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.

	Cutoff	Total	True negatives	False positives	Specificity
Manufacturer instructions	≥1·0	773	706	67	91·3 (89·1–93·2)
Testing positive samples twice	≥1·0	767	725	42	94·5 (92·6–96·0)
Using a higher cutoff level	≥3·0	773	756	17	97·8 (96·4–98·7)
Testing positive samples twice and using a higher cutoff level	≥3·0	767	761	6	99·2 (98·2–99·7)

Data are n or % (95% CI), unless otherwise indicated.

Table: **Specificity of an automated fluorescence immunoassay for SARS-CoV-2 antigen in RT-PCR-negative asymptomatic individuals according to testing strategy**

Published **Online** May 4, 2021 https://doi.org/10.1016/S1473-3099(21)00206-1

than true infections might require a two-tier approach with molecular confirmation,[1] affecting the practicality and acceptance of such a strategy. Here we suggest alternative strategies to optimise the use of Ag-RDTs in asymptomatic populations with low positivity likelihood.

From September, 2020, to January, 2021, we evaluated an Ag-RTD to screen asymptomatic individuals before surgery or childbirth. 773 people were tested in parallel with STANDARD F COVID-19 Ag fluorescence immunoassay (SD Biosensor, Gyeonggi-do, South Korea) and a commercial RT-PCR (COVID-19 Genesig; Primerdesign, Chandler's Ford, UK)[2] using separate nasopharygeal swabs, following the manufacturers' instructions. The antigen assay was read with an automated device (F2400; SD Biosensor), which provides a quantitative immunofluorenscence index. All individuals tested negative by RT-PCR; however, 67 samples (8·7%) were initially positive by the Ag-RDT (table). We examined alternatives to improve test accuracy in our population. First, we repeated the Ag-RDT of positive samples using the same dilution buffer to calculate the average index, resulting in a reduction of false positives to 42 (5·5%). Second, we raised the cutoff for positivity from 1·0 (recommended by the manufacturer) to 3·0, on the basis of a receiver operating characteristic (ROC) curve which demonstrated optimum diagnostic performance at a cutoff of 3·36 (100% sensitivity; 98·5% specificity). To perform the ROC analysis, 30 RT-PCR-positive samples from patients with early COVID-19 from a previous study were included.[3] This approach reduced false positives to 17 (2·2%), and specificity increased significantly (table). The combination of both strategies showed the highest specificity (99·2%; table).

Although further studies are necessary to confirm our results, the presented data suggest that the dilemma of imperfect specificity of Ag-RDTs in asymptomatic populations can be diminished significantly by evaluating testing protocols that maintain the capacity of getting rapid results while increasing the accuracy of the tests.

We declare no competing interests. We thank Gabriel Pizarro for technical assistance.

*Thomas Weitzel, Caricia Pérez, Danixa Tapia, Paulette Legarraga, Lorena Porte
thomas.weitzel@gmail.com

Laboratorio Clínico, Clínica Alemana de Santiago (TW, DT, PL, LP), and Instituto de Ciencias e Innovación en Medicina (TW, CP), Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Santiago, Chile

1 Peeling RW, Olliaro PL, Boeras DI, Fongwen N. Scaling up COVID-19 rapid antigen tests: promises and challenges. *Lancet Infect Dis* 2021; published online Feb 23. https://doi.org/10.1016/S1473-3099(21)00048-7.

2 Porte L, Legarraga P, Vollrath V, et al. Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. *Int J Infect Dis* 2020; **99:** 328–33.

3 Porte L, Legarraga P, Iruretagoyena M, et al. Evaluation of two fluorescence immunoassays for the rapid detection of SARS-CoV-2 antigen-new tool to detect infective COVID-19 patients. *PeerJ* 2021; **9:** e10801.

4 WHO. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays: interim guidance. Sept 11, 2020. https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays (accessed March 16, 2021).

5 Boehme C, Hannay E, Sampath R. SARS-CoV-2 testing for public health use: core principles and considerations for defined use settings. *Lancet Glob Health* 2021; **9:** e247–49.

SARS-CoV-2 rapid antigen detection tests

I read with interest the Personal View by Rosanna Peeling and colleagues[1] on the performance of rapid antigen detection tests (Ag-RDTs) across different SARS-CoV-2 prevalence settings. The authors elegantly show that the negative predictive value (NPV) increases with decreasing disease prevalence and conclude that "for asymptomatic individuals in low prevalence settings, for travel, return to schools, workplaces, and mass gatherings, Ag-RDTs with high negative predictive values can be used with confidence to rule out infection".[1] However, the clinical interpretation of NPVs requires attention.

Independent evaluation of different Ag-RDTs has shown that their sensitivity ranges between 70% and 90% (lower confidence limits 50–80%) in symptomatic individuals,[2] but it deteriorates remarkably (<50%) in asymptomatic close contacts,[3] in those with low nasopharyngeal viral loads,[2] and in paediatric patients.[4] By contrast, the NPV is excellent (>97%) in all instances,[2–4] which has led most investigators to conclude that a negative Ag-RDT might reliably rule out the infection in low-prevalence settings.[1,4]

Predictive values are inherently dependent on disease prevalence and, as such, they can be misleading. When the probability of the disease is low, the NPV of any diagnostic test is high, irrespective of its sensitivity (figure). Assuming a disease prevalence of 2·5%, an Ag-RDT with

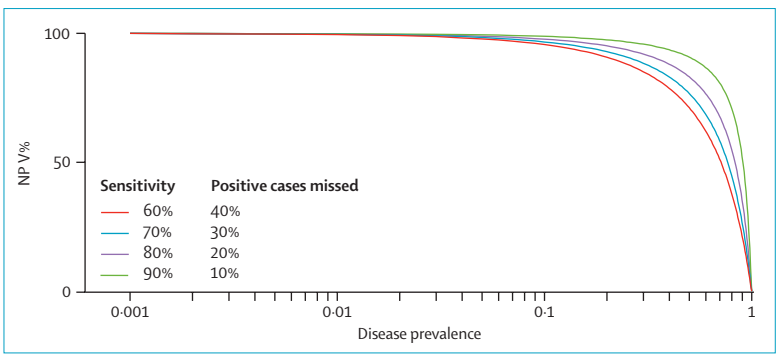

Figure: **NPV in relation to disease prevalence**
NPV=negative predictive value.

80% sensitivity and 97% specificity would result in a NPV of 99·5%, whereas one with 50% sensitivity (same specificity) would yield a NPV of 98·7%. Although both NPVs are excellent, the second test would miss five out of ten infected cases (the false-negative rate is equivalent to 100 minus the sensitivity, which equals 50%).

Current mathematical models suggest that the SARS-CoV-2 pandemic is driven by early and asymptomatic viral transmission and that prompt identification of low-risk and asymptomatic individuals has the strongest effect in controlling viral spread.[5] Thus, if our goal is to ensure SARS-CoV-2-free environments (eg, workplaces, schools, gatherings) by allowing those who test negative to resume their usual activities, false-negative results should not be tolerable; to achieve this goal, screening tools with the highest possible sensitivity are required, since sensitivity is the only parameter that reflects the rate of cases who erroneously test negative, irrespective of the disease prevalence.

Because most of the currently available Ag-RDTs have a considerable false-negative rate,[2–4] health-care professionals should be aware that a single negative test cannot conclusively rule out SARS-CoV-2 infection; this is particularly true in low-prevalence settings, where the typically excellent NPV of Ag-RDTs is misleading.

I report non-financial support from ELPEN and grants from University of Patras, outside the submitted work.

Sotirios Fouzas
sfouzas@upatras.gr

Department of Pediatrics, University Hospital of Patras, Rio, Patras 26504, Greece

1 Peeling RW, Olliaro PL, Boeras DI, Fongwen N. Scaling up COVID-19 rapid antigen tests: promises and challenges. *Lancet Infect Dis* 2021; published online Feb 23. https://doi.org/10.1016/S1473-3099(21)00048-7.

2 FIND. FIND evaluations of SARS-CoV-2 assays. https://www.finddx.org/covid-19/sarscov2-eval/ (accessed March 6, 2021).

3 Torres I, Poujois S, Albert E, Colomina J, Navarro D. Evaluation of a rapid antigen test (Panbio COVID-19 Ag rapid test device) for SARS-CoV-2 detection in asymptomatic close contacts of COVID-19 patients. *Clin Microbiol Infect* 2021; published online Jan 6. https://doi.org/10.1016/j.cmi.2020.12.022.

4 Albert E, Torres I, Bueno F, et al. Field evaluation of a rapid antigen test (Panbio COVID-19 Ag rapid test device) for COVID-19 diagnosis in primary healthcare centres. *Clin Microbiol Infect* 2021; **27:** 472.e7–10.

5 McCombs A, Kadelka C. A model-based evaluation of the efficacy of COVID-19 social distancing, testing and hospital triage policies. *PLoS Comput Biol* 2020; **16:** e1008388.

Authors' reply

We thank Sotirios Fouzas for his interest in our Personal View.[1] Fouzas' conclusion that the excellent negative predictive value of antigen-based rapid diagnostic tests (Ag-RDTs) is misleading has provided us with an opportunity to address common misunderstandings regarding SARS-CoV-2 Ag-RDTs.

The required performance characteristics of a test should be aligned to the purpose of testing. For confirming clinical diagnosis, one needs the most sensitive and specific test possible to ensure the patient is correctly diagnosed and treated. In this case, a molecular test would be the best option. However, when a test is used as a public health tool to ensure a safe environment, then we need an Ag-RDT with a high negative predictive value, while reliably identifying those with high viral loads so that they can be excluded from entry into the safe environment. Infectivity studies showed that the threshold for transmission corresponds to cycle threshold (Ct) values of less than 25, or approximately 10^6 viral copies per mL sample.[2] Ag-RDTs that can reliably detect individuals with these Ct values would be fit for purpose.

It is also important to understand the reasons for the variation in sensitivity of Ag-RDTs in asymptomatic, pre-symptomatic, and symptomatic populations in the published literature. The reference standard for evaluating Ag-RDTs is usually a molecular test, but studies have shown that while infected individuals shed infectious virus up to 9 days after symptom onset, they can remain RNA positive for weeks, when the test is likely detecting RNA fragments rather than infectious virus.[3] Two major sources of variation in sensitivity arise from different molecular tests being used as reference standards and the proportions of patients with high or low viral loads being recruited for the studies. In the end, the most important question is whether an Ag-RDT is sufficiently sensitive to detect those who might be at risk of transmitting SARS-CoV-2 infection. The sensitivity of most Ag-RDTs exceeds 96% in individuals with Ct values of less than 25.[4]

From a practical viewpoint, Ag-RDTs that are affordable, disposable, single-use cassettes that require minimal training and can return results in 15–20 min are much more feasible as a screening tool than are molecular tests, which are more costly,

Published **Online**
May 4, 2021
https://doi.org/10.1016/S1473-3099(21)00250-4