Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Clinical Virology 138 (2021) 104817

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Performance of the RT-LAMP-based eazyplex® SARS-CoV-2 as a novel rapid diagnostic test

Renate Egerer [a], Birgit Edel [a], Bettina Löffler [a], Andreas Henke [b], Jürgen Rödel [a,*]

[a] Institute of Medical Microbiology, Jena University Hospital, Jena, Germany
[b] Section of Experimental Virology, Institute of Medical Microbiology, Jena University Hospital, Jena, Germany

ARTICLE INFO

Keywords:
SARS-CoV-2
RT-LAMP
Without RNA extraction
Rapid diagnostic test
POC

ABSTRACT

Background: Diagnostic assays for severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) that are easy to perform and produce fast results are essential for timely decision making regarding the isolation of contagious individuals.
Objective: We evaluated the CE-approved eazyplex® SARS-CoV-2, a ready-to-use real time RT-LAMP assay for identification of the SARS-CoV-2 N and ORF8 genes from swabs in less than 30 min without RNA extraction.
Study design: Oropharyngeal and nasal swabs from 100 positive and 50 negative patients were inoculated into 0.9 % saline and tested by NeuMoDx™ RT-PCR. An aliquot was diluted fivefold in Copan sputum liquefying (SL) solution and directly analyzed by eazyplex® SARS-CoV-2. In addition, 130 patient swabs were prospectively tested with both methods in parallel. Analytical sensitivity of the assay was determined using virus stock dilutions.
Results: Positive percent agreement (PPA) between the eazyplex® SARS-CoV-2 and RT-PCR was 74 % for samples with Ct values < 35. When using a Ct cut-off ≤ 28 the PPA increased to 97.4 %. In the prospective part of the study overall PPA of the eazyplex® kit was 66.7 % but increased to 100 % when only Ct values ≤ 28 were considered. There were no false positive results. The median time to positivity was 12.5 min for the N gene and 16.75 min for ORF8. Analytical sensitivity was 3.75 $TCID_{50}$/mL. 10^5 virus copies/mL were reproducibly detected.
Conclusion: The eazyplex® SARS-CoV-2 is a rapid assay that accurately identifies samples with high viral loads. It may be useful for near-patient testing outside of a molecular diagnostic laboratory.

1. Background

Reverse transcription (RT)-PCR is considered the reference standard method for the diagnosis of severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) infection and provides high sensitivity and specificity [1–3]. However, the ongoing severe pandemic and the ability of the virus to spread easily through the population have also confronted us with limitations of PCR-based diagnostics in terms of a rapid on-site identification of positive individuals in order to break chains of infection in hospitals, care homes etc. [4]. RT-PCR is often labor-intensive, takes several hours to generate results, and requires special equipment and well-trained personnel [5,6]. In consequence delays in generating diagnostic reports can result from transporting samples to a centralized microbiological laboratory and testing samples in batches [4,7]. Recurring shortages in the supply of RNA extraction and RT-PCR kits during the pandemic are additional problems. There is a need to more rapidly identify individuals with a high viral load and increased risk to transmit the virus [8–10]. In the case of rapid diagnostic tests a lower sensitivity compared to RT-PCR may be acceptable when it is compensated by easy performance and fast results. The increasing use of rapid antigen tests (RAT) at vulnerable sites such as care homes underlines the change in diagnostic needs and strategies [4]. There are a large number of RATs that demonstrate significant differences in sensitivity and specificity, and not all tests are reliable [5,8,11–14]. Fully automated point-of-care (POC) RT-PCR assays are expensive and often in limited supply [2]. An alternative molecular technique is available by loop-mediated isothermal amplification (LAMP) [10]. The LAMP chemistry uses a robust *Bst* DNA polymerase with strand displacement activity, catalyzing high-speed amplification at constant temperature in less than half an hour. In combination with a reverse transcriptase this

* Corresponding author.
E-mail address: juergen.roedel@med.uni-jena.de (J. Rödel).

https://doi.org/10.1016/j.jcv.2021.104817
Received 7 January 2021; Received in revised form 22 March 2021; Accepted 29 March 2021
Available online 1 April 2021

method offers the potential to test samples directly without RNA extraction in a very short turn-around time. Numerous RT-LAMP protocols for identification of SARS-CoV-2 have been published but most of them are in-house methods that rely on liquid reagents and cause too much hands-on time [7,10,15–18]. The recently introduced CE-labeled RT-LAMP assay eazyplex® SARS-CoV-2 (Amplex Diagnostics, Gars Bahnhof, Germany) is based on ready-to-use lyophilized master mixes and intended for testing samples without RNA extraction.

2. Objectives

In this study we evaluated the eazyplex® SARS-CoV-2 as a rapid diagnostic assay using oropharyngeal and nasal swabs. The aim was to examine whether the test reliably identifies samples with high viral loads that are indicative for infectious persons. As reference method the NeuMoDx™ SARS-CoV-2 assay (Qiagen, Hilden, Germany), a fully automated RT-PCR performed on a random-access platform, was used [3,19].

3. Study design

3.1. Virus stock and RNA standard

The SARS-CoV-2 strain Jena/2020/5159 was isolated from a respiratory patient sample in our laboratory in April 2020 and used to initially assess the analytical sensitivity of the eazyplex® assay [20]. The titer of the stock was 10^7 $TCID_{50}$/mL. Two-fold serial dilutions starting at 30 $TCID_{50}$/mL were prepared with 0.9 % saline and Copan sputum liquefying (SL) solution, a phosphate buffer solution containing dithiothreitol (DTT).

The INSTAND reference standard Ch07470 (strain BetaCoV/Munich/ChVir984/2020) was used to determine the limit of detection in relation to RNA copies/mL (INSTAND e.V., Scientific Medical Society for Promotion of Quality Assurance in Medical Laboratories, Düsseldorf, Germany). This standard contained a viral load of 10^6 RNA copies/mL. Virus concentrations of 10^5 and 10^4 copies/mL were prepared in 0.9 % saline and the samples were diluted fivefold in Copan SL solution before testing by eazyplex® RT-LAMP.

3.2. Clinical samples

Clinical samples were oropharyngeal and nasal swabs from symptomatic patients and oropharyngeal swabs from asymptomatic individuals collected for screening purposes. For the retrospective part of the study 150 samples with a positive or negative RT-PCR result were evaluated by the eazyplex® SARS-CoV-2 assay. For the prospective part of the study 130 samples were tested in parallel with both assays. Due to shortages in the supply of swab systems with virus transport media, patient samples were collected with dry cotton, rayon, and nylon-flocked swabs purchased from Greiner Bio-One (Frickenhausen, Germany) and Copan (distributed by Mast Diagnostica, Reinfeld, Germany). The swabs were discharged into 1 mL 0.9 % saline and kept at 4 °C until testing.

3.3. eazyplex® SARS-CoV-2 RT-LAMP assay and NeuMoDx™ reference RT-PCR

The eazyplex® SARS-CoV-2 RT-LAMP assay is a ready-to-use test strip containing lyophilized master mixes for SARS-CoV-2 N gene, SARS-CoV-2 ORF8 gene, inhibition control, sample control and a lysis control (exclusion of unspecific fluorescence during amplification) in one well each. The test kit also contains ready-to-use tubes with resuspension and lysis fluid (RALF) as reaction buffer. Because we could not use swabs with virus transport medium the samples were prediluted fivefold in Copan SL solution and an aliquot of 25 µl was mixed with 500 µl RALF instead of using 5 µl of the original sample directly as recommended by the manufacturer. 25 µl of the prepared suspension was immediately pipetted into each well and the test strip was gently knocked to remove air bubbles. Tests were run on a Genie II Mk2A device (Amplex Diagnostics) at 65 °C for 25 min. Amplification was measured by real-time fluorescence detection using an intercalating dye. Test results were automatically calculated and reported by the integrated eazyReport™ software on the Genie II instrument. A sample was positive if either the N or ORF8 gene or both were detected.

For NeuMoDx™, 700 µl of undiluted original sample was loaded onto the NeuMoDx™ 96 Molecular System. The NeuMoDx™ SARS-CoV-2 assay (Ref. 300,800) targeting the N and Nsp2 gene was used as instructed by the manufacturer's protocol.

3.4. Statistical analysis

The qualitative performance of the assays was assessed by calculating the positive and negative percent agreement (PPA and NPA) as well as positive and negative predictive values (PPV and NPV). NeuMoDx™ test results were defined as the reference. Concordance of the two diagnostic tests was examined by Cohen's κ coefficient analysis.

4. Results

Prior to testing the clinical samples the analytical sensitivity of the eazyplex® assay was examined using two-fold $TCID_{50}$ dilution series of a virus stock. Dilutions were made in 0.9 % saline which was also used for the elution of clinical specimens from dry swabs and in Copan SL solution which has proven to be a reagent well suited for RT-LAMP sample preparation in a previous study [20]. The eazyplex® SARS-CoV-2 achieved reliable detection of SARS-CoV-2 at 3.75 $TCID_{50}$/mL, corresponding to 0.004 $TCID_{50}$/reaction (Table 1). This finding was in good agreement with the analytical sensitivity of an earlier version of this assay based on liquid master mix reagents [20].

The manufacturer of the eazyplex® assay recommends to elute dry swabs in Copan SL solution and to use 25 µl as sample volume for mixing with the RALF reaction buffer. When using the same volume from swabs with saline we observed that several oropharyngeal samples containing a high viral load (RT-PCR Ct values < 25) were inhibitory to RT-LAMP. This could be repealed when samples were five-fold diluted in Copan SL solution and 25 µl of this preparation was mixed with the RALF reaction buffer. This procedure resulted in a sample volume input of 5 µl for testing, corresponding to the manufacturer's instructions for the use of swabs with viral transport medium.

To assess the diagnostic performance 100 RT-PCR-positive and 50 negative swab samples were retrospectively tested by RT-LAMP within 24 h. A sample was interpreted as positive if at least one of the two target genes was detected. The eazyplex® assay showed 100 % NPA and 74 % PPA, compared to NeuMoDx™ reference RT-PCR whereby only samples with Ct $\leq$ 35 were included in this part of the test evaluation (Table 2). PPA increased to 84.1 and 97.4 % when Ct cut-offs of 30 and 28 were defined, respectively (Table 2). At Ct values $\leq$ 28 an almost perfect agreement with RT-PCR was achieved as indicated by a Cohen's k of 0.97. The median threshold time of positive signals was only 12.5 min for the N gene and 14.25 min for the ORF8 gene (Table 3). There was no correlation of the RT-LAMP threshold time with RT-PCR Ct values; however, samples that yielded a positive signal for only one gene in the eazyplex® assay had higher median Ct values [26.7 (IQR 24.4–27.2) for N and 27 (IQR 25.8–28.3) for Nsp2] than those tested positive for both targets [21.3 (IQR 19.6–23.6) for N and 22.5 (20.6–24.8) for Nsp2].

To calculate PPVs and NPVs we prospectively tested 130 swabs in parallel to NeuMoDx™. It should be noted that in our laboratory this fully-automated and rapid RT-PCR is preferentially used for symptomatic patients who have to be hospitalized and contact persons of positive individuals but not for broad screening purposes. The RT-PCR positivity rate of the samples was 12.3 %. Regardless of the RT-PCR Ct values the eazyplex® SARS-CoV-2 showed a PPA of 66.7 % and a NPA of 100 %

Table 1
Limits of detection of the eazyplex® SARS-CoV-2 assay directly conducted on virus stock dilutions without RNA isolation.

Virus concentration		Dilution buffer: Copan SL solution		Dilution buffer: 0.9 % saline	
		Positive replicates, median threshold time, min (IQR)		Positive replicates, median threshold time, min (IQR)	
$TCID_{50}$/mL	$TCID_{50}$/reaction	N gene	ORF8 gene	N gene	ORF8 gene
30	0.036	7/7, 10.5 (10–13.5)	7/7, 11.5 (11–12.25)	5/5, 12 (10.25–12.5)	5/5, 14 (12.75–14.75)
15	0.018	7/7, 12.25 (10.75–17.5)	7/7, 14.25 (12.75–18)	5/5, 12 (11.25–14.75)	5/5, 15.25 (14.5–15.75)
7.5	0.009	7/7, 11.25 (10.5–14.5)	7/7, 14 (13.75–17.75)	5/5, 17.75 (13.25–20.25)	5/5, 18.75 (15.5–20.75)
3.75	0.004	7/7, 16 (13.75–17.5)	7/7, 19 (18–23)	5/5, 12.75 (12.5–18)	5/5, 18.5 (15.25–21.5)
1.875	0.002	5/7, 17.5 (13.25–23)	6/7, 19 (16–24)	2/5, 22.5/23	1/5, 22
0.9375	0.001	1/7, 24.5	0/7	0/7	0/7

IQR, interquartile range.

Table 2
Results of the eazyplex® SARS-CoV-2 assay for samples retrospectively tested in comparison to NeuMoDx™ RT-PCR.

NeuMoDx™		eazyplex®			PPA, % (CI)	NPA, % (CI)	Cohen's κ (CI)
		Positive	Negative	Total			
Ct values <35[a]	Positive	74	26	100	74 (64.3–82.3)	100 (92.9–100)	0.65 (0.54–0.77)
	Negative	0	50	50			
	Total	74	76	150			
Ct values ≤30[a]	Positive	74	14	88	84.1 (74.8–91)	100 (92.9–100)	0.79 (0.69–0.89)
	Negative	0	50	50			
	Total	74	64	138			
Ct values ≤28[a]	Positive	74	2	76	97.4 (90.8–99.7)	100 (92.9–100)	0.97 (0.92–1)
	Negative	0	50	50			
	Total	74	52	126			

PPA, positive percent agreement; NPA, negative percent agreement; CI, 95 % confidence interval.
[a] for N or/and Nsp2 gene.

Table 3
Time to positivity of the eazyplex® SARS-CoV-2 for clinical samples.

Target	Median threshold time, min (IQR)
N gene	12.5 (10.1–16)
ORF8 gene	14.25 (10.75–18)
Sample control (human DNA)	15.5 (12–18.75)
Inhibition control	8.25 (7.75–8.5)

IQR, interquartile range.

(Table 4). When Ct values of 30 and 28 were defined as cut-offs PPAs increased to 84 % and 100 %, respectively (Table 4). The corresponding NPVs were 97.2 % and 100 % (Table 4). In combination with a PPV of 100 % the eazyplex® assay showed a high accuracy of test results for samples containing a high viral load.

When positive and negative eazyplex® results from both parts of the study were combined, the median RT-PCR Ct values of samples detected positive by RT-LAMP were 23 (IQR 20.3–25.2) for N and 23.9 (IQR 21.3–26.3) for Nsp2 whereas RT-LAMP-negative samples had median Ct values of 30.2 (IQR 29.1–33.9) for N and 30.7 (IQR 30–33.2) for Nsp2 (Fig. 1). To establish a relationship between these values and virus RNA copies/mL a laboratory reference standard provided by INSTAND was examined. This standard was processed in the same manner as a clinical sample. At a concentration of 10^5 RNA copies/mL the eazyplex® assay was reliable to identify all parallels at least by a positive signal for one of the two targets. The corresponding median Ct values of NeuMoDx™ RT-PCR were 25.7 for N and 25.9 for Nsp2 (Table 5).

5. Discussion

The ongoing SARS-CoV-2 pandemic with high incidences in many countries stresses the urgent need for rapid diagnostic tests that can be performed on-site or at least technically independent of laboratories with specialized molecular testing facilities. POC-qualified tests may be useful to control the spread of infections if they are reliable to rapidly identify symptomatic and asymptomatic patients who carry a high viral load in the upper respiratory tract (URT) and pose a high risk of transmitting the virus.

The results of this study show that the eazyplex® SARS-CoV-2 is a suitable assay to identify samples with a high viral load in less than

Table 4
Diagnostic performance of the eazyplex® SARS-CoV-2 assay for prospective analysis of nasal and oropharyngeal swabs in comparison to NeuMoDx™ RT-PCR.

NeuMoDx™		eazyplex®			PPA, % (CI)	NPA, % (CI)	PPV, %	NPV, % (CI)
		Positive	Negative	Total				
All Ct values	Positive	16	8	24	66.7 (44.7–84.4)	100 (98.8–100)	100	93 (88.3–95.9)
	Negative	0	106	106				
	Total	16	114	130				
Ct values ≤30[a]	Positive	16	3	19	84.2 (60.4–96.6)	100 (96.6–100)	100	97.2 (92.6–99)
	Negative	0	106	106				
	Total	16	109	125				
Ct values ≤28[a]	Positive	13	0	13	100 (76.8–100)	100 (96.6–100)	100	100
	Negative	0	106	106				
	Total	13	106	119				

PPA, positive percent agreement; NPA, negative percent agreement; CI, 95 % confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[a] for N or/and Nsp2 gene.

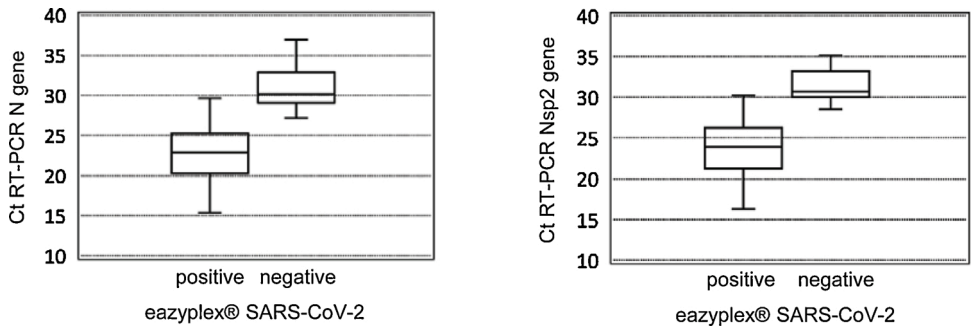

Fig. 1. NeuMoDx™ RT-PCR values for eazyplex® RT-LAMP-positive (n = 90) and negative test results (n = 34) in clinical samples.

Table 5
Detection of different virus concentrations by the eazyplex® SARS-CoV-2 using the INSTAND reference standard.

Copies/mL	eazyplex®				NeuMoDx™	
	Copies/reaction	Positive replicates, median threshold time, min (IQR)		Positive replicates, N or/and ORF8-positive	Positive replicates, median Ct	
		N gene	ORF8 gene		N gene	Nsp2 gene
10^6	240	6/6, 13 (12.75–13.75)	6/6, 15.5 (13.75–20.25)	6/6	4/4, 22.2	4/4, 23
10^5	24	4/6, 19.5 (16.5–20.25)	4/6, 24.5 (23.5–24.75)	6/6	4/4, 25.7	4/4, 25.9
10^4	3	1/6, 24.5	0/6	1/6	4/4, 29.1	4/4, 29.8

IQR, interquartile range.

30 min. It detected SARS-CoV-2-positive samples with NeuMoDx™ RT-PCR Ct values ≤28 with a PPA (sensitivity) of 97 %. Absolute Ct values cannot be used to exactly define a general cut-off for virus concentrations that are characteristic for infectious patients [6]. The estimation of infectivity from the Ct value is dependent on variables of the RT-PCR test system and target genes, and studies that evaluated RT-PCR and virus culture in parallel have produced divergent findings [4,21,22]. The eazyplex® assay reliably detected about 10^5 virus copies/mL. This sensitivity may be sufficient for identifying infectious individuals because several studies have shown that SARS-CoV-2 isolation in culture is typically positive for specimens containing ≥10^6 virus copies/mL [23,24]. This concentration was detected by NeuMoDx™ RT-PCR with Ct values ≤ 24, corresponding to Ct thresholds for infectivity suggested by a recent study [22].

The eazyplex® SARS-CoV-2 reliably detected ≤ 24 virus copies/reaction. Therefore, it can be concluded that the lower sensitivity of the assay for clinical samples compared to RT-PCR is not due to the RT-LAMP amplification reaction itself but to the use of a substantially smaller sample volume for testing. The eazyplex® SARS-CoV-2 opens the possibility to test swab samples without RNA extraction and a minimum of hands-on time that takes less than 5 min for a single specimen. However, although the *Bst* polymerase is very robust the additional reverse transcriptase as a compound of the master mix may be the Achilles heel because inhibitory effects were observed in some samples when used undiluted. This may be due to tissue compounds such as increased sugar and salt concentrations [17]. Similar effects have been described when conducting RT-PCR on raw samples [25,26]. A limitation of this study was that no optimal swab system could be defined and evaluated due the limited availability of several transport systems. Therefore, a compromise for the test performance had to be found and samples were diluted in Copan SL solution before being tested. Unfortunately, the internal and sample control included in the test strip only check the activity of the polymerase and the presence of DNA, respectively.

In principle, the eazyplex® SARS-CoV-2 may be used as a viable alternative to RAT. However, widespread use in POC diagnostics may be hampered by the need for a special device even though the Genie II is a small battery-loaded portable machine and sample preparation does not require any additional equipment other than a calibrated pipette.

There may be two applications in diagnostic workflows. For symptomatic hospitalized patients the test might be used as a first-step screening assay for fast diagnosis of COVID-19 whereby eazyplex® assay should be performed preferentially within the first week of symptoms when high virus concentrations in the URT can be expected. Because the eazyplex SARS-CoV-2 shows a lower sensitivity (PPA) compared to RT-PCR a negative test result for symptomatic patients has to be verified by RT-PCR. A more important application may be the testing of mildly symptomatic individuals who do not need to be hospitalized and asymptomatic contact persons. When it can be assumed that higher infectious viral loads would be reliably identified a negative eazyplex® results would preclude further testing when symptoms do not increase [4].

The eazyplex® SARS-CoV-2 targets two viral genes. As shown here sensitivity was thereby improved [2]. However, it should be noted that one target, the ORF8 gene, is frequently affected by mutations in coronaviruses, occasionally leading to milder infections [27]. The recently emerged SARS-CoV-2 variant B.1.1.7 shows mutations not only in the gene of the spike protein but also in other genes including N and ORF8, but these mutations are not relevant for primer binding (in silico analysis, personal information by Amplex Diagnostics) [28].

In conclusion this study demonstrates the ability of the eazyplex® assay to reliably and quickly detect SARS-CoV-2 from swabs containing high viral loads. The test is robust, easy to perform, and can be used outside a molecular diagnostic laboratory.

Ethical statement

The study protocol for the evaluation of SARS-CoV-2 RT-LAMP assays for clinical samples was reviewed and approved by the ethics committee of the Jena University Hospital (2019−1549_1-MV).

CRediT authorship contribution statement

Renate Egerer: Investigation, Writing - review & editing. **Birgit Edel:** Methodology, Writing - review & editing. **Bettina Löffler:** Writing - review & editing, Project administration. **Andreas Henke:** Methodology, Investigation. **Jürgen Rödel:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft,

Writing - review & editing, Project administration.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

We are grateful to Amplex Diagnostics for providing the eazyplex® SARS-CoV-2 kits. We thank Beate Haschke, Christina Gödicke, Birgit Jahn, Beatrice Sommer-Schmid, and Peggy Plötner for excellent technical assistance. The study was supported by internal funding.

References

[1] Z.C. Brooks, S. Das, COVID-19 testing: impact of prevalence, sensitivity, and specificity on patient risk and cost, Am. J. Clin. Pathol. 154 (2020) 575–584, https://doi.org/10.1093/ajcp/aqaa141.
[2] H.H. Mostafa, J. Hardick, E. Morehead, et al., Comparison of the analytical sensitivity of seven commonly used commercial SARS-CoV-2 automated molecular assays, J. Clin. Virol. 130 (2020), https://doi.org/10.1016/j.jcv.2020.104578, 104578.
[3] H.H. Mostafa, D.M. Lamson, K. Uhteg, et al., Multicenter evaluation of the NeuMoDx™ SARS-CoV-2 test, J. Clin. Virol. 130 (2020), https://doi.org/10.1016/j.jcv.2020.104583, 104583.
[4] F.J. Candel, P. Barreiro, J. San Román, et al., Recommendations for use of antigenic tests in the diagnosis of acute SARS-CoV-2 infection in the second pandemic wave: attitude in different clinical settings, Rev. Esp. Quimioter. 33 (2020) 466–484, https://doi.org/10.37201/req/120.2020.
[5] F.M. Liotti, G. Menchinelli, E. Lalle, et al., Performance of a novel diagnostic assay for rapid SARS-CoV-2 antigen detection in nasopharynx samples, Clin. Microbiol. Infect. (2020), https://doi.org/10.1016/j.cmi.2020.09.030.
[6] Y.-P. Tu, T.J. O'Leary, Testing for severe acute respiratory syndrome-coronavirus 2: challenges in getting good specimens, choosing the right test, and interpreting the results, Crit. Care Med. 48 (2020) 1680–1689, https://doi.org/10.1097/CCM.0000000000004594.
[7] M.F. Österdahl, K.A. Lee, M. Ni Lochlainn, et al., Detecting SARS-CoV-2 at point of care: preliminary data comparing loop-mediated isothermal amplification (LAMP) to polymerase chain reaction (PCR), BMC Infect. Dis. 20 (2020), https://doi.org/10.1186/s12879-020-05484-8, 783.
[8] F. Cerutti, E. Burdino, M.G. Milia, et al., Urgent need of rapid tests for SARS-CoV-2 antigen detection detection: evaluation of the SD-Biosensor antigen test for SARS-CoV-2, J. Clin. Virol. 132 (2020), https://doi.org/10.1016/j.jcv.2020.104654, 104654.
[9] P.-E. Fournier, C. Zandotti, L. Ninove, et al., Contribution of VitaPCR SARS-CoV-2 to the emergency diagnosis of COVID-19, J. Clin, Virol. 133 (2020), https://doi.org/10.1016/j.jcv.2020.104682, 104682.
[10] Y. Kitagawa, Y. Orihara, R. Kawamura, et al., Evaluation of rapid diagnosis of novel coronavirus disease (COVID-19) using loop-mediated isothermal amplification, J. Clin. Virol. 129 (2020), https://doi.org/10.1016/j.jcv.2020.104446, 104446.
[11] E. Albert, I. Torres, F. Bueno, et al., Field evaluation of a rapid antigen test (Panbio™ COVID-19 Ag Rapid Test Device) for COVID-19 diagnosis in primary healthcare centres, Clin. Microbiol. Infect. (2020), https://doi.org/10.1016/j.cmi.2020.11.004.
[12] M. Linares, R. Pérez-Tanoira, A. Carrero, et al., Panbio antigen rapid test is reliable to diagnose SARS-Cov-2 infection in the first days after the onset of symptoms, J. Clin. Virol. 133 (2020), https://doi.org/10.1016/j.jcv.2020.104659, 104659.
[13] G.C.K. Mak, S.S.Y. Lau, K.K.Y. Wong, et al., Analytical sensitivity and clinical sensitivity of the three rapid antigen detection kits for detection of SARS-CoV-2 virus, J. Clin. Virol. 133 (2020), https://doi.org/10.1016/j.jcv.2020.104684, 104684.
[14] T. Toptan, L. Eckermann, A.E. Pfeiffer, et al., Evaluation of a SARS-CoV-2 rapid antigen test: potential to help reduce community spread? J. Clin. Virol. 135 (2021) https://doi.org/10.1016/j.jcv.2020.104713, 104713.
[15] V.L. Fowler, B. Armson, J.L. Gonzales, et al., A highly effective reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the rapid detection of SARS-CoV-2 infection, J. Infect. (2020), https://doi.org/10.1016/j.jinf.2020.10.039.
[16] J.Y.H. Lee, N. Best, J. McAuley, et al., Validation of a single-step, single-tube reverse transcription loop-mediated isothermal amplification assay for rapid detection of SARS-CoV-2 RNA, J. Med. Microbiol. 69 (2020) 1169–1178, https://doi.org/10.1099/jmm.0.001238.
[17] L. Mautner, C.-K. Baillie, H.M. Herold, et al., Rapid point-of-care detection of SARS-CoV-2 using reverse tzranscription loop-mediated isothermal ampolifiaction (RT-LAMP), Virol. J. 17 (2020), https://doi.org/10.1186/s12985-020-01435-6, 160.
[18] B. Schermer, F. Fabretti, M. Damagnez, et al., Rapid SARS-CoV-2 testing in primary material based on a novel multiplex RT-LAMP assay, PLoS One 15 (2020), e0238612, https://doi.org/10.1371/journal.pone.0238612.
[19] A. Lima, V. Healer, E. Vendrone, S. Silbert, Validation of a modified CDC assay and performance comparison with the NeuMoDx™ and DiaSorin® automated assays for rapid detection of SARS-CoV-2 in respiratory specimens, J. Clin. Virol. 133 (2020), https://doi.org/10.1016/j.jcv.2020.104688, 104688.
[20] J. Rödel, R. Egerer, A. Suleyman, et al., Use of the variplex™ SARS-CoV-2 RT-LAMP as a rapid molecular assay to complement RT-PCR for COVID-19 diagnosis, J. Clin. Virol. 132 (2020), https://doi.org/10.1016/j.jcv.2020.104616, 104616.
[21] J. Bullard, K. Dust, D. Funk, et al., Predicting infectious severe acute respiratory syndrome coronavirus 2 from diagnostic samples, Clin. Infect. Dis. 71 (2020) 2663–2666, https://doi.org/10.1093/cid/ciaa638.
[22] B. La Scola, M. Le Bideau, J. Andreani, et al., Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards, Eur. J. Clin. Microbiol. Infect. Dis. 39 (2020) 1059–1061, https://doi.org/10.1007/s10096-020-03913-9.
[23] R.A.P.M. Perera, E. Tso, O.T.Y. Tsang, et al., SARS-CoV-2 virus culture and subgenomic RNA for respiratory specimens from patients with mild coronavirus disease, Emerg. Infect. Dis. 26 (2020) 2701–2704, https://doi.org/10.3201/eid2611.203219.
[24] J.J.A. van Kampen, D.A.M.C. van de Vijver, P.L.A. Fraaij, et al., Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19): duration and key determinants, medRxiv (2020), https://doi.org/10.1101/2020.06.08.20125310.
[25] C. Beltrán-Pavez, L.A. Alonso-Palomares, F. Valiente-Echeverría, et al., Accuracy of a RT-qPCR SARS-CoV-2 detection assay without prior RNA extraction, J. Virol. Methods 287 (2021), https://doi.org/10.1016/j.jviromet.2020.113969, 113969.
[26] N. Merindol, G. Pépin, C. Marchand, et al., SARS-CoV-2 detection by direct rRT-PCR without RNA extraction, J. Clin. Virol. 128 (2020), https://doi.org/10.1016/j.jcv.2020.104423, 104423.
[27] B.E. Young, S.-W. Fong, Y.-H. Chan, et al., Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study, Lancet 396 (2020) 603–611, https://doi.org/10.1016/S0140-6736(20)31757-8.
[28] Public Health England, Investigation of novel SARS-CoV-2 variant: variant of concern 202012/01. https://www.gov.uk/government/publications/investigation-of-novel-sars-cov-2-variant-variant-of-concern-20201201 (accessed 28 December 2020).