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 Plus 2 (2022) 100088

Contents lists available at ScienceDirect

Journal of Clinical Virology Plus

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

Short communication

Direct comparison of Altona-SARS-CoV-2 dual target RT-qPCR Assay with commercial LAMP Assay using throat washes in health care staff testing

Johannes Wanney, Jessica Lüsebrink, Gina Spölgen, Sabrina Demuth, Verena Schildgen, Oliver Schildgen*

Institut für Pathologie, Kliniken der Stadt Köln gGmbH, Klinikum der Privaten Universität Witten/Herdecke, Ostmerheimer Str. 200, Cologne D-51109, Germany

ARTICLE INFO

Edited by Catherine Moore

ABSTRACT

Background: Rapid molecular diagnostics by PCR has a crucial role in handling the global SARS-CoV-2 pandemic. As diagnoses are time-sensitive and global supply chains are susceptible to various factors alternative detection methods would be an important backup.

Objectives: During the study the performance of a commercially available isothermal LAMP method for SARS-CoV-2 detection was compared to a IVD RT-PCR Assays using throat wash specimens that were routinely taken in our hospital setting.

Study design: Throat wash specimens of hospital staff (n = 174) previously tested positive for SARS-CoV-2 by the Altona Diagnostics RealStar SARS-CoV-2 RT-PCR (Altona Diagnostics, Hamburg, Germany) was tested for SARS-CoV-2 also by the SARS-CoV-2 Rapid Colorimetric LAMP Assay (NEB Germany GmbH, Frankfurt a.M., Germany).

Results: The sensitivity of the colorimetric LAMP Assay compared to RT-qPCR was 78.74%, and the specificity was determined to 88.24% with a positive predictive value of 0.986 and a negative predicitve value of 0.882. The positive and negative likelihood ratio for LAMP was 6.693 and 0.241, respectively, while the diagnostic odds ratio was 27.77.

Conclusions: In times of limited PCR test ressources and in settings with limited PCR capacities, the colorimetric LAMP Assay could serve as an alternative, if a calculable loss of sensitivity is acceptable from the Public Health perspective in certain settings.

1. Short communication

1.1. Background

The regular testing for SARS-CoV-2 infections among health care staff has been an important tool to maintain the operational performance of our institution by reducing the risk of nosocomial transmissions in either direction and by reducing the number of individuals being set into quarantine preventing personnel shortages. Therefore, we have used throat washes to setup a broad and rapid staff member testing scheme. The reason why throat washes were used for our medical staff member testing was simple. Around two days after the official national pandemic emergency status was claimed by the German government, swabs have become a highly limited resource, not at least as one of the worl leading vendors for those goods has its headquarter in the city of Brescia in Northern Italy, which was the geographic region where the first European lockdown took place. Thus, we rapidely had to establish an alternative route of sampling and thus reactivated an old virological technique, i.e. the throat washing, for which soley sterile saline and sterile urine cups were required. All throat washes obtained have been analysed with a commercial IVD qPCR Assay detecting the E- and S-gene of SARS-CoV-2 [1,2].

Beside several external factors (i.e. issues throughout supply chains regarding sampling material and lab consumables) the peak periods during the pandemic led to delayed sample processing based on limited numbers of available nucleic acid extraction devices resulting in an hangover of specimens that could not be tested timely.

For this reason we evaluated a colorimetric LAMP Assay as an alternate method due to availability, independency of supply chain issues or limited lab devices.

1.2. Objectives

Due to the lack of PCR reagents at the beginning of the pandemic alternate methods for nucleic acids detection were required in order to continously perform routine diagnostic testing for SARS-CoV-2 timely. In this context the sensitivity, specificity as well as the positive and neg-

* Corresponding author.
E-mail address: schildgeno@kliniken-koeln.de (O. Schildgen).

https://doi.org/10.1016/j.jcvp.2022.100088
Received 19 April 2022; Received in revised form 24 May 2022; Accepted 1 June 2022

Table 1
4-field contingency matrix summarizing LAMP and RT-qPCR results for the two SARS-CoV-2 nucleic acids detection kits used in this study. The Altona RT-qPCR method was set as standard method.

	Altona PCR was positive	Altona PCR was negative
LAMP was positive	137	2
Lamp was negative	37	15

ative predictive values of a colorimetric LAMP Assay (New England BioLabs Inc., SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit) were analyzed to evaluate its usage for routine testing of hospital staff.

1.3. Study design

Throat washes were routinely collected during staff testing from individuals, who had either SARS-Cov-2 specific symptoms or contact to SARS-CoV-2 positive persons.

In total, 174 qPCR positive throat wash specimens were collected between March 2020 and August 2021 in a hospital setting. In addition, 17 negatively tested specimens were included as negative controls. Total RNA was extracted with the Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega, Darmstadt, Germany) and was tested for SARS-CoV-2 by the Altona Diagnostics RealStar SARS-CoV-2 RT-PCR kit (Altona Diagnostics, Hamburg, Germany). Afterwards, all samples were tested for SARS-CoV-2 by the New England BioLabs Inc., SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit (NEB Germany GmbH, Frankfurt a.M., Germany), determining specificity, sensitivity, and positive and negative predictive values. Thereby, the results obtained by the Altona-Real-Star qPCR test were set as reference standard to which the predictive values and ratios of the colorimetric LAMP Assay were calculated. The sensitivity and specificity were calculated with a standard contingency table (Table 1).

For the calculation of the sensitivity's and specificity's 95%-confindence intervals the Wilson score method was applied [3], whereas for the determination of the confidence intervals for the positive and negative likelyhood ratios the method by Simel et al. was used [4]. The confidence interval for the diagnostic odds ratio was calculated according to the method published by Armitage and Berry assuming equal variancies (Chapter 4 of [5]). All tests were two-sided.

2. Results

A total number of 137 specimens (78.7%) was tested positive by qPCR and LAMP, whereas 37 specimens (21.3%) tested positive for SARS-CoV-2 by PCR but negative by LAMP. However, of those 37 LAMP false negative specimens 21 specimens displayed qPCR ct-values >35 in the respective positive tested gene. As this finding may suggest that the ct-value >35 is a determing factor for LAMP-negativity, the range of the ct-values of false negative LAMP results were analysed. The Altona RT-qPCR ct-value range of LAMP-false-negative specimens was 18.1 to 43.63, with a mean of 33.34 and a median of 33.04, thus being two ct-value below the mark of ct-value 35 and not being indicative for a correlation between ct 35 and LAMP-negativity.

Table 1 summarizes the results and was the basis for the determination of specificity, sensitivity, positive and negative predictive values and diagnostic odd ratio.

Two specimens (11.8%) previously tested negative by PCR were tested positive by LAMP, while 15 specimens (88.2%) tested truly negative in both Assays.

The sensitivity for the colorimetric LAMP Assay compared to RT-qPCR was 78.74% (CI: 0.7207 – 0.8416) and the specificity was determined to 88.24% (CI: 0.6566 – 0.9671) with a positive likelyhood ratio for LAMP of 6.693 (CI: 1.816 – 24.658), a negative likelyhood ratio of 0.241 (CI: 0.172 – 0.337). The diagnostic odds ratio was 27.77 (CI: 6.077 – 126.901).

3. Discussion

The colorimetric LAMP Assay has some advantages compared to qPCR based methods. First, the handling is rather simple and the time needed for completion is below 40 min. Additionally, the reaction can be analysed without any technical equipment and reactions can be started individually without the need to collect a minimum of specimen to get diagnostics economically justifiable.

However, the major challenge in our setting is to detect SARS-CoV-2 infections in medical staff as early as possible to prevent nosocomial transmissions. As the very early phase of infection is often characterized by very low SARS-CoV-2 copy numbers and fluctuating high Ct-values before the real replicative phase with viral shedding starts, highly sensitive detection methods are needed. In this regard, the colorimentric LAMP Assay evaluated in this study was not sensitive enough to replace the established routine PCR Assay.

The fact that the majority of LAMP-false negative specimens displayed a ct-value >35 suggests, that this mark is a sensitivity cut-off of the LAMP-Assay. While for community surveillance purpose this cut-off may reflect an appropriate cut-off to discriminate between putatively infectious and non/less-infectious individuals or the respective risks of tranmission thereof, in medical staff the highest possible sensitivity was required for SARS-CoV-2 testing as still no reliable cut-off values were defined as surrogate markers for infectivity [6]. Consequently, this phenomenon was not further investigated.

Nevertheless, the likelyhood ratios and the diagnostic odds ratio open the possibility to use the colorimetric LAMP Assay in settings like rural laboratories with limited equipment and qPCR capacities or in situations with a need of high test coverage due to high numbers of expected positively cases, because sensitivity and specificity of the LAMP Assay are for sure higher than that of many rapid antigen tests [7–10] and are in the same range as for the Abbott ID Now IVD real time LAMP Assay [11,12]. Thereby, it would be worth to test if the Assay sensitivity may be improved by automatic read out systems.

Critcially, it has to be discussed that the sensitivity and the specificity obtained in the present study would not be acceptable when strictly adhering to the ECDC technical guidance [13]. There it was concluded that „*ECDC agrees with the minimal criteria set by WHO and advocates for the use of tests with a performance closer to NAAT,* i.e. *≥90% sensitivity (for samples with Ct98% specificity*“, thus the LAMP overall sensitivitiy of 78.74% and the specificity of 88.24% do not meet these requirements set for rapid antigen testings. As LAMP in contrast is a nucleic acid detection Assays the specificity and sensitivity required is even higher than for rapid antigen tests.

Finally, the usage of throat washes should be shortly discussed. We have used these specimens fort wo reasons. First, the sampling technique is long known and follows the KISS principle (keep it simple and stupid), thus the sampling itself is not error-prone and covers the entire throat, which is important in the early phase of the infection when the replication of the virus starts focally. Second, the method was rapidely available and independent of the most limited resource in the early phase of the pandemics besides PCR reagents, namely swabs. Meanwhile several studies have been published that also made use of throat washes or saliva, although these studies suggested to the community that this sampling procedure was entirely novel [14–18].

However, furthermore, most importantly and in summary, the colotimetric LAMP Assay could become true alternative whenever a qualitative nucleic acid diagnostics is required while qPCR reagents are a limited ressource, such as observed during the very first phase of the current pandemics [19].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Johannes Wanney: Investigation, Writing – review & editing. **Jessica Lüsebrink:** Investigation. **Gina Spölgen:** Investigation. **Sabrina Demuth:** Investigation. **Verena Schildgen:** Conceptualization, Writing – review & editing, Supervision. **Oliver Schildgen:** Conceptualization, Writing – review & editing, Supervision.

Acknowledgments

The authors declare no conflicts of interest.

References

[1] V. Schildgen, J. Lusebrink, P. Thomaidis, P. Meibert, O. Schildgen, Diagnostic stumbling blocks in the COVID-19 monitoring of medical staff while putting hygiene requirements into practice, New Microbes New Infect. 37 (2020) 100735.
[2] M. Malecki, J. Lusebrink, S. Teves, A.F. Wendel, Pharynx gargle samples are suitable for SARS-CoV-2 diagnostic use and save personal protective equipment and swabs, Infect. Control Hosp. Epidemiol. 42 (2) (2021) 248–249.
[3] R.G. Newcombe, Two-sided confidence intervals for the single proportion–Comparison of seven methods, Stat. Med. 17 (8) (1998) 857–872.
[4] D.L. Simel, G.P. Samsa, D.B. Matchar, Likelihood ratios with confidence–Sample size estimation for diagnostic test studies, J. Clin. Epidemiol. 44 (8) (1991) 763–770.
[5] Armitage P., Berry G., Matthews J.N.S. Statistical Methods in Medical Research. 4th ed. Armitage P, Berry G, Matthews JNS, editors. Oxford, UK: Blackwell; 2002.
[6] M. Platten, D. Hoffmann, R. Grosser, F. Wisplinghoff, H. Wisplinghoff, G. Wiesmuller, et al., SARS-CoV-2, CT-values, and infectivity-conclusions to be drawn from side observations, Viruses 13 (8) (2021) 1459, doi:10.3390/v13081459.
[7] V. Schildgen, S. Demuth, J. Lusebrink, O. Schildgen, Limits and opportunities of SARS-CoV-2 antigen rapid tests–An experienced-based perspective, Pathogens 10 (1) (2021) 38, doi:10.3390/pathogens10010038.
[8] A. Osterman, H.M. Baldauf, M. Eletreby, J.M. Wettengel, S.Q. Afridi, T. Fuchs, et al., Evaluation of two rapid antigen tests to detect SARS-CoV-2 in a hospital setting, Med. Microbiol. Immunol. 210 (1) (2021) 65–72, doi:10.1007/s00430-020-00698-8.
[9] S. Iyer, M. Kumar, ASSURED-SQVM diagnostics for COVID-19–Addressing the why, when, where, who, what and how of testing, Expert Rev. Mol. Diagn. 21 (4) (2021) 349–362 in press, doi:10.1080/14737159.2021.1902311.
[10] J. Dinnes, J.J. Deeks, S. Berhane, M. Taylor, A. Adriano, C. Davenport, et al., Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection, Cochrane Database Syst. Rev. 3 (2021) CD013705.
[11] D. Pattnaik, N. Poddar, B.K. Pathi, K. Panigrahi, S. Sabat, A. Roy, et al., Comparative evaluation of cartridge-based Abbott ID now test with probe-based real-time reverse transcription polymerase chain reaction assay for detection of SARS-CoV-2, Cureus 14 (2) (2022) e22470.
[12] A.S. James, J.I. Alawneh, COVID-19 infection diagnosis–Potential impact of isothermal amplification technology to reduce community transmission of SARS-CoV-2, Diagnostics 10 (6) (2020) 399, doi:10.3390/diagnostics10060399.
[13] ECDC. 2022. Options for the use of rapid antigen detection tests for COVID-19 in the EU/EEA ECDC: ECDC; 2021 [cited 2022 24.05.2022]. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Options-for-the-use-of-rapid-antigen-tests-for-COVID-19-first-update.pdf.
[14] R. Utama, R. Hapsari, I. Puspitasari, D. Sari, M. Hendrianingtyas, N. Nurainy, Self-collected gargle specimen as a patient-friendly sample collection method for COVID-19 diagnosis in a population context, Sci. Rep. 12 (1) (2022) 3706.
[15] E. Poukka, H. Makela, L. Hagberg, T. Vo, H. Nohynek, N. Ikonen, et al., Detection of SARS-CoV-2 infection in gargle, spit, and sputum specimens, Microbiol. Spectr. 9 (1) (2021) e0003521.
[16] C.K. Lai, G.C. Lui, Z. Chen, Y.Y. Cheung, K.C. Cheng, A.S. Leung, et al., Comparison of self-collected mouth gargle with deep-throat saliva samples for the diagnosis of COVID-19–Mouth gargle for diagnosis of COVID-19, J. Infect. 83 (4) (2021) 496–522.
[17] D.M. Goldfarb, P. Tilley, G.N. Al-Rawahi, J.A. Srigley, G. Ford, H. Pedersen, et al., Self-collected saline gargle samples as an alternative to health care worker-collected nasopharyngeal swabs for COVID-19 diagnosis in outpatients, J. Clin. Microbiol. 59 (4) (2021) e02427–20.
[18] A. Biber, D. Lev, M. Mandelboim, Y. Lustig, G. Harmelin, A. Shaham, et al., The role of mouthwash sampling in SARS-CoV-2 diagnosis, Eur. J. Clin. Microbiol. Infect. Dis. 40 (10) (2021) 2199–2206.
[19] M.P. Cheng, J. Papenburg, M. Desjardins, S. Kanjilal, C. Quach, M. Libman, et al., Diagnostic testing for severe acute respiratory syndrome-related Coronavirus 2–A narrative review, Ann. Intern. Med. 172 (11) (2020) 726–734.