

## **Molecular diagnosis of severe acute respiratory syndrome virus 2 (SARS-CoV-2) Covid-19: State of the art and where we go from here**

**James B. Mahony**

Department of Pathology & Molecular Medicine, McMaster University, and The Research Institute of St. Joe's Hamilton, St. Joseph's Healthcare Hamilton, Hamilton, Ontario Canada

**\*Corresponding author:** James B. Mahony, Department of Pathology & Molecular Medicine, McMaster University, and The Research Institute of St. Joe's Hamilton, St. Joseph's Healthcare Hamilton, Hamilton, Ontario Canada; E-mail: [mahonyj@mcmaster.ca](mailto:mahonyj@mcmaster.ca)

### **ABSTRACT**

There are a large number of PCR tests available from a variety of manufacturers that have received EUA by the FDA. These PCR tests together with research-based tests have been used to quantitate viral RNA during the course of infection in a variety of Covid-19 patients. The most commonly used molecular test for diagnosing SARS-CoV-2 has been the RT-qPCR test. None of the molecular tests including RT-qPCR, LAMP, CRISPR or NGS however, can distinguish between live and dead virus and provide this important piece of information to public health authorities to help control the pandemic. Similarly, detection of viral antigens or antibodies do not provide information on infectivity which is important for people returning to the workplace after quarantine. Some newly developed saliva tests including the BinaxNOW rapid COVID-19 test (Abbott Diagnostics) for nasal swabs from symptomatic individuals up to 7 days post onset of symptoms utilize lateral flow of lateral flow technology to provide rapid easy to read test results in under 30 minutes and should help to increase testing volumes. Saliva antigen tests that measure viral proteins represent the first POC tests that do not require laboratories to perform the testing and can be performed by non-healthcare workers. The advantage of saliva antigen tests is that they can be self-administered and used at home or in a variety of settings including the workplace. Saliva RNA testing on the other hand, usually requires a laboratory to perform RNA extraction and nucleic acid amplification and therefore do not provide rapid resulting. The next major and long-awaited development in Covid-19 diagnostics will be the development of an RNA POCT providing a rapid result that can be performed at the point-of-need without the need for any instrumentation or equipment. A rapid POCT for viral RNA will provide additional information on infectivity beyond what the antigen test provides and may replace the antigen POCT for determining whether a person is infectious.

### **Keywords**

RT-PCR  
RT-LAMP  
NGS  
point-of-care (POC) testing

### **ABBREVIATIONS**

**SARS-CoV-2:** Severe acute respiratory syndrome virus 2

**Covid-19:** SARS-CoV-2 infection

**NAATs:** Nucleic acid amplification tests

**RT-qPCR:** Quantitative reverse transcriptase polymerase chain reaction

**RT-LAMP:** Reverse transcriptase loop-mediated isothermal amplification

**NGS:** Next generation sequencing

**LoD:** Limit of detection

**POC (T):** Point-of-care (test)

**CRISPR:** Clustered regularly interspaced short palindromic repeats

**EUA:** Emergency use authorization

## Review Article

### RT-PCR

There are a number of NAATs that have been used for the molecular detection of bacterial and viral pathogens. RT-qPCR is the most commonly used molecular test and has become the workhorse in molecular diagnostics for SARS-CoV-2 [1]. It can be performed using a range of clinical specimens including nasopharyngeal swabs, nasal swabs, BAL specimens, throat swabs, blood, feces and more recently saliva. A typical RT-qPCR can take 4-6 hours from sample collection to resulting and in high volume labs up to one week. A variety of gene targets have been used for RT-qPCR including one or more of the envelope (E), nucleocapsid (N), RNA-dependent RNA polymerase (RdRp), or spike (S) genes [2]. Nalla et al. (2020) compared the performance of primers-probes for various genes and found that the CDC N2 and Corman E gene primers gave the highest sensitivity and reproducibility with a LoD of 6 genome equivalents [3]. The amount of virus present in clinical specimens is quantitated by the RT-PCR cycle threshold (cutoff of positivity usually 40 cycles) and viral loads can be followed during the course of infection. SARS-CoV-2 RNA can be detected as early as 1 day post onset of symptoms and peaks within the first week of symptom onset. This positivity starts to decline around week 3 and subsequently becomes undetectable usually after 5 weeks [2,3]. Copy number varies across different patient populations, specimen types and days post infection. For example, stool specimens generally have lower copy numbers by several logs compared with NP specimens while anal swabs appear to be positive more often in later stages of infection compared with NP specimens and the value of testing feces has not been systematically evaluated.

As of March 30 the FDA had approved over 22 *in vitro* Emergency Use Authorization diagnostic tests. Few comparisons of these commercial tests have been performed. Lieberman et al. (2020) compared four commercial RT-qPCR tests: Cepheid's Xpert Xpress SARS-CoV-2, Hologic's Panther Fusion SARS-CoV-2 Assay, DioSorin's Simplexa COVID-19 Direct RT-PCR kit, and Roche's cobas SARS-CoV-2 test [4]. All tests were equally specific (100%) but Cepheid's Xpert Xpress had higher sensitivity with a lower LoD. The limitation of this study was that only 26 specimens were evaluated.

Although RT-qPCR has been extremely useful in the study of Covid-19 it has significant limitations in that it requires extraction of RNA, expensive laboratory equipment, trained personnel, and usually takes several hours or even days to provide results. For these reasons, RT-qPCR is unlikely to be used in POC settings where other tests such as RT-LAMP and clustered regularly interspaced short palindromic repeats (CRISPR) are better suited. A major drawback of RT-qPCR and all molecular tests is that a positive result only reflects the presence of viral RNA and does not distinguish between viable or dead virus which is important to public health

authorities trying to control the spread of the pandemic. Virus viability can only be ascertained by culturing the virus. Despite these limitations RT-qPCR has been the workhorse of molecular diagnostics and has contributed immensely to our understanding of the natural history of Covid-19 infection.

### Loop-mediated isothermal amplification

Although RT-qPCR is the gold standard for the detection of SARS-CoV-2 because of its high sensitivity and specificity, there are significant caveats as mentioned above including the requirement for laboratory facilities, expensive instruments and trained personnel to perform the test. To overcome these limitations isothermal amplification methods have been developed and these can be applied to POC settings. There are a number of isothermal amplification methods that have been applied to the detection of pathogenic bacteria and viruses. These include loop-mediated amplification (LAMP), strand displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA), transcription mediated amplification (TMA), rolling circle amplification (RCA), recombinase polymerase amplification (RPA), helicase-dependent amplification (HDA), signal mediated amplification of RNA technology (SMART), and specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) [5], however, only three of these methods have been applied to Covid-19 detection. LAMP has been used for the detection of a number of viruses including SARS-CoV-1, MERS, Dengue, Ebola, Zika virus and others. We have developed multiplex LAMP assays for detecting respiratory syncytial virus (RSV) A and B and another for influenza A/H1, A/H3 and B that can provide results in 20 minutes [6,7]. Both of these LAMP assays had analytical sensitivities of 1 genome equivalent for each virus.

Following the success of RT-PCR, the second most often used method to detect SARS-CoV-2 RNA has been RT-LAMP. LAMP utilizes 6 primers (2 inner, 2 outer, and 2 loop primers) and a strand displacement polymerase such as *Bst* polymerase and is performed at a constant temperature of 62-65° C. Specimen preparation in particular RNA extraction can often take up to an hour to perform in laboratories that perform a large volume of molecular tests and extraction usually takes longer than the time required for amplification which can be as little as 15-20 minutes. RT-LAMP is well suitable for POC testing including home testing and other non-clinical settings because it does not require thermal cycling and can be more sensitive than RT-qPCR.

RT-LAMP assays have been described for the detection of three coronaviruses including SARS-CoV-1, MERS and SARS-CoV-2 [8]. Park et al. (2020) developed an RT-LAMP assay for SARS-CoV-2 that had a LoD of 100 copies of SARS-CoV-2 RNA with no cross reactivity to other human coronaviruses [9]. These authors compared the performance of 16 LAMP primer sets targeting the Nsp3, Orf8, and S genes. The 100 copy LoD

was achieved with two primer pairs targeting the Nsp3 gene. This assay used leuco crystal violet colorimetric detection and generated results in 30 minutes that could be read visually without any equipment [9]. The authors suggest that the leuco crystal violet detection method is well suited for use in POC tests.

Yu et al. (2020) developed a sensitive RT-LAMP assay (iLACO) targeting the ORF1ab gene for rapid and colorimetric detection of SARS-CoV-2 [10]. The assay had a LoD of 10 copies, used a pH dye providing a visual color change (red to yellow), and the results could be read in 15-40 minutes. Jiang et al. (2020) developed an RT-LAMP with primers targeting ORF1ab, E and N genes. The assay had a sensitivity of 91.4% and a specificity of 99.5% based on testing 47 patients with and 213 patients without SARS-CoV-2 infection [11]. In another study of 17 and 191 patients by Yang et al. (2020) similar sensitivity and specificity were obtained [12]. These authors suggest that the major drawback of RT-LAMP is that it has a low throughput as only one sample can be processed at a time compared to RT-PCR where up to 96 specimens can be tested in one run on specific pieces of lab equipment. This is true if the testing is performed in a lab but the advantage of LAMP is the rapid resulting time which lends itself to POCT testing of one specimen without batching of specimens.

In another study of two separate cohorts of patients Hu et al. (2020) compared the performance of RT-LAMP with RT-qPCR using specimens from 81 Covid-19 positive patients and 400 clinically negative controls [13]. The LoD of RT-LAMP was 4 copies/reaction [13] while RT-qPCR had an LoD of 42 copies/reaction similar to findings seen in two other studies [14,15]. The RT-LAMP assay developed by Hu et al. had an overall sensitivity of 88.89% and specificity of 99.0% and a higher sensitivity compared with RT-qPCR 88.89% vs. 81.48% [13]. Their RT-LAMP assay targeted the S gene, used a visual readout but took 1 hour to perform. The strength of this study is that it used 481 clinical respiratory specimens from two different cohorts of suspected Covid-19 patients. RT-LAMP has shown a 10-fold higher analytical sensitivity than RT-qPCR in several studies in different clinical settings.

Jinzhao Song and colleagues at the University of Pittsburgh described a novel closed tube Penn-LAMP assay, an innovative two stage RPA followed by RT-LAMP [16]. The assay performed in a single tube used leucocrystal violet dye providing a deep violet color easily read by the naked eye [16]. The tube was incubated for 15-20 min at 38° C for RPA followed by 40 min at 63° C for LAMP. The Penn-LAMP process provided 100 times greater sensitivity than RT-PCR or LAMP alone. They claim that this one tube assay can be run at home and offers true POC testing; but is the need for two different incubation temperatures and vortexing amenable to POC testing? By comparison, Yang et al. (2020) combined RT-LAMP technology with lateral flow paper detection and smart

phone connectivity to provide a true POC test and facilitate result sharing with healthcare providers [15]. Commercial manufacturers clearly need to incorporate these applications in the development of rapid and inexpensive POC tests to facilitate containment of Covid-19.

Investigators at Columbia University (Wei et al. 2020) have developed a high performance LAMP assay that can detect 1 copy RNA/1  $\mu$ L in saliva specimens [17]. This HP-LAMP assay uses a pre-loaded microfuge tube containing all the necessary reagents for amplification and had a sensitivity and specificity of 97% and 100% compared with the Roche RT-PCR test. Using this HP-LAMP test saliva specimens gave concordant results with NP specimens.

Most of the LAMP and CRISPR isothermal amplification tests have not yet been approved by health care authorities worldwide nor have they been independently evaluated. Atila BioSystems LAMP COVID-19 Detection Kit is approved for emergency use by FDA and reports a low LoD (4 copies/ $\mu$ L), 100% sensitivity and 1 h turn-around time. Moore et al. (2020) compared the Abbott RealTime SARS-CoV-2 RT-qPCR with the ID NOW™ COVID-19 test which received EUA approval in March 2020. The ID NOW™ COVID-19 test utilizes isothermal amplification, has a fast turn-around time of <13 minutes for nasal swabs and throat swabs and uses the portable the ID NOW instrument. They showed that the RealTime SARS-CoV-2 assay was more sensitive and reliable than the ID NOW™ COVID-19 test [18]. In another study by Basu et al. (2020) the ID NOW test was less sensitive than the Cepheid Xpress Xpert giving negative results for one third of NP swabs that tested positive by Cepheid Xpress Xpert [19].

### CRISPR/Cas diagnostic assays

Prokaryotes are known to keep several copies of foreign pathogenic genetic elements in genomic loci called clustered regularly interspaced short palindromic repeats (CRISPR). The prokaryotic CRISPR/Cas system captures and inserts specific DNA sequences into DNA and uses the Cas endonuclease to remove foreign nucleic acid. The CRISPR/Cas technology was originally developed as a third generation genome editing technology. The promiscuous cleavage activities of a unique group of Cas nucleases have been harnessed for *in vitro* nucleic acid detection.

A recent addition to Covid-19 RNA molecular diagnostic armamentarium is the SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) protocol described by Joung et al. (2020) that uses a two-step target amplification and CRISPR-mediated detection in a one tube testing algorithm. This SHERLOCK testing in one pot (STOP) combines simplified extraction of viral RNA with isothermal amplification and CRISPR-mediated detection [20]. A CRISPR based diagnostic kit has been developed by Sherlock Biosciences in Cambridge Massachusetts. This test has been used for screening in Thai

## Review Article

hospitals and shows rapid, sensitive and accurate detection of SARS-CoV-2. The first version of the test released in January 2020 was called STOP (SHERLOCK testing in one pot) while the current test is called STOPCovid. The Sherlock™ CRISPR test was the first CRISPR-based test approved for EUA by the FDA. It provides results in 1 h and has a reported specificity of 100% with a LoD of 6 copies/uL [20]. The second test version (STOPCovid.v2) uses CRISPR enzymes that are modified to detect coronavirus RNA with an LoD of 100 copies of RNA. This test uses a lateral flow test strip for ease of reading the result. Sherlock Biosciences validated the test with NP swabs and it had a 97% sensitivity and 100% specificity compared to the CDC RT-qPCR test. It is hoped that this test which has been shown to work with a variety of clinical specimens including saliva and gives results in under an hour will help meet the growing need for ramped up testing. The company is working to create a single cartridge test that can be used in POC settings. Other laboratories in California and Argentina are also developing CRISPR assays.

Broughton et al. (2020) combined LAMP and Cas endonuclease to develop a CRISPR/Cas12 based lateral flow assay that detects SARS-CoV-2 RNA using extracted RNA. The assay had an LoD of 10 copies/uL compared with 1 copy/uL for the CDC RT-PCR test [21]. Extracts were first amplified by RT-LAMP followed by Cas12 and lateral flow detection. The assay involved RNA extraction (10 minutes), RT-LAMP (40 minutes) and lateral flow detection and took about an hour to provide results. The assay was evaluated with 80 respiratory swab specimens (36 Covid-19 patients and 42 other respiratory virus infected patients) and showed a 95% sensitivity and 100% specificity compared with RT-qPCR. This was the second CRISPR technology-based assay to be granted EUA status by the US FDA and is being produced by Mammoth Biosciences in California.

Abbott et al. (2020) first used CRISPR technology to destroy SARS-CoV-2 sequences in eukaryotic cells [22]. Lucia et al reported a CRISPR/Cas12 assay capable of detecting SARS-CoV-2 RNA with a LoD of 10 copies/uL and an estimated cost of \$1-2 USD per reaction [23]. Hou et al. (2020) described a modified CRISPR/Cas13 assay that combined a Recombinase Polymerase Amplification (RPA) step, followed by T7 transcription and Cas13 detection. This assay had a single copy LoD and a sensitivity of 100% detecting 52/52 Covid-19 positive cases [24]. Collectively, these results highlight the potential of CRISPR-based diagnostic methods. Despite these promising results CRISPR/Cas-based diagnostics are not widely used by clinical laboratories and need further evaluation.

### Next-generation sequencing

Next generation sequencing (NGS) is a genomics technology that enables the simultaneous sequencing of billions of DNA fragments. Several applications of NGS include mutation and pathogen discovery, infection control surveillance, outbreak

tracking, and diagnosis of infectious diseases. As of today, there are 12 sequencing platforms from Illumina and Oxford Nanopore Technologies. Metagenomics NGS was first used by Zhou et al. (2020) to detect the presence of SARS-CoV-2 in five Covid-19 patients in Wuhan [25]. The sequence of this virus is 79.6% identical to SARS-CoV identified in 2003 and 96% identical to a bat coronavirus [25]. NGS categorized SARS-CoV-2 as a *Betacoronavirus* with its closest viral relatives being two bat coronaviruses. Although useful in identifying related organisms the analytical sensitivity of NGS for pathogen detection has not clearly been established and is limited due to the resident microbiota which can result in high background [26]. In addition, large-scale population testing may be limited by the high cost of NGS instrumentation.

One application of NGS has been described which combines RT-LAMP and NGS to make possible the testing of a very large number specimens in a single run as described by Schmid-Burgk et al. [27]. This test called LAMP-Seq involves testing an individual specimen by LAMP using specially designed barcoding primers, then sending the amplified material to the lab for additional PCR amplification, followed by NGS testing. The authors suggest that this approach could in theory test millions of specimens in a single day. If true this would certainly provide a significant increase in population-scale testing. Further studies will be required to confirm this diagnostic approach.

## CONCLUSION

Although this is a review on molecular diagnostic testing a few words need to be said about the recently released antigen tests. At the time of writing four rapid antigen-based tests have received EUA by the FDA (the Veritor System by Becton Dickinson, a Quidel test to be run on the Sofia, A third test from LumiraDx, and the BinaxNOW COVID-19 nasal swab test from Abbott). These tests give results in 15-30 minutes. The LumiraDx test is a microfluidic immunofluorescence assay that requires an instrument to provide results in 12 minutes. The BinaxNOW rapid antigen card test is designed for use on nasal swabs from symptomatic individuals with symptom onset <7 days. It received EUA on August 26, 2020 and is the first rapid test that does not require any special equipment. It is inexpensive (\$5/test), can provide results in 15 minutes and has a sensitivity of 97% compared with RT-PCR. The Yale SalivaDirect "protocol" is an extraction free PCR protocol that can be used with a variety of vendor's reagents and equipment. It is the fifth saliva test to be given EUA by the FDA. The SalivaDirect protocol does not require a swab while the BinaxNOW test requires a nasal swab to be collected by a healthcare provider. Both of these tests require trained individuals to run and cannot be performed by the lay public at home. Viral titers determined by RT-PCR in saliva specimens are similar to or slightly lower than that found in NP specimens [28]. In another study Wyllie et al. (2020) reported that saliva specimens were positive in 13 asymptomatic healthcare workers and 7 of these individuals had negative self-

collected NP specimens [29]. More studies will be required to validate the usefulness of saliva RNA testing in symptomatic and asymptomatic individuals. In general, the rapid antigen tests are less sensitive than RNA tests and a negative antigen result may require a follow up lab-based RNA test for confirmation. Several companies are developing rapid at-home tests but none have yet received EUA status. Despite these limitations, implementation of rapid antigen tests should allow the number of tests performed per day to surpass the 850,000 performed in the United States in August 2020 and approach the desired number of 4 million tests per day by October.

RNA tests for POC use should combine ease of specimen collection that does not require swabbing (example saliva or gargle/spit specimens) with the ease of reading lateral flow results so that they can be used by untrained health care professionals and the lay public [30]. More than 190 coronavirus tests have received EUA by US regulators in an effort to provide testing at a pace that can keep up with the pandemic but at the time of writing there are no home based tests that have received EUA approval. Saliva specimens used for antigen testing do not require extensive processing other than solubilization for reaction with antibodies. RNA testing on saliva specimens on the other hand will require virus lysing and/or RNA extraction which is usually the rate limiting step prolonging the turn-around time for resulting. Alternatives to RNA extraction such as heat or alkaline lysis are being explored. The development of molecular POCTs is certainly possible and several commercial companies are working on molecular tests that use paper-based detection suitable for POC use. At the time of writing there are no paper based RNA tests have received FDA approval.

Our understanding of the natural history of SARS-CoV-2 especially it's pathogenesis *viz.* multiple organ system involvement and the subsequent outcome for patients has evolved quickly largely in part to the use of molecular testing. The availability of sensitive and specific NAATs such as RT-qPCR has been essential to our understanding of the pathogenesis and epidemiology of SARS-CoV-2 virus infections. The rapid growth in our knowledge of Covid-19 pathogenesis will be exceeded only by the commendable effort of the international scientific community in its efforts to develop a Covid-19 vaccine.

## REFERENCES

1. Afzal A (2020) Molecular diagnostic technologies for COVID-19: Limitations and challenges. *Journal of Advanced Research*, <https://doi.org/10.1016/j.jare.2020.08.002>.
2. Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, Singh KP, Chaicumpa W, Bonilla-Aldana DK, Rodriguez-Morales AJ. (2020) Coronavirus disease 2019- Covid-19. *Clin Microbiol Rev* 33:e00028-20, <https://doi.org/10.1128/CMR.00028-20>.
3. Nalla AK, Casto AM, Huang MW, Perchetti GA, Sampoleo R, Shrestha L, Wei Y, Zhu H, Jerome KR, Greninger AL. (2020) *J Clin Microbiol* 58: e00557-20, <https://doi.org/10.1128/JCM.00557-20>.
4. Lieberman JA, Pepper G, Naccache SN, Huang ML, Jerome KR, Greninger AL (2020) Comparison of Commercially Available and Laboratory Developed Assays for *in vitro* Detection of SARS-CoV-2 in Clinical Laboratories. *J Clin Microbiol*.doi: <https://doi.org/10.1128/JCM.00821-20>.
5. Stone CB, Mahony JB. (2014). Molecular detection of bacterial and viral pathogens - Where do we go from here? *Clin Microbiol* 3(6); 3:(6) 175 <https://doi.org/10.4172/2327-5073.1000175>.
6. Mahony J, Chong S, Bulir D, Ruyter A, Mwawasi K, Waltho D (2013) Development of a sensitive loop-mediated isothermal amplification (LAMP) assay providing specimen- to-result diagnosis of RSV infections in 30 minutes. *J. Clin. Microbiol*; 51(8): 2696-2701.
7. Mahony J, Chong S, Bulir D, Ruyter A, Mwawasi K, Waltho D (2020) Multiplex loop-mediated isothermal amplification (M-LAMP) for the detection of Influenza A/H1, A/H3 and B can provide a specimen-to-result diagnosis in forty minutes with a single genome copy sensitivity. *J Clin Virol* 2013 58:127-131 Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. (2020). Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA* March 11, 2020, <https://doi.org/10.1001/jama.3786>.
8. Rabiee N, Bagherzadeh M, Ghasemi A, Zare H, Ahmadi S, Fatahi Y, Binarvand R, Rabiee M, Ramakrishna S, Shokouhimehr M, Varma RS. (2020). Point-of-use rapid detection of SARS-CoV-2: Nanotechnology-enabled solutions for the COVID-19 pandemic. *Int J Mol Sci*; 21 5126-5160.
9. Park GS, Ku K, Baek SH, Kim SJ, Kim SI, Kim BT, Maeng JS. (2020). Development of reverse transcriptase loop-mediated isothermal amplification assays targeting severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). *J Mol Diagn*; 22(6)
10. Yu L, Wu S, Hao X, Li X, Liu X, Ye S, Han H, Dong X, Li X, Li J (2020) Rapid colorimetric detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform: iLACO. *medRxiv* <https://10.1101/2020.02.20.20025874>.
11. Jiang M, Fang W, Aratehfar A, et al. (2020) Development and validation of a rapid single-step reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) system potentially to be used for reliable and high-throughput screening of COVID-19. *MedRxiv*.
12. Yang W, Dang X, Wang Q, et al. (2020) Rapid detection of SARS-CoV-2 using reverse transcriptase RT-LAMP method. *MedRxiv*.

## Review Article

13. Hu X, Deng Q, Li J, Chen J, Wang Z, Zhang X, Fang Z, Li H, Zhao Y, Yu P, Li W, Wang X, Li S, Zhang L, Hou T. (2020) Development and clinical application of a rapid and sensitive loop-mediated isothermal amplification test for SARS-CoV-2 infection. *mSphere* 5:e00808-20. <https://doi.org/10.1128/mSphere.00808-20>.
14. Huang WE, Lim B, Hsu CC, Xiong D, Wu W, Yu Y, Jia H, Wang Y, Zeng Y, Ji M, Chang H, Zhang X, Wang H, Cui Z. 2020. RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. *Microb Biotechnol* 13:950–961.
15. Yang T, Wang YC, Shen CF, Cheng CM (2020) Point-of-care RNA-based diagnostic device for COVID-19. *Diagnostics* 10:165. <https://doi.org/10.3390/diagnostics10030165>
16. El-Tholoth M, Bau HH, Song JZ (2020) A single and two-stage, closed-tube molecular test for the 2019 novel coronavirus (COVID-19) at home, clinic, and points of entry. *ChemRxiv*.
17. Wei S, Kohl E, Djandji A, Morgan S, Whittier S, Mansukhani M, Yeh R, Alejaldre JC, Fleck E, D'Alton M, Suh Y, Williams Z. (2020). Field-deployable, rapid diagnostic testing of saliva samples for SARS-CoV-2. *medRxiv* doi: <https://doi.org/10.1101/2020.06.13.20129841>. Posted on 2020-06-16.
18. Moore NM, Li H, Schejbal D, Lindsley J, Hayden MK (2020) Comparison of two commercial molecular tests and a laboratory-developed modification of the CDC 2019-nCoV RT-PCR assay for the detection of SARS-CoV-2. *J Clin Microbiol*. doi: <https://doi.org/10.1128/JCM.00938-20>.
19. Basu A, Zinger T, Inglima K, Woo K-M, Atie O, Yurasits L, See B, Aguero-Rosenfeld ME (2020) Performance of Abbott ID Now COVID-19 rapid nucleic acid amplification test using nasopharyngeal swabs transported in viral transport media and dry nasal swabs in a New York City academic institution. *J Clin Microbiol* 58:e01136-20. <https://doi.org/10.1128/JCM.01136-20>.
20. Joung J, Ladha A, Saito M, Kim N, Woolley AE, Segel M, Barretto RP, Ranu A, Macrae RK, Ioannidi EI, Krajeski RN, Bruneau R, Huang MW, Yu, XG, Li JZ, Walker BD, Hung DT, Greninger AL, Jerome, KR, Gootenberg JS, Abudayyeh OO, Zhang F (2020). Detection of SARS-CoV-2 with SHERLOCK one-pot testing. *N Engl J Med*, September 16, <https://doi.org/10.1056/NEJMc2026172>.
21. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, Miao X, Streithorst JA, Granados A, Sotomayor-Gonzalez A, Zorn K, Gopez A, Hsu E, Gu W, Miller S, Pan CY, Guevara H, Wadford DA, Chen JS, Chiu CY. 16 (2020) CRISPR-Cas12- based detection of SARS-CoV-2. *Nat Biotechnol* <https://doi.org/10.1038/s41587-020-0513-4>.
22. Abbott (2020) Abbott launches molecular point-of-care test to detect novel coronavirus in as little as five minutes. <https://abbott.mediaroom.com/2020-03-27-Abbott-Launches-Molecular-Point-of-Care-Test-to-Detect-Novel-Coronavirus-in-as-Little-as-Five-Minutes>. Accessed 2 April 2020.
23. Lucia C, Federico PB, Alejandra, GC (2020) An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12. *bioRxiv*.
24. Hou T, Zeng W, Yang M, Chen W, Ren L, Ai J, Wu J, Liao Y, Gou X, Li Y (2020) Development and evaluation of a CRISPR-based Diagnostic For 2019-novel Coronavirus. *medRxiv*.
25. Zhou P, Yang XL, Wang XG et al. (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*; 579:270-273.
26. Gu W, Miller S, Chiu CY (2019) Clinical metagenomics next-generation sequencing for pathogen detection. *Annu Rev Pathol*; 14:319-338.
27. Schmid-Burgk J, Li D, Feldman D, et al. LAMP-Seq: population-scale COVID-19 diagnostics using a compressed barcode space. *bioRxiv*. 2020. Nguyen T (2020). The race to develop paper-based tests for Coronavirus - *Medscape* - , August 26, 2020 <https://www.medscape.com/viewarticle/936355>.
28. Williams E, Bond K, Zhang B, Putland M, Williamson DA. (2020) Saliva as a noninvasive specimen for detection of SARS-CoV-2. *J Clin Microbiol* 58(8)e00776-20. <https://doi.org/10.1128/JCM.00776-20>.
29. Wyllie AL, et al. (2020) Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs. *medRxiv*, 2020.2004.2016.20067835, [doi.org/10.1101/2020.04.16.20067835](https://doi.org/10.1101/2020.04.16.20067835).
30. Stone C, Mahony J (2018) Point-of-care (POC) tests for infectious diseases – the next generation! *Annals of Infectious Disease and Epidemiology*: 3(1): 1025.