Technical and Prospective Clinical validation of the SARS-CoV-2 RT SmartAmp assay on the GenPad device

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BACKGROUND

A previous batch of the GenPad SARS-CoV-2 assay and the GenPad analyser was validated/ The performance was shown to be comparable to the reference Corman PCR. In summary, all performance standard for sensitivity, specificity and reproducibility as well and the performance standard for the clinical validation were met. There was no cross-reactivity with other respiratory viruses included in the specificity panel and there were no false-positive GenPad results in the technical and clinical validation samples. All SARS-CoV-2 variants included in the variants panel were detected with the GenPad. The lower limit of detection of the previous batch of the GenPad assay was shown to be less than 200 copies/mL sample (see validation report in APPENDIX-1).

Recently, the manufacturer released a new version of the GenPad SARS-CoV-2 NAAT assay with a claim of improved performance in general.
The current validation study is designed to verify and demonstrate the technical and clinical performance of the new test kit. The following characteristics will be studied:

- Technical and Clinical Specificity and Cross-Reactivity
- Technical and Clinical Sensitivity

STUDY GOAL

Evaluation of the technical (laboratory) performance.
Prospective evaluation of the clinical performance of GenPad COVID-19 Test on Nasopharyngeal / Oropharyngeal swab in a public health setting.

OUTLINE OF THE STUDY

A previous validation study showed good technical sensitivity and specificity (see appendix-1). Recently, an improved GenPad device and test cartridge became available. This protocol describes a repeat technical validation of the improved system as well as a prospective clinical evaluation.
Technical validation was performed using well characterized and quantitated sensitivity-, specificity- and SARS-CoV-2 variants-panels from the RIVM. These samples are part of the external quality assessment program for laboratories performing SARS-CoV-2 diagnostics in The Netherlands (https://tinyurl.com/ypwaa52k).
For the prospective clinical validation, patients referred to a Public Health testing facility in The Netherlands (GGD Kennemerland, Schiphol XL) were included. Separate patient samples (combined NP/OP swabs) were collected simultaneously for the GenPad assay and reference PCR by the same person in the same way. So, from each patient referred to the Public Health testing facility, two NP/OP swabs were collected:

1. Standard of care swab: Standard NP/OP swab were collected in Hologics' UTM and tested using Hologic's Panther SARS-CoV-2 TMA assay (reference method). The Panther PCR provides a positive or negative results without cycle-threshold (Ct) value. The Hologic's Panther TMA assay is known to be a highly sensitive PCR. *(Gorzalski AJ, Tian H, Laverdure C, Morzunov S, Verma SC, VanHooser S, Pandori MW. High-Throughput Transcription-mediated amplification on the Hologic Panther is a highly sensitive method of detection for SARS-CoV-2. J Clin Virol. 2020 Aug;129:104501).*
2. From each patient a second NP/OP swab was collected in the SSB UTM provided with the GenPad tests. SSB UTM samples were only used for study purposes and were only tested using the GenPad system. SSB UMT vials did not contain sufficient sample for repeat testing or additional testing using other reference methods.

To minimize sampling bias, both NP/OP swabs were taken at the same time in the same way and by the same trained healthcare personnel. The reference PCR method (Panther) was performed on the same day in the laboratory by trained laboratory personnel at Streeklab Haarlem. The GenPad COVID-19 tests was performed preferably on the same day as sampling and the reference method, but at a maximum of 48 hours after sampling at Labonovum laboratories (https://labonovum.nl/en/). Samples were transported and stored at 4°C until testing. The results of the reference PCR and the GenPad were compared to evaluate the performance of the GenPad.
This study was approved by the institutional ethics review board of the VU University Medical Center (VUMC), Amsterdam, the Netherlands, and had not been found to be subject to Social Support Act (Wet maatschappelijke ondersteuning, Wmo).

MATERIALS

- GenPad:
  - All reagents, devices, and test cartridges were provided by the manufacturer.
  - Laboratory facilities were provided by Labonovum.
- Reagents and test kits for the Panther Aptima SARS-coV-2 PCR:
  - Were made available by Streeklab Haarlem as standard of care.

METHODS

Reference test method

Specimen collection and tests: A standard NP/OP swab in Hologic's Panther UTM was taken from patients referred to the Public Health testing facility of region Kennemerland (The Netherlands) for SARS-CoV-2 testing with the reference method. The Panther UTM was not compatible with the GenPad system and the SSB UTM could also not be used with the Panther PCR. Therefore, two separate NP/OP swabs were collected from these patients: one in the Panther UTM and the second swab was simultaneously taken by the same trained health care personnel, in the GenPad SSB UMT.

Reference test method: Aptima™ SARS-CoV-2 Assay (Panther™ System; Hologic) is a high-throughput NAAT. This assay combines the technologies of target capture, Transcription Mediated Amplification (TMA), and Dual Kinetic Assay (DKA) to target 2 separate regions of the SARS-CoV-2 ORF1ab gene and an intern control amplicon: https://tinyurl.com/2p87ttra. The reference test method was performed on the same day of sampling.

Unique patient barcodes on the Panther UTM and SSB UTM tubes were scanned in Lab-Dx software program to build a secure study database. Results from the reference method were primarily used for standard care purposes and additionally to assess the clinical performance of the GenPad assay.

Study test method

The GenPad test procedures was performed according to the manufacturers' instructions for use (Appendix 2: GenPad IFU).

Specimen collection and study test: Eligible patients referred to the Public Health testing facility of region Kennemerland for SARS-CoV-2 testing were asked to perform an additional OP/NP swab using SSB UTM provided with the GenPad test kit. These study swabs were only used for the GenPad assay. OP/NP swabs in SSB UTM were transported to the laboratory, together with the NP/OP swab in Hologic's UTM. The SSB UTM samples were analyzed with the GenPad device at Labonovum laboratories and preferably on the same day of sampling, but with a delay of maximum 48 hours after sampling. SSB UTM samples were kept at 4°C until GenPad testing.

Validation protocol

I. Technical validation:

The 2021.5 EQA (external quality assessment) panel SARS-CoV-2 (LEQA5 RIVM panel) was used for the technical validation. These samples are part of the external quality assessment program for laboratories performing SARS-CoV-2 diagnostics in The Netherlands (https://tinyurl.com/ypwaa52k). The LEQA-5 panel consisted of 10 simulated clinical specimens containing either whole infectious human respiratory seasonal viruses, or heat-inactivated SARS-CoV-2 viruses or no virus. SARS-CoV-2 was isolated from clinical specimens on VERO E6 cells and heat-inactivated by heat treatment at 60 °C for two hours. The number of detectable copies of SARS-CoV-2 positive strand RNA in the stocks of SARS-CoV-2 was back-calculated from determination of the copy number after extraction of RNA by digital SARS-CoV-2 E-gene and RdRP-gene PCR. Virus dilutions were made in MEM with Hanks' salts. HEp2 cells were added to the dilution at a concentration of 10.000 cells per ml panel specimen to simulate a clinical specimen.

In Table 1, all specimens are listed together with the expected target specific Cp values obtained at RIVM with routinely used diagnostic RT-qPCRs for the respective pathogens and the expected conclusion for SARS-CoV-2 detection in the specimens. The digital copies of RdRP-gene and E-gene are also listed in Table 1 for the SARS-CoV-2 containing specimens.

Table-1 LEQA5 panel composition and Ct values in real-time RT-PCR. The SARS-CoV-2 RT-PCRs were performed in 4-fold; after the average of the Ct value the number of times found positive is given between brackets (). LEQA5 panel was used for the technical validation of the GenPad system.

Panel coding	Virus[1]	Number of copies E target/ml specimen, determined with dPCR[2]	Number of copies RdRP target/ml specimen, determined with dPCR[2]	Target specific Ct[3]	E-gene (Sarbeco) Ct	RdRP-gene (SARS-CoV-2) Ct	Conclusion SARS-CoV-2
LEQA5_CoV21-1	SARS-CoV-2 BA.2 (d2)	32046	17300	-	31.33 (4/4)	31.93 (4/4)	POSITIVE
LEQA5_CoV21-2	SARS-CoV-2 BA.2 (d3)	3205	1730	-	33.99 (4/4)	34.87 (4/4)	POSITIVE
LEQA5_CoV21-3	Influenza A(H3N2) + SARS-CoV-2 Delta	550000	394000	34.25 (4/4)	28.20 (4/4)	28.64 (4/4)	POSITIVE
LEQA5_CoV21-4	SARS-CoV-2 BA.1 (d2)	19921	17300	-	33.35 (4/4)	33.26 (4/4)	POSITIVE
LEQA5_CoV21-5	SARS-CoV-2 BA.2 (d1)	320456	173000	-	27.52 (4/4)	28.11 (4/4)	POSITIVE
LEQA5_CoV21-6	SARS-CoV-2 BA.1 (d1)	199200	173000	-	29.24 (4/4)	29.75 (4/4)	POSITIVE
LEQA5_CoV21-7	SARS-CoV-2 BA.2 (d3)	3205	1730	-	33.11 (4/4)	34.85 (4/4)	POSITIVE
LEQA5_CoV21-8	hCoV-OC43	-	-	27.68 (4/4)	-	-	Negative
LEQA5_CoV21-9	No virus	-	-	-	-	-	Negative
LEQA5_CoV21-10[4]	SARS-CoV-2 BA.2 (d4)	320	173	-	34.74 (1/4)	36.20 (3/4)	Weakly POSITIVE

Samples from the RIVM panel, were evaluated using the provided GenPad cartridges with 100 uL sample volume being added to 1mL SBB-buffer of which 500 uL was loaded onto the cartridge after mixing well.

Performance standards for technical validation

Sensitivity and specificity: the results of the 10 LEQA5 samples should be identical to the RIVM results, except for educational samples. Educational samples are weakly positive and contain a virus concentration below the LLOD of most detection systems.

II. Clinical validation

Study population and design

Patients with or without specific complaints, referred to a Public Health SARS-CoV-2 testing facility (GGD Kennemerland location Schiphol-XL, The Netherlands) and with an age of 16 years and over, were recruited. Patient samples (OP/NP swabs) were collected simultaneously for both tests (the reference methods and the GenPad SARS-CoV-2 Test). Panther TMA samples were tested immediately, according to the standard of care procedure. GenPad samples were tested immediately or with a delay of maximum 48 hours, during which the samples were stored at 4°C. Sensitivity and specificity were calculated with 2x2 contingency tables, 95% confidence intervals were calculated with the Wilson confidence interval (if the proportion falls in the range [5% -95%], or with the Exact confidence interval. Statistical analyses were performed with R and RStudio (R version 4.0.3).

Number of samples

The intention was to collect at least 100 positive samples with varying range of Ct values in the reference method and at least 75 negative samples.

Inclusion criteria, recruitment and informed consent

Voluntary participants with an age of 16 years or older visiting the Public Health facility for SARS-CoV-2 testing. He or she must be able to read and understand the informed consent form. Eligible candidates were given at least 2 hours for consideration of participation by receiving an e-mail with the informed consent form at least 2 hours before their appointment. Participants were able to end their participation in the study at any time without explanation, and thus to withdraw their consent without any consequences.

Handling and storage of data, documents and study samples

To ensure privacy, all collected data was encrypted and each participant received a participant-specific identification code. All data was stored in a safe place only accessible for the study coordinators. All OP/NP samples were stored according to standard procedures and national regulations.

Performance standard for clinical validation

GenPad results of positive and negative patient samples should be comparable to those of the reference methods.

1. Specificity: agreement on samples tested negative with GenPad and the reference Panther PCR should be at least 95%. There must be no positives with GenPad, that cannot be confirmed by means of clinical follow-up analyses, or by repetition or a different NAAT method or with higher input volume.
2. Sensitivity: agreement on samples tested positive with GenPad and the reference PCR (Panther) should be at least 95%. Hence, the reference PCR (Panther) is known to be a highly sensitive PCR compared to other widely used SARS-CoV-2 PCR detection systems *(Gorzalski AJ, Tian H, Laverdure C, Morzunov S, Verma SC, VanHooser S, Pandori MW. High-Throughput Transcription-mediated amplification on the Hologic Panther is a highly sensitive method of detection for SARS-CoV-2. J Clin Virol. 2020 Aug;129:104501).* Therefore, the agreement of samples tested positive with GenPad and the Panther PCR may be lower than 95%, but not lower than 90%.

RESULTS

Technical validation

The results of the RIVM LEQA-5 panel are shown in table 1. The GenPad assay detected all positive LEQA-5 samples, except the educational samples LEQA5-CoV2_10. Educational samples contain low concentrations of SARS-CoV-2 virus which are around the lower limit of detection of commonly used SARS-CoV-2 diagnostic PCR systems.
For comparison reasons, previous results obtained with the Panther SARS-CoV-2 TMA and the GeneXpert SARS-CoV-2 kit, using the same LEQA-5 panel are shown in table 1. Results obtained with the GenPad are comparable to Panther and GeneXpert with the difference that Panther and GeneXpert also detects educational sample LEQA5-CoV2_10. However, GenPad samples were diluted 10 times before testing, whereas GeneXpert and Panther tested undiluted LEQA-5 samples. Sample 10 contains 320 E-gene target/ml sample. Sample 7 and sample 2 contain a 10-fold higher concentration of 3205 E-gene target/ml sample and were teste positive with the GenPad. The 10-fold dilution could account for the negative GenPad result obtained with sample 10.
Therefor it is apparent that the lower limit of detection of the GenPad assay is comparable to the GeneXpert and Panther SARS-CoV-2 assays.
Despite the 1:10 dilution of GenPad samples all performance standard for technical validation were met.

Table 1: GenPad results with the RIVM LEQA-5 panel. Columns designated as "RIVM data" shows the characteristics of the samples including the quantitated number of the SARS-CoV-2 E-gene targets copies/mL specimen and the expected Ct-values for E-gene targets. GenPad results (with 1:10 diluted samples) as well as results for the reference Panther PCR are shown. For comparison, previous results with the GeneXpert SARS-CoV-2 kit using the same LEQA-5 panel are also shown. LEQA samples were tested with the Panther and GeneXpert assay without dilution.

RIVM data					LIFEPAD			Panther SARS-CoV-2 TMA		GeneXpert SARS-CoV-2	
Panel Coding	Virus	Number of copies of E target/ml specimen, determined with dPCR	E-gene (Sarbeco) Ct	Conclusion SARS-CoV-2	IPC	TARGET (time to positivity)	Conclusion	Target RLU	Conclusion	Target Ct	Conclusion SARS-CoV-2
LEQA5_CoV2_1	SARS-CoV-2 BA.2 (d2)	32046	31.33	Positive	23	32	Positive	1166	Positive	32.4	Positive
LEQA5_CoV2_2	SARS-CoV-2 BA.2 (d3)	3205	33.99	Positive	26	32	Positive	1140	Positive	35.3	Positive
LEQA5_CoV2_3	Influenza A (H3N2) + SARS-CoV-2 Delta	550000	28.20	Positive	28	29	Positive	1166	Positive	27.2	Positive
LEQA5_CoV2_4	SARS-CoV-2 BA.1 (d2)	19921	33.35	Positive	26	32	Positive	1137	Positive	33.5	Positive
LEQA5_CoV2_5	SARS-CoV-2 BA.2 (d1)	320456	27.52	Positive	29	30	Positive	1131	Positive	28.0	Positive
LEQA5_CoV2_6	SARS-CoV-2 BA.1 (d1)	199200	29.2	Positive	25	34	Positive	1176	Positive	30.1	Positive
LEQA5_CoV2_7	SARS-CoV-2 BA.2 (d3)	3205	33.11	Positive	29	33	Positive	1154	Positive	35.0	Positive
LEQA5_CoV2_8	hCoV-OC43	N/A	N/A	Negative	29	N/A	Negative	282	Negative	N/A	Negative
LEQA5_CoV2_9	No virus	N/A	N/A	Negative	31	N/A	Negative	276	Negative	N/A	Negative
LEQA5_CoV2_10	SARS-CoV-2 BA.2 (d4)	320	34.74	Weakly positive	24	N/A	Negative	559	Positive	38.7	Positive

Clinical validation

General

From all patients referred to a Public Health testing facility in The Netherlands (GGD Kennemerland, Schiphol XL) between March 31, 2022 until April 07, 2022 and who were eligible to participate in this study a total of 212 were included. 181 of these included persons were considered complete cases with complete set of swabs (Panther UMT and SSB UTM) and complete set of results (Panther PCR, and GenPad PCR) available. 24 out of 212 (11.3%) included cases were considered incomplete cases due to technical error with the GenPad Software and insufficient SSB sample volume for repeat analysis. Software issues were resolved by installing the proper update and inclusion was continued. An additional 7 samples (3.3%) were considered incomplete due to invalid

results with the GenPad assay. Further analysis was done with results from the 181 complete cases.

GenPad Performance before discrepant analysis

106 out of 181 (59%) patients tested positive with the Panther TMA and 75 (41%) were negative. With GenPad, similar positivity and negativity rates were seen: 107 out of 181 cases (59%) were tested positive and 74 (41%) were tested negative. Sensitivity and specificity for GenPad were 100% and 98.7% respectively (table 1).

Table 1a: Performance of the GenPad assay compared to the reference Panther TMA assay, before discrepant analysis

		REFERENCE TEST METHOD (PANTHER TMA)		
STUDY TEST METHOD (GENPAD)		POSITIVE	NEGATIVE	**TOTAL**
	POSITIVE	106	1	107
	NEGATIVE	0	74	74
	TOTAL	106	75	181

	GenPad vs PANTHER TMA
Sensitivity	100 %, (106/106)
Specificity	98.7 %, (74/75)
PPV	99.1 % (106/107)
NPV	100 %, (74/74)

Discrepancy analysis

1 out of 181 complete cases were tested negative with Panther TMA (RLU: 272) but positive with GenPad with time to positivity of 31 minutes. Unfortunately, there was insufficient SSB sample for repeat analyses or additional analyses. The patient was a fully vaccinated (Pfizer/Moderna) 49 years old male with complaints of a common cold (runny nose and sneezing) starting 3 days before sampling. For further analysis, this sample was considered negative and thus false positive with GenPad. However, false negative Panther result, for instance due to misidentification of Panther UTM, cannot be excluded. Follow-up analysis after discrepancy analysis did not change the performance results.

CONCLUSION AND DISCUSSION

The GenPad SARS-CoV-2 assay met the criteria for technical validation. Correct results were obtained with all LEQA-5 panel samples, except for the educational sample LEQA5-CoV2_10, containing a viral concentration approximating the lower limit of detection of commonly used diagnostic SARS-CoV-2 PCR assays. The Panther and GeneXpert SARS-CoV-2 assays also detected the educational sample. However, this difference with the GenPad may not be significant since the GenPad samples were diluted 10 times before testing, whereas GeneXpert and Panther tested undiluted LEQA-5 samples. Hence, the education sample (320 E-gene copies/ml sample) is only a 1:10 dilution different compared to samples LEQA5-CoV2_2 and LEQA5-CoV2_7 (both with 3205 E-gene copies/ml). It is therefore likely that the GenPad assay would have detected the educational sample if the 1:10 dilution of the EQA samples was not required.

In the clinical validation the GenPad assay showed excellent performance compared to the Panther SARS-CoV-2 TMA. The Panther TMA assay is known as one of the most sensitive SARS-CoV-2 detection assays *(Gorzalski AJ, Tian H, Laverdure C, Morzunov S, Verma SC, VanHooser S, Pandori MW. High-Throughput Transcription-mediated amplification on the Hologic Panther is a highly sensitive method of detection for SARS-CoV-2. J Clin Virol. 2020 Aug;129:104501).*

Based on 181 patients of which 59% tested positive and 41% negative with the Panther TMA, the sensitivity and specificity of the GenPad system was 100% and 98.7% respectively. One sample was negative with the Panther TMA but positive with the GenPad. This sample was considered false negative with GenPad. However, based on the complaints of the patient and timing of the test 3 days after start of symptoms, a positive result would not have been surprising. Hence a false negative Panther result, for instance due to misidentification of the Panther UTM, cannot be excluded.

Summary

The GenPad SARS-CoV-2 assay showed excellent performance compared to the highly sensitive and specific Panther TMA assay in both the technical and clinical validation.

Considering the 1:10 dilution of GenPad samples for the technical validation, it is likely, that the performance of GenPad was similar to the Panther SARS-CoV-2 TMA. This stellar performance is reflected by the results in the clinical validation: compared to the Panther SARS-CoV-2 TMA, the sensitivity and specificity of the GenPad system were 100% and 98.7% respectively.