Approaches to Deployment of Molecular Testing for SARS-CoV-2 in Resource-Limited Settings

Gama Bandawe, PhD[a,*], Moses Chitenje, BSc[b,c], Joseph Bitiliyu-Bangoh, MSc[d], Elizabeth Kampira, PhD[e]

KEYWORDS

• SARS-CoV-2 • Molecular testing • Laboratory strengthening
• National reference laboratory • Cost reduction

KEY POINTS

- Deployment of molecular testing in resource-limited settings needs to be approached in the broader context of laboratory strengthening.
- Scale-up of molecular testing was built on existing pathogen control programs for human immunodeficiency virus and tuberculosis.
- National reference laboratories have an essential role to play in successful roll out of molecular testing.
- Pooled testing and direct-to-polymerase chain reaction methods have great potential for cost saving and increasing access to molecular testing.

INTRODUCTION

Less than a month after severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was described as the causative agent of COVID-19,[1,2] molecular diagnostic assays based on reverse transcriptase qualitative polymerase chain reaction (RT-qPCR) were rapidly developed.[3] In the absence of other sensitive and reliable methods, these assays became the primary method that enabled countries around the globe to identify and the

[a] Biological Sciences Department, Academy of Medical Sciences, Malawi University of Science and Technology, P. O. Box 5196, Limbe, Malawi; [b] International Teaching and Education Centre for Health (ITECH), PO Box 30369, Capital City Lilongwe 3, Plot 13/14, 1st Floor ARWA House, City Center, Lilongwe, Malawi; [c] Public Health Institute of Malawi, Ministry of Health, Lilongwe, Malawi; [d] Ministry of Health, P.O. Box 30377, Lilongwe 3, Malawi; [e] Centres for Disease Control and Prevention, P. O. Box 30016, NICO House, City Centre, Lilongwe 3, Lilongwe, Malawi
* Corresponding author.
E-mail address: gbandawe@must.ac.mw

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disease, conduct surveillance, and mount a response to the pandemic. Use of this assay as a routine diagnostic tool in many parts of the world was limited, as it is relatively specialized requiring some complex machinery, infrastructure, and training to conduct competently and routinely with high throughput. The World Health Organization (WHO) published testing guidelines,[4–6] which included biosafety level 2 conditions for handling of specimens for molecular testing. Although these requirements pose a challenge even in the most affluent countries, in resource-limited settings they presented an even more significant challenge. Despite this, molecular testing capacity had to be rapidly scaled up to meet the testing needs in every part of the world. Here the authors outline some of the key considerations, partnerships, and activities that were required and draw on several specific examples from Malawi in Southern Africa. Lessons drawn from this experience can be informative for continued laboratory strengthening and preparation for any future outbreaks of novel zoonotic or reemerging pathogens of public health concern.

SYSTEMS STRENGTHENING

Low- and middle-income countries are often those with the highest disease burden, and lack of adequate laboratory capacity presents a further barrier in provision of appropriate diagnosis, care, and treatment of existing diseases and emerging pathogens.[7–9] The establishment of fully equipped testing laboratories that fulfill WHO guidelines required huge investment, expertise, and time, which are limited by the many other competing priorities and requirements of a national COVID-19 response. Laboratory systems in many low- and middle-income countries were already struggling under the weight of a myriad of systemic challenges including lack of laboratory supplies, lack of essential equipment, limited numbers of skilled personnel, lack of educators and training programs, inadequate logistical support, deemphasis of laboratory testing, insufficient monitoring of test quality, decentralization of laboratory facilities, and lack of government standards for laboratory testing.[9] Efforts to scale-up any disease response would need to therefore be conducted using a broader systems strengthening approach that attempts to address many of these issues concurrently.

In the last 2 decades, a significant amount of funding and investment has flowed into strengthening of laboratories[10,11] mainly aligned with specific pathogen control programs especially human immunodeficiency virus (HIV), tuberculosis (TB), and malaria. With HIV control programs, improved laboratory capacity has resulted from the need to provide comprehensive laboratory diagnostic services for monitoring patients on antiretroviral therapy with CD4, chemistry, hematology, testing for HIV-1 drug resistance mutations and testing for opportunistic infections.[12] Importantly, there was also a need for molecular tests for detecting and measuring plasma RNA levels via RT-qPCR for early infant diagnosis and detection of treatment failure or viremic control as part of the treatment cascade.[13] With the TB control programs, the need to provide rapid molecular point-of-care detection as well as detection of rifampicin resistance[14] was essential. Both of these programs proved to be invaluable in providing a meaningful platform that the deployment of molecular testing for SARS-CoV-2 could build on.

The exigency of using existing infrastructure and capacity to pivot onto the COVID response reinforced the need for continued emphasis on integration of laboratory services and capacity to meet a diversity of needs, which we have now learned can evolve rapidly. This integrated laboratory system approach, in contrast to the disease-specific programs, moves toward provision of quality-assured basic

laboratory testing through the use of common specimen collection, reporting and diagnostic platforms that can be used across diseases, and disease control programs, and it increases capacity for introducing and using new and more complex technologies.[12]

ROLE OF THE NATIONAL REFERENCE LABORATORY

Globally, national reference laboratories play a central role in the implementation of any disease response and especially the scale-up of diagnostic capacity. In the context of resource-limited settings where capacity may not have existed or needed to be significantly boosted, the importance of this facility is heightened further. National Reference Laboratories are at the pinnacle of diagnostic service provision and play pivotal roles in diagnosis, disease surveillance, and statistical analysis of epidemiologic data. In 2009 the Southern Africa Development Community set out the functions and minimum standards for national reference laboratories that must be achieved and maintained by all its member states. The functions included general diagnostics (specialized testing services especially molecular testing), development and implementation of diagnostic policy, maintaining diagnostic standards, training and skills transfers, servicing and maintenance of equipment, provision of quality management systems, information management, and public health functions. The main public health functions of national reference laboratories that were specified involve coordination of the following: surveillance and epidemic response, training, qualifications and continuing professional education, operational research for health, laboratory health and safety, specimen handling, and transportation.[15]

In Malawi, the Public Health Institute of Malawi (PHIM), under the Malawi Ministry of Health alongside its department of Health Technical Support Services, activated the Public Health Emergency Operations Center to coordinate the national COVID-19 response. Under PHIM, the Public Health Reference Laboratory (PHL) is the coordinating body for the tiered laboratory system that includes national and reference laboratories in the upper tier, central laboratories based in the country's major referral hospitals, and a lower tier of peripheral laboratories in district hospitals and health centers. PHL also coordinates private sector and academic laboratories within the country.

One of the most important roles spearheaded by the PHL was the coordination of multiple partners in the many activities required to successfully capacitate the health system to conduct high-throughput molecular testing and make it as widely available as possible. **Table 1** gives a snapshot of some of the most important activities undertaken and the partners involved. Alongside the Ministry of Health, at least 10 different international organizations and regional partners were involved in supporting the various activities under the thematic areas of equipment, infrastructure, personnel, procurement of reagents and consumables, training, sample transportation, data management, and quality management.

Other key functions of the PHL were decisions on the scale of the diagnostic response, determination and development of guidelines, and validation and approval of which specific tests, reagents, and platforms would be used. This particular issue took on magnified importance for several reasons. The disruptions to global supply chains brought about by lockdowns and international travel bans as well as unprecedented demand for molecular testing supplies and reagents meant that the demand for testing was never going to be matched by the supply. Data from the Association of Supply Chain Management and the American Society for Microbiology showed that worldwide shortages of media, reagents, collection devices, and consumables

Table 1
Summary of some of the activities undertaken to achieve successful roll out of molecular testing services for SARS-CoV-2 in Malawi under 8 thematic areas

Thematic Area	Activities	Partners	Main Outcomes/Highlights
Equipment	Inventory of available platforms	MoH	Updated inventory available at central level Identification of 22 high-throughput platforms across the country for molecular testing facilitated planning, procurement, and distribution of supplies
	Servicing and calibration of equipment	MoH CDC Malawi through UMB	Auxiliary equipment with valid calibration certificates. Securing of service contracts Ensuring availability of calibrators through implementing partners
	Biosafety cabinets	CDC Malawi through UMB	Functional and fully serviced biosafety cabinets in every testing site
	Procurement of Abbott m2000 platform GeneXpert machines Quant Studio 5	CDC Malawi Thermofisher USAID	Scaled up capacity for molecular testing
Infrastructure	Demarcation/partitioning of laboratories for molecular testing	MoH, CDC Malawi through UMB	All sites partitioned to accommodate separate molecular testing.
personnel	Recruitment of additional laboratory	MoH CDC Malawi through UMB	Additional 150 laboratory personnel recruited. Repurposing of UMB staff to support COVID testing. Uninterrupted service for other molecular assays due to adequate personnel

Procurement of reagents and consumables	Determining needs	MoH CDC Malawi through UMB and I-TECH	Constant supply of reagents Sufficient supply of reagents
	Coordination with development partners	MoH	Ensured collaborative effort and maximum resource allocation Monitoring of reagents and distribution to various testing sites
	Supply chain and logistics	UNICEF/WFP Central Medical Stores Trust CHAI, I-TECH	Ensured delivery of reagents, supplies, and PPE amid global supply chain constraints
Training	Training in sample collection and processing	MoH CDC Malawi through I-TECH CDC Zambia WHO, World Bank	Training of laboratory officers in SARS-CoV-2 testing to scale-up testing capacity Supported initial TOT for PCR testing 25,000 health workers trained in sample collection
Sample transportation	Development of a transportation system	UMB/Riders for Health	Successful transportation of 231,850 samples to molecular laboratories. Transportation from ports of entry and hard to reach areas.
Data management	Production of case-based surveillance form	MoH	Standardization of data
	Development of a national dashboard	I-TECH	Stakeholders are able to access data through the dashboard
	connectivity	EGPAF	Majority (85%) of testing sites are connected to the dash board

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Thematic Area	Activities	Partners	Main Outcomes/Highlights
Quality management	Approval of laboratories to perform SARS-CoV-2 PCR testing	MoH-HTSS	15 molecular laboratories and 320 antigen testing sites have been approved
	Validation of different platforms	PHL	Validated 3 molecular platforms and 4 antigen test kits All molecular assays in use validated in county against available platforms
	EQA	PHIM supported by UMB	Ensured accurate result generation using annual EQA with score of 94%

Full names of key partners and organisations are given below the table

Abbreviations: CDC, Centers for Disease Control and Prevention; CHAI, Clinton Health Access Initiative; EGPAF, Elizabeth Glazer Paediatric AIDS Foundation; HTSS, Health Technical Support Services; I-TECH, International Training and Education Center for Health; PHL, Public Health Laboratory; MoH, Ministry of Health; UMB, University of Maryland Baltimore; UNICEF, United Nations Children's Fund; USAID, United States Agency for International Development; WFP, World Food Program; WHO, World Health Organization.

significantly affected day-to-day testing for both COVID-19 and other infectious diseases.[16] These shortages were more acute in resource-limited settings, and many countries had to make do with whatever they could get access to. At the same time, there was a flood of newly developed tests reagents and consumables that were yet to be validated that became available on the market. Sensitivity of molecular tests is greatly affected by proper specimen collection, and a myriad of swabs, specimen collection kits, and viral transportation media also became available and were aggressively marketed. In addition to issuing comprehensive guidelines in sample collection, much work had to be put in to validate the performance of and approve which product could be used by health workers and laboratory staff to ensure the quality of molecular diagnostic results.

POLYMERASE CHAIN REACTION PLATFORMS

Molecular diagnostic (PCR) systems for SARS-CoV-2 provide extremely sensitive, specific, and often quantitative detection of the SARS-CoV-2 RNA. However, they are complex, expensive, and slow to deliver. A single RT-PCR test kit may cost more than 100USD, whereas setting up a diagnostic/processing laboratory requires more than 15,000 USD, whereas the analysis time is 4 to 6 hours, and sample-to-result turn-around time is often more than 24 hours.[17,18] A PCR system includes PCR kit, PCR machine, and PCR software, and all RT-PCR systems are different due to differences in kit chemistry, thermal profile, PCR kinetics, and so forth.[19] An additional issue is the fact that different kits are compatible with different machines, and they have specific versions and software. Scale-up of molecular testing needed to account for all of these differences and circumvent issues related to this. Procurement of testing kits and receipt of donations needed to be done based on an up-to-date inventory of the available systems and their compatibility with different machines. Compatibility of different test kits with instruments along with sensitivities, limits of detection, cycle threshold value cut-offs, and the required consumables are detailed by FIND[20] and the Global Fund.[21]

In Malawi, 4 laboratories were initially optimized to perform RT-qPCR using US-CDC ThermoFisher TaqMan and DaanGene protocols on Applied Biosystem 7500 and Abbott m2000sp/m2000rt instruments.[22] The DaanGene kits were part of a donation of 1.5 million laboratory diagnostic test kits and more than 100 tons of infection prevention and control commodities from the Jack Ma and Alibaba Foundations made in March 2020.[23] An initial 20,000 kits were donated to each member state, and for many African countries this was the most widely available kit. The kit is manufactured by the DaAn Gene Co., Ltd. of Sun Yat-sen University, Guangdong, China and is based on one-step RT-PCR technique. It contains an endogenous internal standard detection system, which was used for monitoring the processes of specimen collection, RNA, and PCR amplification, thereby reducing false-negative results. The kit is compatible with ABI PRISM 7500 SDS and LightCycler480 II instruments.

An important consideration is the maintenance of the cold chain when shipping test kits from the manufacturer as well as when distributing the kits to central and peripheral laboratories. In the face if logistical challenges associated with this, kits that have lyophilized components were favored in procurement processes. Kits such as the TIB Molbiol (Berlin GmbH/Roche Diagnostics) were preferred because the product is dried and is stored at 4°C to 25°C enabling shipping without temperature control. Although some challenges persist with instability of enzymes once reconstituted, advances in development of room-temperature–storable PCR mixes for SARS-CoV-2 detection[24]

offer some promise in this regard and would be a welcome boost to molecular testing in resource-limited settings.

Abbott Platform

The Abbott RealTime HIV-1 Qualitative test (Abbott Diagnostics, Inc., Chicago Illinois, USA) is an RT-PCR–based assay for the qualitative detection of HIV type 1 (HIV-1) nucleic acids from human plasma and dried blood spots. The RealTime HIV-1 Qualitative test is intended to be used as an aid in the diagnosis of HIV-1 infection in pediatric and adult subjects. It is designed to be run on the Abbott RealTime m2000rt amplification system or the fully automated m24 system. The RealTime HIV-1 is included in a Global Fund framework agreement as part of an expanded assay menu—together with HIV early infant diagnosis, mycobacterium tuberculosis (MTB), hepatitis B virus, hepatitis C virus, human papillomavirus, and Chlamydia trachomatis/Neisseria gonorrhoeae—at the same low access price. Abbott offers scale-up planning as well as assistance with scale-up, including training and performance monitoring based on country needs.[25] For this reason, it has become a key component in the global HIV program as well as laboratory systems strengthening programs and thus has a presence in most countries supported by PEPFAR and Global Fund programs.

The FDA-approved Abbott's RealTime SARS-CoV-2 assay is a dual-target RT-PCR assay for the quantitative recognition of RdRp and N genes. It uses an unrelated RNA sequence as an internal control (IC) to validate the PCR and detects the RdRp, N, and IC target sequences via specific fluorescent-labeled probes. Different fluorophores are used for SARS-CoV-2–specific and IC-specific probes to allow simultaneous detection of these targets[26]; this became one of the first tests that was deployed in Malawi and was used in the detection of the first case in the country in March 2020 at the National reference laboratory and College of Medicine and the Malawi Liverpool Wellcome Trust laboratories. By April 2020, additional molecular laboratories were activated to extend testing to additional districts using the Abbott test kit and m2000sp/m2000rt instruments (**Fig. 1**A).[22]

GeneXpert

GeneXpert (Cepheid, Inc., Sunnyvale, CA) is a cartridge-based PCR machine that is used to diagnose TB and detect rifampicin resistance. Following its endorsement of Xpert MTB/RIF by the WHO in 2010, its implementation across the globe has

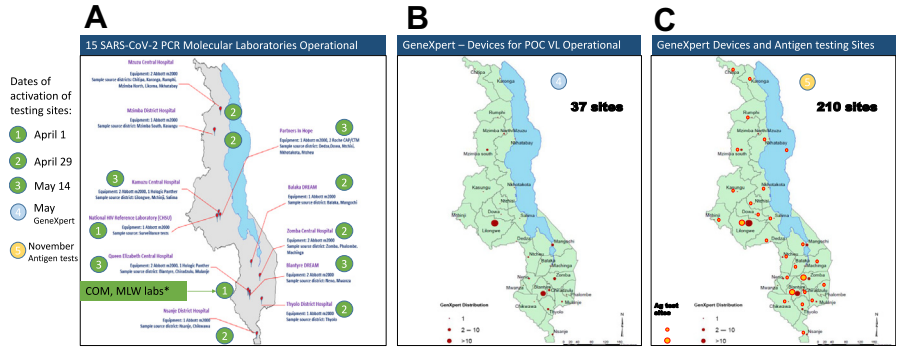

Fig. 1. Map of testing sites for SARS-CoV-2 in Malawi. (*A*) The 15 RT-qPCR sites activated in the first 2 months from April 2020 to May 2020. (*B*) The activation of GeneXpert sites (37) in all districts of the country. (*C*) The rapid antigen testing sites activated in November of 2020 (210 sites).

revolutionized management of TB and has become the bedrock of many national TB control programs.[27] The cartridge-based modular diagnostic tool has enabled the rapid diagnosis of critically ill cases and assessment of suspected patients, allowing for a specific epidemiologic management. The biggest advantage has been the transfer diagnostics to point-of-care scenarios including smaller peripheral laboratories. Public health experts in low- and middle-income countries were quick to see its potential in expanding testing capacity and called for production of cartridges for SARS-CoV-2 detection.[28] The Xpert Xpress SARS-CoV-2 cartridge was granted emergency use authorization in March 2020.

In Malawi there was at least one GeneXpert platform in each of the country's 26 districts, and by the May of 2020 SARS-CoV-2 testing on this platform was available in each district (**Fig. 1**B). By August 2020 there were 37 sites across the country.[29] The GeneXpert platform became the most important tool enabling the establishment of near point-of-care molecular testing capacity at ports of entry where local laboratories had limited capacity but the need for accurate testing with rapid turn-around time was greatest.

IMPACT ON TUBERCULOSIS AND HUMAN IMMUNODEFICIENCY VIRUS CONTROL PROGRAMS

The negative impact of COVID-19 on health care systems in general as well as on pathogen control programs was certainly expected and anticipated.[30] The lockdowns and health facility closures seen early in the pandemic were especially damaging to mass vaccination campaigns for measles, polio, and meningitis and left millions of children at increased risk.[31] The shift in focus resulted in massive redeployment of human and financial resources, delays, and disruptions in supply chains of essential medicines and equipment. The most direct impact of the scale-up in molecular testing for SARS-CoV-2 was in the shifting of testing platforms and skilled personnel from HIV and TB control programs.[32] This process had to be managed in a circumspect manner to mitigate any negative impacts, and policy makers and planners were acutely aware of this. In many facilities, machines were shared and rotated between testing for HIV/TB and testing for SARS-CoV-2, and this situation continues today. Some countries' TB programs saw up to a 70% reduction in new TB case detection[31] but this was mainly attributed to factors such as decreased patient flows.[33] For HIV programs despite some countries experiencing decreases of greater than 50% in HIV testing and greater than 10% increase in deaths from opportunistic infections,[34] in PEPFAR-supported countries there was only a 7% drop in provision of viral load testing services.[32] In several countries many HIV testing services experienced minimal disruption, and viral load testing coverage levels were restored to prepandemic levels or better due to swift measures taken by health officials. In Malawi, routine viral load testing was suspended from March to June of 2020 but quickly saw rebounds to prepandemic levels once the suspension was lifted. Some specific and impactful government measures included providing guidance on continuing essential services, increasing the number of viral load specimen pick-ups at testing facilities, expanding collection of dried blood spot specimens (which can be stored and transported without refrigeration) relative to plasma specimens, and integrating viral load testing with antiretroviral therapy distribution[32] and implementation of remote viral load supervision using mobile phones.[35]

TRANSITION TO ANTIGEN TESTS AND USE CASES FOR MOLECULAR TESTS

The development of and transition to rapid antigen tests provided relief to strained central and peripheral testing sites relying on RT-qPCR and enabled significant

decentralization and scale-up of testing. The public health impact was massive, as most of the individuals suspecting COVID-19 infection go first to the local clinics where RT-qPCR was most often not available. In Malawi the use of rapid antigen testing resulted in an increase of testing sites from 37 (with conventional PCR and GeneXpert) to 210 across the country (Fig. 1C). Numerous studies have been conducted on the performance of these tests relative to PCR-based tests and have consistently found reductions in sensitivity particularly in asymptomatic subjects.[36–38] This is considered an acceptable trade-off for the high number of tests being conducted and for the fact that patients who are the most infectious are more likely to test positive. The huge reduction in turn-around time and the ability to conduct more frequent and repeated test use are also seen a compensating for the loss in sensitivity.[38,39]

In spite of all the foregoing, several use cases remain for PCR-based testing. Most countries around the world have a requirement for an RT-qPCR negative result both for entry and exit, and airlines will not allow passengers to board without it.[40] Many countries are loosening guidelines for isolation in an attempt to reduce the amount of economic disruption of COVID infections[41,42] and do not require a negative PCR test for discharge, opting rather for a negative antigen test. In Malawi, the guidelines do not rely on PCR-based or antigen tests for discharge[43] but require a minimum of 10 days in isolation, 3 of which have to be symptom free; this is mainly because it is known that some individuals may continue to test positive for months[44] and providing a second antigen test may prove difficult even with the current availability of antigen testing.

Another use of PCR testing is in environmental monitoring for SARS-CoV-2. Because of extended shedding and excretion of SARS-CoV-2 RNA in fecal matter, water-based epidemiology is now recognized as a potentially important means of surveillance of SARS-CoV-2 transmission and real-time trend monitoring.[45] The methodologies used are based on RT-qPCR analysis of sewage or waste water.[46] This method of surveillance can predict surges in cases with a lead time of up to 2 weeks, and in densely populated urban areas in developing countries this approach could be superior to clinical surveillance for real-time monitoring of disease trends.[45] However, much methodological development still needs to take place in this area before it can be deployed on a routine basis especially in resource limited settings. Unlike clinical samples, detection of viruses in environmental samples is challenging due to the low-concentration virus present, and this makes it necessary to concentrate the sample, and the presence of fecal and suspended solids and chemicals induced by domestic usage, urban and rural runoffs, and industrial activities makes amplification difficult.[46,47]

APPROACHES TO COST REDUCTION

Several approaches have been considered in an effort to reduce costs of molecular testing and thereby widen access to testing in resource-limited settings. Pooling of samples, direct-to-PCR testing procedures, usage of simpler sampling methods such as saliva, and technologies such as isothermal PCR reactions and colorimetric PCR-based viral detection methodologies have all been proposed.[48–51] The last 2 approaches are promising and are reviewed elsewhere in this edition. The former 2 however seem to offer rapid and immediate relief on already stretched resources.

Pooled Testing

Pooling involves pipetting equal amounts of multiple samples into one tube, enabling one to screen multiple patients at a go in batches that can then be retested to identify

positive samples within each batch. This sort of sample pooling strategy has routinely been used for detection of the HIV and hepatitis B and C viruses in blood bank donor screening in many countries and dating back many years.[52] By some estimates using a pooling strategy for SARS-CoV-2 detection can reduce cost by 69% and requires 10-fold fewer tests.[53] Pools of up to 32 samples can successfully be used with a 10% false-negative rate.[54] However, a balance must be struck between increasing the group size and retaining test sensitivity, as sample dilution increases the likelihood of false-negative test results for individuals with low viral load at the time of the testing.[55] Similarly, minimizing the number of tests to reduce costs must be balanced against minimizing the time that testing takes, as the process is quite labor intensive.[56] Realistically, smaller batches of 10 or fewer give an acceptable false-negative rate samples, and high intra- and interassay variability is observed especially where low viral load samples are present.[53,57] In addition, the pooling is only cost-effective when prevalence is low[57,58] and for screening natural groupings with correlated risks of infection amenable to repeated mass testing such as workplaces, prisons, schools, and other institutions. None the less, this strategy has been used with varying degrees of success in Ghana,[56] Uganda,[59] Rwanda, and South Africa.[58]

Simplified Extraction and Straight to Polymerase Chain Reaction Methods

In currently used RT-qPCR methods RNA extraction constitutes a major bottleneck that requires a significant quantity of consumable plastics and chemical reagents to complete.[60] Few laboratories' resource-limited settings have automated extraction robots, and RNA extraction kits are among those reagents affected by increased demand and global supply chain challenges. One solution is to bypass the RNA extraction step, which would result in reduction of analysis time, savings in reagents and consumables, reductions in waste, and possibly expand the number of nonspecialized laboratories able to perform COVID-19 diagnosis.[61–63] Studies have shown good sensitivity with extraction-free PCR assays, especially for high viral loads[62] and others have shown that the extraction step can be bypassed if samples are stored in universal transport medium or molecular grade water but not when stored in saline or Hanks medium.[61] One study conducted in Malawi using a direct-to-PCR methodology was able to achieve significant savings in cost and processing time by using a 30-second mechanical homogenization step versus an hour-long reagent heavy extraction procedure.[63] This approach is particularly promising and has potential to be scaled up to all molecular laboratories.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Significant challenges have had to be surmounted in the deployment of molecular testing for SARS-CoV-2 in resource-limited setting but it has played an essential role in the global response to COVID-19. Continued progress in strengthening of laboratory systems, integration, and development of increased technical and human capacity will ensure that gains made in this area are not lost and will safeguard the ability of health care systems and medical laboratory scientists to be better prepared and equipped for future pandemics. There is also a need to intensify research into development of platforms, and more cost-saving methodologies that are better suited for lower- and middle-income countries are fully harnessed to effectively address gaps and challenges that remain. In particular, field trials and implementation research coupled with robust qualitative studies that can lead to scale-up of some of these approaches are emphasized. There is also a role for increased partnerships and technology transfer to enable building of local manufacturing capacity to allow more countries

to develop their own capacity to supply their health sectors with much needed reagents and supplies for molecular diagnostics. Some encouraging examples of this have been illustrated in countries such as South Africa,[64] Senegal, and Brazil where agencies such as FIND and UNITAID have partnered with local forms to achieve this in antigen testing.[65] Similar endeavors related to molecular testing would be a welcome development.

CLINICS CARE POINTS

- Molecular testing for SARS-CoV-2 in many settings is dependent on GeneXpert and Abbott platforms.
- Scale up of testing has pargely been well handled to minimise the impact on HIV and TB control programs.
- Introduction of rapid antibody testing has enabled increases in testing capacity and reach and it has simutlaneously releived pressure on infrastructure, equipment and personnel.

DISCLOSURE

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REFERENCES

1. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579(7798):270–3.
2. Gorbalenya AE, Baker SC, Baric RS, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5(4):536–44.
3. Chan JF-W, Yip CC-Y, To KK-W, et al. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse Transcription-PCR assay validated in Vitro and with clinical specimens. J Clin Microbiol 2020;58(5). https://doi.org/10.1128/JCM.00310-20.
4. Cao W, Liu X, Bai T, et al. High-dose intravenous immunoglobulin as a therapeutic option for deteriorating patients with Coronavirus Disease 2019. Open Forum Infect Dis 2020;48:1–6.
5. World Health Organization. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases, *WHO*. Interim Guidance 2020.
6. World health Organization. Laboratory testing strategy recommendations for COVID-19. World heal organ. 2020. Available at: https://apps.who.int/iris/bitstream/handle/10665/331509/WHO-COVID-19-lab_testing-2020.1-eng.pdf.
7. Vitoria M, Granich R, Gilks CF, et al. The global fight against HIV/AIDS, tuberculosis, and Malaria: current Status and future Perspectives. Am J Clin Pathol 2009;131(6):844–8.
8. Birx D, de Souza M, Nkengasong JN. Laboratory challenges in the scaling up of HIV, TB, and Malaria programs: the Interaction of health and laboratory systems, clinical research, and service delivery. Am J Clin Pathol 2009;131(6):849–51.
9. Petti CA, Polage CR, Quinn TC, et al. Laboratory medicine in Africa: a barrier to effective health care. Clin Infect Dis 2006;42(3):377–82.

10. Ravishankar N, Gubbins P, Cooley RJ, et al. Financing of global health: tracking development assistance for health from 1990 to 2007. Lancet 2009;373(9681): 2113–24.
11. Yu D, Souteyrand Y, Banda MA, et al. Investment in HIV/AIDS programs: Does it help strengthen health systems in developing countries? Glob Health 2008;4(1): 8. https://doi.org/10.1186/1744-8603-4-8.
12. Parsons LM, Somoskovi A, Lee E, et al. Global health: integrating national laboratory health systems and services in resource-limited settings. Afr J Lab Med 2011;1(1):1–5.
13. UNAIDS. Fast-Track Ending the AIDS Pandemic by 2030.; 2014.
14. Steingart KR, Schiller I, Horne DJ, et al. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev 2014;1. https://doi.org/10.1002/14651858.CD009593.pub3.
15. SADC. Functions and Minimum Standards for National Reference Laboratories in the SADC Region. Published online 2009.
16. American Society for Microbiology. Supply shortages impacting COVID-19 and non-COVID testing Title. 2021. Available at: https://asm.org/Articles/2020/September/Clinical-Microbiology-Supply-Shortage-Collecti-1. Accessed January 10, 2022.
17. Ramdas K, Darzi A, Jain S. 'Test, re-test, re-test': using inaccurate tests to greatly increase the accuracy of COVID-19 testing. Nat Med 2020;26(6):810–1.
18. Sheridan C. Fast, portable tests come online to curb coronavirus pandemic. Nat Biotechnol 2020;38(5):515–8.
19. Das P, Mondal S, Pal S, et al. COVID diagnostics by molecular methods : a systematic review of nucleic acid based testing systems. Indian J Med Microbiol 2020;39(3):271–8.
20. Find. Find EVALUATION UPDATE: SARS-COV-2 molecular diagnostics. 2022. Available at: https://www.finddx.org/covid-19/sarscov2-eval-molecular/.
21. Global Fund. List of SARS-CoV-2 Diagnostic test kits and equipments eligible for procurement according to Board Decision on Additional Support for Country Responses to COVID-19 (GF/B42/EDP11) SARS-CoV-2 Nucleic Acid Amplification Technologies (only sequencing e 2022;19:1–74.
22. Ministry of Health of Malawi. MALAWI SARS-COV-2 DIAGNOSIS NATIONAL LABORATORY GUIDELINE, Ministry of Health Malawi, Manual, 1, 2020.
23. Africa CDC. Jack Ma and Alibaba Foundations donate COVID-19 medical equipment to African union member states. 2020. Available at: https://africacdc.org/news-item/jack-ma-and-alibaba-foundations-donate-covid-19-medical-equipment-to-african-union-member-states/. Accessed January 15, 2022.
24. Xu J, Wang J, Zhong Z, Su X, Yang K, Chen Z. Room-temperature-storable PCR mixes for SARS-CoV-2 detection. *Clinical Biochemistry* (84) 73-78;2020.
25. Mazzola LT, Perez-Casas C. HIV/AIDS Diagnostics Technology Landscape. *UNITAID*. 5th edition. Report; 2015.
26. Abbott. Abbott RealTime SARS-C0V-2 assay. 2021. Available at: https://www.molecular.abbott/int/en/products/infectious-disease/RealTime-SARS-CoV-2-Assay. Accessed February 22, 2022.
27. Brown S, Leavy JE, Jancey J. Implementation of GeneXpert for TB testing in low- and middle-income countries: a systematic review. Glob Heal Sci Pract 2021; 9(3):698. https://doi.org/10.9745/GHSP-D-21-00121. LP - 710.
28. Oladimeji O, Atiba BP, Adeyinka DA. Leveraging polymerase chain reaction technique (GeneXpert) to upscaling testing capacity for SARS-CoV-2 (COVID-19) in

Nigeria: a game changer. Pan Afr Med J 2020;35(Supp 2):8–9. https://doi.org/10.11604/pamj.2020.35.2.22693.
29. Public Health Institute of Malawi. COVID-19 Daily situation report - 30th. 2020;(August):1-8.
30. Togun T, Kampmann B, Stoker NG, et al. Anticipating the impact of the COVID-19 pandemic on TB patients and TB control programmes. Ann Clin Microbiol Antimicrob 2020;19(1):21.
31. Roberts L. How COVID hurt the fight against other dangerous diseases. Nature 2021;592(7855):502–4.
32. Lecher SL, Naluguza M, Mwangi C, et al. Notes from the field: impact of the COVID-19 response on scale-up of HIV viral load testing — PEPFAR-supported countries, January–June 2020. MMWR Morb Mortal Wkly Rep 2021;70(21):794–5.
33. Chopra KK, Matta S, Arora VK. Impact of second wave of Covid-19 on tuberculosis control. Indian J Tuberc 2021;68(3):311–2.
34. Medina N, Alastruey-Izquierdo A, Bonilla O, et al. Impact of the COVID-19 pandemic on HIV care in Guatemala. Int J Infect Dis IJID Off Publ Int Soc Infect Dis 2021;108:422–7.
35. Masiano S, Dunga S, Tembo T, et al. Implementing remote supervision to improve HIV service delivery in rural Malawi. J Glob Heal Rep 2020;(VI):1–11.
36. Muthamia E, Mungai S, Mungai M, et al. Assessment of performance and implementation characteristics of rapid point of care sars-cov-2 antigen testing in kenya. medRxiv 2021. https://doi.org/10.1101/2021.06.03.21258290.
37. Amer RM, Samir M, Gaber OA, et al. Diagnostic performance of rapid antigen test for COVID-19 and the effect of viral load, sampling time, subject's clinical and laboratory parameters on test accuracy. J Infect Public Health 2021;14(10):1446–53.
38. American Society for Microbiology. HomeArticlesReal-world performance of COVID-19 rapid antigen tests real-world performance of COVID-19 rapid antigen tests. 2021. Available at: https://asm.org/Articles/2021/December/Real-World-Performance-of-COVID-19-Rapid-Antigen-T. Accessed January 21, 1022.
39. Kahanec M, Lafférs L, Schmidpeter B. The impact of repeated mass antigen testing for COVID-19 on the prevalence of the disease. J Popul Econ 2021;34(4):1105–40.
40. Travelbans.org. TRAVEL BAN, NEW RULES AND UNEXPECTED FLYING RESTRICTIONS: WHAT will TOURISM be like after CORONAVIRUS?. 2022. Available at: https://travelbans.org/. Accessed February 12, 2022.
41. Limb M. Covid-19: Self-isolation after infection cut to seven days in England. BMJ 2021;375:n3137. https://doi.org/10.1136/bmj.n3137.
42. CDC. CDC Updates and Shortens Recommended isolation and Quarantine Period for general population. 2021. Available at: https://www.cdc.gov/media/releases/2021/s1227-isolation-quarantine-guidance.html. Accessed February 1, 2022.
43. Ministry of Health of Malawi. Covid - 19 Case Management Manual, *Ministry of Health*, Manual; september 2020.
44. Henderson DK, Weber DJ, Babcock H, et al. The perplexing problem of persistently PCR-positive personnel. Infect Control Hosp Epidemiol 2021;42(2):203–4.
45. Kumar M, Joshi M, Kumar A, et al. Unravelling the early warning capability of wastewater surveillance for COVID-19 : a temporal study on SARS-CoV-2 RNA detection and need for the escalation. Environ Res 2021;196(December 2020):110946. https://doi.org/10.1016/j.envres.2021.110946.

46. Hamouda M, Mustafa F, Maraqa M, et al. Science of the Total Environment Wastewater surveillance for SARS-CoV-2 : lessons learnt from recent studies to de fi ne future applications. Sci Total Environ 2021;759:143493.
47. Haramoto E, Kitajima M, Hata A, et al. A review on recent progress in the detection methods and prevalence of human enteric viruses in water. Water Res 2018; 135:168–86.
48. Bokelmann L, Nickel O, Maricic T, et al. Point-of-care bulk testing for SARS-CoV-2 by combining hybridization capture with improved colorimetric LAMP. Nat Commun 2021;12(1):1–8.
49. Garcia-Venzor A, Rueda-Zarazua B, Marquez-Garcia E, et al. SARS-CoV-2 direct detection without RNA isolation with Loop-Mediated isothermal amplification (LAMP) and CRISPR-Cas12. Front Med 2021;8(February):1–9. https://doi.org/10.3389/fmed.2021.627679.
50. Morehouse ZP, Proctor CM, Ryan GL, et al. A novel two-step, direct-to-PCR method for virus detection off swabs using human coronavirus 229E. Virol J 2020;17(1):129.
51. Pijuan-galito S, Tarantini FS, Tomlin H, et al. Saliva for COVID-19 testing : Simple but Useless or an Undervalued resource 2021;1(July):1–6.
52. Van TT, Miller J, Warshauer DM, et al. Pooling nasopharyngeal/throat swab specimens to increase testing capacity for influenza viruses by PCR. J Clin Microbiol 2012;50(3):891–6.
53. Mahmoud SA, Ibrahim E, Thakre B, et al. Evaluation of pooling of samples for testing SARS-CoV- 2 for mass screening of COVID-. *BMC Infect Dis* Published Online 2021;1–9.
54. Yelin I, Aharony N, Tamar ES, et al. Evaluation of COVID-19 RT-qPCR test in Multi sample Pools. Clin Infect Dis 2020;71(16):2073–8.
55. Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, et al. False-negative results of initial RT-PCR assays for COVID-19: a systematic review. PLoS One 2020;15(12):e0242958.
56. Asante IA, Adusei-poku M, Bonney HK, et al. Molecular diagnosis for the novel coronavirus SARS-CoV-2 : lessons learnt from the Ghana experience. Ghana Med J 2020;54(4).
57. Nianogo RA, Emeruwa IO, Gounder P, et al. Optimal uses of pooled testing for COVID-19 incorporating imperfect test performance and pool dilution effect: an application to congregate settings in Los Angeles County. J Med Virol 2021; 93(9):5396–404.
58. Mutesa L, Ndishimye P, Butera Y, et al. A pooled testing strategy for identifying SARS-CoV-2 at low prevalence. Nature 2021;589(January). https://doi.org/10.1038/s41586-020-2885-5.
59. Bogere N, Bongomin F, Katende A, et al. Performance and cost-effectiveness of a pooled testing strategy for SARS-CoV-2 using real-time polymerase chain reaction in Uganda. Int J Infect Dis 2021;113:355–8.
60. Tang Y, Schmitz JE, Persing DH, et al. Laboratory diagnosis of COVID-19: current issues and challenges. Am J Clin Microbiol 2020;(May).
61. Merindol N, Pépin G, Marchand C, et al. SARS-CoV-2 detection by direct rRT-PCR without RNA extraction. J Clin Virol 2020;128(May):104423.
62. Visseaux B, Collin G, Houhou-fidouh N, et al. Evaluation of three extraction-free SARS-CoV-2 RT-PCR assays : a feasible alternative approach with low technical requirements. J Virol Methods 2021;291(November 2020):8–11.
63. Morehouse ZP, Samikwa L, Proctor CM, et al. Validation of a direct-to-PCR COVID-19 detection protocol utilizing mechanical homogenization: a model for

reducing resources needed for accurate testing. PLoS One 2021;16(8 August):1–9.
64. South African Government. SAHPRA approves affordable, locally developed Coronavirus COVID-19 antigen test. 2021. Available at: https://www.gov.za/speeches/sahpra-approves-affordable-locally-developed-coronavirus-covid-19-antigen-test-8-dec-2021.
65. Reuters. Global agencies sign tech transfer deals to boost COVID testing in Africa, Latam. 2021. Available at: https://www.reuters.com/business/healthcare-pharmaceuticals/emb-tech-transfers-boost-antigen-testing-africa-latin-america-2021-07-15/. Accessed December 12, 2021.