinical sample, the QIA P&A kit showed good performance for sample preparation and amplification of SARS-CoV-2 and can contribute as a rapid molecular testing strategy in managing the CoV-2 pandemic.

1. Background

The SARS-CoV-2 pandemic has been and still is a challenge for the world. Although the initial identification [1] and subsequent implementation of diagnostic methods [2] was extremely fast, the pandemic could not be contained, in contrast to the 2002–2003 SARS-CoV-1 outbreak [3]. One of the reasons is the efficient spread of SARS-CoV-2, partly because also asymptotically infected individuals or patients prior clinical symptoms had been found to be contagious [4].

The worldwide spread of the virus also resulted in major complications for diagnostic laboratories, partly by insufficient access to reagents and even plastics provided by diagnostic companies. Initially lysis buffers, required for inactivation of the virus and crucial for nucleic acid

(NA) extraction from clinical samples, were the main limitation, but later also availability of plastics and even pipet tips became problematic because of the worldwide demand [5]. As a result, most laboratories have spread the risk by investing in implementation of alternative platforms for diagnosing SARS-CoV-2.

Recently, a new sample preparation solution was launched by Qiagen, the Qiaprep& Viral RNA UM (QIA P&A) kit, that combined a short, liquid based sample preparation with one-step real-time PCR amplification and detection of SARS-CoV-2, including two internal controls; one synthetic RNA IC and a sampling control amplifying transcripts of human B2M and RNaseP genes. The Research Use Only version of the kit, has been used in combination with the primers and probes developed by the CDC [6] which are included in the SARS-CoV-2

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N1 + N2 assay (Qiagen) as well as the oligos published by Corman et al. [2].

2. Objectives

In this study, the performance of the QIA P&A kit was investigated by comparing the result to those of routine diagnostic workflows, that include NA extraction procedures, in four Dutch hospital laboratories.

3. Study design

Clinical samples submitted for clinical diagnosis of SARS-CoV-2 infection to the hospital laboratories, were used for comparison between the primary diagnostic results and the QIA P&A kit. All samples were anonymized, so no approval by the Medical Ethics Committee was required as only diagnostic test results have been compared. For the QIA P&A test, 8 μ L of clinical sample in transport medium was added to 2 μ L of sample preparation buffer in a PCR plate. After 2 min of incubation, 10 μ L of reaction mixture was added and after mixing, the RNA PCR amplification protocol was started. Two centres managed to automate the workflow.

4. Results (Table 1)

The Leiden University Medical Centre (LUMC) retrospectively selected 89 clinical samples of which 36 positives as determined by the Alinity-m SARS-COV-2 assay (Abbott, USA). All samples that were positive with a C_T up to 35 could be confirmed. Of five positives with $C_T > 35$, three samples tested negative in the QIA P&A. A prospective run of 60 samples simultaneously to the Alinity-m showed complete concordance, including 5 positive samples.

The Diaconessenhuis (DIAK) selected 104 samples that had been tested in real-time PCR [2]. Of the 84 positive samples 75 were positive in the QIA P&A. Of 11 samples with C_T values over 35, only 2 were detected by the QIA P&A (18 %). Only two of the nine discrepant samples could be confirmed as positive in the GeneXpert® (Cepheid, USA) assay.

A subset of 11 positive samples was tested in two separate runs for

Table 1
Performance of QIA P&A as compared to the routine diagnostic procedures.

Manual procedure		QIA P&A vs Alinity m		Missed C_T
LUMC	N=	Concordance		
POS Cq <30	33	33	100 %	
POS Cq 30–35	3	3	100 %	
POS Cq >35	5	2	40 %	35.2, 35.9, 38.8
NEGATIVE	75	75	100 %	
DIAK		QIA P&A vs LDT/GeneXpert®		Missed C_T
	N=	Concordance		
POS Cq <30	64	64	100 %	
POS Cq 30–35	9	9	100 %	
POS Cq >35	11	2	18 %	range 36.0–39.9
NEGATIVE	20	20	100 %	
Automated procedures		QIAP&A vs LDT on QIASymphony		Missed C_T
ETZ	N=	Concordance		
QS SP/AS				
POS Cq <30	38	38	100 %	
POS Cq 30–35	16	14	87.5 %	33.7, 34.1
POS Cq >35	2	1	50 %	35.5
NEGATIVE	8	7	87.5 %	37.9
MAASSTAD		QIA P&A vs LDT on NeuMoDx96		Missed C_T
Tecan	N=	Concordance		
POS Cq <30	20	20	100 %	
POS Cq 30–35	2	1	50 %	31.0
NEGATIVE	72	63	87.5 %	9x Inhibition*

* C_T IC = 0.

analysis of the reproducibility of the QIA P&A results (data not shown). The difference in C_T values ranged from 0 (perfect duplicate) up to 2.8, without an association to viral load: one duplicate had C_T values of 23.7 and 26.5 whereas another sample (initial C_T of 32.5) resulted in C_T 35.3 and 34.9 (0.4 difference) by QIA P&A.

As automation of pipetting volumes of 2 μ L and 8 μ L may be challenging, the Elisabeth Tweesteden (ETZ) hospital managed to automate the procedure on the QIASymphony SP/AS (Qiagen) platform, using increased volumes of 20 μ L sample preparation buffer and 80 μ L clinical sample. In this way they tested 64 samples, compared to diagnostic real-time PCR targeting the E gene [2] on the QIASymphony SP/AS. The results showed that only 3 samples with C_T values of 33.3, 33.7 and 35.5 were not detected. However, one sample previously tested negative was positive in the QIA P&A with a C_T of 37.9. The Alinity m confirmed this sample to be true positive (C_T 34.7).

Maasstad hospital managed to automate the QIA P&A procedure using small pipetting volumes of 2 μ L and 8 μ L, using a Tecan Fluent 480 pipetting platform (Tecan, Switzerland). A total of 94 samples were tested using real-time PCR with sarbeco E primers [2] in the QIA P&A and the NeuMoDx™ 96 (Qiagen) as reference assay. The NeuMoDx™ is a fully automated microfluidics based PCR platform [7].

Out of 22 positive samples, 21 (95.5 %) were positive in the QIA P&A. One sample with an initial C_T value of 31.0 was missed. This sample was confirmed in an LDT with a C_T of 37.1. Out of 72 negative samples, 63 were confirmed and nine samples were inhibited based on a failed internal control, also after retesting.

Three centres tested an EQA panel provided by the Dutch National Institute of Health and Environment (RIVM, Bilthoven). Table 2 shows the results of this panel for both their routine diagnostic procedure and the QIA P&A. Although some variation in the high C_T value samples, very comparable results have been obtained. The observed specificity is depending on the primer set used. All assays gave negative results with other circulating human coronaviruses, but the E-gene primers are beta-coronavirus group specific and therefore also detected SARS-CoV-1 (sample 10).

5. Conclusions

Nucleic extraction is a crucial part of molecular diagnostic procedures and results in highly purified DNA or RNA, which may take from 30 min. up to several hours. This study shows the evaluation of a two-

Table 2
Comparative results of the 4th Dutch EQA panel for SARS-CoV-2 testing.

EQA panel	HCoV	LUMC	MAASSTAD	Tecan	ETZ	QS-AS
		DX [#]	QIA P&A	DX [#]	QIA P&A	DX [#] QIA P&A
1	SARS-CoV-2	27.1	28.9	26.9	33.7	29.1 25.5
2	HCoV-NL63	0	0	0	0	0 0
3	HCoV-229E	0	0	0	0	0 0
4	SARS-CoV-2	39.5	37.5	0	0	0 34.8
5	SARS-CoV-2	34.2	33.4	31.1	0	35.7 32.0
6	HCoV-OC43	0	0	0	0	0 0
7	SARS-CoV-2	36.7	35.2	31.1	40.8	34.7 32.3
8	Negative	0	0	0	0	0 0
9	Negative	0	0	0	0	0 0
10	SARS-CoV-1	0	0	27.7*	38.5*	29.0 *

[#] DX = routine diagnostic test.

* sarbeco PCR: reactive to betacoronaviruses, not specific to SARS-CoV-2.

minute, liquid based, sample preparation method for SARS-CoV-2 testing in comparison to different diagnostic workflows at four hospital laboratories in the Netherlands. The overall conclusion is that the QIA P&A procedure provided excellent results, given the small amount of sample that is being used. Performing the QIA P&A on 185 positive samples with C_T values up to 35 samples as determined with different platforms, resulted in 182 positive results. Only three samples with C_T values of 31.0, 33.3 and 33.7 from the two centres using automated procedures were not detected in QIA P&A. Of 18 samples with $C_T > 35$, only 5 (28 %) were detected, but the clinical relevance of this kind of results is an ongoing point of discussion.

It should be noted that the sample preparation buffer does not inactivate the SARS-CoV-2, so for proper sample handling a BSL-2 facility is required. This can be overcome by heat inactivation of the sample in transport buffers, for which 50 μ L sample at 70 °C for 10 min. would be sufficient according to the manufacturer's instructions. The QIA P&A can handle a broad range of non-fixating buffers and transport media, but is incompatible with inactivating lysis buffers containing detergents or guanidinium thiocyanate. Potentially, the inhibited samples observed in one centre, were performed on swab in lysis buffer. Unfortunately, the original sample tubes were unavailable for reinspection.

The QIA P&A can be used in combination with lab developed primer sets for other viruses as well. Early 2021, the company will launch a CE/IVD version of the kit containing specific viral target primers and probes. Altogether the QIA P&A can be considered a valuable addition to the diagnostic laboratory, either as back-up when extraction reagents or plastics are poorly delivered or, when automated, to further increase the diagnostic capacity.

Declaration of Competing Interest

None of the authors reports a conflict of interest. The QIA P&A kit

was provided by Qiagen, without further involvement in experimental design and data analysis.

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