



INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

EVALUATION OF RT-LAMP AND DRY SWAB RNA EXTRACTION FREE METHOD FOR DETECTION OF SARS-COV-2 INFECTION

Kyaw Khine Win¹, Aung Yan Lin², Kyaw Wunna², Khine Khine Su¹

1. Defence Services Medical Academy
2. Military Institute of Nursing and Paramedical Sciences

ABSTRACT

The pandemic coronavirus SARS CoV-2 in the world has caused a large infected population suffering from COVID-19. To curb the spreading of the virus, WHO urgently demanded an extension of screening and testing; thus, a rapid and simple diagnostic method is needed. The ease-of-use and rapid turnaround time of reverse transcription loop mediated isothermal amplification (RT-LAMP) and dry swab RNA extraction free method offer the potential to expand access to testing and decrease delays in diagnosis. In this study, ninety-four nasopharyngeal swabs were collected and tested for the presence of SARS-CoV-2 infection by RT-PCR (gold standard), RT-LAMP and dry swab method. In this study, 68 (72.3%) out of 94 patients were positive for SARS CoV-2 infection by RT-PCR. The overall sensitivity, specificity, positive predictive value and negative predictive value of RT-LAMP was 76.5%, 100%, 100% and 62%. The overall sensitivity, specificity, positive predictive value and negative predictive value of dry swab method was 66%, 100%, 100% and 53%. Hundred percent sensitivity was occurred in RT-LAMP and Dry swab method with Ct<20. In Ct 20-30, sensitivity of RT-LAMP and dry swab method was 87.5% and 82.5%. RT-PCR method required molecular facility; trained technician; and it takes several hours to get the results. Although sensitivity and specificity of RT-LAMP and dry swab methods are inferior to RT-PCR, they can be performed easily in the short period of time (less than 2 hours). Therefore, rapid and simple alternative molecular diagnostic methods should be considered for diagnosis of SARS CoV-2 infection.

Keywords: SARS-CoV-2, RT-PCR, RT-LAMP, dry swab RNA extraction free method, Cycle threshold

1. INTRODUCTION

SARS-CoV-2 was discovered in late December 2019 and, since then, has spread out from Wuhan, Hubei province, People's Republic of China, into numerous countries worldwide, becoming a global pandemic (Guan et al, 2020). To slow down and limit the spread, it is crucial to rapidly identify infected people, followed by strict public health measures. The current recommended testing method for potentially infected people by the Center of Disease Control and Prevention (CDC) and other relevant departments worldwide is the detection of SARS-CoV-2 nucleic acid via reverse transcription real-time polymerase chain reaction (RT-PCR) (Corman et al, 2020).

Although RT-PCR methods are the gold standard for the SARS-CoV-2 detection due to their high sensitivity and specificity, there are still some caveats. To perform this method, one needs a molecular biological laboratory facility with highly trained personnel. There is an urgent demand for a rapid, simple, sensitive and specific molecular assay, to facilitate faster detection of SARS-CoV-2, which can reduce or avoid further spread (Notomi et al, 2000).

Loop-mediated isothermal amplification (LAMP) is a technology that provides nucleic acid amplification in a short time using 4 to 6 specially designed primers and a DNA polymerase with chain displacement activity. Since the LAMP method only needs one constant temperature (usually 65°C). If the template is RNA, the amplification reaction can be accomplished in one step by adding a reverse transcriptase, and is therefore called reverse transcription LAMP (RT-LAMP) (Notomi et al, 2000).

In the dry swab RNA extraction free method, elimination of RNA extraction step in one-step RT-PCR method reduced the cost of test significantly saved time and other resources. Direct RT-PCR assay with heat inactivated or lysed samples using buffers such as Tris or Tris-EDTA (TE) served as an effective alternative method (Smyrlaki et al, 2020). A similar approach to this method, using heat-inactivated TE buffer extract of nasopharyngeal swabs transported in a dry tube from the sample collection site to the laboratory, has been described in India (Kiran et al, 2020).

During the outbreak, detection of positive patients is one of the key points to controlling the outbreak. The gold standard for clinical diagnostic detection of SARS-CoV-2 remains RT-PCR. In the current pandemic, a more rapid and high throughput method is in growing concern. Generally, the ease-of-use and rapid turnaround time of RT-LAMP and dry swab RNA extraction free RT-PCR method offer the

potential to expand access to testing and decrease delays in diagnosis by shifting to decentralized testing of patients with early symptoms. The performance of RT-LAMP and dry swab RNA extraction free RT-PCR method are determined by the sensitivity and specificity of the test to detect a SARS-CoV-2 infection compared with a reference standard, the real time polymerase chain reaction (RT-PCR). Therefore, our study is to evaluate the performance of RT-LAMP and dry swab RNA extraction free method in the diagnosis of SARS CoV-2 infection.

2. Materials and methods

It was a laboratory based cross-sectional descriptive study was carried out from September 2022 to October 2022 at molecular laboratory of No (1) Defence Services General Hospital, Yangon. After getting written informed consent, nasopharyngeal swabs were collected using nylon flocked swabs. All technicians had completed a training course that was prepared according to established guidelines on swab collection (Mosi et al, 2021). Two nasopharyngeal swabs were collected from each patient; one was placed in a 3ml viral transport media (VTM) (Himedia, India) and another one was placed into the dry tubes. Ninety-four nasopharyngeal swabs were collected from patient suspicious of SARS CoV-2 infection. All nasopharyngeal swab samples were tested for the presence of SARS CoV-2 infection by RT-PCR (gold standard), RT-LAMP and dry swab method.

2.1 Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR based SARS-CoV-2 detection was done at No (1) Defence Services General Hospital, molecular laboratory, a reference laboratory for SARS-CoV-2 accredited by national health laboratory from Myanmar and successfully accomplished in the External Quality Assurance Program of the Royal College of Pathologists of Australia (RCPA) and World Health Organization (WHO) in 2021.

Viral RNA extraction was done using the MegaBio plus Virus DNA/RNA purification kit (Bioer, China) and Bioer Automatic Nucleic Acid purification machine (Gene Pure Pro, China). SARS-CoV-2 RNA detection was done by bio Perfectus Nucleic Acid Detection Kit (bio Perfectus, Jiangsu bio Perfectus Biotech Co., Ltd, China). BioPerfectus SARS-CoV-2 detection kit contain primer and probes targeting the ORF1ab gene and Nucleocapsid gene target. The target RNA was amplified in the 7500 fast Thermocycler instrument (Thermofisher, USA). Thermal cycling condition was 50°C for 10 mins (reverse transcription), 97°C for 1 min (polymerase activation), followed by 45 cycles of 97°C for 5sec (denaturation) and 58°C for 30 sec (annealing). Samples were reported as SARS CoV-2 detected when two targets or only one of two target is positive with Cycle threshold (Ct) less than 37.

2.2 Reverse transcription loop mediated isothermal amplification (RT-LAMP)

RT-LAMP detection for SARS-CoV-2 was carried out using the RT-LAMP COVID-19 detection kit (real time). The RT-LAMP detection kit contains 6 primers selectively detect specific genes (RdRP gene and N gene) of SARS-CoV-2. The sample was amplified in the 7500 fast Thermocycler instrument (Thermofisher, USA). PCR program consisted of 40 cycles of 58°C for 30 sec and final reaction volume of 25 µl (12.5 µl of 2xReaction buffer; 1 µl of enzyme mix; 2 µl of detection primer; 5 µl of extracted RNA and 4.5 µl of distilled water). Sample was considered as SARS-CoV-2 positive when only one of two target was detected with Ct less than 40.

2.3 Dry swab-based RNA extraction free method

Hi-PCR Covid-19 detection kit (Dry Swab Method) was used for SARS-CoV-2 detection. After nasopharyngeal swab collection as dry swab (without VTM), dry swab was transferred to the 1.5ml tube. Added the 400µl of TE-PK buffer (1X TE buffer 360µl + PK 40µl) and incubated for 30 mins at room temperature. After incubation, 50µl of TE-PK buffer extract was transferred to the PCR tubes and incubated at 98 °C for 6 mins. TE-PK buffer extract from PCR tubes was directly used as RNA template for SARs CoV-2 detection. PCR master mix preparation was 10 µl of 2x One step buffer mix, 0.8 µl of One-step RT Enzyme Mix, 1.5 µl of SARS-CoV-2 Primer-Probe Mix, 0.2 µl of water and 7.5 µl of extracted RNA. PCR program was 50°C for 15 mins (cDNA synthesis), 95°C for 30 sec (Initial denaturation), followed by 45 cycles of 95°C for 03 sec (Denaturation) and 58°C for 30 sec (Annealing). Samples were considered as SARS-CoV-2 detected when two targets or only one of two target is positive with Cycle threshold (Ct) less than equal 38.

3. Results

Ninety-four participants were involved in this study. The mean age was 36.3 years; 68 (72.3%) individuals were male and 26 (27.7%) were female. The minimum age was 18 for males and 20 for female, the maximum age was 86 for male and 66 for female. Table 1 shows age distribution of study participants (Table 1).

Table 1. Age distribution of study participants

| Age interval (year) | Frequency (%) |
|---------------------|---------------|
| 18-30 | 36 |
| 31-40 | 24 |
| 41-50 | 19 |
| 51-60 | 10 |
| >60 | 5 |
| Total | 94 |

Among the study participants, 29.8% (28/94) were presented with symptoms consistent with COVID-19. All symptomatic participants were tested positive with RT-PCR, RT-LAMP and dry swab method. Fever, cough and breathlessness were most common presenting symptoms.

In this study, RT-PCR was positive in 68 (72.3%) participants, corresponding to the prevalence of 72.3%. RT-LAMP was positive in 50 (53.2%) cases and negative in 44 (46.8%) cases (Figure 1). The clinical performance of RT-LAMP was 55.3% (52/94) true positive (RT-PCR positive/RT-LAMP positive), 27.7% (26/94) true negative (RT-PCR negative/RT-LAMP negative) and 17% (16/94) false negative (RT-PCR positive/RT-LAMP negative). The overall sensitivity of RT-LAMP was 76.5%, specificity was 100%, positive predictive value

was 100% and negative predictive value was 62%.

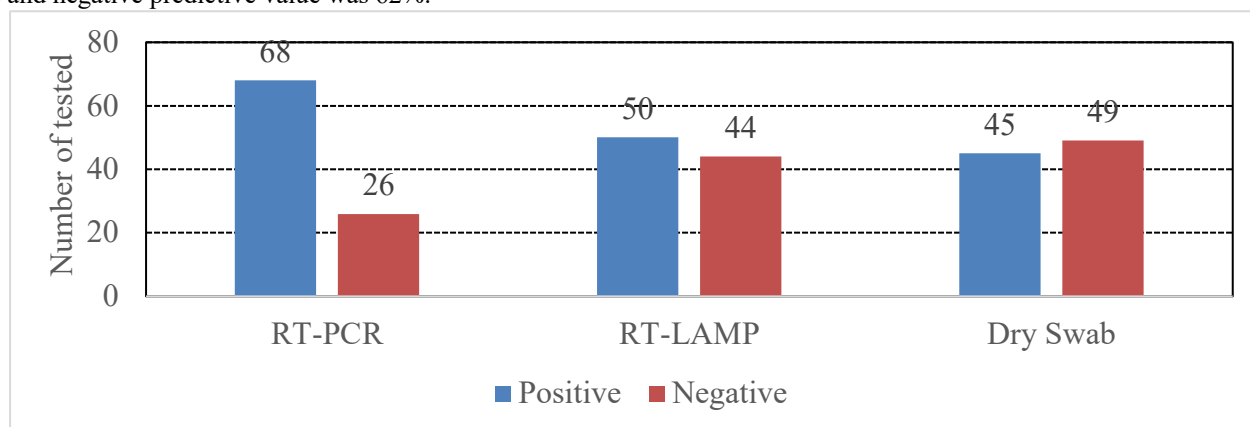


Figure 1. Positivity of RT-PCR, RT-LAMP and dry swab method

In the dry swab RNA extraction free method, 45 cases (48%) were positive and 49 cases (52%) were negative. True positive was 45 (47.9%), true negative was 26 (27.7%) and false negative was 23 (24.4%). The overall sensitivity was 66%, specificity was 100%, positive predictive value was 100% and negative predictive value was 53%. No false positive case in RT-LAMP and dry swab RNA extraction free method.

In RT-LAMP and dry swab method, no false positive cases were noted and sensitivity was increased in symptomatic cases. In symptomatic cases, sensitivity and specificity were 100% and low cycle threshold value were noted in RT-LAMP (mean Ct = 21.6) and dry swab method (mean Ct = 22.3). In this study, result turnaround time of RT-PCR was 5-6 hours but less than 2 hours in RT-LAMP and dry swab method (Table 2).

Table 2. Diagnostic performance of RT-LAMP and dry swab RNA extraction free method

| Index | RT-LAMP | | Dry swab method | |
|---------------------------|-------------------|------------|-------------------|------------|
| | Positive | Negative | Positive | Negative |
| Standard | | | | |
| RT-PCR positive | 52 (55.3%) | 16 (17%) | 45 (47.9%) | 23 (24.4%) |
| RT-PCR negative | 0 | 26 (27.7%) | 0 | 26 (27.7%) |
| Sensitivity | 76.5% | | 66% | |
| Specificity | 100% | | 100% | |
| Positive predictive value | 100% | | 100% | |
| Negative predictive value | 62% | | 53% | |
| Turnaround time | Less than 2 hours | | Less than 2 hours | |

Sensitivity increased with high viral load (viral load is inversely related to the Ct value). Therefore, sensitivity variation was noted in different Ct value. Hundred percent sensitivity were occurred in Ct value less than 20 in both RT-LAMP and dry swab method. In Ct value 20-30, 87.5% and 82.5% sensitivity were seen in RT-LAMP and dry swab method. Sensitivity decreased to 35% and 20% in RT-LAMP and dry swab method with Ct value more than 30.

Table 3. Positivity of RT-LAMP and dry swab method in different Ct value

| Ct value | | RT-PCR Ct value | | |
|----------|---|-----------------|------------|----------|
| | | <20 | 21-30 | >30 |
| RT-LAMP | + | 8 (100%) | 35 (87.5%) | 7 (35%) |
| | - | 0 | 5 (12.5%) | 13 (65%) |
| Dry swab | + | 8 (100%) | 33 (82.5%) | 4 (20%) |
| | - | 0 | 7 (17.5%) | 16 (80%) |

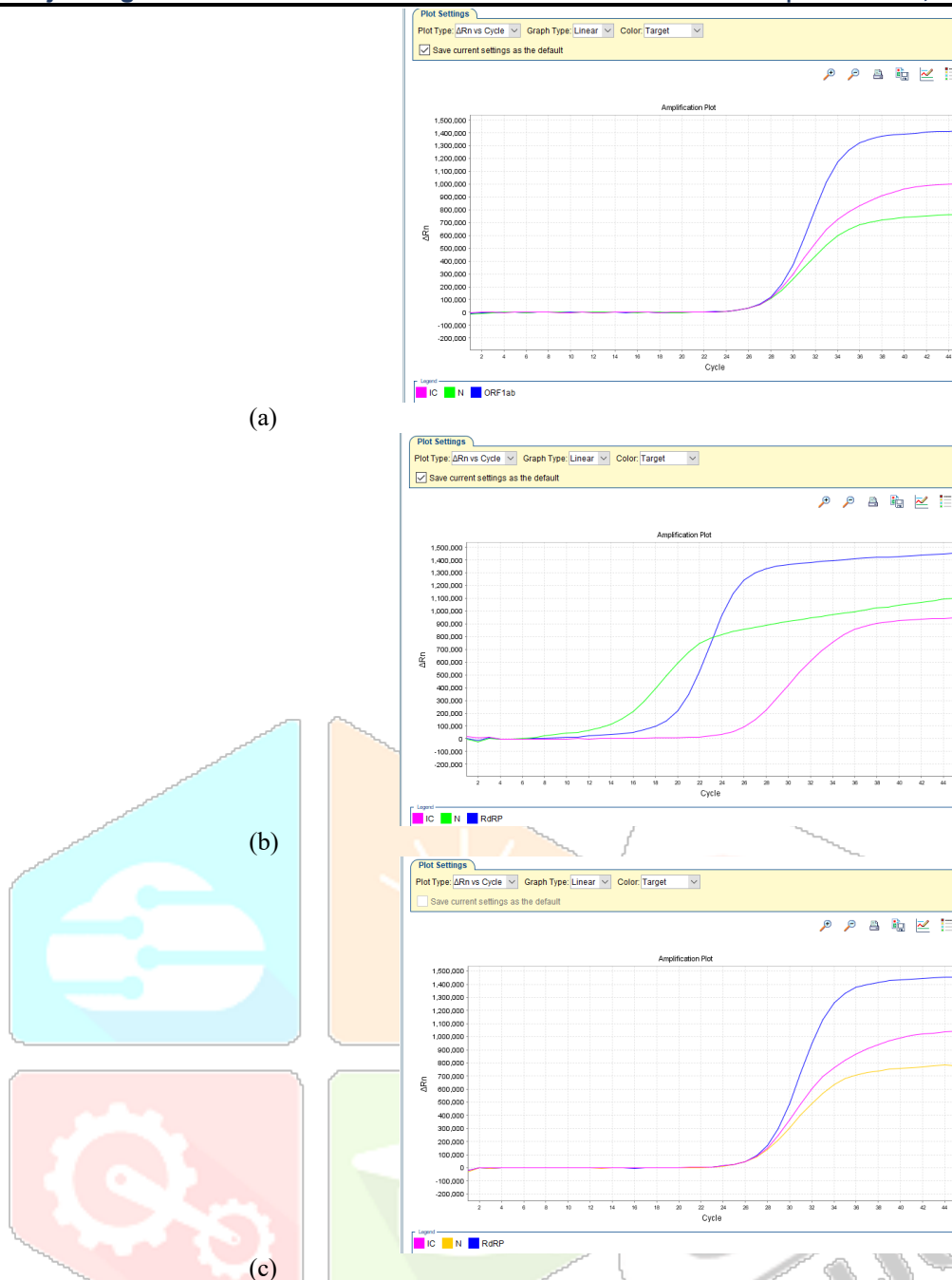


Plate (1). Amplification curves (a) RT-PCR shows ORF1ab gene (Ct=29), Nucleocapsid gene (Ct=29) and Internal control gene (Ct=29) targets, (b) RT-LAMP show RdRp gene (Ct=20), Nucleocapsid gene (Ct=17) and Internal control gene (Ct=28) targets and (c) Dry swab method show RdRp gene (Ct=28), Nucleocapsid gene (Ct=28) and Internal control gene (Ct=28) targets

4. Discussion

The ongoing COVID-19 pandemic has created an unprecedented need for rapid diagnostic testing. The World Health Organization (WHO) recommends a standard assay that includes an RNA extraction step from a nasopharyngeal (NP) swab followed by reverse transcription polymerase chain reaction (RT-PCR) to detect the purified SARS-CoV-2 RNA (Bruce et al, 2020).

This study was designed to evaluate the performance of RT-LAMP and dry swab RNA extraction free method for detection of SARS-CoV-2 infection. In this study, ninety-four participants were involved and 29.8% (28/94) was symptomatic cases. In most of the studies, SARS-CoV-2 found more in male than female (Badawi et al, 2016)(Chen et al, 2020). In our study, 68 (72.3%) individuals were male and 26 (27.7%) were female. According to this finding, male predominant may be due to this study was carried out in military population at tertiary military hospital.

SARS-CoV-2 infects people of all ages. However, there are two main groups at a higher risk of developing severe disease: older people and people with underlying co-morbidities (Wang et al, 2020). The mean age of the patients in this study was 36.3 years, ranging from 18 years – 86 years. A study by Chen et al, (2020) observed that the average age of the patients was 55.5 years. Another study by Dhakad et al (2021) showed the mean age was 36.5 years (SD=13.4 years).

COVID-19 presents varied clinical features, ranging from asymptomatic to ARDS. The most common symptoms at onset of COVID-19 include fever, cough, and shortness of breath (Singhal, 2020). In this study, 29.8% (28/94) of the participants were symptomatic. All symptomatic participants were tested positive with RT-PCR, RT-LAMP and dry swab method. Among the symptomatic participants 100% sensitivity and specificity were noted with low Ct value in RT-LAMP (mean Ct=21.6) and dry swab RNA extraction free method (mean

Ct=22.3). The sensitivity and specificity were better with symptomatic cases who were at the early stage of the disease course that means patients with low Ct value have high viral load (Mishra et al, 2022).

According to this study, RT-LAMP was positive in 50 (53.2%) cases and negative in 44 (46.8%) cases. The overall sensitivity of RT-LAMP was 76.5%, specificity was 100%, positive predictive value was 100% and negative predictive value was 62%. In the dry swab RNA extraction free method, 45 cases (48%) were positive and 49 cases (52%) were negative. The overall sensitivity was 66%, specificity was 100%, positive predictive value was 100% and negative predictive value was 53%. No false positive case in RT-LAMP and dry swab RNA extraction free method. One of the reasons for lower positivity in dry swab-based method may be due to the viral RNA present in the more dilute swab sample can be concentrated in VTM based RT-PCR method (Esbin et al, 2020); direct heating of samples at 95°C for 10 minutes may delayed the detection of viral RNA; direct addition of unprocessed swab samples decreases the test sensitivity (Esbin et al, 2020).

In this study, the positive predictive value of dry swab-based RT-PCR method was 100% and negative predictive value was 53%. The positive predictive value and negative predictive value of RT-LAMP was 100% and 62%. In this study, no false positive cases were noted. In a study by Bruce et al, 2020 the positive predictive value was 100%, given that no false positives were observed and the negative predictive value was ranging from 97.4% to 99.8%.

In this study, diagnostic performance was highly dependent on the viral load. Hundred percent sensitivity were occurred in Ct < 20 in both RT-LAMP and dry swab method. In Ct value 20-30, 87.5% and 82.5% sensitivity were seen in RT-LAMP and dry swab method. Sensitivity decreased to 35% and 20% in RT-LAMP and dry swab method with Ct > 30. Viral load and cycle threshold (Ct) values are inversely related that means low Ct value have high viral load. Progressive decrease in performance of RT-LAMP and dry swab method was observed as Ct values of different SARS-CoV-2 genes are increased (Mishra et al, 2022). Similarly, sensitivity was greatest in strong positives. Recent work has shown that SARS-CoV-2 could not be cultured from samples with Ct values greater than 24 and/or longer than 8 days past symptom onset (Bullard et al, 2020).

In conclusion, scalable rapid turn-around time tests, may efficiently detect individuals with high viral loads at the point of care. Although sensitivity and specificity of RT-LAMP and dry swab method are inferior to the RT-PCR, they can be performed easily in the short period of time (less than 2 hours). Therefore, faster and easier alternative molecular diagnostic methods should be considered for prioritized samples within the existing test chain, reliably identifying those with highest virus concentrations ahead of the standard RT-PCR workflow, and able to be scaled to any required number of tests per day.

5. Acknowledgements

We give special thanks to all personnel who participated in this study.

6. CONFLICT OF INTEREST

There has no conflict of interest.

7. Conflict of interest

There has no conflict of interest.

8. Funding

This research did not receive any specific grant from funding agencies.

9. References

- Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, Liu XQ, Chen RC, Tang CL, Wang T, Ou CQ, Li L, Chen PY, Sang L, Wang W, Li JF, Li CC, Ou LM, Cheng B, Xiong S, Ni ZY, Xiang J, Hu Y, Liu L, Shan H, Lei CL, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Cheng LL, Ye F, Li SY, Zheng JP, Zhang NF, Zhong NS, He JX; China Medical Treatment Expert Group for COVID-19. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. *Eur Respir J.* 2020 May 14;55(5):2000547. doi: 10.1183/13993003.00547-2020. PMID: 32217650; PMCID: PMC7098485.
- Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MP, Drosten C. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 2020 Jan;25(3):2000045. doi: 10.2807/1560-7917.ES.2020.25.3.2000045. Erratum in: *Euro Surveill.* 2020 Apr;25(14): Erratum in: *Euro Surveill.* 2020 Jul;25(30): Erratum in: *Euro Surveill.* 2021 Feb;26(5): PMID: 31992387; PMCID: PMC6988269.
- Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* 2000 Jun 15;28(12):E63. doi: 10.1093/nar/28.12.e63. PMID: 10871386; PMCID: PMC102748.
- Smyrlaki I, Ekman M, Lentini A, Lentini A, de Sousa NR, Papanicolaou N, Vondracek M, Aarum J, Safari H, Muradrasoil S, Rothfuchs AG, Albert J, Hogberg B and Reinius B. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. *Nat Commun* 11, 4812 (2020). <https://doi.org/10.1038/s41467-020-18611-5>
- Kiran U, Gokulan CG, Kuncha SK, Vedagiri D, Chander BT, Sekhar AV, Dontamala S, Reddy AL, Tallapaka KB, Mishra RK, Harshan KH. Easing diagnosis and pushing the detection limits of SARS-CoV-2. *Biol Methods Protoc.* 2020 Aug 20;5(1):bpaa017. doi: 10.1093/biomethods/bpaa017. PMID: 33072873; PMCID: PMC7454390.
- Mosi L, Sylverken AA, Oyebola K, Badu K, Dukhi N, Goonoo N, Mante PK, Zahouli J, Amankwaa EF, Tolba MF, Fagbamigbe AF, De Souza DK & Matoke-Muhia D. (2021) Correlating WHO COVID-19 interim guideline 2020.5 and testing capacity, accuracy, and logistical challenges in Africa. *PanAfrican Medical Journal.* 39(89) <http://hdl.handle.net/20.500.11910/16379>
- Bruce EA, Huang ML, Perchetti GA, et al. Direct RT-qPCR detection of SARS-CoV-2 RNA from patient nasopharyngeal swabs without an RNA extraction step. *Plos Biology.* 2020 Oct;18(10):e3000896. DOI: 10.1371/journal.pbio.3000896. PMID: 33006983; PMCID: PMC7556528.

- 8 Badawi A, Ryoo SG. Prevalence of comorbidities in the Middle East respiratory syndrome coronavirus (MERS-CoV): a systematic review and meta-analysis. *Int J Infect Dis.* 2016 Aug;49:129-33. doi: 10.1016/j.ijid.2016.06.015. Epub 2016 Jun 21. PMID: 27352628; PMCID: PMC7110556.
- 9 Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* 2020 Feb 15;395(10223):507-513. doi: 10.1016/S0140-6736(20)30211-7. Epub 2020 Jan 30. PMID: 32007143; PMCID: PMC7135076.
- 10 Wang B, Li R, Lu Z, Huang Y. Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. *Aging (Albany NY).* 2020 Apr 8;12(7):6049-6057. doi: 10.18632/aging.103000. Epub 2020 Apr 8. PMID: 32267833; PMCID: PMC7185114.
- 11 Dhakad MS, Gogoi S, Kumari A, Singh AK, Jais MB, Prakash A, Pangtey G, Kaur R. Comparative evaluation of cost effective extraction free molecular technique for detection of SARS-CoV-2 with reference to standard VTM based RT-qPCR method. *Iran J Microbiol.* 2021 Dec;13(6):748-756. doi: 10.18502/ijm.v13i6.8073. PMID: 35222851; PMCID: PMC8816692.
- 12 Singhal T. A Review of Coronavirus Disease-2019 (COVID-19). *Indian J Pediatr.* 2020 Apr;87(4):281-286. doi: 10.1007/s12098-020-03263-6. Epub 2020 Mar 13. PMID: 32166607; PMCID: PMC7090728.
- 13 Mishra, B., Ranjan, J., Purushotham, P., Saha, S., Payal, P., Kar, P., Das, S., & Deshmukh, V. (2022). High proportion of low cycle threshold value as an early indicator of COVID-19 surge. *Journal of medical virology*, 94(1), 240–245. <https://doi.org/10.1002/jmv.27307>
- 14 Esbin MN, Whitney ON, Chong S, Maurer A, Darzacq X, Tjian R. Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection. *RNA.* 2020 Jul;26(7):771-783. doi: 10.1261/rna.076232.120. Epub 2020 May 1. PMID: 32358057; PMCID: PMC7297120.
- 15 Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, Boodman C, Bello A, Hedley A, Schiffman Z, Doan K, Bastien N, Li Y, Van Caesele PG, Poliquin G. Predicting Infectious Severe Acute Respiratory Syndrome Coronavirus 2 From Diagnostic Samples. *Clin Infect Dis.* 2020 Dec 17;71(10):2663-2666. doi: 10.1093/cid/ciaa638. PMID: 32442256; PMCID: PMC7314198.

