Editorial

Keeping up with the Spread of SARS-CoV-2: a Review of the Global Response to the Need for Innovative Tests for Global Population Screening

Sophie Mavrikou[1,2] and Spyridon Kintzios[1,2]
[1]Laboratory of Cell Technology, Department of Biotechnology, Agricultural University of Athens, Athens, Greece
[2]EU-CONEXUS European University, Athens, Greece

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Correspondence should be addressed to Sophie Mavrikou; Tel: +30 210 5294292; E-mail: sophie_mav@aua.gr

Abstract

Respiratory viral infections constitute a global problem in current medicine, veterinary, and plant pathology. Up to now, a vast number of techniques based on microbiology, biochemistry, molecular biology, and immunology have been proposed for virus determination. However, most of these assays are time-consuming, require complicated procedures for sample preparations and sophisticated instrumentation. The recent COVID-19 pandemic has demonstrated the need for the development of more accurate, rapid, early and cost-effective bioassays for high-throughput measurements. Therefore, the deployment of biosensors as well as lateral flow assays provides an alternative means to reliable and fast viral detection with tremendous future perspectives.

One of the major challenges – if not the main – presented by the COVID-19 pandemic is the application of extreme pressures on healthcare systems, the economy and general society. A key issue for epidemiologists to model the continual spread of the novel coronavirus is the ability for accurate estimation of the level of infection. It is generally agreed, however, that these models largely underestimate the number of positive cases, possibly by a tenfold order (Lipsitch *et al.* 2015). In the same way, as most models, the predictions of how the outbreak will spread out, the percentage of the population that will be infected and/or die, are subject to the scientific information they rest on. Up to now, most scientists working on epidemic modeling focus on data improvement, rather than making premature predictions. Nonetheless, several challenges to such analyses have to be confronted, especially in real-time. For instance, delays to confirmation of cases can occur, as consequences of limited detection and testing capacity or due to a delay to symptom appearance as a result of a long incubation period (Aylward *et al.* 2014). Thus, the major disadvantages of modelling methodologies are the delays and uncertainty that could arise just by the incorporation of the delays that derive from the natural history of infection and reporting processes (Nishiura *et al.* 2009). Furthermore, individual data sources might be inadequate, biased, or only conceive certain aspects of the outbreak dynamics. Therefore, evidence synthesis methods, which fit to multiple data sources rather than a single dataset (or datapoint) are recommended as more powerful assessments of the underlying dynamics of transmission from noisy data. Consequently, the accuracy of epidemic modelling emerges the need for the development of high-throughput, reliable tests for the early detection of the infection.

Current molecular tests for SARS-CoV-2 identification in suspect infected individuals are operated in certified reference laboratories (such as the CDC-designated public health laboratories), with assays mainly based on real-time reverse-transcriptase-based PCR (RT-PCR), with a limit of detection (LOD) of 4-10 copies/μl of sample and requiring at least a couple of hours for the completion of the assay process, not including in this period the time required for sample collection, shipment, processing and data registration. The lack of technical means to test the majority of the population, including potential asymptomatic patients, has forced the use of epidemiologic algorithms as a means to forecast the spread of the disease. The global effort to foster the urgent development of high throughput, portable diagnostic

kits for SARS-CoV-2 is additionally reflected on the establishment of transnational non-profit organizations and joint initiatives for the purpose of supporting expedient evaluation of emerging assays, such as the Geneva-based Foundation for Innovative New Diagnostics (FIND) (2020).

On the positive side, the medical diagnostics community has responded with remarkable speed to the urgent need for developing Point-of-Care (POC), rapid, reliable and, as much as possible, affordable tests for SARS-CoV-2 infection monitoring (Sheridan 2020). The following list is a set of most representative products classified according to the different assay principles and/or assay formats:

A number of tests have emerged for the detection of gene sequences specific to SARS-CoV-2. An example is the DETECTR system by Mammoth Biosciences (CA), provided as a reconfigured visual lateral flow strip assay (LAF) format. The assay employs a high-fidelity CRISPR detection enzyme and designed sets of gRNAs that can either 1) differentiate SARS-CoV-2 or 2) provide multi-coronavirus strain detection (Broughton *et al.* 2020). The system has a LOD of 70-300 copies/µl of sample and generates a clear visible signal within 30 minutes of total duration (sample to result). Not included is the time required for patient RNA extraction and the creation of RNP complexes (30 min). A positive result relates to the identification of both the N-gene (SARS-CoV-2 specific) and E-gene (SARS-CoV, bat-SARS-like-CoV, and SARS-CoV-2) coronavirus sequences.

A similar approach targeting the S gene and Orf1ab gene coronavirus sequences is under development by the collaborative efforts between Cepheid (CA, USA) and Sherlock Biosciences (MA, USA). SHERLOCK, which stands for Specific High Sensitivity Enzymatic Reporter, is a method for identifying specific sequences of genetic material in a sample using CRISPR, in particular CRISPR-Cas12 and Cas13. The POC test will be able to deliver results in approximately 45 minutes with an LOD of 10-100 copies/µl of sample, but has a low capacity of parallel sample testing (Herper 2020). Quite recently, Curti *et al.* (2020) reported the proof-of-concept, though not yet clinically validated CRISPR-Cas12 based diagnostic tool to detect synthetic SARS-CoV-2 RNA sequences in saliva, an obviously ideal sample source. The assay is planned to be commercialized by CASPR (CA, USA). The visualization of the test results was achieved using a fluorescence spectrophotometer and the LOD for *ORF1ab* coronavirus sequences was estimated to be up to 10 copies/µl at a very low cost (USD 1-2/ sample).

An alternative to coronavirus-related gene sequence identification is targeting antibodies raised against the viral envelope proteins. Compared with CRISP-based, gene sequence-detecting methods, serological assay tests have the advantage of lower cost and higher speed. On the other hand, the determination of antibodies in a patient is not reliably associated with the viral load and/or the stage of virus replication in the host. That said, the antibody-targeting approach offers the capability to discriminate between infected (acutely or past) and non-infected patients. In particular, testing for patient immunoglobins allows for a comparative discrimination between early infection stage (IgM, 4-10 days) or late one (IgG, 11 days or later). The identification of infected patients at an even earlier stage (0-3 days) is currently not feasible with the antibody detection assays. An example of antibody screening, PharmACT (Berlin, Germany) has developed a rapid blood test for IgM and IgG necessitating just two drops of capillary blood and 20 min for the colorimetric result to get developed (PharmACT 2020). The assay has been 100% correlated with RT-PCR and validated in respect for zero false positive results. Beyond blood testing, Israel-based BATM and Novamed have joined forces to develop a ultra-rapid, home testing kit for sputum analysis against SARS-CoV-2 (Marom 2020).

It is small wonder that Asian countries, having previous experience with coronavirus outbreaks, have been able to rapidly respond to the current challenge by developing a number of commercially available SARS-CoV-2 diagnostic tests. For example, a LAF test is being currently developed at the Genomics Research Center, Academia Sinica (Taipei, Taiwan) employing monoclonal antibodies against the nucleocapsid (N) protein of SARS-CoV-2 (Yang *et al.* 2020). Also Wondfo Biotech (Guangzhou, China) with branches in San Diego, Chicago and Bern, has announced the commercial release of its own, cost-efficient LAF kit based on IgM and IgG antibodies directed against SARS-CoV-2 (Finecare™), along with its own RT-PCR based test (2020). The same company was responsible for the development of the first colloidal gold antigen assay against SARS in 2003. In line with this conceptual approach, Snibe Diagnostic (Shenzhen, China) has released its 30 min, fully automated MAGLUMI 2019-nCoV IgM/IgG Kits, requiring 10 µl of blood sample

(2020).

The ability to screen for viral antigens instead of host antibodies raised against the virus is quite attractive and also important from an epidemiologic point view, since it would allow for a more accurate monitoring of the virus replication and spread potential. Furthermore, serological tests bear the inherent risk of false positive results due to non-specific occurring infections. One test targeting the S1 domain of the SARS-CoV-2 S1 protein is currently being developed by Sona Nanotech (Canada) in collaboration with GE Healthcare Life Sciences, based on Sona's proprietary nanorod technology in a LAF format. The test is estimated to deliver results within 15 min or less (Evans 2020).

Electrochemical techniques could also demonstrate a promising potential for the fabrication of low-cost and easy-to-use portable devices for medical diagnosis of the infection. One of their key features is that inexpensive electrodes can be relatively easily integrated with electronics and biorecognition elements in order to perform at the point-of-care real-time measurements in portable systems in a very short time (Hammond *et al.* 2016). Our own research team is also engaged in the proof-of-concept development of an ultra-rapid (3 min) test for the SARS-CoV-2 S1 protein based on measuring the bioelectric responses of cells bearing target-specific antibodies on their surface, a principle known as Molecular Identification by Membrane Engineering (Kokla *et al.* 2013).

In conclusion, precise and mass-screening of SARS-CoV-2 incidence in the global general population constitutes one of the few indispensable solutions to the control of this 21st century pandemic. Worldwide, expert developers of in vitro diagnostic methods have promptly addressed this challenge by developing novel assay principles and complete tests, whereas exemplary cases of private-public collaborations have paved the way for commercialization and wide distribution of test kits, reagents and assorted devices. A key issue to the successful and fast transition of these approaches to the market as well as to the site of final application (home or primary care unit), is the accelerated validation and eventual approval of the best assays. This can only be realized in the framework of an international collaboration between national regulatory authorities and the continuous exchange of information among the global research community.

Authors' Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

References

Broughton JP, Deng X, Yu G, Fasching CL, Singh J, Streithorst J, 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 2020 Rapid Detection of 2019 Novel Coronavirus SARS-CoV-2 Using a CRISPR-based DETECTR Lateral Flow Assay. *medRxiv* [Preprint]. 2020.03.06.20032334

Curti L, Pereyra-Bonnet F, & Gimenez Carla A 2020 An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12. *bioRxiv* [Preprint]. 2020.02.29.971127

Evans D 2020 Sona Develops Rapid Screening Test for Coronavirus. Retrieved 03/26/2020, from https://sonanano.com/sona-develops-rapid-screening-test-for-coronavirus/

Foundation for Innovative New Diagnostics 2020 COVID-19 diagnostics. Retrieved 03/25/2020, from https://www.finddx.org/covid-19

Hammond J L, Formisano N, Estrela P, Carrara S, & Tkac J 2016 Electrochemical biosensors and nanobiosensors. *Essays in biochemistry* **60** 69-80.

Herper M 2020 New coronavirus test returns results in 45 minutes, but it may be limited to hospitals. Retrieved 3/21/2020

Kokla A, Blouchos P, Livaniou E, Zikos C, Kakabakos SE, Petrou PS & Kintzios S 2013 Visualization of the membrane-engineering concept: evidence for the specific orientation of electroinserted antibodies and selective binding of target analytes. *J Mol Recognit* **26** 627-232

Lipsitch M, Donnelly CA, Fraser C, Blake IM, Cori A, Dorigatti I, Ferguson NM, Garske T, Mills HL, Riley S, Van Kerkhove MD & Hernán MA 2015 Potential Biases in Estimating Absolute and Relative Case-Fatality Risks during Outbreaks. *PLoS Negl Trop Dis* **9** e0003846

Marom Z 2020 BATM partners with Novamed for at-home COVID-19 diagnostics kit., from http://www.batm.com/media/1387/collaboration-with-novamed-for-at-home-covid-19-diagnostic-kit-190320.pdf

Nishiura H, Klinkenberg D, Roberts M, & Heesterbeek J A P 2009 Early Epidemiological Assessment of the Virulence of Emerging Infectious Diseases: A Case Study of an Influenza Pandemic. *PLOS ONE,* **4**(8) e6852

PharmACT HOLDING 2020 SARS CoV-2 rapid test. Retrieved 03/24/2020, from https://pharmact-health.com/en/sars-cov-2-rapid-test/

Sheridan C 2020 Fast, portable tests come online to curb coronavirus pandemic. *Nat Biotechnol* **38** 515-518

Snibe Diagnostic 2020 The world's first 2019-nCoV CLIA Kits received CE mark. Retrieved 03/16/2020, from http://www.snibe.com/zh_en/en_newsView.aspx?id=576

Wondfo Biotech 2020 Fight against COVID-19. Retrieved 03/22/2020, from https://en.wondfo.com.cn/covid-19-3/

WHO Ebola Response Team, Aylward B, Barboza P, Bawo L, Bertherat E, Bilivogui P, Blake I, Brennan R, Briand S, Chakauya JM, Chitala K, Conteh RM, Cori A, Croisier A, Dangou JM, Diallo B, Donnelly CA, Dye C, Eckmanns T, Ferguson NM, Formenty P, Fuhrer C, Fukuda K, Garske T, Gasasira A, Gbanyan S, Graaff P, Heleze E, Jambai A, Jombart T, Kasolo F, Kadiobo AM, Keita S, Kertesz D, Koné M, Lane C, Markoff J, Massaquoi M, Mills H, Mulba JM, Musa E, Myhre J, Nasidi A, Nilles E, Nouvellet P, Nshimirimana D, Nuttall I, Nyenswah T, Olu O, Pendergast S, Perea W, Polonsky J, Riley S, Ronveaux O, Sakoba K, Santhana Gopala Krishnan R, Senga M, Shuaib F, Van Kerkhove MD, Vaz R, Wijekoon Kannangarage N & Yoti Z 2014 Ebola virus disease in West Africa--the first 9 months of the epidemic and forward projections. *N Engl J Med* **371** 1481-1495

Yang A-S, Huang S-W, & Kuo P-C 2020 Catching Virus Fast! Academia Sinica discovered useful antibodies for developing rapid immune based test kit of SARS-CoV-2 coronavirus. Retrieved 03/12/2020, from https://www.sinica.edu.tw/en/news/6505