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Use of the variplex™ SARS-CoV-2 RT-LAMP as a rapid molecular assay to complement RT-PCR for COVID-19 diagnosis

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ABSTRACT

Background: Molecular assays based on reverse transcription-loop-mediated isothermal amplification (RT-LAMP) may be useful for rapid diagnosis of the severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) because of the easy performance and the option to bypass RNA extraction.
Objectives: This study was designed to evaluate the clinical performance of the CE-labeled variplexTM real time SARS-CoV-2 RT-LAMP assay in comparison to commercial RT-PCRs.
Study design: RNA extracted from pharyngeal swabs was tested by variplex™ RT-LAMP and Corman's LightMix™ E gene RT-PCR as reference. Samples of respiratory secretions from Coronavirus infection disease (COVID-19) and negative control patients were analyzed by variplex™ without RNA extraction and tested in parallel with the Allplex™ and VIASURE BD MAX RT-PCRs.
Results: Using isolated RNA variplex™ RT-LAMP showed a sensitivity of 75 % compared to LightMix E gene RT-PCR but contrary to the latter it produced no false-positive results. For the evaluation of samples from respiratory secretions concordance analysis showed only a moderate agreement between the variplex™ RT-LAMP conducted on unprocessed samples and Allplex™ and VIASURE RT-PCRs (Cohen's κ ranging from 0.52−0.56). Using the approach to define a sample as true-positive when at least two assays gave a positive result the clinical sensitivities were as follows: 76.3 % for variplex™, 84.2 % for Allplex™ and 68.4 % for VIASURE. However, when results of RT-PCR and RT-LAMP were combined diagnostic sensitivity was increased to 92–100 %.
Conclusion: The variplex RT-LAMP may serve as a rapid test to be combined with a RT-PCR assay to increase the diagnostic accuracy in patients with suspected COVID-19 infection.

1. Background

The severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) pandemic has already caused an enormous burden on healthcare systems worldwide [1]. Because the virus can be transmitted rapidly by direct contact and aerosols and it causes a severe life-threatening syndrome in a part of the patients timely and accurate diagnosis of Coronavirus infection disease (COVID-19) is essential to reduce virus spread and save patients' lives by starting appropriate treatment and care as soon as possible [2–4] Chest computed tomography (CT) imaging is a sensitive method to identify lung infection by SARS-CoV-2 in an early stage but it is not specific [1,3,5]. Reverse transcription (RT)-PCR actually serves as the gold standard laboratory test for confirmation of clinical infection and screening of contact persons [6]. A large number of assays adapted on commonly used technical platforms are currently flooding the market [4]. As viral targets of RT-PCR assays sequences of the envelope (E), nucleocapsid (N), spike (S), membrane protein (M), open reading frame 1ab (ORF1ab), and RNA-dependent RNA polymerase (RdRP) genes are used [2,6]. Although RT-PCR offers high analytical sensitivity several studies reported on false-negative as well as fluctuating results in patients whose clinical diagnosis using chest CT was in accordance with COVID-19 [2,7]. Problems with clinical sensitivity of nucleic acid amplification tests can be due to analytical errors of RNA isolation procedures and choose of inadequate primers. Other challenges in diagnostics are associated with the significantly increased requests for testing, resulting in time delays to generate diagnostic reports [8,9].

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Moreover, mass testing has rapidly caused serious shortages in the supply of RNA purification kits in many countries [9,10]. For a rapid diagnosis of SARS-CoV-2 cost-effective methods with low hands-on time that circumvent limitations of RT-PCR may be helpful tools for a routine diagnostic workflow [9,11]. RT-loop-mediated isothermal amplification (LAMP) may offer the possibility to be established as an alternative diagnostic technique [12–14]. The combination of RT with *Bst* polymerase possessing a DNA strand displacement activity allows amplification of target genes at a constant temperature in less than one hour. RNA purification can be bypassed depending on the sample type and different transport media because of the robustness of the polymerase.

2. Objectives

There are several studies that demonstrated satisfying sensitivity and specificity of RT-LAMP for SARS-CoV-2 detection but little is known about its performance of testing clinical samples directly without RNA extraction [11–14]. In this study we evaluated the newly introduced CE-labeled variplex™ SARS-CoV-2 LAMP assay and compared the clinical performance with commercial RT-PCR tests. Testing was performed using pharyngeal washes and samples from respiratory secretions, including sputum, endotracheal secretions, and bronchoalveolar lavage.

3. Study design

3.1. Pharyngeal swabs, RNA extraction, and envelope (E) gene screening RT-PCR

Pharyngeal specimens were collected using eSwab™ transport systems (Copan, Brescia, Italy). Total viral RNA was extracted from 200 µl of the sample medium using the QIAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Hilden, Germany. Extraction was performed on the automated Qiasymphony SP instrument (Qiagen). Purified RNA was eluted in 60 µl AVE buffer and divided into two parts for testing. To rule out cross-reactivity with human coronaviruses 229E and OC43 external quality assessment samples (INSTAND e.V., Düsseldorf, Germany) were processed in a similar manner. Reference RT-PCR was performed using the LightMix® Modular SARS-CoV E-gene primers (TIB Molbiol, Berlin, Germany) and the LightCycler® Multiplex RNA Virus Master (Roche, Penzberg, Germany) [15]. RT-PCR was run on a LightCycler 480 (Roche, Penzberg, Germany).

3.2. Variplex™SARS-CoV-2 RT-LAMP assay using extracted RNA

The variplex™ SARS-CoV-2 is a qualitative molecular assay using a mix of 6 oligonucleotide primers targeting a 282-bp sequence of the membrane protein (M) gene. For a single test 15 µl of RT master mix and 8 µl of eluted RNA were pipetted into two wells of a Genie® test strip (Amplex Diagnostics). 2 µl of the primer mixes for SARS-CoV-2 or the inhibition control were added to one each well. Tests were run at 65 °C for 40 min using a Genie II Mk2A device (Amplex Diagnostics). Amplification was measured by real-time fluorescence detection using a DNA intercalating dye. Data interpretation and calculations were automatically performed by the integrated eazyReport™ software (Amplex Diagnostics).

3.3. Direct testing of respiratory samples by variplex™SARS-CoV-2 RT-LAMP and comparison with the VIASURE and Allplex™ RT-PCR assays

Respiratory samples, including sputum, endotracheal secretions, bronchoalveolar lavages, and pharyngeal washes, collected form COVID-19 patients with an initially positive E gene screening RT-PCR were used for direct RT-LAMP without RNA purification. Specimens from patients with multiple negative E gene RT-PCR results served as negative controls. In a first step all samples were mixed in a ratio of ≤1:1 with Copan sputum liquifying (SL) solution containing dithiothreitol (1 mL ready-to use tubes, Copan). 75 µl of the suspension was pipetted into 500 µl of LPTV lysis buffer (Amplex Diagnostics) and gently mixed. From this mixture 8 µl were pipetted into two wells of a Genie test strip. 15 µl of RT master mix and 2 µl of primer mixes for SARS-CoV-2 or the inhibition control were added. An additional well consisted of 10 µl sample/LPTV buffer and 15 µl of RT master mix only and served as a lysis control to exclude the occurrence of unspecific fluorescence during amplification.

For comparative RT-PCR analysis the suspensions were processed using the Allplex™ 2019-nCoV assay which includes the E, N and RdRP genes as viral targets, and the VIASURE SARS-CoV-2 (S gene)-BD MAX™ system. For the Allplex™ assay RNA was isolated from 200 µl of the sample using the QIAsymphony DSP Virus/Pathogen Mini Kit. 8 µl of RNA was added to 17 µl of the master mix as described in the manufacturer's instructions. RT-PCR was run on a CFX96 Real-Time PCR Detection System (Bio-Rad, Feldkirchen, Germany). For the VIASURE assay 200 µl of the sample was used for RNA extraction. VIASURE rehydration buffer and gene reaction tubes containing a ready-to-use master mix were loaded onto BD MAX™ ExK™ TNA-3 reagent strips. Nucleic acid extraction and real time RT-PCR were performed on the automated BD MAX™ system (BD).

3.4. Virus stock dilutions

To assess the analytical sensitivity of the assays the SARS-CoV-2 isolate Jena/2020/5159 propagated and titrated on Vero-76 cells was used. 10-fold serial dilutions of a virus stock of 10^7 $TCID_{50}$/mL in a pharyngeal wash were mixed with Copan SL solution and processed for the different assays as described above.

3.5. Statistical analysis

The qualitative performance of the assays was assessed by calculating the specificity, sensitivity, negative and positive prospective values, and accuracy. For reference a sample was defined as true-positive when at least two different tests gave a positive result. Concordance of two diagnostic tests was examined by Cohen's κ coefficient analysis. Correlation between RT-PCR Ct values and RT-LAMP threshold time was estimated using Pearson coefficient analysis.

4. Results

First, we analyzed a panel of pharyngeal swabs sent to the laboratory for routine SARS-CoV-2 diagnostics performed by LightMix® E gene RT-PCR. An aliquot of the RNA eluate was applied to the variplex™ RT-LAMP M gene assay. 96 PCR-positive and 41 negative RNA aliquots were tested. Samples with divergent results between LightMix® RT-PCR and RT-LAMP were verified by VIASURE and Allplex™ assays in order to identify false-positively tested specimen. 10 out of 96 RNA eluates that were LightMix® E-positive could not be confirmed by a second test and were defined as false-positive. Their median Ct value was 36.6 (IQR 36.1–37.6). In contrast, no false-positive results were observed using the variplex™ RT-LAMP. However, the sensitivity of RT-LAMP was only 75 % (Table 1). When only samples with an E gene RT-PCR Ct value <35 as indicative for potentially infectious patients were considered for analysis, sensitivity of RT-LAMP reached 86.4 % (Table 1). The median threshold time of positive signals was 15.75 min (IQR 13.25–24).

To verify the sensitivity of RT-LAMP extracted RNA from a log-dilution series of a virus stock was tested. The variplex™ assay achieved a reliable detection at 1 $TCID_{50}$/mL, corresponding to 0.03 $TCID_{50}$/reaction. In comparison LightMix® RT-PCR showed 100 % detection down to 0.1 $TCID_{50}$/mL. This concentration was positive by RT-LAMP in 33 % of the samples (Table 2).

Next, we investigated a panel of clinical samples that were tested by RT-LAMP without RNA extraction. Only samples from respiratory secretions and pharyngeal washes were used because in preliminary

Table 1
Diagnostic performance of the variplex™ RT-LAMP assay using isolated RNA from pharyngeal swabs.

	True-positive	True-negative	False-positive	False-negative	Sensitivity, % (CI[a])	Specificity, % (CI[a])	PPV, % (CI[a])	NPV, % (CI[a])	Accuracy, % (CI[a])
All Ct[b] values	72	41	0	24	75 (65.1−83.3)	100 (91.4−100)	100	63.1 (57.7−70.7)	82.5 (75.1−88.4)
Ct[b] values <35	70	41	0	11	86.4 (77−93)	100 (91.4−100)	100	78.8 (68.3-86.6)	91 (84.4−95.4)

[a] CI, 95 % confidence interval.
[b] LightMix® E-gene RT-PCR, reference method.

Table 2
Limits of detection of the variplex™ RT-LAMP and LightMix® RT-PCR conducted on RNA eluates from SARS-CoV-2 virus stock dilutions.

Virus concentration		Variplex™ M	LightMix® E
$TCID_{50}$/mL	$TCID_{50}$/reaction	Positive replicates M gene, mean threshold time [min] (SD)	Positive replicates E gene, mean Ct (SD)
100	3	6/6, 8.5 (1.25)	6/6, 25.4 (0.5)
10	0.3	6/6, 11.5 (1.75)	6/6, 28.8 (0.5)
1	0.03	6/6, 22.25 (6.25)	6/6, 33.3 (1)
0.1	0.003	2/6, 36	6/6, 36.1 (1.5)
0.01	0.0003	–	2/6, 37.1
0.001	0.00003	–	–

experiments we observed inhibitory effects by transport media of swabs on RT. A total of 43 specimens collected from 20 patients were included. From 6 patients 3 or more samples were obtained during the course of the disease. As controls we examined 30 samples from patients that were repeatedly tested negative by LightMix™ screening RT-PCR. Respiratory secretions from COVID-19 patients were often highly viscous and tough. To homogenize the specimens they were mixed with Copan SL solution. This procedure was applied to all samples to standardize the methodology. Homogenized samples diluted in LPTV buffer were directly pipetted into the master mix for RT-LAMP. For comparative analysis two aliquots were subjected to RNA isolation and RT-PCR using the Allplex™ and VIASURE BD MAX™ assays. All tests did not produce false-positives results in the group of control patients. From the samples of COVID-19 patients heterogeneous results were obtained. As expected a high agreement of results was found for the three different targets of the Allplex™ assay (Table 3). The results obtained with the variplex™ RT-LAMP only showed a moderate agreement to both the Allplex™ and VIASURE RT-PCR results (Table 3). To calculate how the moderate Cohen's κ concordance coefficients were related to different sensitivities of the assays we defined a sample as true-positive when at least two target genes of the virus were detected. When only one target gave a positive signal the sample was tested by the LightMix® RT-PCR to verify the result. Using this approach, all assay had sensitivities <90 % (Table 4). The Allplex™ RdRP assay offered the highest sensitivity of 84 %, followed by E and N gene tests from the same kit. Combining the three targets of Allplex™ did not result in a higher positive rate of the samples. The sensitivity of the VIASURE assay was only 68.4 % and that of the variplex™ RT-LAMP was in between, at 76.3 % (Table 4). However, when results of the variplex™ RT-LAMP were combined with those of the VIASURE S or Allplex™ RdRP RT-PCR diagnostic sensitivity was increased to 92 and 100 %, respectively (Table 4).

The median Ct values of the RT-PCRs were as follows: 28.4 for Allplex™ E (IQR 22.5–31.4), 30.7 for Allplex™ RdRP (IQR 25.2–33.9), 31 for Allplex™ N (IQR (IQR 26.3–34.2), and 33.2 (IQR 27.5–35.9) for VIASURE S. The median threshold time of a positive variplex™ RT-LAMP was 33.5 min (IQR 27.75–38.5). Ct values of different RT-PCRs showed a high degree of correlation with Pearson coefficients ranging from 0.96−0.99 ($P < 0.05$). In contrast, threshold times of RT-LAMP showed a weak correlation with RT-PCR Ct values (Person coefficients ranging from 0.44−0.47, $P < 0.05$). Table 5 shows the course of testing an ICU patient over 30 days, illustrating the fluctuating results by different assays.

In comparison the different sensitivities of the assays were also examined using simulated samples. For these experiments we started at 300 $TCID_{50}$/mL because of the dilution of the samples in LPTV buffer for direct RT-LAMP testing. As shown in Table 6 the Allplex RT-PCRs reached higher sensitivities than the other assays. The lower sensitivity of the variplex™ RT-LAMP was probably caused by the relatively high dilution of the sample in LPTV buffer because the limit of detection of 0.004 $TCID_{50}$/reaction was satisfying in comparison to the Allplex RT-PCR™.

5. Discussion

Timely and accurate laboratory diagnosis of patients with the suspicion of SARS-CoV-2 infection is important for optimizing patient treatment and preventing transmission to other persons [3]. RT-PCR is the standard method to detect an acute infection and is also used to identify asymptomatic carriers [16,17]. However, several studies have reported false-negative results in initial testing of symptomatic patients as well as during the course of the disease in no small measure that can have an impact on isolation or discharge of patients [2,7]. It has been suggested that a single RT-PCR assay should not be the only laboratory diagnostic marker [7,16]. The data of this study demonstrate that the variplex™ LAMP SARS-CoV-2 assay may be suitable as an additional tool to close gaps in COVID-19 diagnosis.

By using extracted RNA the variplex™ RT-LAMP assay showed a lower sensitivity, compared to our screening E gene RT-PCR, but performance was acceptable when only E gene Ct values <35 were considered. This cut-off has been chosen because on one hand it has been proposed that patients diagnosed with high Ct values are rather non-infectious and on the other hand we could identify several false-

Table 3
Comparison of RT-LAMP and RT-PCR assays conducted on respiratory samples.

Compared assays	P/P	P/N	N/P	N/N	% agreement	Cohen's κ (CI[a])	Scale
Variplex™ M/Allplex™ E	21	8	9	35	76.7	0.52 (0.32−0.72)	Moderate agreement
Variplex™ M/Allplex™ RdRP	22	7	9	35	78.1	0.55 (0.35−0.74)	Moderate agreement
Variplex™ M/Allplex™ N	23	7	8	35	79.4	0.58 (0.39−0.77)	Moderate agreement
Variplex™ M/VIASURE S	20	9	6	38	79.4	0.56 (0.37−0.77)	Moderate agreement
Allplex™ RdRP/Allplex™ N	30	1	1	41	97.3	0.94 (0.87−1)	Almost perfect agreement
Allplex™ RdRP/Allplex™ E	29	1	2	41	95.5	0.92 (0.82−1)	Almost perfect agreement

[a] CI, 95 % confidence interval.

Table 4
Diagnostic performance of the variplex™ LAMP assay directly conducted on respiratory samples in comparison and combination with RT-PCR.

	True-positive	True-negative	False-positive	False-negative	Sensitivity, % (CI[a])	Specificity, % (CI[a])	PPV[b], % (CI[a])	NPV[b], % (CI[a])	Accuracy, % (CI[a])
Variplex™ M	29	35	0	9	76.3 (59.8-88.6)	100 (90–100)	100	79.6 (68.7-87.3)	87.7 (77.9-94.2)
VIASURE S	26	35	0	12	68.4 (51.4–82.5)	100 (90–100)	100	74.5 (64.8–82.3)	83.6 (73–91.2)
Allplex™ E	30	35	0	8	80 (62.7–90.4)	100 (90–100)	100	81.4 (70.3–89)	89 (79.5–95.2)
Allplex™ RdRP	32	35	0	6	84.2 (68.8–94)	100 (90–100)	100	85.4 (73.7–92.4)	91.8 (82–96.9)
Allplex™ N	31	35	0	7	81.6 (65.7–92.3)	100 (90–100)	100	83.3 (71.9–90.7)	90.4 (81.2–96.1)
Allplex™ E + RdRP + N	32	35	0	6	84.2 (68.8–94)	100 (90–100)	100	85.4 (73.7–92.4)	91.8 (82–96.9)
Allplex™ RdRP + VIASURE S	33	35	0	5	86.8 (71.9–95.6)	100 (90–100)	100	87.5 (75.6–94.1)	93.2 (84.7–97.7)
Variplex™ M + VIASURE S	35	35	0	3	92.1 (78.6–98.3)	100 (90–100)	100	92.1 (79.8–97.2)	95.9 (88.5–99.1)
Variplex™ M + Allplex™ RdRP	38	35	0	0	100 (90.8–100)	100 (90–100)	100	100	100 (95.1–100)

[a] CI, 95 % confidence interval.
[b] PPV, positive predicitive value; NPV, negative predictive value.

Table 5
Fluctuating RT-LAMP and RT-PCR results of SARS-CoV-2 in an ICU patient.

Day	Material	Variplex™ M (threshold time, min)	Allplex™ E (Ct)	Allplex™ RdRP (Ct)	Allplex™ N (Ct)	VIASURE S (Ct)
0	Bronchoalveolar lavage	Negative	28	30.7	30.9	34.5
5	Endotracheal secretion	39.75	31.2	32.8	33.9	35.9
12	Bronchoalveolar lavage	30.25	Negative	38.2	Negative	Negative
19	Endotracheal secretion	35	33.4	Negative	35.8	37.8
26	Bronchoalveolar lavage	Negative	Negative	Negative	Negative	Negative
30	Endotracheal secretion	39.75[a]	Negative	Negative	Negative	Negative

[a] Confirmed by LightMix® E-gene RT-PCR.

Table 6
Limits of detection of the variplex™ RT-LAMP directly conducted on SARS-Cov-2 stock dilutions without RNA isolation and comparison to RT-PCR assays[a].

Virus concentration ($TCID_{50}$/mL)	Variplex™ M		VIASURE S		Allplex™			
	$TCID_{50}$/reaction	Positive replicates M gene, mean threshold time [min] (SD)	$TCID_{50}$/reaction	Positive replicates S gene, mean Ct (SD)	$TCID_{50}$/reaction	Positive replicates E gene, mean Ct (SD)	Positive replicates RdRP gene, mean Ct (SD)	Positive replicates N gene, mean Ct (SD)
300	0.4	5/5, 10.5 (0.75)	3	5/5, 28.3 (2.1)	8	5/5, 27.1 (0.8)	5/5, 28 (1.1)	5/5, 27.6 (2.1)
30	0.04	5/5, 17 (5)	0.3	5/5, 31.5 (1.8)	0.8	5/5, 30.5 (0.8)	5/5, 30.8 (0.7)	5/5, 31.2 (1.5)
3	0.004	5/5, 30 (3)	0.03	5/5, 34.4 (1.1)	0.08	5/5, 33.2 (0.9)	5/5, 33.9 (1)	5/5, 34.2 (1.7)
0.3	0.0004	–	0.003	2/5, 37.8	0.008	5/5, 35.4.(0.8)	5/5, 36.6 (0.6)	5/5, 37.1 (1.9)
0.03	0.00004	–	0.0003	–	0.0008	2/5, 37	1/5, 37.3	1/5, 35.8
0.003	0.000004	–	0.00003	–	0.00008	–	–	–

[a] The virus stock was diluted in a pharyngeal wash mixed with Copan SL solution.

positive RT-PCR tests that were associated with a high Ct value [18].

A major advantage of RT-LAMP is that it allows a simple testing of specimens when unprocessed samples are used, bypassing the bottleneck of RNA extraction [9,19]. Against the background of irregularities regarding the delivery of RNA isolation kits by many manufacturers RT-LAMP would be highly attractive as an alternative easy-to-use technology [9,13]. For direct testing we focused on samples from respiratory secretions and pharyngeal washes instead of swabs because several transport media can inhibit or reduce RT activity, as reported in recent studies [10,11]. Another reason was that the supply of swabs with fluid transport media was running into a critical shortage in a phase of significantly increased demand for testing.

The direct comparison of the variplex™ RT-LAMP with commercial RT-PCR assays showed that no method was able to detect all positive samples and fluctuating results during the course of the disease were observed in several patients. It soon became obvious during this study that there is only a moderate agreement between RT-LAMP and RT-PCR results in COVID-19 patients. Combining both techniques led to a sensitivity of 92–100 %. The complementation of the methods may be due to the difference in sample preparation. RNA extraction has the advantage that viral RNA is concentrated in a RT-PCR compatible buffer [8]. However, column-based extraction as used in many commercial tests can also result in a loss of RNA [8]. By using RT-LAMP to test unprocessed samples this problem is avoided but RT activity may be

inhibited by carbohydrates and salts depending on the sample composition [10]. In this context suitable specimen types have to be carefully evaluated. Saliva which has been described to contain high virus copy numbers may also represent a potential specimen type for direct RT-LAMP testing [13,20].

In conclusion this study shows that the variplex™ SARS-CoV-2 RT-LAMP assay may serve as an easy-to perform rapid molecular test to be combined with RT-PCR in order to ensure an efficient workflow of timely and accurate diagnosis even at times of high work load and increased testing requests. The major limitation of this work was the relatively small sample size due to low numbers of COVID-19 patients in our hospital. Future studies are needed to examine the utility of RT-LAMP under routine conditions with high sample throughput.

Ethical statement

The study protocol for the evaluation of the variplex™ SARS-CoV-2 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

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

Declaration of Competing Interest

The authors report no declarations of interest.

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References

[1] H. Ge, X. Wang, X. Yuan, et al., The epidemiology and clinical information about COVID-19, Eur. J. Clin. Microbiol. Infect. Dis. 39 (2020) 1011–1019, https://doi.org/10.1007/s10096-020-03874-z.

[2] H. Wang, X. Li, T. Li, et al., The genetic sequence, origin, and diagnosis of SARS-CoV-2, Eur. J. Clin. Microbiol. Infect. Dis. (2020), https://doi.org/10.1007/s10096-020-03899-4.

[3] D. Wu, T. Wu, Q. Liu, Z. Yang, The SARS-CoV-2 outbreak: what we know, Int. J. Infect. Dis. 94 (2020) 44–48, https://doi.org/10.1016/j.ijid.2020.03.004.

[4] N. Younes, D.W. Al-Sadeq, H. Al-Jighefee, et al., Challenges in laboratory diagnosis of the novel coronavirus SARS-CoV-2, Viruses 12 (2020) e582, https://doi.org/10.3390/v12060582.

[5] J.-L. He, L. Luo, Z.-D. Luo, et al., Diagnostic performance between CT and initial real-time RT-PCR for clinically suspected 2019 coronavirus disease (COVID-19) patients outside Wuhan, China, Respir. Med. 168 (2020), 105980, https://doi.org/10.1016/j.rmed.2020.105980.

[6] P.B. van Kasteren, B. van der Veer, S. van den Brink, et al., Comparison of seven commercial RT-PCR diagnostic kits for COVID-19, J. Clin. Virol. 128 (2020), 104412, https://doi.org/10.1016/j.jcv.2020.104412.

[7] Y. Li, L. Yao, J. Li, et al., Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19, J. Med. Virol. 92 (2020) 903–908, https://doi.org/doi:10.1002/jmv.25786.

[8] M.N.Esbin O.N. Whitney, S. Chong, et al., Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection, RNA 26 (2020) 771–783, https://doi.org/10.1261/rna.076232.120.

[9] J. Kashir, A. Yaqinuddin, Loop mediated isothermal amplification (LAMP) assays as a rapid diagnostic for COVID-19, Med. Hypotheses 141 (2020), 109786, https://doi.org/10.1016/j.mehy.2020.109786.

[10] N. Merindol, G. Pèpin, C. Marchand, et al., SARS-CoV-2 detection by direct rRT-PCR without RNA extraction, J. Clin. Virol. 128 (2020), 104423, https://doi.org/10.1016/j.jcv.2020.104423.

[11] L.E. Lamb, S.N. Bartolone, E. Ward, M.B. Chancellor, Rapid detection of novel coronavirus/Severe acute respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by reverse transcription-loop-mediated isothermal amplification, PLoS One 15 (2020), e0234682, https://doi.org/10.1371/journal.pone.0234682.

[12] Y.H. Baek, J. Um, K.J.C. Antigua, et al., Development of a reverse transcription-loop-mediated isothermal amplification as a rapid early-detection method for novel SARS-CoV-2, Emerg. Microb. Infect. 9 (2020) 998–1007, https://doi.org/10.1080/22221751.2020.1756698.

[13] N. L'Helgouach, P. Champigneux, F.S. Schneider, et al., EasyCOV: LAMP-based rapid detection of SARS-CoV-2 in saliva, medRxiv (2020), https://doi.org/10.1101/2020.05.30.20117291.

[14] C. Yan, J. Cui, L. Huang, et al., Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay, Clin. Microb. Infect. 26 (2020) 773–779, https://doi.org/10.1016/j.cmi.2020.04.001.

[15] V.M. Corman, O. Landt, M. Kaiser, et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Euro Surveill. 25 (2020), 2000045, https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

[16] E. Farfour, P. Lesprit, B. Visseaux, et al., The Allplex 2019-nCoV (Seegene) assay: which performances are for SARS-CoV-2 infection diagnosis? Eur. J. Clin. Microbiol. Infect. Dis. (2020) https://doi.org/0.1007/s10096-020-03930-8.

[17] P.L. Bulterys, N. Garamani, B. Stevens, et al., Comparison of a laboratory-developed test targeting the envelope gene with three nucleic acid amplification tests for detection of SARS- CoV-2, J. Clin. Virol. 129 (2020), 104427, https://doi.org/10.1016/j.jcv.2020.104427.

[18] 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.

[19] M.A. Lalli, X. Chen, J.J. Langmade, et al., Rapid and extraction-free deetction of SARS-CoV-2 from saliva with colorimetric LAMP, medRxiv (2020), https://doi.org/10.1101/2020.05.07.20093542, 2020.05.07.20093542.

[20] A. Bosworth, C. Whalley, C. Poxon, et al., Rapid implementation and validation of a cold-chain free SARS-CoV-2 diagnostic testing workflow to support surge capacity, J. Clin. Virol. 128 (2020), 104469, https://doi.org/10.1016/j.jcv.2020.104469.