Opinion Paper

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Laboratory medicine in the COVID-19 era: six lessons for the future

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Abstract: The lockdown due to the coronavirus disease 2019 (COVID-19), a major healthcare challenge, is a worldwide threat to public health, social stability, and economic development. The pandemic has affected all aspects of society, dramatically changing our day-to-day lives and habits. It has also changed clinical practice, including practices of clinical laboratories. After one year, it is time to rethink what has happened, and is still happening, in order to learn lessons for the future of laboratory medicine and its professionals. While examining this issue, I was inspired by Italo Calvino's famous work, "Six memos for the next millennium".But I rearranged the Author's six memos into "Visibility, quickness, exactitude, multiplicity, lightness, consistency".

Keywords: consistency; COVID-19; laboratory medicine; quality; quickness; SARS-CoV-2; visibility.

Introduction

The lockdown due to the coronavirus disease 2019 (COVID-19), a major healthcare challenge, is a worldwide threat to public health, social stability, and economic development. The pandemic has affected all aspects of society, dramatically changing our day-to-day lives and habits, and forcing us to become "experts" in virology, learning terms such as droplets, lockdown and social distancing. It has also changed clinical practice, months being spent in the safety of homes, and in-person clinical examinations and hospital treatment being cancelled or modified in accordance with government-imposed quarantine rules. In response, novel approaches, such as telemedicine services and electronic diaries, have been introduced to enable patients to receive medical care in the absence of face-to-face services [1]. After one year, it is time to rethink what has happened, and is still happening, in order to learn lessons for the future of laboratory medicine and its professionals. While examining this issue, I was inspired by Italo Calvino's famous work, "Six memos for the next millennium" [2], but I rearranged the Author's six memos into "Visibility, quickness, exactitude, multiplicity, lightness, consistency".

Visibility

One of the most striking aspects of COVID-19 is the stark and ill-explained variation in experience of the disease. Some people are asymptomatic whereas others, originally healthy, can develop severe, sometimes fatal, pneumonia. COVID-19 cases, in fact, include asymptomatic carriers, patients with mild symptoms and fulminant disease characterized by sepsis and acute respiratory failure [3]. Irrespective of any initial reluctance to identify all possible transmission routes and mistaken initiatives undertaken to restrict the risk of human transmission of the virus from symptomatic patients, further evidence has highlighted the role of asymptomatic and pre-symptomatic subjects in transmitting the disease. In particular, the outbreak of COVID-19, which unfolded on board a Princess Cruises' ship, the Diamond Princess, represented a milestone in our understanding of both the risk of transmission by asymptomatic subjects, and the role of laboratory testing. Shortly after arriving in Yokohama, Japan, the ship was under quarantine orders as from 5 February 2020, after a former passenger tested positive for the virus (i.e. severe acute respiratory syndrome coronavirus 2; SARS-CoV-2), after disembarking in Hong Kong [4]. A body of evidence shows that SARS-CoV-2 can be transmitted by healthy carriers, viral transmission from asymptomatic and pre-symptomatic subjects underpinning the current pandemic [5, 6]. It was soon recognized that SARS-CoV-2 can be detected in

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symptomatic and pre-symptomatic subjects only by diagnostic (laboratory) tests, and millions of people worldwide now understand the key value of molecular testing on swabs as a tool (gold standard) for diagnosis, safe return to the workplace and the resumption of social activities. Further evidence has highlighted the value of "simple" clinical biochemistry, haematology, and coagulation tests for disease monitoring, prognostication and guidance for appropriate therapy [7]. The COVID pandemic dramatically challenged the capacity of laboratory medicine to cope with an unexpected increase in workload and the need for consolidated and innovative laboratory testing, but offered the opportunity to increase its visibility to the population at large. The key message from the World Health Organization was "test, test, test", with Dr Tedros Adhanom Ghebreyesus calling for more testing to tackle the coronavirus pandemic. This call resulted in an impressive acknowledgment by lay individuals of the central role played by laboratory medicine in modern medicine and health care (https://www.bbc.com/news/av/world-51916707).

Before the pandemic, several documents, papers and initiatives emphasized the importance of laboratory testing in numerous clinical pathways [8, 9], but the pandemic further raised awareness of the essential contribution made by clinical laboratories to diagnostic reasoning and the management of cases of suspected or confirmed SARS-CoV-2 infection. These include etiological diagnosis, patient monitoring, and epidemiological surveillance [7]. Further evidence of the importance of laboratory medicine is being gained thanks to serological testing for SARS-CoV-2 antibodies in vaccine(s) clinical trials, in properly monitoring vaccinated subjects (eventually with different vaccines and different clinical histories), and in better understanding the effects of virus variants from both diagnostic and clinical viewpoints [10, 11].

In addition, due to the development of rapid, point-of-care or better near-patient testing devices, it was predicted that "testing should become a part of life: in the morning you take your cereals, your vitamins, and you quickly check your health status" [12]. The relationship between quickness and quality will be discussed further on in this paper, but here it is important to be aware of the dramatic increase in the public demand for laboratory tests and, irrespective of previous evidence, the public's awareness of the key role played laboratory medicine in health care has certainly improved, largely due to its performance in the current pandemic.

First lesson

The first lesson to take home is therefore "do not miss the opportunity, thanks to the visibility gained from the pandemic, to provide further evidence of the central role played by laboratory medicine in modern, personalized medicine".

Quickness

Timeliness is a fundamental attribute of all laboratory tests [13], but in the case of COVID-19 it is a real matter of concern. Delays in test reporting may negatively impact both patient outcomes and delay isolation, the key to reducing spread of SARS-CoV-2. Data collected during the pandemic demonstrate that effective screening depends largely on frequency of testing and speed of reporting [14]. Yet the rapid communication of a wrong result is worse than a delayed test result, as highlighted by Gray and colleagues in their interesting article entitled "No test is better than a bad test?" [15]. In the case of the COVID-19 diagnosis, for example, a first generation rapid antigenic test did not assure a valuable sensitivity and specificity, as highlighted in numerous reports. In particular, the CDC COVID-19 Surge Laboratory Group described a very poor test sensitivity (41.2%) in samples from asymptomatic SARS-CoV-2 rRT-PCR positive individuals leading to an estimated predictive positive value (PPV) of 33.3%, while it improved to 80% and PPV of 94.1% in patients who reported one or more symptoms at specimen collection [16]. As shown in Figure 1, one in five symptomatic patients with confirmed COVID-19 received a negative rapid antigen test result. Deeks and Raffle highlighted the risk of missing the diagnosis of COVID-19 by using rapid antigen tests based on lateral flow devices and the additional poor performance of the same devices when testing was performed by untrained or poorly trained personnel. In particular, the authors reported the data from two studies performed in the UK. Detection rates (sensitivity) were 73% (95% confidence interval 64–85%) when tested by skilled NIHR research nurses and 79% (73–85%) when tested by Porton Down laboratory scientists. But testing by Boots test centre employees (following written instructions) achieved sensitivity of just 58% (52–63%) [17]. Even for point-of-care tests (POCT) used for SARS-CoV-2 antibody assay, a systematic review and meta-analysis of available methods,

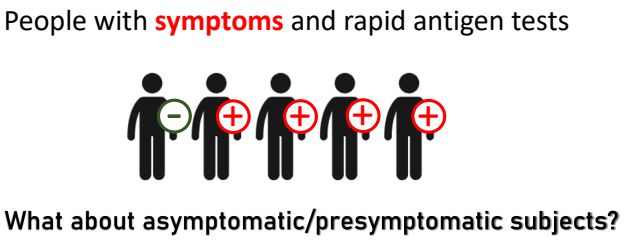

Figure 1: False negative results of rapid antigenic tests in symptomatic patients (from reference [16], modified).

concluded that "currently, available evidence does not support the continued use of existing point-of-care serological tests" [18]. Overall these data indicate that speed and accuracy should go hand in hand, and that the presumed advantages of rapid testing need to be balanced against its lower sensitivity (sometimes even specificity) and lower PPV, especially in asymptomatic cases. In the COVID-19 pandemic, another aspect of quickness involving laboratory medicine is related to the emergency use authorization (EUA) adopted mainly in the US. On February 2020, the U.S. secretary of health and human services declared that emergency use of diagnostics for SARS-CoV-2 was justified, triggering emergency authority for the Food and Drug Administration (FDA) to grant an emergency use authorization (EUA) for a device if it reasonably believes that it may be effective, rather than waiting to grant full approval when it has reasonable assurance that the device is safe and effective. This mechanism should expedite access to accurate diagnostic tests during emergencies, when information gaps and false results may adversely affect patient care and public health decision-making. This step enabled molecular and diagnostic tests to be developed, validated and deployed within weeks rather than months or over a year, as traditionally required. Shuren and Stenzel concluded "although this approach resulted in earlier test availability, the EUA's less-rigorous evidence standard, coupled with delayed FDA review, allowed the use of several LDTs that ultimately proved to have performance problems or to be poorly validated" [19]. The Authors also stated that "when a public health threat warrants large-scale testing, it would be more effective to authorize a small number of well-designed, well-developed, and validated tests run on common high-throughput platforms, followed by a few point-of-care tests, all of which are manufactured in large quantities, than to simultaneously develop and authorize scores of diagnostics. Such diffuse efforts are an inefficient use of resources". This again highlights the key role played by the correct evaluation and validation of laboratory methods, platforms and diagnostic systems in assuring reliability, accuracy and patient safety and counteracting the opinion that quality in laboratory testing can be taken for granted.

A final aspect of quickness in the COVID-19 pandemic is related to scientific publications: at least 40 articles on SARS-CoV-2 and COVID-19 have been retracted from peer-reviewed journals and preprint servers [20]. Moreover, the retractions involved some articles that appeared in two of the most prestigious medical journals, The Lancet [21] and New England Journal of Medicine [22]. Also in this case, the time for an appropriate peer-review process, adequate evaluation of the quality of the papers should be granted, referees and editors being forced to accelerate the publication workflow. Moreover, it should always be borne in mind that, for some studies, it is advisable for journals to mandatorily ask the authors to share their primary data. It is of vital importance to ensure data integrity and transparency of the research findings, and to combat publication frauds, particularly in this dire socio-economical pandemic.

Second lesson

Speed must never compromise quality, but a marriage between accuracy, reliability and quickness should be made, if need be by adopting and managing valuable POCT and near patient testing.

Exactitude

Much of the dialogue around laboratory testing has focused on how Countries and individual laboratories can boost the capacity to meet the sudden, enormous increase in workload particularly regarding molecular tests. A survey promoted by the American Society for Clinical Chemistry (AACC) demonstrated that more than 70% of respondent laboratories report that they are unable to obtain supplies necessary for running SARS-CoV-2 tests, 57% report difficulties in securing test kits, and 21% expect to be unable to process all requested tests (https://www.aacc.org/science-and-research/covid-19-resources/aacc-covid-19-testing-survey/full-survey-results. Accessed March 10th, 2021). Yet uncertainty in testing, particularly in the case of serological tests, has not received adequate attention. Aloisio and Coll. emphasized that "… it is, however, embarrassing to note the extremely low number of papers that at the same time dealt with the real-life analytical performance of SARS-CoV-2 serologic assays… and even for anti-SARS-CoV-2 serology bad (even if cheaper)

assays will always be clinically (and socially) counterproductive" [23].

In addition, while the focus in the past was on the analytical phase, a body of evidence accumulated in the last few decades has highlighted the current greater vulnerability of the extra-analytical phases [24, 25]. In SARS-CoV-2 testing, irrespective of the satisfactory results of molecular testing in external quality assessment (EQA) schemes [26], several articles have documented errors in the pre-analytical phase, particularly related to problems in sample collection and handling [27, 28]. This, in particular, has been seen to affect the accuracy of molecular testing in swabs due to several factors, including poor training and technique in the collection of nasopharyngeal specimens [29], inadequate procedures for handling, transport and storage of the swabs, sample cross-contamination and contamination of reagents [27].

Regarding the post-analytical phase, particular concern has finally been raised concerning the need to interpret any laboratory (and diagnostic) test result in the context of the pre-test probability of disease. For COVID-19, pre-test probability assessment includes symptoms, previous medical history of the disease and any potential exposure to SARS-CoV-2 and must be considered for both molecular testing, SARS-CoV-2 antibody results, and all other clinical chemistry, haematology and coagulations results [30, 31]. Table 1 shows the pre- and post-test probabilities for SARS-CoV-2 rRT-PCR tests, with calculations based on a 70% sensitivity and 95% specificity, and the value of an additional test repetition to rule out the disease. The need to assure quality in all steps of the testing cycle has finally been acknowledged in several papers and guidelines dealing with COVID-19 diagnosis [28, 32], thus confirming previously published articles on the changing paradigm of quality in laboratory medicine [33–35]. In addition, the Working Group of the IFCC "Laboratory Errors and Patient Safety" has developed a Model of Quality Indicators (MQI) necessary for measuring mistakes, errors and non-conformities in all steps of the testing process, to be used as a benchmark with other laboratories worldwide, and to integrate current external quality assessment (EQA)/proficiency testing (PT) schemes dealing with only analytical quality [36, 37]. In laboratory medicine, great attention is currently focused on measurement uncertainty (MU). The international vocabulary of metrology (VIM) has defined MU as a "non-negative quantity that characterizes the dispersion of the values that could reasonably be attributed to the measurand" [38]. For a given test result, MU thus represents the interval associated with a defined probability in which the true result should lie. In addition, this interval should fall within limits that guarantee fitness for the clinical purpose of the tests in question [39]. The usefulness of MU can be summarized as follows: (a) gives objective information on the quality of individual laboratory performance; (b) serves as a management tool for the medical laboratory and for IVD manufacturers, obliging them to investigate and eventually fix problems identified; (c) helps the manufacturers of 'superior' products and measuring systems to demonstrate the superiority of their products; (d) identifies analytes requiring analytical improvement for their clinical use, and encourages IVD manufacturers to work toward improving the quality of assay performance; (e) leads to the discontinuation of poor quality assays (with demonstrated insufficient quality). However, we have highlighted the need to comply with a basic clause of ISO 15189: 2012, the International Standard for Medical laboratories accreditation that recommends the notification of MU to laboratory users. In fact, the footnote to the above-cited clause 5.5.1.4 states that: "upon request, the laboratory shall make its estimates of measurement uncertainty available to laboratory users" [40]. The notification of MU should facilitate the appropriate interpretation of laboratory results, particularly when they are close to the upper (or lower) reference value or to the decision level (cut-off) thus, if communicated effectively, strengthening the relationship between clinicians, patients and medical laboratories. Laboratory professionals should clearly inform all users (physicians and patients) that MU is only a part of the broader uncertainty of laboratory information, which takes into account both pre- and post-analytical issues, such as the quality of both sample/specimen and comparator (reference interval/decision limits) [41].

In the COVID-19 pandemic, it is widely accepted that the interpretation of diagnostic test results depends on understanding the accuracy of the test, and the probability that the patient has the condition before testing. This, in turn, has led to a better understanding of the true meaning of uncertainty in laboratory tests and of the fact that, because nearly all tests are imperfect, errors in interpretation most often arise from overestimating the effect of a test result on the post-test probability of a condition [42]. The appropriate use of diagnostic tests in clinical decision making should be viewed as a key skill for all physicians, since the correct selection, and interpretation, of diagnostic tests is crucial to diagnostic error reduction and improvement in clinical decision making.

Third lesson

Assure and monitor quality in all phases of the testing process and measure clinical and economical outcomes to

Table 1: Pre- and post-test probabilities for SARS-CoV-2 rRT-PCR tests: calculations based on a sensibility of 70% and specificity of 95% (from reference [30], modified).

Pre-test probability	Post-test probability, negative test	Post-test probability, positive test	Post-test probability, two independently negative tests
5	1.6	42	0.5
15	5	71	2
25	10	82	3
50	24	93	9
75	49	98	23
90	74	99	47

provide evidence of the effectiveness of laboratory services. The IFCC Model of Quality Indicators (MQI) is a valuable tool for achieving this goal.

Multiplicity

COVID-19 is the paradigm of the need for a real and valuable integration between the different "subdisciplines" of laboratory medicine and other diagnostic tests. The demand is now high for diagnosis, prognostication, monitoring, guidance for effective and personalized therapy and, finally, epidemiological surveillance. Therefore, competences and skills in clinical biochemistry, immunology, coagulation, haematology, microbiology and virology and pathology are highly requested. Moreover, to enable clinicians to correctly evaluate complex clinical pictures and heterogenous pathological lesions, laboratory information should be successfully integrated with imaging (e.g., conventional chest X-rays only, computed tomography [CT] scan) and, if necessary, data should be analysed using machine-learning/artificial intelligence tools [43].

Numerous innovative techniques are currently being developed for the accurate, rapid and effective diagnosis of COVID-19. Figure 2 shows the state-of-the-art, which is continuously changing. In particular, in addition to the currently more frequently used technique for SARS-CoV-2 testing, the rRT-PCR, recent papers report that digital PCR (RT-ddPCR) significantly improves accuracy and reduces the false negative rate for SARS-CoV-2 in pharyngeal swab specimens, which is more convenient and simpler to sampling. Furthermore, ddPCR is more sensitive and suitable for low viral load specimens from the patients under isolation and observation who may be asymptomatic. Finally, RT-ddPCR could be used for quantitative monitoring of convalescents in order to evaluate disease progression [44].

Reportedly, a panel of sensitive, quantitative RT-ddPCR-based SARS-CoV-2 assays has been developed to collectively span the genome and target non-genic and genic regions, including important enzymes transcribed only as genomic RNA and structural genes that are also transcribed as different sub-genomic RNAs. These assays can serve as novel molecular tools to investigate SARS-CoV-2 infection, replication dynamics, and gene expression thus enhancing our understanding of the viral dynamics and pathogenesis of SARS-CoV-2 over the course of infection. In particular, sensitive RT-ddPCR assays could be of great utility in studying the course of infection two or more weeks after the resolution of acute symptoms in order to better understand whether prolonged viral shedding after resolution of symptoms is really related to the risk of disease transmission [45].

Other recently reported promising approaches to COVID-19 diagnosis use clustered regularly interspaced short palindromic repeats (CRISPR). In particular, Broughton and Coll. reported the development of a rapid (<40 min), user-friendly and accurate CRISPR–Cas12-based lateral flow assay for SARS-CoV-2 detection in respiratory swab RNA extracts with 95% positive predictive agreement and 100% negative predictive agreement with respect to conventional rRT-PCR [46]. The recently developed CRISPR-Cas12a with RT-recombinase polymerase amplification reaction (RT-RPA) has further advantages: a) it can directly target samples for detecting SARS-CoV-2 without requiring complicated procedures (RNA extraction); b) the whole process of the test takes about 60 min, and c) the test takes place at a constant temperature of 42 °C, thus enabling POC testing, as it acts as a real one-step detection of SARS-CoV-2. In their paper, the authors reported that positive predictive agreement and negative predictive agreement of the CRISPR-Cas12a assay in relation to clinical RT-PCR diagnostic testing were 100% for the detection of SARS-CoV-2 [47].

High-throughput serum peptidome profiling methods based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have been

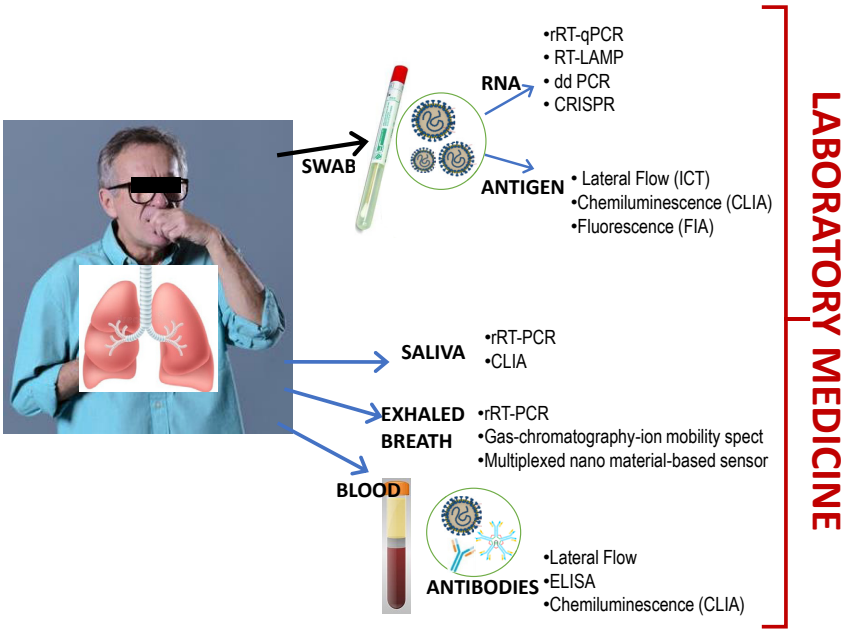

Figure 2: Available methods for SARS-CoV-2 diagnosis.

developed for the efficient detection of COVID-19 [48]. The serum metabolome of COVID-19 patients, assayed using gas chromatography-mass spectrometry, was found to be distinctive, and is of considerable value in investigating pathogenesis, determining a diagnosis, predicting severe cases, and improving treatment [49]. These techniques, as well other approaches for diagnosing COVID-19 and/or better understanding its pathophysiology, call for artificial intelligence and machine learning-based analysis to achieve the integrated interpretation of multiple and complex data [43, 50]. Serological testing is another non-invasive diagnostic approach to COVID-19 pandemic management: SARS-CoV-2 antibody tests are valuable tools for understanding the immune response, evaluating the spread of the infection in the population and investigating seroprevalence in sub-populations at a higher risk of infection, healthcare workers in particular, providing evidence of efficacy in vaccine trials, and now in monitoring response in vaccinated subjects. Although not well suited for early COVID-19 diagnosis, serological tests should be used for the so-called "late diagnosis" in subjects with no, or minor, symptoms who have not undergone molecular testing [51]. Anti–SARS-CoV-2 antibodies should answer several questions: what is the prevalence, level, and durability of detectable anti–SARS-CoV-2 antibodies among patients who have or have recovered from reverse transcriptase polymerase chain reaction (RT-PCR)–diagnosed SARS-CoV-2 infection? [52]. Numerous immunoassays have been developed to detect SARS-CoV-2 antibodies. However, a standard approach to testing in terms of antibody subtypes and timing has not yet been established. Most immunoassays detect antibodies to the viral spike protein, receptor-binding domain (i.e. part of the spike protein), or the nucleocapsid protein [53]. Since neutralizing antibodies, which most commonly act against the receptor-binding domain region of the viral spike protein, bind to the virus and prevent infection, they are of particular interest in determining whether antibodies confer protective immunity. Therefore, a fundamental issue is that levels of SARS-CoV-2 antibodies detected by immunoassays should be closely correlated with neutralizing antibodies and only if this correlation is demonstrated, should they be used as a "surrogate" measure of neutralizing ability [54].

A very recently published review emphasizes that current evidence is still limited due to both the poor evaluation of the analytical performances of numerous assays, and the fact that most studies to date have not been designed to evaluate whether the presence of antibodies in subjects who have recovered from SARS-CoV-2 confers natural immunity, or whether the absence of post-infection antibodies is clinically meaningful [53].

Further studies are needed to answer the above-cited open questions, and to finally establish the true value of SARS-CoV-2 antibodies testing in clinical practice. We have emphasized, however, the importance of SARS-CoV-2 antibodies testing for the appropriate prioritisation of vaccination candidates, and for deferring vaccination in individuals less likely to be infected, re-infected and/or develop more aggressive COVID-19 illness until the more vulnerable individuals have acquired a sufficient degree of protective immunity [55]. Recently published articles confirm the importance of SARS-CoV-2 antibodies testing

before and after vaccination to provide evidence of effective protection, obviate any systemic reactions (reactogenicity) in subjects with pre-existing immunity and prioritise the administration of booster doses for individuals with no previous infection [56, 57].

Lesson four

Evaluate all well-developed and promising technologies, validate and deploy them according to established guidelines and recommendations focusing on patient needs. Integrate different diagnostic approaches in a clear and reliable report so that the information is conducive to diagnostic accuracy, effective therapy and the best possible clinical outcome.

Lightness

Nasopharyngeal swabs (NPS) are the recommended specimen for COVID-19 diagnosis. However, NPS testing requires trained personnel and handling of specially designed swabs, and the technique cannot be easily performed in all populations. There is an urgent need for sampling strategies using a non-invasive, painless approach allowing easy sample collection in children, the disabled and the elderly. Innovative and interesting developments for diagnosing COVID-19 by means of less invasive and painless testing entail the use of exhaled breath and saliva as alternative samples. It has been known for several decades that endogenously produced volatile organic compounds (VOCs), present in exhaled breath, are frequent targets of breath diagnostics research, representing metabolic endpoints that can be quickly assessed for health information [58]. Grassin-Delyle and coll. developed real-time, online, proton transfer reaction time-of-flight mass spectrometry for the metabolomic analysis of expired air from adults undergoing invasive mechanical ventilation in the intensive care unit for severe COVID-19 or non-COVID-19 acute respiratory distress syndrome (ARDS). The detection of methylpent-2-enal, 2,4-octadiene 1-chloroheptane, and nonanal in exhaled breath allowed the authors to identify ARDS patients with COVID-19 [59].

In another study, exhaled breath condensate (EBC) RT-PCR was found to be an effective, non-invasive method for identifying SARS-CoV-2 from lower respiratory tract samples, and should be considered an aid in the diagnosis of COVID-19 [60]. A further paper by Shan and coll. described an innovative breath device. Consisting of a nanomaterial-based hybrid sensor array with multiplexed detection capability, it can detect disease-specific biomarkers from exhaled breath, thus enabling rapid and accurate diagnosis. The training and test set data exhibited, respectively, 94 and 76% accuracy in differentiating patients from controls as well as 90 and 95% accuracy in differentiating between COVID-19 and non-COVID-19 lung infection [61].

Saliva-based sampling for SARS-CoV-2 detection via rRT-PCR can potentially address many of the barriers associated with nasopharyngeal swab sampling [62–64]. Saliva samples can be self-collected, instructions being given by lower-cadre health care professionals or other personnel. The main advantages of saliva collection for sampling are that the approach: a) is non-invasive, b) is painless (entailing no patient discomfort or anxiety), c) involves easy collection, storage and shipping, d) is suitable for children, anxious, disabled and elderly individuals, e) is cost-effective for screening large populations [65].

We developed an active control program for employees of the University of Padova for SARS-CoV-2 detection on salivary samples using molecular testing (rRT-PCR), which started on October 8th 2020 and is on-going. The program, offered to all employees (n=6,500) on a voluntary basis and followed by 86% of candidates, was based on self-collection of salivary samples and molecular testing, and was found to be a reliable, well-accepted and effective tool for the early detection of SARS-CoV-2 in asymptomatic subjects. It allowed immediate contact tracing and containment, thus obviating further viral spread in the community, and creating a protected island. The reported preliminary data, collected between October and late December 2020, highlight the excellent accuracy (94% agreement between salivary and nasopharyngeal swab samples). Importantly, the overall incidence among employees in active surveillance was 1.8% (62 positive cases on saliva test and 32 identified by symptoms/close contact). Among the employees not under surveillance, the overall percentage of cases positive for symptoms/close contact was 6.1% (70/1150) [66].

More recently, we evaluated and validated an automated chemiluminescent assay for SARS-CoV-2 antigen on salivary samples that allows us to obtain accurate and reliable results, thus providing further confirmation of the value of the use of saliva in large population screening programs [67].

Testing programmes with more frequent and rapid tests across communities coupled with isolation of individuals with confirmed infection are essential for mitigating the COVID-19 pandemic [68]. Essential requirements

are not only test accuracy, but also non-invasive sampling strategies, appropriate turnaround time and effective contact tracing based on the timely and correct acknowledgment/utilization of laboratory information. Testing alone is not enough.

Lesson five

Make sample collection as simple as possible, ensure timely acknowledgment and facilitate the right interpretation of the laboratory information provided. Change laboratory organization and processes, in line with a patient-centred focus

Consistency

While the importance of testing in mitigating and suppressing further viral spread is indisputable, the need for more, and better, testing has often been stressed [69]. First, to assure consistency in testing strategies, it is necessary to carefully validate and verify the analytical and diagnostic performance characteristics of laboratory tests, and to impose stricter standards in laboratory testing, including the mandatory adoption of internal quality systems and participation in external quality assessment/proficiency testing schemes, also for POCT. Second, testing should be always accompanied by appropriate standard operating procedures (SOP) for assuring quality in sample collection, handling and transportation as well as comprehensible information for allowing the timely and right interpretation and utilization of laboratory results. In this context, yet again it is important to be aware of the disease prevalence and pre-test probability for the right interpretation of laboratory tests. Training and continuing education should be guaranteed to enhance physicians' understanding of test performance, interpretation and related limitations, and appropriate training should be offered to nurses and other health care operators. Third, as more tests are produced, turnaround time, correct and intelligible reporting of test results and close connection with physicians and health care authorities are fundamental requirements, in addition to analytical accuracy. Indeed, testing alone does not prevent viral transmission in the community, or mitigate the pandemic. Testing in the absence of other proven prevention strategies cannot prevent outbreaks [70]. The COVID-19 pandemic is the paradigm of proof that laboratory testing should be considered an essential but not isolated step in disease prevention, early diagnosis, valuable monitoring, personalized therapy and epidemiological surveillance. This, in turn, reinforces the need to change the vision of clinical laboratories: rather than being factories focussing on large volumes of data production, they should be seen as integrated practice units based on patient needs and the knowledge of diagnostic-therapeutic pathways. As already reported, "quality in laboratory medicine has two dimensions, which can no longer be separated" (Figure 3). The "internal dimension", performed and assured within the laboratory environment to provide efficiency, is based on accuracy and reliability of analytical results, timeliness in their production and communication and, finally, on cost-containment activities. The "external dimension" is assured by diagnostic accuracy, value in test-treatment pathways, effect on clinical and economical outcomes and, finally, on patient safety. Indeed, the effectiveness of a test depends not only on the result being delivered according to set standards (e.g. accuracy, timeliness and acceptable costs), but also on the result being acted upon appropriately and in a timely fashion to assure valuable outcomes [71].

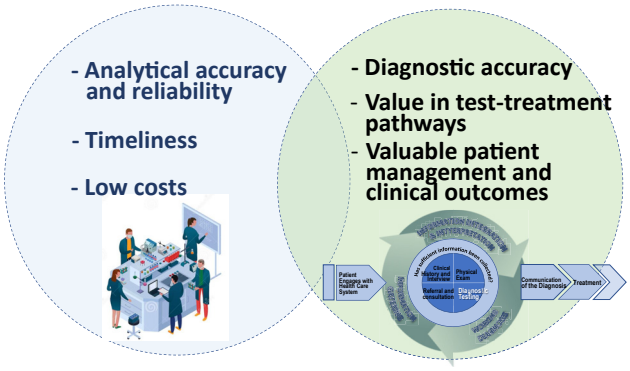

Figure 3: Internal and external dimensions of quality in laboratory medicine (from reference [71], modified).

Lesson six

Move your laboratory out of the silo, avoid isolation and integrate laboratory testing in diagnostic and clinical pathways that effectively prevent disease, and provide early diagnosis, valuable monitoring, personalized therapy and epidemiological surveillance. The ultimate goal is effectiveness, not just efficiency.

Conclusions

Several studies, including the recently published article by Du and Colleagues [68], conclude that "screening SARS-CoV-2 once a week or every 15 days, coupled with

strict enforcement of a two-week isolation period for confirmed cases should be considered justified on cost-effectiveness grounds", and the same benefits have been reported in our experience based on molecular testing of saliva samples [66]. Both studies, however, highlight the need to avoid the principal policy objections to mass screening: imperfect test performance and poor adherence to both testing and isolation strategies. This is the main lesson for the future of laboratory medicine: the ability to combine analytical quality with a more involvement in diagnostic-therapeutic pathways, a better knowledge of patient/user needs and preferences. The key role of laboratory testing in modern medicine requires laboratory professionals to take on more, ever new responsibility with the aim of improving not only analytical performance but appropriate requesting and utilization of laboratory tests, as well as the integration of laboratory information with all other diagnostic and clinical data. As Manabe and Colleagues [69] state "even the perfect test cannot go it alone"; and this should be assured not only for COVID-19, but also for all other clinical conditions. A further lesson for laboratory professionals is that the COVID-19 pandemic has tested their preparedness and response to extraordinary events. Disasters, both natural and man-made, including armed conflict, can lead to humanitarian emergencies (HE), and diagnostic testing is a crucial tool in achieving fast response and containment. Thanks to advances in science and technology, diagnostic testing is readily available, but serious considerations should be made about how technology can be integrated into handling future healthcare crises. Laboratory professionals are called upon not only to manage increased workloads in traditional centralized facilities, but also to provide advice on the effective utilization of rapid diagnostic tests, point-of-care and near-patient care technologies, and on the integration of laboratory information with communication technology to enable real-time data reporting and transmission 72[72]. COVID-19 will not be the last pandemic to emerge. Only if preparedness is improved, will a better management of future disasters and healthcare crises be achieved.

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