Clarifying the evidence on SARS-CoV-2 antigen rapid tests in public health responses to COVID-19



Published Online February 17, 2021

The use of rapid lateral flow antigen testing (LFT) for SARS-CoV-2 has been questioned1-3 with uncorroborated4 reports of poor LFT sensitivity. The debate surrounding the use of the Innova Lateral Flow SARS-CoV-2 Antigen Test in the UK risks confusing policy makers internationally and potentially stalling deployment of LFTs in other countries.⁵ As scientists and health professionals evaluating some of the world's largest pilots of LFT, we wish to challenge those interpretations and clarify the evidence on how such testing might be used to detect SARS-CoV-2 in minutes and improve COVID-19 control measures.

Testing for SARS-CoV-2 is central to COVID-19 management and has relied on quantitative reverse transcriptase polymerase chain reaction (PCR) technology. PCR seeks the genetic code of the virus from nose or throat swabs and amplifies it over 30-40 cycles, doubling each cycle, enabling even miniscule, potentially single, copies to be detected. PCR is thus a powerful clinical test, specifically when a patient is, or was recently, infected with SARS-CoV-2. Fragments of RNA can linger for weeks after infectious virus has been cleared,6 often in people without symptoms or known exposures.7

However, for public health measures, another approach is needed. Testing to help slow the spread of SARS-CoV-2 asks not whether someone has RNA in their nose from earlier infection, but whether they are infectious today. It is a net loss to the health, social, and economic wellbeing of communities if post-infectious individuals test positive and isolate for 10 days. In our view, current PCR testing is therefore not the appropriate gold standard for evaluating a SARS-CoV-2 public health test.

Most people infected with SARS-CoV-2 are contagious for 4-8 days.7 Specimens are generally not found to contain culture-positive (potentially contagious) virus beyond day 9 after the onset of symptoms, with most transmission occurring before day 5.78 This timing fits with the observed patterns of virus transmission (usually 2 days before to 5 days after symptom onset), which led public health agencies to recommend a 10-day isolation period.9 The short window of transmissibility contrasts with a median 22-33 days of PCR positivity (longer with severe infections and somewhat shorter among asymptomatic individuals).10 This suggests that 50-75% of the time an individual is PCR positive, they are likely to be post-infectious. 11,12

Once SARS-CoV-2 replication has been controlled by the immune system, RNA levels detectable by PCR on respiratory secretions fall to very low levels when individuals are much less likely to infect others. 13-15 The remaining RNA copies can take weeks, or occasionally months, 16,17 to clear, during which time PCR remains positive.7

A public health test for detecting someone who might be contagious is, by logical deduction, expected to have a sensitivity of around 30-40% versus PCR when testing a random sample of asymptomatic people in a steady-state outbreak.¹⁸ Furthermore, the asymmetry of RNA reflecting more infectiousness nearer to the time of exposure, means that the sensitivity of the ideal test of infectiousness when measured against PCR may vary across the epidemic curve, from as high as 50-60% when an outbreak is surging to 20-30% or less as infections decline.19

LFT and the UK testing programme have been criticised1-3,5 for poor sensitivity in people without symptoms. In our view, these criticisms misinterpreted data from the interim report on the pilot of community testing in Liverpool, UK.20,21 When paired LFT and PCR testing was done in Liverpool, the epidemic curve was declining.20 At this point, a priori one should expect a





public health test that is highly sensitive for detecting infectious virus to show low overall sensitivity relative to PCR in people without symptoms or known exposures.

Further confusion reigns over PCR cycle threshold (Ct) values, viral loads, and infectiousness. In the Liverpool pilot, Innova LFT picked up 19 of 24 (79%) samples with Ct below 20 and ten of 11 (91%) samples with Ct below 18.20 The 66% sensitivity in the Liverpool interim report20 was based cautiously on Ct below or equal to 25 indicating viable virus. For the laboratory processing of the Liverpool samples, Ct values of 21–18 most likely reflect the 100 000 to 1 million RNA copies per mL, quantities below which virus cultures usually become negative and transmission risks are low.^{22–24} Other laboratories place this threshold at a Ct of 30.24 There is no international standardisation between laboratories and assays, leaving Ct calibration with viral load poorly reported and easy to misunderstand.

Early findings, widely reported,3 from a study by Ferguson and colleagues,25 suggested that LFT had only 3% sensitivity for detecting SARS-CoV-2 among PCR-positive students at Birmingham University. Test underperformance was implied to explain finding only two positive results among 7189 individuals tested with Innova LFT.25 In that study,25 in a random sample of 710 (10%) LFT-negative individuals there were six PCR-positive results. That finding was extrapolated to 60 cases in the whole cohort, giving an extrapolated sensitivity of two of 62 (3.2%). The Ct values from the six PCR-positive samples were projected to Ct values for the 60 cases (54 unobserved plus six observed); in all six observed cases, viral loads were very low (Ct ≥29 reflecting around <1000 RNA copies per mL in the laboratory used)—when LFT should be negative. By comparison, the Liverpool pilot saw virus levels 1000 to 1 million times higher.20 In our view, the Birmingham study showed that PCR-positive asymptomatic students at a time of falling COVID-19 incidence had low viral loads compared with symptomatic members of the public attending testing centres and that LFT was working as expected.26

We wholeheartedly support healthy scientific debate to inform policies promptly. The COVID-19 road ahead looks challenging; therefore, we need big, bold actions across science and society, such as the Liverpool community testing pilot. The prompt evidence from such pilots can inform policies and help maintain public

confidence in the public health responses needed to navigate this pandemic's onward path.

IEB, MG-F, and MGS received grant funding from the UK Department of Health and Social Care to evaluate LFT in the Liverpool pilot that is discussed in this Comment. IEB reports fees from AstraZeneca as chief data scientist adviser via Liverpool University and a senior investigator grant from the National Institute for Health Research (NIHR) outside the submitted work. MGS is Chair of the Infectious Disease Scientific Advisory Board and a minority shareholder in Integrum Scientific LLC, Greensboro, NC, USA, a company that has interests in COVID-19 testing but not with lateral flow technology, and reports grants from the NIHR, the Medical Research Council, and the Health Protection Research Unit in Emerging and Zoonotic Infections, University of Liverpool. MJM reports research funding by the US National Institutes of Health Director's Early Independence Award DP5-OD028145 and from Open Philanthropy and Good Ventures. TEP is supported by the NIHR Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance at Oxford University in partnership with Public Health England (PHE), the NIHR Biomedical Research Centre, Oxford, and worked with PHE Porton on validation of LFT.

Michael J Mina, Tim E Peto, Marta García-Fiñana, Malcolm G Semple, *lain E Buchan buchan@liverpool.ac.uk

Center for Communicable Disease Dynamics, Department of Epidemiology and Department of Immunology and Infectious Diseases, Harvard T H Chan School of Public Health and Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA (MJM); Nuffield Department of Medicine, University of Oxford, Oxford, UK (TEP); Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, UK (MGS); Institute of Population Health, University of Liverpool, Liverpool L36 3GF, UK (MG-F, IEB)

- Deeks J, Raffle A, Gill M. Covid-19: government must urgently rethink lateral flow test roll out. BMJ Opinion, Jan 12, 2021. https://blogs.bmj.com/ bmj/2021/01/12/covid-19-government-must-urgently-rethink-lateral-flowtest-roll-out (accessed Feb 12, 2021).
- 2 Deeks J. Lateral flow tests cannot rule out SARS-CoV-2 infection. BMJ 2020; 371: m4787.
- 3 Armstrong S. Covid-19: tests on students are highly inaccurate, early findings show. BMJ 2020; 371: m4941.
- Fearon E, Davis E, Stage H, et al. A response to "Covid-19: Controversial rapid test policy divides doctors and scientists". BMJ 2021; published online Jan 12. https://doi.org/10.1136/bmj.n81.
- 5 Kmietowicz Z. Covid-19: controversial rapid test policy divides doctors and scientists. BMJ 2021; published online Jan 12. https://doi.org/10.1136/ bmj.n81.
- 6 van Kampen JJA, van de Vijver DAMC, Fraaij PLA, et al. Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-2019 (COVID-19). Nat Commun 2021; 12: 267.
- 7 Cevik M, Tate M, Lloyd O, et al. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. Lancet Microbe 2021; 2: e13–22.
- 8 Jefferson T, Spencer EA, Brassey J, Heneghan C. Viral cultures for COVID-19 infectious potential assessment—a systematic review. Clin Infect Dis 2020; published online Dec 3. https://doi.org/10.1093/cid/ciaa1764.
- 9 WHO. Criteria for releasing COVID-19 patients from isolation: scientific brief. 2020. https://www.who.int/news-room/commentaries/detail/criteria-for-releasing-covid-19-patients-from-isolation (accessed Feb 12, 2021).
- 10 Sun J, Xiao J, Sun R, et al. Prolonged persistence of SARS-CoV-2 RNA in body fluids. Emerg Infect Dis 2020; 26: 1834–38.
- Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis 2020; published online May 22. https://doi.org/10.1093/cid/ciaa638.
- 12 Eyre DW, Lumley SF, O'Donnell D, et al. Differential occupational risks to healthcare workers from SARS-CoV-2 observed during a prospective observational study. Elife 2020; 9: e60675.
- 13 Basile K, McPhie K, Carter I, et al. Cell-based culture of SARS-CoV-2 informs infectivity and safe de-isolation assessments during COVID-19. Clin Infect Dis 2020; published online Oct 24. https://doi.org/10.1093/cid/ciaa1579.
- 14 Huang CG, Lee KM, Hsiao MJ, et al., Culture-based virus isolation to evaluate potential infectivity of clinical specimens tested for COVID-19. J Clin Microbiol 2020; 58: e01068–20.

- Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020; 581: 465–69.
- 16 Cevik M, Marcus JL, Buckee C, Smith TC. SARS-CoV-2 transmission dynamics should inform policy. Clin Infect Dis 2020; published online Sept 23. https://doi.org/10.1093/cid/ciaa1442.
- 17 Cevik M, Kuppalli K, Kindrachuk J, Peiris M. Virology, transmission, and pathogenesis of SARS-CoV-2. *BMJ* 2020; **371**: m3862.
- 18 Cleary B, Hay JA, Blumenstiel B, et al. Using viral load and epidemic dynamics to optimize 2 pooled testing in resource constrained settings. *medRxiv* 2021; published online Jan 15. https://doi.org/10.1101/2020.05.01.20086801 (preprint).
- 19 Hay J, Kennedy-Shaffer L, Kanjilal S, Lipsitch M, Mina M. Estimating epidemiologic dynamics from single cross-sectional viral load distributions. medRxiv 2020; published online Oct 13. https://doi.org/10.1101/ 2020.10.08.20204222 (preprint).
- 20 University of Liverpool. Buchan I, ed. Liverpool Covid-19 Community Testing Pilot interim report. University of Liverpool. 2020. https://www.liverpool.ac. uk/media/livacuk/coronavirus/Liverpool,Community,Testing,Pilot,Interim,Ev aluation.pdf (accessed Feb 12, 2021).
- 21 Ashton M, Beale R, Buchan I, et al. Response to: Deeks et al. Briefing note for journalists on harm from continued rollout of the Innova Lateral Flow Test. University of Liverpool. Jan 22, 2021. https://news.liverpool.ac.uk/2021/01/22/covid-19-liverpool-experts-challenge-flawed-reports-on-lateral-flow-tests/ (accessed Feb 12, 2021).

- 22 Lee L, Rozmanowski S, Pang M, et al. An observational study of SARS-CoV-2 infectivity by viral load and 2 demographic factors and the utility lateral flow devices to prevent 3 transmission. University of Oxford, 2021. http://modmedmicro.nsms.ox.ac.uk/wp-content/uploads/2021/01/infectivity_manuscript_20210119_merged.pdf (accessed Feb 12, 2021).
- 23 Marks M, Millat-Martinez P, Ouchi D, et al. Transmission of COVID-19 in 282 clusters in Catalonia, Spain: a cohort study. Lancet Infect Dis 2021; published online Feb 2. https://doi.org/10.1016/51473-3099(20)30985-3.
- 24 Pray IW, Ford L, Cole D, 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: 1642–47.
- Ferguson J, Dunn S, Best A, et al. Validation testing to determine the effectiveness of lateral flow testing for asymptomatic SARS-CoV-2 detection in low prevalence settings. medRxiv 2020; published online Dec 24. https://doi.org/10.1101/2020.12.01.20237784 (preprint).
- 26 Crozier A, Rajan S, Buchan I, McKee M. Put to the test: use of rapid testing technologies for covid-19. BMJ 2021; 372: n208.