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.

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Current Opinion in Virology

Test it earlier, result it faster, makes us stronger: how rapid viral diagnostics enable therapeutic success

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The COVID-19 pandemic has entailed simultaneous revolutions in virology diagnostics, clinical trials management, and antiviral therapy and vaccinology. Over the past year, SARS-CoV-2 diagnostic testing has moved from highly centralized laboratories to at-home and even over the-counter. This transition has been lionized for its potential public health impact via isolation, but has been less examined for its effect on individual health and therapeutics. Since early initiation of antiviral therapy routinely has been associated with greater treatment efficacy for viral infections, these diagnostic testing innovations offer new opportunities for both clinical testing as well as clinical trials for antiviral therapy. Given a rapidly growing antiviral therapeutic pipeline and the profound impact of individual beneficiary outcomes on sculpting reimbursement policy, the therapeutic benefits associated with rapid viral testing may lead to significant adoption beyond potential public health impacts.

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Introduction

The COVID-19 pandemic has been a hurricane of worldwide disruption. If any silver lining can be found to the pandemic, it is in the rapid and successful implementation of incipient technologies, such as mRNA vaccines, prefusion glycoprotein immunogens, monoclonal antibody generation pipelines, and a variety of new diagnostic testing platforms along with a dramatically expanded capacity for viral diagnosis. As of writing, more than 450 million diagnostic tests for SARS-CoV-2 have been performed in the United States and more than 350 *in vitro* diagnostics have been authorized by the FDA. Viral testing turnaround times have explicitly been written into Medicare reimbursement policy. These gains do not necessarily have to involute due to laboratory utilization management if they can work in tandem with new therapeutic antiviral agents by offering earlier, more rapid, and decentralized diagnosis for viral infection.

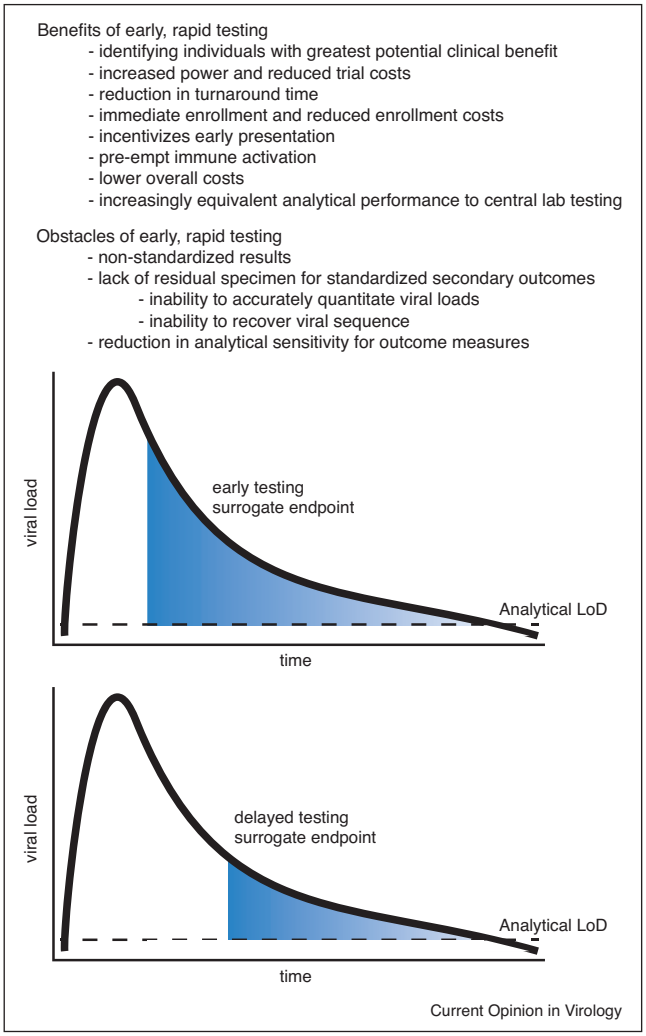
Early diagnosis and treatment are paramount for antiviral therapeutics

As has long been appreciated for influenza virus treatment [1,2••], early diagnosis and initiation of treatment significantly increases the chances of clinical benefit and reaching primary and secondary outcomes in COVID-19 therapeutic trials. In the double-blind, placebo-controlled ACTT-1 trial, patients who received remdesivir earlier had significantly greater clinical benefit which was greatest in patients who received the drug before six days after symptom onset [3,4]. The mortality benefit of anti-SARS-CoV-2 convalescent plasma has also been shown to be dependent on early initiation, albeit in a non-randomized fashion [5]. In support of the early treatment hypothesis, the two monoclonal antibody therapies that have been authorized for COVID-19 treatment were only successful as outpatient therapy [6••,7]. In contrast, multiple anti-SARS-CoV-2 therapeutic monoclonal antibody trials in hospitalized patients have now been suspended due to futility, indicating that waiting until hospital admission may be too late [8]. The clinical benefit of early antiviral initiation for respiratory diseases may in part be due to reducing widescale immune activation, in part evidenced by the clinical benefit of steroid treatment in individuals hospitalized with severe illness [9]. Furthermore, initiating treatment early gives a potential greater benefit for surrogate outcomes such as viral load reduction, given that SARS-CoV-2 viral loads are often highest at symptom onset (Figure 1) [10].

Faster viral diagnostics for clinical trials are critical and achievable

The timescales by which diagnostic testing can help antiviral therapy are indeed quite brief. Initial COVID-19 symptoms are non-specific (save anosmia) and early in the pandemic patients often did not present to the hospital for testing for 4–8 days after symptom onset [11]. Time from symptom onset to testing in outpatient drive-through clinics locally was routinely 5–6 days. After a specimen is collected, median turnaround times in a central laboratory are often 6−24 hours, but may take up to 72 hours to return a test result, depending on a myriad

Figure 1



Summary of benefits and obstacles to early, rapid testing for clinical trial design for therapeutic antivirals, as it relates to COVID-19. Most important, the early detection of viral infection increases power by identifying those with greatest potential clinical benefit from therapy thus reducing overall trial costs. Early detection of cases also maximizes potential cumulative reductions in surrogate outcomes such as changes in viral load or O_2 demand (highlighted in blue).

of factors such as platform used, location of the testing laboratory, staffing levels, day of the week, and overall demand nationwide for testing and availability of reagents. Once a positive result is identified by an outside laboratory, it can take an additional 1–5 days to contact, enroll, and randomize a patient.

Shortening each of these time frames is critical for clinical trial design in order to have a chance at preventing hospitalization and disease progression. Reducing time from symptom onset to testing is probably the area where the most gains can be made and is most dependent on availability and cost of testing. Reducing turnaround times to hours can significantly reduce the hassle of enrollment by combining diagnosis and enrollment into one visit. In addition to identifying patients with the most potential clinical benefit, rapid, decentralized testing can also reduce trial costs at multiple touchpoints, eliminating central lab transit and accessioning costs, enrollment costs, even if decentralized testing is accompanied by occasional higher reagent costs. Most critical is the ability to run smaller, more highly powered studies by maximizing the potential effect size of a given therapy, as described above.

Of course, the initiation of therapy in individuals with mild symptoms or even no symptoms in the case of prophylactic therapy presents a prevention paradox, wherein specific populations with the potential greatest clinical benefit will have to be defined *ex ante* in the absence of pathogenesis, save the presence of virus. Here, age, comorbidity, and exposure status indications are most commonly used to determine clinical benefit, as illustrated by a number of prophylactic nursing home-based or household transmission trials during SARS-CoV-2 [12,13].

New availability of rapid viral diagnostics

With a $100/test reimbursement and seemingly infinite testing demand, the COVID-19 pandemic presented nearly all clinical laboratories the opportunity to adopt highly automated sample-to-answer platforms, save for the supply chain of the platforms themselves. Testing platforms such as the Roche cobas 6800/8800, Hologic Panther(Fusion)/Aptima, Abbott Alinity-m, and Cepheid Infinity each provide the ability to perform high hundreds to low thousands of tests a day with analytical sensitivities in the low hundreds of copies/mL, minimal hands-on time, and in-lab turn-around time under 4 hours [14•,15–19]. Compared to a typical laboratory-developed testing workflow — which requires discrete steps for nucleic acid extraction, PCR amplification, and reporting — these platforms can themselves be considered rapid testing platforms, especially if they can be performed in a random-access fashion [16,20]. These high-throughput platforms performed the lion's share of the two million tests a day across the United States and are now firmly entrenched in clinical and hospital laboratories across the country. The main limitation of the platforms other than the high reagent costs is the need to locate them in a central laboratory, which requires additional timing and logistics to physically move hundreds of samples to the instrument's location. Nonetheless, when discussing rapid viral testing and its future influence on clinical testing and trials, the widespread availability of high-throughput, sample-to-answer qRT-PCR platforms in 2020–21 is a major story, offering the possibility of same-day testing within every metropolitan area across the United States.

Beyond the canonical central laboratory testing, a number of new technologies and approaches emerged that offer more point-of-care testing opportunities. Significant growth was seen in the past year in point-of-care qRT-PCR testing platforms such as the Cepheid GeneXpert, Roche Liat, BioFire Respiratory Panel 2.1-EZ, or Mesa BioTech Accula systems, among others [18,21–23]. Isothermal technologies such as RPA and LAMP have allowed extraordinarily rapid (subhalf hour) turnaround times that are now FDA authorized for at-home testing with direct-to-consumer and over the-counter availability [24–27]. Thousands of clinics now own small Abbott IDNow toaster ovens that can perform limited multiplex testing with turnaround times of less than 15 min and low thousands of copies/mL analytical sensitivities [28••,29,30]. Though the test menu for many of these point-of-care instruments is currently somewhat limited compared to many of the centralized testing platforms, we will no doubt see new waived assays on these instruments since so many clinics now own them and prefer to garner the testing reimbursement rather than paying an outside laboratory. The main limitation with these rapid molecular assays, save the Cepheid GeneXpert, is the moderate compromise in analytical sensitivity [28••,31]. Provided an adequate specimen is obtained, these platforms may specifically select for individuals with the greatest potential benefit of antiviral therapy. However, currently most trials still require central laboratory confirmation of results from these more distributed testing platforms.

Finally, though fewer clinical trials have employed these to date, COVID-19 has seen the return of viral antigen testing with Quidel, Veritor, and BinaxNow assays in the United States market and a plethora of new rapid antigen tests abroad [32–35]. The limited analytical sensitivity of antigen testing fell out of favor after the 2009 H1N1 pandemic, when these tests had below 50% sensitivity for the novel reassortant influenza virus [36,37]. New antigen readers and rapid lateral flow technologies combined with specific development of reagents for SARS-CoV-2 and its comparably low genetic diversity brought more respectable analytical and clinical sensitivities for these assays, approaching Ct ~30 or viral loads in the mid-tens of thousands of copies/mL [38,39]. Depending on whether antigen testing was performed on asymptomatic or early symptomatic individuals and when testing was performed relative to peak community transmission, clinical sensitivities of SARS-CoV-2 antigen testing could range from as low as 56–77% in asymptomatic to >96% in individuals in their first week of symptoms [39–42]. The success has led to a new FDA pathways for authorization of at-home, over the-counter, and/or serial rapid antigen testing, with multiple rapid antigen tests now authorized for over the-counter purchase that can be run at home. The potential <$5 cost of the rapid antigen tests combined with the challenge of combating asymptomatic spread has brought focus on the public health potential of rapid testing [43•,44]. However, performance characteristics of these assays should continue to be monitored as new viral variants arise [45].

Obstacles to rapid testing for clinical trials

Despite the growth in rapid, point-of-care diagnostics available for clinical testing, there are unique requirements associated with clinical trials that complicate the use of distributed diagnostics as a complete testing solution. Additional testing is required to confirm primary endpoints on a standardized test as well as for secondary outcomes such as viral load quantitation or viral whole genome sequencing. Many of the new distributed testing platforms may make use of the entire sample to ensure adequate analytical sensitivity, requiring additional samples to be used for these secondary outcomes since no residual sample is available. By virtue of being located at the point-of-care, too many testing instruments are required to execute a clinical trial such that meeting regulatory requirements for cross-validation of each instrument is not possible. The potential variability in these assays creates a problem in comparing local site testing, often requiring a single standard assay to confirm primary outcomes.

Viral load quantitation

Since changes in viral loads are often used as a secondary outcome in trials, quantitative tests for SARS-CoV-2 viral levels are often required. All SARS-CoV-2 molecular tests for diagnostic use on the market to date have only been authorized by the FDA for qualitative detection, these assays are not set up to return viral loads. This complicates both the additional validation work required for quantitative testing for clinical trials as well as the testing workflow, which can lengthen turnaround time. Furthermore, international standards have been late to arrive for SARS-CoV-2. Distributed testing platforms described above cannot necessarily meet the need for standardized, quantitative testing that has been rigorously cross-validated across different testing units.

Real-time sequence-specific information

With more than a million genomes sequenced in a single year, SARS-CoV-2 is the most sequenced virus in the history of humankind. The surfeit of data combined with the increased potential for adaptation to humans of a new zoonosis and immune escape has brought heightened attention to viral evolution. Viral sequencing for sieve analysis for vaccine studies or resistance monitoring in therapeutic trials has traditionally been performed well after a clinical trial has released top-line numbers or even subgroup analyses and secondary endpoints [46]. With COVID-19, these analyses are being performed in near real-time with the release of primary outcome data. For specific therapeutic monoclonal antibodies in the context of highly diverse viruses, extremely rapid sequence-specific information may be required to determine eligibility.

Already specific lineages of SARS-CoV-2 demonstrate resistance to single monoclonal antibody therapies [47]. Again, none of the available authorized distributed COVID-19 diagnostic platforms currently provide the high-resolution, sequence-specific information required for these secondary analyses (Illumina COVIDSeq which would not count as a rapid test) [48]. While the emergency use authorization of CRISPR-based viral diagnostics is promising for potential rapid sequence-specific diagnosis, it does not offer sufficient resolution and requires high-consequence mutations to be determined before the development of the test [49,50]. For high viral load specimens, diagnostic antigen tests could make use of the very therapeutic monoclonals they are meant to determine susceptibility to, finally bringing some truth to the term 'companion diagnostic'.

Conclusions

An old saw in laboratory medicine is that you can only choose two when it comes to cost, speed, and accuracy in clinical testing. The COVID-19 pandemic significantly altered the landscape of clinical virology testing in a way not seen since HIV. With the growing pipeline of small molecule and monoclonal antibody therapies, there is a chance that costs of highly accurate, rapid testing may be covered by a growing reimbursement for the clinical benefit associated with these new diagnostic platforms, allowing clinicians, patients, and laboratorians to have their cake and eat it too. The simultaneous revolutions in widespread accessibility for diagnostics and newfound availability of therapeutics for respiratory viral infections creates a promising future for clinical trials and real-world clinical care in the coming years.

Conflict of interest statement

ALG reports contract testing from Abbott and research support from Merck and Gilead.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hayden FG, Sugaya N, Hirotsu N, Lee N, de Jong MD, Hurt AC, Ishida T, Sekino H, Yamada K, Portsmouth S *et al.*: **Baloxavir marboxil for uncomplicated influenza in adults and adolescents**. *N Engl J Med* 2018, **379**:913-923.

2. •• Aoki FY, Macleod MD, Paggiaro P, Carewicz O, El Sawy A, Wat C, Griffiths M, Waalberg E, Ward P, IMPACT Study Group: **Early administration of oral oseltamivir increases the benefits of influenza treatment**. *J Antimicrob Chemother* 2003, **51**:123-129

Impactful study of administration of oral antiviral for influenza in the first 12 hours of symptoms versus first 48 hours of symptoms.

3. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S *et al.*: **Remdesivir for the treatment of Covid-19 — final report**. *N Engl J Med* 2020, **383**:1813-1826.

4. Aleissa MM, Silverman EA, Acosta LMP, Nutt CT, Richterman A, Marty FM: **New perspectives on antimicrobial agents: remdesivir treatment for COVID-19**. *Antimicrob Agents Chemother* 2020, **65**.

5. Salazar E, Christensen PA, Graviss EA, Nguyen DT, Castillo B, Chen J, Lopez BV, Eagar TN, Yi X, Zhao P *et al.*: **Significantly decreased mortality in a large cohort of coronavirus disease 2019 (COVID-19) patients transfused early with convalescent plasma containing high-titer anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein IgG**. *Am J Pathol* 2021, **191**:90-107.

6. •• Chen P, Nirula A, Heller B, Gottlieb RL, Boscia J, Morris J, Huhn G, Cardona J, Mocherla B, Stosor V *et al.*: **SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with Covid-19**. *N Engl J Med* 2021, **384**:229-237

First therapeutic monoclonal antibody to be authorized by the FDA for SARS-CoV-2 in an outpatient setting.

7. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, Musser BJ, Soo Y, Rofail D, Im J *et al.*: **REGN-COV2, a neutralizing antibody cocktail, in outpatients with Covid-19**. *N Engl J Med* 2021, **384**:238-251.

8. ACTIV-3/TICO LY-CoV555 Study Group, Lundgren JD, Grund B, Barkauskas CE, Holland TL, Gottlieb RL, Sandkovsky U, Brown SM, Knowlton KU, Self WH *et al.*: **A neutralizing monoclonal antibody for hospitalized patients with Covid-19**. *N Engl J Med* 2021, **384**:905-914 http://dx.doi.org/10.1056/NEJMoa2033130.

9. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A *et al.*: **Dexamethasone in hospitalized patients with Covid-19**. *N Engl J Med* 2021, **384**:693-704.

10. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C *et al.*: **Virological assessment of hospitalized patients with COVID-2019**. *Nature* 2020, **581**:465-469.

11. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X *et al.*: **Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China**. *Lancet* 2020, **395**:497-506.

12. Barnabas RV, Brown ER, Bershteyn A, Stankiewicz Karita HC, Johnston C, Thorpe LE, Kottkamp A, Neuzil KM, Laufer MK, Deming M *et al.*: **Hydroxychloroquine as postexposure prophylaxis to prevent severe acute respiratory syndrome coronavirus 2 infection: a randomized trial**. *Ann Intern Med* 2021, **174**:344-352 http://dx.doi.org/10.7326/M20-6519.

13. Eli Lilly and Company: *A Phase 3 Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate the Efficacy and Safety of LY3819253 Alone and in Combination With LY3832479 in Preventing SARS-CoV-2 Infection and COVID-19 in Skilled Nursing and Assisted Living Facility Residents and Staff; a NIAID and Lilly Collaborative Study*. 2021clinicaltrials.gov.

14. • Lieberman JA, Pepper G, Naccache SN, Huang M-L, Jerome KR, Greninger AL: **Comparison of commercially available and laboratory developed assays for in vitro detection of SARS-CoV-2 in clinical laboratories**. *J Clin Microbiol* 2020, **58** http://dx.doi.org/10.1128/JCM.00821-20 e00821-20

Early demonstration of the high comparability of commercial assays for SARS-CoV-2, with slight edge to Cepheid GeneXpert.

15. Kohmer N, Rabenau HF, Hoehl S, Kortenbusch M, Ciesek S, Berger A: **Comparative analysis of point-of-care, high-throughput and laboratory-developed SARS-CoV-2 nucleic acid amplification tests (NATs)**. *J Virol Methods* 2021, **291**:114102.

16. Dust K, Hedley A, Nichol K, Stein D, Adam H, Karlowsky JA, Bullard J, Van Caeseele P, Alexander DC: **Comparison of commercial assays and laboratory developed tests for detection of SARS-CoV-2**. *J Virol Methods* 2020, **285**:113970.

17. Wirden M, Feghoul L, Bertine M, Nere M-L, Le Hingrat Q, Abdi B, Boutolleau D, Ferre VM, Jary A, Delaugerre C *et al.*: **Multicenter**

comparison of the Cobas 6800 system with the RealStar RT-PCR kit for the detection of SARS-CoV-2. *J Clin Virol* 2020, **130**:104573.

18. Broder K, Babiker A, Myers C, White T, Jones H, Cardella J, Burd EM, Hill CE, Kraft CS: **Test agreement between Roche Cobas 6800 and Cepheid GeneXpert Xpress SARS-CoV-2 assays at high cycle threshold ranges**. *J Clin Microbiol* 2020, **58**.

19. Chandler CM, Bourassa L, Mathias PC, Greninger AL: **Estimating the false positive rate of highly automated SARS-CoV-2 nucleic acid amplification testing**. *J Clin Microbiol* 2021 http://dx.doi.org/10.1128/JCM.01080-21. online ahead of print. PMID 33972455.

20. Nalla AK, Casto AM, Huang M-LW, Perchetti GA, Sampoleo R, Shrestha L, Wei Y, Zhu H, Jerome KR, Greninger AL: **Comparative performance of SARS-CoV-2 detection assays using seven different primer/probe sets and one assay kit**. *J Clin Microbiol* 2020, **58** http://dx.doi.org/10.1128/JCM.00557-20 e00557-20.

21. Hansen G, Marino J, Wang Z-X, Beavis KG, Rodrigo J, Labog K, Westblade LF, Jin R, Love N, Ding K *et al.*: **Clinical performance of the point-of-care cobas Liat for detection of SARS-CoV-2 in 20 minutes: a multicenter study**. *J Clin Microbiol* 2021, **59**.

22. Creager HM, Cabrera B, Schnaubelt A, Cox JL, Cushman-Vokoun AM, Shakir SM, Tardif KD, Huang M-L, Jerome KR, Greninger AL *et al.*: **Clinical evaluation of the BioFire® Respiratory Panel 2.1 and detection of SARS-CoV-2**. *J Clin Virol* 2020, **129**:104538.

23. Hogan CA, Garamani N, Lee AS, Tung JK, Sahoo MK, Huang C, Stevens B, Zehnder J, Pinsky BA: **Comparison of the Accula SARS-CoV-2 test with a laboratory-developed assay for detection of SARS-CoV-2 RNA in clinical nasopharyngeal specimens**. *J Clin Microbiol* 2020, **58**.

24. Xia S, Chen X: **Single-copy sensitive, field-deployable, and simultaneous dual-gene detection of SARS-CoV-2 RNA via modified RT-RPA**. *Cell Discov* 2020, **6**:37.

25. Mohon AN, Oberding L, Hundt J, van Marle G, Pabbaraju K, Berenger BM, Lisboa L, Griener T, Czub M, Doolan C *et al.*: **Optimization and clinical validation of dual-target RT-LAMP for SARS-CoV-2**. *J Virol Methods* 2020, **286**:113972.

26. Yu CY, Chan KG, Yean CY, Ang GY: **Nucleic acid-based diagnostic tests for the detection SARS-CoV-2: an update**. *Diagnostics (Basel)* 2021, **11**.

27. Dao Thi VL, Herbst K, Boerner K, Meurer M, Kremer LP, Kirrmaier D, Freistaedter A, Papagiannidis D, Galmozzi C, Stanifer ML *et al.*: **A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples**. *Sci Transl Med* 2020, **12**.

28. •• Serei VD, Cristelli R, Joho K, Salaru G, Kirn T, Carayannopoulous MO, Uprety P: **Comparison of Abbott ID NOW COVID-19 rapid molecular assay to Cepheid Xpert Xpress SARS-CoV-2 assay in dry nasal swabs**. *Diagn Microbiol Infect Dis* 2021, **99**:115208

Beautiful demonstration of the enhanced analytical sensitivity of the Cepheid GeneXpert SARS-CoV-2 assay in the context of low viral load specimens.

29. Tworek JA, Khan F, Sekedat MD, Scheidel C, Malani AN: **The utility of rapid nucleic acid amplification testing to triage symptomatic patients and to screen asymptomatic preprocedure patients for SARS-CoV-2**. *Open Forum Infect Dis* 2021, **8**:ofaa607.

30. Yoon SH, Yang S, Cho H, Eun S, Koo CM, Kim MK: **Point-of-care testing for the detection of SARS-CoV-2: a systematic review and meta-analysis**. *Eur Rev Med Pharmacol Sci* 2021, **25**:503-517.

31. Harrington A, Cox B, Snowdon J, Bakst J, Ley E, Grajales P, Maggiore J, Kahn S: **Comparison of Abbott ID now and Abbott m2000 methods for the detection of SARS-CoV-2 from nasopharyngeal and nasal swabs from symptomatic patients**. *J Clin Microbiol* 2020, **58**.

32. Jääskeläinen AE, Ahava MJ, Jokela P, Szirovicza L, Pohjala S, Vapalahti O, Lappalainen M, Hepojoki J, Kurkela S: **Evaluation of three rapid lateral flow antigen detection tests for the diagnosis of SARS-CoV-2 infection**. *J Clin Virol* 2021, **137**:104785.

33. Merino P, Guinea J, Muñoz-Gallego I, González-Donapetry P, Galán JC, Antona N, Cilla G, Hernáez-Crespo S, Díaz-de Tuesta JL, Gual-de Torrella A *et al.*: **Multicenter evaluation of the PanbioTM COVID-19 rapid antigen-detection test for the diagnosis of SARS-CoV-2 infection**. *Clin Microbiol Infect* 2021, **27**:758-761 http://dx.doi.org/10.1016/j.cmi.2021.02.001.

34. Pray IW, Ford L, Cole D, Lee C, Bigouette JP, Abedi GR, Bushman D, Delahoy MJ, Currie D, Cherney B *et al.*: **Performance of an antigen-based test for asymptomatic and symptomatic SARS-CoV-2 testing at two university campuses - Wisconsin, September-October 2020**. *MMWR Morb Mortal Wkly Rep* 2021, **69**:1642-1647.

35. Young S, Taylor SN, Cammarata CL, Varnado KG, Roger-Dalbert C, Montano A, Griego-Fullbright C, Burgard C, Fernandez C, Eckert K *et al.*: **Clinical evaluation of BD veritor SARS-CoV-2 point-of-care test performance compared to PCR-based testing and versus the Sofia 2 SARS antigen point-of-care test**. *J Clin Microbiol* 2020, **59**.

36. Ginocchio CC, Zhang F, Manji R, Arora S, Bornfreund M, Falk L, Lotlikar M, Kowerska M, Becker G, Korologos D *et al.*: **Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak**. *J Clin Virol* 2009, **45**:191-195.

37. Vasoo S, Stevens J, Singh K: **Rapid antigen tests for diagnosis of pandemic (Swine) influenza A/H1N1**. *Clin Infect Dis* 2009, **49**:1090-1093.

38. Perchetti GA, Huang M-L, Mills MG, Jerome KR, Greninger AL: **Analytical sensitivity of the Abbott BinaxNOW COVID-19 Ag CARD**. *J Clin Microbiol* 2021, **59** http://dx.doi.org/10.1128/JCM.02880-20 e02880-20.

39. Pollock NR, Jacobs JR, Tran K, Cranston AE, Smith S, O'Kane CY, Roady TJ, Moran A, Scarry A, Carroll M *et al.*: **Performance and implementation evaluation of the Abbott BinaxNOW rapid antigen test in a high-throughput drive-through community testing site in Massachusetts**. *J Clin Microbiol* 2021, **59** http://dx.doi.org/10.1128/JCM.00083-21 e00083-21.

40. Shah MM, Salvatore PP, Ford L, Kamitani E, Whaley MJ, Kaitlin M, Currie DW, Morgan CN, Segaloff HE, Lecher S *et al.*: **Performance of repeat BinaxNOW SARS-CoV-2 antigen testing in a community setting, Wisconsin, November-December 2020**. *Clin Infect Dis* 2021 http://dx.doi.org/10.1093/cid/ciab309. ciab309.

41. Sood N, Shetgiri R, Rodriguez A, Jimenez D, Treminino S, Daflos A, Simon P: **Evaluation of the Abbott BinaxNOW rapid antigen test for SARS-CoV-2 infection in children: implications for screening in a school setting**. *PLoS One* 2021, **16**:e0249710.

42. Homza M, Zelena H, Janosek J, Tomaskova H, Jezo E, Kloudova A, Mrazek J, Svagera Z, Prymula R: **Five antigen tests for SARS-CoV-2: virus viability matters**. *Viruses* 2021, **13**.

43. • Larremore DB, Wilder B, Lester E, Shehata S, Burke JM, Hay JA, Tambe M, Mina MJ, Parker R: **Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening**. *Sci Adv* 2021, **7**

Modeling detailing the potential public health impact of rapid testing in the absence of therapies. A greater impact with the addition of viral therapies would no doubt be seen.

44. Peeling RW, Olliaro PL, Boeras DI, Fongwen N: **Scaling up COVID-19 rapid antigen tests: promises and challenges**. *Lancet Infect Dis* 2021 http://dx.doi.org/10.1016/S1473-3099(21)00048-7. S1473-3099(21)00048-7.

45. Bourassa L, Perchetti GA, Phung Q, Lin MJ, Mills MG, Roychoudhury P, Harmon KG, Reed JC, Greninger AL: **A SARS-CoV-2 nucleocapsid variant that affects antigen test performance**. *medRxiv* 2021 http://dx.doi.org/10.1101/2021.05.05.21256527.

46. Benkeser D, Juraska M, Gilbert PB: **Assessing trends in vaccine efficacy by pathogen genetic distance**. *J Soc Fr Statistique (2009)* 2020, **161**:164-175.

47. Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, Wang M, Yu J, Zhang B, Kwong PD *et al.*: **Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7**. *Nature* 2021, **593**:130-135 http://dx.doi.org/10.1038/s41586-021-03398-2 PMID 33684923.

48. Bhoyar RC, Jain A, Sehgal P, Divakar MK, Sharma D, Imran M, Jolly B, Ranjan G, Rophina M, Sharma S *et al.*: **High throughput detection and genetic epidemiology of SARS-CoV-2 using COVIDSeq next-generation sequencing**. *PLoS One* 2021, **16**: e0247115.

49. Joung J, Ladha A, Saito M, Kim N-G, Woolley AE, Segel M, Barretto RPJ, Ranu A, Macrae RK, Faure G *et al.*: **Detection of SARS-CoV-2 with SHERLOCK one-pot testing**. *N Engl J Med* 2020, **383**:1492-1494.

50. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, Miao X, Streithorst JA, Granados A, Sotomayor-Gonzalez A *et al.*: **CRISPR-Cas12-based detection of SARS-CoV-2**. *Nat Biotechnol* 2020, **38**:870-874.