The implementation of antibody Rapid Diagnostic Testing vs. rRT-PCR sample pooling in the screening of COVID-19: A case of different testing strategies in Africa

Authors: Tinashe K. Nyazika [a,b,c¶], Rabelani Kaela [d], Mathias Mugoni [e], Kudakwashe Musomekwa [f], Eric Kyei-Baafour [g], Simbarashe Chiwanda [h], Prichard T. Mapondera [d], Tatenda S. Makawa [i], Elliot M. Sithole [j], George Mavunganidze [k], Justen Manasa [l], Kondwani C. Jambo [a,b], Cuthbert Musarurwa [m]

Affiliations

[a] Malawi-Liverpool-Wellcome Trust Clinical Research Programme, University of Malawi College of Medicine, Blantyre, Malawi

[b] Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

[c] Department of Pathology, College of Health Sciences, University of Malawi, Blantyre, Malawi

[d] African Society of Laboratory Medicine, PHIA Lab Corps, Addis Ababa, Ethiopia

[e] Department of Microbiology, Optimum Health Medical Laboratories, Gaborone, Botswana

[f] Aids Healthcare Foundation, Zimbabwe; Mpilo Center of Excellence, Mpilo Central Hospital, Zimbabwe

[g] Department of Immunology, Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana

[h] Department of Epidemiology and Disease Control, Ministry of Health and Child Care, Zimbabwe

[i] Department of Paraclinical Veterinary Studies, Faculty of Veterinary Studies, University of Zimbabwe, Zimbabwe

[j] Department of Health Sciences, School of Public Health, University of South Wales, Wales, United Kingdom

[k] Zimbabwe Association of Medical Laboratory and Clinical Scientists, Zimbabwe

[l] Department of Medical Microbiology, College of Health Sciences, University of Zimbabwe, Zimbabwe

[m] Department of Chemical Pathology, College of Health Sciences, University of Zimbabwe, Zimbabwe

[¶]**Correspondence**: Tinashe K. Nyazika, Malawi-Liverpool-Wellcome Trust Clinical Research Programme, University of Malawi College of Medicine, Blantyre, Malawi. Email: tknyazika@mlw.mw

(The views expressed in this article do not necessarily reflect the views of the journal or of ASM.)

Abstract

COVID-19 has wreaked havoc across the globe, although cases in Africa remain lower than other regions but they are gradually on an upward trajectory. To date, COVID-19 cases have been reported in 54 countries. However, due to limited SARS-COV-2 rRT-PCR testing capacity and scarcity of testing reagents, it is probable that the total number of cases could far exceed published statistics. In this viewpoint, using Ghana, Malawi, South Africa and Zimbabwe as examples of countries that have implemented different testing strategies, we argue that the implementation of sample pooling for rRT-PCR over antibody rapid diagnostic testing could have a greater impact in assessing disease burden. Sample pooling offers huge advantages compared to single test rRT-PCR, as it lowers experimental costs, personnel time, reduces burnout and analytical run-times. Africa is already strained in terms of testing resources for COVID-19, hence cheaper alternative ways need to be implemented to conserve resources, maximise on mass testing and reduce transmission in the wider population.

Keywords

Antibody rapid diagnostic test, sample pooling, rRT-PCR, COVID-19, Africa

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak was first reported in Wuhan, China, in December 2019, and has spread throughout the world with an unprecedented impact on humanity (1). The disease later termed coronavirus disease 2019 (COVID-19), has now been reported to have affected more than 6.2 million people worldwide culminating in over 376,000 deaths, by the 2nd June 2020 (2). In Africa, COVID-19 has been reported in 54 countries, with a cumulative 155,610 confirmed cases and 4,429 deaths (3). With over 3 billion people under COVID-19 induced lockdown worldwide, accompanied by the attendant negative impact on global economies, governments are racing to flatten the epidemic curve of COVID-19 in both space and time (4, 5). Some of the policies imposed by governments to flatten the curve include; travel bans, lockdowns, quarantining and widespread mass testing using real time reverse transcription polymerase chain reaction (rRT-PCR) (4, 5).

In order to achieve gains from these implemented measures and to reduce the burden of COVID-19 cases on societies and economies, it is critical to implement diagnostic strategies with high analytical sensitivity and specificity to enable early detection of SARS-CoV-2 cases for isolation, contact tracing, care and management. International collaborative efforts initiated after the emergence of the SARS-CoV-2 pandemic, led to the development of rRT-PCR diagnostic assays to support case ascertainment and outbreak tracking (5, 6). The rRT-PCR is reported to have a diagnostic sensitivity of 88 – 99% and a diagnostic specificity of 77 – 100% (7). This development thus, accelerated the definitive diagnosis of asymptomatic, pre-symptomatic and symptomatic cases (6).

Due to limited SARS-CoV-2 rRT-PCR testing capacity and scarcity of testing reagents in many geographical regions including Africa, it is clear that the total number of COVID-19 cases is probably much higher than published confirmed statistics (8). Using Ghana, Malawi, South Africa and Zimbabwe as examples of countries that have implemented different testing strategies, we argue that the implementation of sample pooling for rRT-PCR over antibody rapid diagnostic testing (RDT) could have a greater impact in the early detection of the active COVID-19 cases, and assist in curbing the spread of the disease, whereas antibody based RDTs may help to ascertain community seroprevalence and estimate potential herd immunity benefits.

Case studies of COVID-19 diagnostic strategies in Africa

A case study of Ghana

Ghana is one of the few countries that implemented sample pooling strategy for COVID-19 rRT-PCR testing and confirmation (9, 10). However, at the onset of the pandemic, the country implemented single sample rRT-PCR testing before scaling it upwards through sample pooling (10, 11). As of 31st May 2020, a cumulative 219,825 tests had been conducted by the seven government affiliated laboratories spread across Ghana (12). Through sample pooling approximately ~0.71% of the total population have been tested, giving rise to 8,297 confirmed COVID-19 cases (positivity rate 3.77%) (12). Ghana is the 2nd highest ranked African country in terms of testing capacity. The COVID-19 testing strategy implemented, pools, at most 10 different patient samples for amplification prior to testing. This strategy has tremendously improved Ghana's COVID-19 testing capacity and definitive diagnosis.

A case study of Malawi

As of 1st June 2020, Malawi had conducted 5,505 rRT-PCR tests yielding 358 COVID-19 cases (13). Malawi has the highest SARS-CoV-2 positivity rate (6.50%) in the region, despite its low testing rate of only ~0.03% of the population tested so far (13). The country recently increased its COVID-19 testing sites to 14, in order to expand geographic coverage. Owing to the low testing capacity, Malawi needs to be more restrictive in choosing who to test, in order to maximise benefits from its limited resources.

A case study of South Africa

South Africa has conducted 742,742 rRT-PCR tests yielding 35,812 COVID-19 cases and a positivity rate of 4.82% (14). It is the leading African country in terms of testing capacity, with 1.25% of its population having been tested for COVID-19 by the 1st June 2020. South Africa is currently conducting individual sample testing using several in-house and commercial PCR assays to test for the presence of SARS-CoV-2 RNA (15). However, most diagnostic laboratories are overwhelmed as the country is conducting targeted community symptom screening and testing for both symptomatic and asymptomatic individuals in some provinces (15, 16). Novel screening and testing strategies need to be adopted in order to manage and improve the capacity and sample turnaround testing time in diagnostic laboratories.

A case study of Zimbabwe

Recently, the Ministry of Health and Child Care (MoHCC) of Zimbabwe, introduced antibody RDT as a screening test strategy for SARS-CoV-2, to complement the rRT-PCR assays being offered in government reference laboratories, state universities and private laboratories.

Despite this development, the country still has limited capacity to conduct mass testing (17). As of 1st June 2020, a cumulative 46,021 COVID-19 tests (28,112 RDTs and 18,709 RT-PCR) had been conducted, with 203 confirmed cases (18). The current testing coverage translates to approximately ~0.13% of the population tested by rRT-PCR/ Xpert® Xpress for SARS-CoV-2 with a 1.09% positivity rate. Furthermore, many of the RDTs in current use have not been validated or verified for use within this population. Thus, the current rRT-PCR under-testing raises the concern that many infected individuals are probably going undiagnosed.

Implementation of different testing strategies for COVID-19

Sample pooling for rRT-PCR

This technique was first suggested by Robert Dorfman in 1943 as a way of optimising syphilis testing, and since then, has been applied in surveillances and screening for infections such as human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), malaria and influenza virus (19–25). Sample pooling has popularly been employed in serological work but also in molecular diagnostics and research. It helps to lower experimental costs, personnel time and reduces analytical run-times whilst maximising the level of surveillance and accuracy of the diagnostic test (19, 21, 24, 26). In public health emergency responses, such as the COVID-19 pandemic, rapidly growing workloads can outstrip laboratory testing capacity, resulting in reagent shortages and personnel burnout (16, 27). Thus, in large populations with a low disease prevalence (<10%), sample pooling provides a cost-effective strategy for screening asymptomatic and pre-symptomatic individuals (25, 27–29). Two different sample pooling strategies can be used namely; single-step pools, where prevalence and confidence intervals are calculated based on pool results (30), or multiple-step pools, where individual samples from a positive pool

are retested to identify the number of positive individuals per pool (31, 32). The latter being more useful for COVID-19 as the individuals need to be identified for isolation, contact tracing, care and treatment.

The use of a single test to screen groups of more than two people though labour intensive, provides a comprehensive, rapid and localised mass testing strategy required to identify cases acutely and minimise spread of disease. However, the anticipated cost reduction only becomes meaningful if statistical equivalence between the pooled and non-pooled experimental setups is achieved and maintained (33). *In vitro* and *in silico* studies of pooling nasopharyngeal samples for rRT-PCR assays, have reported the optimal pool size (*p*), to be five (5) samples per pool (28, 29). On account of variances in different PCR assay limits-of-detection (LoD), it is paramount to validate the use of pooled samples and rule out false negatives as a result of sample dilution. Of note, *p* can be up-scaled to *p*=32 (29, 34–36), where concentration of RNA by elution/lyophilisation becomes necessary to curb the risk of false negatives due to sample dilution (37). Fundamental principles for successful application of sample pooling are subject to the assay's sensitivity and specificity, limit-of-detection and the prevalence of disease in the population.

COVID-19 is estimated to have a high effective reproductive number R_0 (ranging from 1.4 to 6.49, with a median 2.79) (38). Employing sample pooling for contact tracing ensures swift case identification, with less resources used to arrest community transmissions (38–40). Meanwhile, for frontline healthcare workers (HCW) interfacing with COVID-19 patients, sample pooling can be modelled to cluster high-risk individuals, in order to concentrate positivity rate to a few pools thereby further conserving resources.

Sample pooling is also associated with limitations such as; reduced test sensitivity in settings with very low infection prevalence (21, 41). Thus, a diagnostic laboratory has to optimise the pool size based on the prevalence of the infection within the society. Additionally, it is also critical for laboratories to understand that a negative pool result, would not distinguish between a true negative and an indeterminate or inconclusive result due to poor specimen collection or handling (24).

Antibody RDTs testing for COVID-19

Any acceptable screening or first line test should ideally have a high clinical diagnostic sensitivity, whereas the consecutive confirmatory test needs to have a high specificity (5, 7, 8, 42, 43). Despite this, some low-medium income countries (LMICs) in Africa have implemented unverified RDTs for COVID-19 into their testing and surveillance protocols. Whether these RDTs are yielding intended results remains to be verified. However, cheaper but well validated serological assays are essential to complement the fairly expensive rRT-PCR diagnostic assays to accurately assess the COVID-19 disease burden within communities and mapping global exposure (6). Samples can also be pooled as is applied when screening blood products for such infections as HBV and HCV to achieve maximum testing benefit (44).

Testing of specific antibodies against SARS-CoV-2 in patient blood is a good choice for rapid and simple diagnosis of COVID-19 (45). However, early studies have reported that the majority of COVID-19 patients seroconvert between days 7 and 11 post-exposure to the SARS-CoV-2 thus rendering antibody testing questionable in the setting of an acute illness

(43, 46, 47). Moreover, RDTs kits for SARS-CoV-2 antibodies have an analytical sensitivity of 69 – 88% for IgM and 90 – 99% for IgG (7, 8, 42, 45). Therefore, RDTs pose a challenge of; When to test? Whom to test? What to test? How often to test? and, what to do with test results? (43). Although validated RDTs can be used in sero-prevalence studies, data generated from use of RDT's may not be reliable unless conducted systematically and periodically due to the wide variation in duration of time to seroconversion among individuals (16).

Scenarios where rRT-PCR sample pooling or antibody RDT may be employed.

Establishing the status of an asymptomatic individual.

COVID-19 follows an asymptomatic disease course before symptoms appear thus, causing increased spread of the disease (48, 49). Since antibodies can only be detected 6-29 days after symptom onset, antibody RDT's may result in false negative COVID-19 status which will in turn delay management and contact tracing thus allowing propagation of infections within communities. On the contrary, various studies using rRT-PCR, have demonstrated asymptomatic carriage in obstetric patients (13.7%), Diamond Princess ship passengers (17.9%), skilled nursing facility residents (35.6%) (48–50). It should, however, be stressed that in the highlighted studies, the asymptomatic carriers contributed ~50% of the COVID-19 cases.

Testing Healthcare workers and other employees providing essential services.

Healthcare workers (HCW) and essential service employees showing no symptoms but continuously interfacing with the general population have been shown to be capable of transmitting SARS-CoV-2, as demonstrated in the skilled nursing facility residents in New

York (48). Mass testing for asymptomatic HCWs and employees in the essential services is therefore critical in order to mitigate workforce depletion by unnecessary quarantine; reduce spread of atypical, mild, or asymptomatic cases; and protect the healthcare and essential service workforce (51). Thus, it is crucial to set a reasonable testing schedule and frequency using pooled sampling rRT-PCR after assessing their risk profile to allow early detection and intervention in asymptomatic and pre-symptomatic individuals. This can be done by splitting employees working in the same department into groups and staggering testing of these groups to help identify any potential circulation (thus need of contact tracing) of the disease among staff while minimising once off use of resources.

Asymptomatic and pre-symptomatic HCWs and essential service workers are an underappreciated potential source of infection and worthy of testing to reduce in-hospital transmission and community spread (51, 52). Workers returning to work may be tested by validated RDTs as a means of tracing possible missed asymptomatic/presymptomatic and symptomatic cases. Furthermore, routine temperature checks must be conducted daily, collectively, this will aid in reducing community spread.

Contact tracing

Elucidation of the chain of infection and identification of the source of COVID-19 infections are crucial for effective disease containment (52, 53). Although the rRT-PCR option offers a diagnostic solution and is important for establishing infection status in contacts of an index case, this approach might not be diagnostically useful in recovered patients who are no longer shedding the virus (53). The duration of viral shedding for COVID-19 remains uncertain (54), but data from SARS-CoV indicate that 21 days after symptom onset, 53% of

cases achieved viral clearance in nasopharyngeal aspirate samples (55). Thus, serological tests are more useful in identifying convalescent cases, ascertaining seroprevalence and an accurate denominator for case fatality rate (53).

Establishing status in symptomatic patients

It is critical to establish the status of any person exhibiting COVID-19 related symptoms as soon as possible to enable appropriate management. As previously emphasised, RDTs have a limitation as far as detection at early onset is concerned thus, may not be very useful.

Establishing past exposure and immunity to COVID-19

Detecting individuals with past exposure or those that would have recovered from the SARS-CoV-2, by testing for IgM/IgG exposure is critical. However, whether the immune response following exposure to SARS-CoV-2 is long-lasting and protective against reinfection remains an issue of debate (5, 47, 56). Furthermore, laboratory tests that detect antibodies to SARS-CoV-2, including rapid immunodiagnostic tests, need further validation to determine their clinical utility (57).

Conclusions

The use of sample pooling for rRT-PCR testing particularly in Africa, to screen for active COVID-19 cases has a great advantage over single test rRT-PCR, as it helps lowering experimental costs, personnel time, burnout and also reduces analytical run-times (27, 29, 37, 48, 58). Africa is already strained in terms of testing resources for COVID-19, hence cheaper alternatives need to be implemented to conserve resources, maximise on mass testing and reduce transmission in the wider population. Currently, WHO does not

recommend the use of antibody and antigen-detecting rapid diagnostic tests for patient care but encourages the continuation of work to establish their usefulness in disease surveillance and epidemiologic research (57, 59). Healthcare workers and other essential services workers, particularly those working in cities and towns with confirmed cases are a key reservoir for the transmission of COVID-19, due to their interface with patients and the wider population, respectively. Thus, it is crucial to set a reasonable testing schedule and frequency using pooling of samples for rRT-PCR after assessing their risk profile to allow early detection and intervention in asymptomatic and pre-symptomatic individuals. Blanket testing of asymptomatic frontline staff is a futile exercise that will not add value to this fight.

Acknowledgements

The authors thank the Zimbabwe Association of Medical Laboratory and Clinical Scientists for its technical input. TKN is supported by training grant awarded as part of the Wellcome Strategic award number 101113/Z/13/Z084 to The Malawi-Liverpool-Wellcome Trust Clinical Research Programme. KCJ is supported by an MRC/DFID African Research Leader award number MR/T008822/1.

We declare no competing interests.

Key terms and definitions

Asymptomatic - laboratory-confirmed COVID-19 case who does not develop symptoms.

Pre-symptomatic – an individual who has been exposed to the virus (becoming infected) but hasn't developed symptom yet.

Seroconversion – It is the transition from a seronegative condition—where no antibodies are detectable in the serum or are present at titres below the limit of detection— to a seropositive condition, in which antibodies are detectable in serum samples.

Symptomatic - a case who has developed signs and symptoms compatible with COVID-19 virus infection

Sensitivity – the ability of a diagnostic test to correctly identify all patients with the disease.

Specificity – the ability of a diagnostic test to correctly identify all patients who do not have a disease.

316 **References:**

317 1. Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, Su X, Cao B. 2020. SARS-CoV-2 and viral sepsis:
318 observations and hypotheses. The Lancet 395:1517–20.

319 2. World Health Organization. 2020. Coronavirus disease (COVID-19) Situation Report –
320 134.

321 3. World Health Organization African Region. 2020. COVID-19 Outbreak Situation Report
322 14.

323 4. Parmet WE, Sinha MS. 2020. Covid-19 — The Law and Limits of Quarantine. N Engl J
324 Med 382:e28.

325 5. Petherick A. 2020. Developing antibody tests for SARS-CoV-2. The Lancet 395:1101–
326 1102.

327 6. Okba NMA, Muller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, Lamers MM,
328 Sikkema RS, de Bruin E, Chandler FD, Yazdanpanah Y, Le Hingrat Q, Descamps D,
329 Houhou-Fidouh N, Reusken CBEM, Bosch B-J, Drosten C, Koopmans MPG, Haagmans
330 BL. 2020. Severe Acute Respiratory Syndrome Coronavirus 2−Specific Antibody
331 Responses in Coronavirus Disease 2019 Patients. Emerg Infect Dis 26:1478–1488.

332 7. Castro R, Luz PM, Wakimoto MD, Veloso VG, Grinsztejn B, Perazzo H. 2020. COVID-19: a
333 meta-analysis of diagnostic test accuracy of commercial assays registered in Brazil. Braz
334 J Infect Dis 24:180–187.

335 8. Hoffman T, Nissen K, Krambrich J, Rönnberg B, Akaberi D, Esmaeilzadeh M, Salaneck E,
336 Lindahl J, Lundkvist Å. 2020. Evaluation of a COVID-19 IgM and IgG rapid test; an

efficient tool for assessment of past exposure to SARS-CoV-2. Infect Ecol Epidemiol 10:1754538.

9. Ihekweazu C, Agogo E. 2020. Africa's response to COVID-19. BMC Med 18:151.

10. Simons B. 2020. Should African countries embrace Ghana's Covid-19 'pooled testing'?

11. Dzansi J. 2020. Ramping up early detection of COVID-19 with limited resources: The role of pool testing.

12. Ghana Health Service. 2020. Situation Update, COVID-19 Outbreak in Ghana as at 31 May.

13. UNICEF Malawi. 2020. Malawi COVID-19 Situation Report 3 June.

14. COVID-19 Statistics in SA. 2020. Update on Covid-19 (1st June).

15. National Institute of Communicable Diseases. 2020. COVID-19 weekly epidemiology brief: Week 19.

16. Mendelson M, Madhi SA. 2020. South Africa's coronavirus testing strategy is broken and not fit for purpose: It's time for a change. S Afr Med J 110.

17. Ministry of Health and Child Care of Zimbabwe. 2020. Strategy to increase active screening for COVID-19 in Zimbabwe. Harare, Zimbabwe.

18. Ministry of Health and Child Care of Zimbabwe. 2020. 27th May COVID-19 update. Harare, Zimbabwe.

19. Abel U, Schosser R, Süss J. 1999. Estimating the prevalence of infectious agents using pooled samples: Biometrical considerations. Zentralblatt Für Bakteriol 289:550–563.

20. Dorfman R. 1943. The detection of defective member of large populations. Ann Math Stat 436–440.

21. Ladman BS, Spackman AE, Gelb JJ. 2011. Comparison of Pooling 11 or 5 Oropharyngeal Swabbings for Detecting Avian Influenza Virus by Real-Time Reverse Transcription-PCR in Broiler Chickens. Avian Dis 56:227–229.

22. Novack L, Shinar E, Safi J, Soliman H, Yaari A, Galai N, Pliskin JS, Sarov B. 2007. Evaluation of pooled screening for anti-HCV in two blood services set-ups: Evaluation for pooled screening for anti-HCV. Trop Med Int Health 12:415–421.

23. Ssematimba A, Malladi S, Bonney PJ, Flores-Figueroa C, Muñoz-Aguayo J, Halvorson DA, Cardona CJ. 2018. Quantifying the effect of swab pool size on the detection of influenza A viruses in broiler chickens and its implications for surveillance. BMC Vet Res 14:265.

24. Van TT, Miller J, Warshauer DM, Reisdorf E, Jernigan D, Humes R, Shult PA. 2012. Pooling Nasopharyngeal/Throat Swab Specimens To Increase Testing Capacity for Influenza Viruses by PCR. J Clin Microbiol 50:891–896.

25. Zhou Z, Mitchell RM, Gutman J, Wiegand RE, Mwandama DA, Mathanga DP, Skarbinski J, Shi YP. 2014. Pooled PCR testing strategy and prevalence estimation of submicroscopic infections using Bayesian latent class models in pregnant women receiving intermittent preventive treatment at Machinga District Hospital, Malawi, 2010. Malar J 13:509.

26. Nguyen NT, Aprahamian H, Bish EK, Bish DR. 2019. A methodology for deriving the sensitivity of pooled testing, based on viral load progression and pooling dilution. J Transl Med 17:252.

27. Lohse S, Pfuhl T, Berkó-Göttel B, Rissland J, Geißler T, Gärtner B, Becker SL, Schneitler S, Smola S. 2020. Pooling of samples for testing for SARS-CoV-2 in asymptomatic people. Lancet Infect Dis.

28. Abdalhamid B, Bilder CR, McCutchen EL, Hinrichs SH, Koepsell SA, Iwen PC. 2020. Assessment of Specimen Pooling to Conserve SARS CoV-2 Testing Resources. Am J Clin Pathol 153:715–718.

29. Deckert A, Bärnighausen T, Kyei N. 2020. Pooled-sample analysis strategies for COVID-19 mass testing: a simulation study. Bull World Health Organ.

30. Cowling DW, Gardner IA, Johnson WO. 1999. Comparison of methods for estimation of individual-level prevalence based on pooled samples. Prev Vet Med 39:211–225.

31. Brookmeyer R. 1999. Analysis of multistage pooling studies of biological specimens for estimating disease incidence and prevalence. Biometrics 55:608–12.

32. Chen CL, Swallow WH. 1990. Using group testing to estimate a proportion, and to test the binomial model. Biometrics 46:1035–1046.

33. Kendziorski C, Irizarry RA, Chen K-S, Haag JD, Gould MN. 2005. On the utility of pooling biological samples in microarray experiments. Proc Natl Acad Sci 102:4252–4257.

34. Aprahamian H, Bish DR, Bish EK. 2016. Residual risk and waste in donated blood with pooled nucleic acid testing. Stat Med 35:5283–5301.

35. Zhang S-D, Gant TW. 2005. Effect of pooling samples on the efficiency of comparative studies using microarrays. Bioinformatics 21:4378–4383.

36. Yelin I, Aharony N, Tamar ES, Argoetti A, Messer E, Berenbaum D, Shafran E, Kuzli A, Gandali N, Shkedi O, Hashimshony T, Mandel-Gutfreund Y, Halberthal M, Geffen Y, Szwarcwort-Cohen M, Kishony R. 2020. Evaluation of COVID-19 RT-qPCR test in multi-sample pools. Clin Infect Dis 10.1093/cid/ciaa531.

37. Edouard S, Prudent E, Gautret P, Memish ZA, Raoult D. 2015. Cost-effective pooling of DNA from nasopharyngeal swab samples for large-scale detection of bacteria by real-time PCR. J Clin Microbiol 53:1002–1004.

38. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. 2020. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J Travel Med 27:1–4.

39. Hellewell J, Abbott S, Gimma A, Bosse NI, Jarvis CI, Russell TW, Munday JD, Kucharski AJ, Edmunds WJ, Funk S, Eggo RM, Sun F, Flasche S, Quilty BJ, Davies N, Liu Y, Clifford S, Klepac P, Jit M, Diamond C, Gibbs H, van Zandvoort K. 2020. Feasibility of controlling COVID-19 outbreaks by isolation of cases and contacts. Lancet Glob Health 8:e488–e496.

40. Yuan J, Li M, Lv G, Lu ZK. 2020. Monitoring transmissibility and mortality of COVID-19 in Europe. Int J Infect Dis 95:311–315.

41. Arnold ME, Slomka MJ, Coward VJ, Mahmood S, Raleigh PJ, Brown IH. 2013. Evaluation of the pooling of swabs for real-time PCR detection of low titre shedding of low pathogenicity avian influenza in turkeys. Epidemiol Infect 141:1286–1297.

418 42. Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M. 2020. Analytical performances
419 of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics.
420 Clin Chem Lab Med CCLM 1–8.

421 43. Patel R, Babady E, Theel ES, Storch GA, Pinsky BA, St. George K, Smith TC, Bertuzzi S.
422 2020. Report from the American Society for Microbiology COVID-19 International
423 Summit, 23 March 2020: Value of Diagnostic Testing for SARS–CoV-2/COVID-19. mBio
424 11:mBio.00722-20, e00722-20.

425 44. Dwyre DM, Fernando LP, Holland PV. 2011. Hepatitis B, hepatitis C and HIV transfusion-
426 transmitted infections in the 21st century: Transfusion transmission of hepatitis B,
427 hepatitis C, and HIV. Vox Sang 100:92–98.

428 45. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, Sun R, Wang Y, Hu B, Chen W, Zhang Y, Wang J,
429 Huang B, Lin Y, Yang J, Cai W, Wang X, Cheng J, Chen Z, Sun K, Pan W, Zhan Z, Chen L,
430 Ye F. 2020. Development and clinical application of a rapid IgM-IgG combined antibody
431 test for SARS-CoV-2 infection diagnosis. J Med Virol 1–7.

432 46. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, Zhang
433 X, Zhang M, Wu S, Song J, Chen T, Han M, Li S, Luo X, Zhao J, Ning Q. 2020. Clinical and
434 immunological features of severe and moderate coronavirus disease 2019. J Clin Invest
435 130:2620–2629.

436 47. To KK-W, Tsang OT-Y, Leung W-S, Tam AR, Wu T-C, Lung DC, Yip CC-Y, Cai J-P, Chan JM-
437 C, Chik TS-H, Lau DP-L, Choi CY-C, Chen L-L, Chan W-M, Chan K-H, Ip JD, Ng AC-K, Poon
438 RW-S, Luo C-T, Cheng VC-C, Chan JF-W, Hung IF-N, Chen Z, Chen H, Yuen K-Y. 2020.
439 Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum

antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 3099:30196–1.

48. Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, Taylor J, Spicer K, Bardossy AC, Oakley LP, Tanwar S, Dyal JW, Harney J, Chisty Z, Bell JM, Methner M, Paul P, Carlson CM, McLaughlin HP, Thornburg N, Tong S, Tamin A, Tao Y, Uehara A, Harcourt J, Clark S, Brostrom-Smith C, Page LC, Kay M, Lewis J, Montgomery P, Stone ND, Clark TA, Honein MA, Duchin JS, Jernigan JA. 2020. Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility. N Engl J Med 382:2081–2090.

49. Mizumoto K, Kagaya K, Zarebski A, Chowell G. 2020. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. Eurosurveillance 25:2000180.

50. Sutton D, Fuchs K, D'Alton M, Goffman D. 2020. Universal Screening for SARS-CoV-2 in Women Admitted for Delivery. N Engl J Med NEJMc2009316.

51. Black JRM, Bailey C, Przewrocka J, Dijkstra KK, Swanton C. 2020. COVID-19: the case for health-care worker screening to prevent hospital transmission. The Lancet 395:1418–1420.

52. Ferretti L, Wymant C, Kendall M, Zhao L, Nurtay A, Abeler-Dörner L, Parker M, Bonsall D, Fraser C. 2020. Quantifying SARS-CoV-2 transmission suggests epidemic control with digital contact tracing. Science 368:eabb6936.

459 53. Yong SEF, Anderson DE, Wei WE, Pang J, Chia WN, Tan CW, Teoh YL, Rajendram P, Chen
460 MI-C, Vasoo S, Ong B, Leo YS, Wang L, Lee VJM. 2020. Connecting clusters of COVID-19:
461 an epidemiological and serological investigation. Lancet Infect Dis 20:809–15.

462 54. Vetter P, Eckerle I, Kaiser L. 2020. Covid-19: a puzzle with many missing pieces. BMJ 368.

463 55. Peiris JSM, Chu CM, Cheng VCC, Chan KS, Hung IFN, Poon LLM, Law KI, Tang BSF, Hon
464 TYW, Chan CS, Chan KH, Ng JSC, Zheng BJ, Ng WL, Lai RWM, Guan Y, Yuen KY. 2003.
465 Clinical progression and viral load in a community outbreak of. The Lancet 361:6.

466 56. Zhu F-C, Li Y-H, Guan X-H, Hou L-H, Wang W-J, Li J-X, Wu S-P, Wang B-S, Wang Z, Wang L,
467 Jia S-Y, Jiang H-D, Wang L, Jiang T, Hu Y, Gou J-B, Xu S-B, Xu J-J, Wang X-W, Wang W,
468 Chen W. 2020. Safety, tolerability, and immunogenicity of a recombinant adenovirus
469 type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised,
470 first-in-human trial. The Lancet 6736:31208–3.

471 57. World Health Organization. 2020. “Immunity passports” in the context of COVID-19.

472 58. Kimball A, Hatfield KM, Arons M, James A, Taylor J, Spicer K, Bardossy AC, Oakley LP,
473 Tanwar S, Chisty Z, Bell JM, Methner M, Harney J, Jacobs JR, Carlson CM, McLaughlin
474 HP, Stone N, Clark S, Brostrom-Smith C, Page LC, Kay M, Lewis J, Russell D, Hiatt B, Gant
475 J, Duchin JS, Clark TA, Honein MA, Reddy SC, Jernigan JA, Public Health – Seattle & King
476 County, CDC COVID-19 Investigation Team, Public Health – Seattle & King County, Baer
477 A, Barnard LM, Benoliel E, Fagalde MS, Ferro J, Smith HG, Gonzales E, Hatley N, Hatt G,
478 Hope M, Huntington-Frazier M, Kawakami V, Lenahan JL, Lukoff MD, Maier EB,
479 McKeirnan S, Montgomery P, Morgan JL, Mummert LA, Pogosjans S, Riedo FX,
480 Schwarcz L, Smith D, Stearns S, Sykes KJ, Whitney H, CDC COVID-19 Investigation Team,

Ali H, Banks M, Balajee A, Chow EJ, Cooper B, Currie DW, Dyal J, Healy J, Hughes M, McMichael TM, Nolen L, Olson C, Rao AK, Schmit K, Schwartz NG, Tobolowsky F, Zacks R, Zane S. 2020. Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility — King County, Washington, March 2020. MMWR Morb Mortal Wkly Rep 69:377–381.

59. World Health Organization. 2020. Advice on the use of point-of -care immunodiagnostic tests for COVID-19. Geneva, Switzerland.