AfriDx Policy Brief

the potential role of isothermal nucleic acid tests in addressing COVID-19 diagnostics challenges in Ghana

EDCTP

AfriDx Diagnostic Project

Executive Summary

Nucleic Acid Testing (NAT) via reverse-transcription quantitative polymerase chain reaction (RT-qPCR, also known as PCR testing) is the gold standard for early-stage detection of COVID-19. However, antigen rapid diagnostic tests have taken over in many LMICs due to their affordability, speed and ability to be used in the home or at the point of care without laboratory infrastructure.

AfriDx explored the potential for a NAT which is closer in sensitivity to RT-qPCR but can be used in a resource limited laboratory or health centre in Ghana. This was based on reverse-transcription loop-mediated amplification (RT-LAMP) which requires comparatively simple equipment and can be freeze-dried for long-term storage.

Through collaboration with AfriDx partners and a survey of the diagnostics community, we identified that RT-LAMP tests have potential to provide more affordable, easier to use and accessible NATs within both clinical and non-clinical settings. However, adoption is currently non-existent in Ghana for any disease target. This is partly due to the early stage of development of many tests, with limited options on the market worldwide and none with regulatory approval in Ghana. In particular, the lack of isothermal NATs with WHO approval through their Emergency Use Licence or prequalification (PQ) programmes is a major barrier. There are also concerns about higher false positive rates and lower sensitivity in RT-LAMP compared to RT-qPCR. Moreover, successful integration of new workflows and data collection modes within the health system was a concern raised by surveyed clinicians. This illustrates that a systems approach is needed to diagnostics innovation which includes optimising the technology alongside operational research, stakeholder engagement and support at all levels of the health system.

The following three policy recommendations could ensure that the potential benefits of access to RT-LAMP tests for SARS-CoV-2 and other diseases are better understood and realised:

1. A national pathway for translating research on NATs out of academic research labs through to clinical trials phase, alongside guidance for preparation for regulation by the Ghana FDA would be beneficial.

2. Integration of isothermal NATs with existing testing and reporting within the health system is essential. This is particularly critical where use of point of care or near point of care NATS introduces a new tier of data collection at clinics and non-clinical sites. Any roadmap for adoption needs to consider digital infrastructures alongside the NAT technology itself.

3. The Ghana Ministry of Health should play a key role in informing diagnostic innovators and manufacturers of national diagnostics demand, which will be critical to ensure there is a business case to adopt new innovations.

Types of COVID-19 Diagnostics

Diagnostic testing of symptomatic, asymptomatic and recovered COVID-19 patients is critical to slowing down the spread of the virus and new viral variants.

In the first week of COVID-19 infection, nucleic acid testing (NAT) is the most reliable method to identify the causative agent (viral RNA), but these tests are expensive and usually require well-resourced laboratories. The RNA diagnostic involves sample collection followed by RNA extraction, specific amplification of the viral RNA after reverse transcribing it to DNA and finally detection of this DNA. Detection is usually performed by

measuring changes in fluorescence intensity or colour of a dye although multiple readout technologies exist, including optical and electrochemical sensing.

Most of the COVID-19 testing procedures have been based on reverse-transcription quantitative polymerase chain reaction (RT-qPCR) but availability in LMICs is limited because it is not suitable for deployment outside a sophisticated laboratory environment with necessary equipment and skilled technicians. It is also time-consuming (i.e. one to two days from sample collection, transport to central laboratories, preparation to analysis and result).

For this reason, there has been significant market shaping activity since the start of the AfriDx Consortium's work that has shifted demand towards antigen rapid tests (RDTs) rather than nucleic acid testing. This is partly a result of large investments by the ACT-Accelerator led by FIND and Unitaid. ACT-Accelerator estimated that 500 million COVID-19 diagnostic tests would be needed in LMICs during 2021, of which 75% would be deployed in decentralised settings such as primary healthcare and community care[1]. Antigen RDTs were thus targeted for investment in scale up and the ACT-Accelerator procured over 156 million COVID-19 tests for LICs and LMICs, achieving price reductions bringing the cost per test down to US$1-2[2].

However, nucleic acid testing (NAT or NAAT) via quantitative real-time PCR continues to be the most widely used in most African countries and constitutes WHO's recommended reference standard. For example, in South Africa only 28% of tests in the week to 9 April 2022 were antigen RDTs9[3]. On the other hand, in Zimbabwe rapid testing has overtaken PCR testing, increasing by 88% between April and December 2021[4]. As of Oct 2021, Ag-RDTs made up 44% of the total volume of demand for diagnostics through UNICEF, whereas rRT-PCR tests for manual use and rRT-PCR tests for automated use constituted 40% and 16 %, respectively[5].

Nucleic Acid Testing Capacity in Ghana

In Ghana, testing was initially performed only at two sites: Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) and Noguchi Memorial Institute for Medical Research (NMIMR) with a combined capacity to process 4500 tests/day but there are now 40 accredited SARS-CoV-2 diagnostics labs in the country according to the Ghanaian Government[6]. Testing peaked at 0.2 daily tests per thousand people and as of May 2022 was at 0.01 tests per thousand people and a positive rate of <2%[7]. While SARS-CoV-2 is currently at low levels in Ghana, variants with higher transmission rates are likely and this capacity is also indicative of Ghana's current ability to address other disease outbreaks or diagnostic requirements for endemic disease.

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[1] https://www.finddx.org/covid-19/eoi-covid19-ag-rdt/
[2] WHO, The ACT-Accelerator: Two Years of Impact.
https://www.who.int/publications/m/item/the-act-accelerator--two-years-of-impact
[3] https://www.nicd.ac.za/wp-content/uploads/2022/04/COVID-19-Testing-Summary-Week-14-2022.pdf
[4] https://www.afro.who.int/news/africa-track-control-covid-19-pandemic-2022
[5]
https://www.unicef.org/supply/media/9776/file/COVID-19-In-Vitro-Diagnostics-Supply-Assessment-and-Outlook-Update-October-2021.pdf
[6] https://www.ghs.gov.gh/covid19/accredited_labs.php
[7] Hannah Ritchie, Edouard Mathieu, Lucas Rodés-Guirao, Cameron Appel, Charlie Giattino, Esteban Ortiz-Ospina, Joe Hasell, Bobbie Macdonald, Diana Beltekian and Max Roser (2020) - "Coronavirus Pandemic (COVID-19)". Published online at OurWorldInData.org. Retrieved from: 'https://ourworldindata.org/coronavirus' [Online Resource]

Affordability and Supply of NATs

While RT-qPCR is available in Ghana, the costs to the health care system are prohibitive for scaling up to higher testing rates, in addition to the availability of appropriate facilities. Currently, the price of RT-qPCR tests for manual use through UNICEF range from 2.80 USD to11.67 USD[8], plus extraction kits ranging from 1.05 USD to 3.57 USD per extraction. Automated RT-qPCR kits which include extraction are more expensive, starting at 10 USD per assay which UNICEF is setting as the target price for all such kits. It is clear that RT-LAMP tests need to fall significantly below this price to be competitive given the changes in training, workflow and infrastructure required to adopt them and this was reflected in responses to the AfriDx survey of key opinion leaders.

Box: Examples of Isothermal NATs

RT-LAMP

This technique uses a reverse transcriptase and a DNA polymerase as per RT-qPCR but there is no requirement to heat the sample to 95 C to denature the DNA as the enzyme displaces the DNA strands to access a single strand template itself. LAMP therefore works at a single temperature and is known as “isothermal”. Unlike RT-qPCR which requires a fluorescent readout, LAMP has a range of possible detection technologies and formats including colorimetric detection by eye and can be adapted for high-throughput central processing in addition to POC testing.

RPA/Cas systems

These techniques amplify RNA isothermally using Recombinase polymerase amplification (RPA) and then use an RNA-guided enzyme (Cas12 or Cas13) to increase specificity of the reaction. Cas12/13 detects the target sequence and then cleaves single-stranded DNA linked to fluorophores which activate on cleavage producing detectable fluorescence. While this mode requires fluorescent detection systems, it can be paired with a lateral flow assay for visual detection.

The Potential of Isothermal NATs

Ag-RDTs are less sensitive than NATs so a NAT that combines the convenience of antigen RDT with a sensitivity closer to RT-qPCR could have broad potential for more affordable and distributed diagnostics. Isothermal amplification techniques (see Box) are tests that operate at a single temperature as opposed to the rapidly cycling temperatures required by PCR. These methods are typically much less prone to inhibition than RT-qPCR and therefore have been shown in lab and initial clinical studies to allow direct amplification from swabs or saliva and some other samples with minimal processing beyond heating the sample in a buffer. This means that isothermal NATs are amenable to point of care testing formats and many current designs incorporate this with easy-to-use cartridges and a reader or lateral flow systems. As isothermal techniques typically involve shorter amplification steps than RT-qPCR at < 60 mins, combined with the

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8 https://www.unicef.org/supply/media/9776/file/COVID-19-In-Vitro-Diagnostics-Supply-Assessment-and-Outlook-Update-October-2021.pdf

shorter sample preparation steps this offers the prospect of significantly shorter workflows and higher throughput testing.

However, these assays are extremely sensitive and produce very large amounts of amplified DNA for even one starting molecule, making them prone to contamination. Particularly due to the mechanism of LAMP which produces a length of DNA containing many copies of the original target sequence, it is absolutely critical for lab-based tests that appropriate controls are put in place to avoid contamination and thus false positives. POC devices are typically sealed to avoid this problem occurring but are not amenable for centralised, high-volume testing so both routes should be explored with this challenge in mind.

Supply of Isothermal NAATs

There is a supply challenge for isothermal NATs in Ghana for multiple reasons. Firstly, there are far fewer companies worldwide with RT-LAMP or other isothermal amplification kits on the market and none that we could find which were regulated by the Ghana FDA. Clinical use of RT-LAMP tests that are available on the open market is to our knowledge undocumented in Africa, although there may be some laboratory developed tests in use.

Some RT-LAMP tests have been validatedby the US FDA and other stringent regulatory bodies but their design is typically complex for self-testing at home rather than in a clinic or lab, which is not appropriate to the Ghaianan context. Most antigen RDTs and RT-qPCR kits that are approved in Africa have been prequalified or sold under Emergency Use License from the World Health Organisation. These are mainly manufactured in the US, Europe, China or South Korea. Diagnostic kits that are supplied by UNICEF[8] or the African Medical Supplies Platform[9], which are working directly with governments and donors, are likely to see greatest adoption in African countries including Ghana.

It is therefore imperative for wide adoption that RT-LAMP kits are manufactured at low-cost and transition through both the national and WHO regulatory systems.

Key Challenges and Opportunities for Adoption of Isothermal NATs in LMICs

AfriDx conducted a survey with key opinion leaders in the diagnostics and clinical fields as well as drawing together our own learning through the consortium to identify the top challenges and opportunities for adoption of isothermal NATs in LMICs.

Key Opportunities for Adoption of Isothermal NATs in LMICs

1. Users of RT-qPCR in Ghana have found it to be complex, requiring significant repetitive manual preparation steps and producing results after several hours. The speed and simplicity of RT-LAMP was preferred by AfriDx partners.
2. The lower cost of RT-LAMP was an attractive feature compared to RT-qPCR and while there are still issues of affordability, it has potential to expand access to NAT technology.
3. The PATHPOD device and cartridge system deployed by AfriDx expanded the potential sites of testing to smaller health clinics and potentially airports or other non-clinical settings.

[9] https://amsp.africa/

Key Challenges for Adoption of Isothermal NATs in LMICs:

1. Isothermal NATs have not been approved through the regulatory system for many infectious diseases and are still in the process of development and approval for SARS-CoV-2. This means that they are not on WHO-approved lists which form the basis of most diagnostics policies in LMICs and particularly in Africa. More data is needed around the clinical and business value proposition to bring manufacturers, investors and other stakeholders to the table to accelerate translation of the technology

2. Clinicians are concerned about the risks of false positives and negatives. Laboratory infrastructure and test design requires careful consideration as LAMP is typically much more prone to amplicon contamination and non-specific amplification in the absence of a pathogen.

3. Clinicians and procurement departments noted a concern about the integration and data flow using rapid NATs in a clinical environment as well as quality control and training of non-specialist local staff with the range of non-standardized protocols and platforms.

Key Policy Recommendations

Taking into account the AfriDx experience with researching RT-LAMP assays for manufacture and deployment in Ghana, the following three policy recommendations were formulated. These steps by the Ghanaian government and international health bodies like the WHO could ensure that the potential benefits of access to RT-LAMP tests for SARS-CoV-2 and other diseases are better understood and realised:

1. A national pathway for translating research on NATs through to clinical trials and towards future regulation by the Ghana FDA would be beneficial. AfriDx was not the only clinical trial of an RT-LAMP diagnostic taking place concurrently in Ghana. Coordination of access to samples, standardised control materials and clinical trial training could improve the scientific outputs of trials and the pathway to regulatory approval.

2. Integration of isothermal NATs with existing testing and reporting within the health system is essential and introduces a new tier of data collection at clinics and non-clinical sites. Any roadmap for adoption needs to consider digital infrastructures alongside the NAT technology itself.

3. The Ghana Ministry of Health should play a key role in informing diagnostic innovators and manufacturers of national diagnostics demand, which will be critical to ensure there is a business case to adopt new innovations.

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Funder: EDCTP Contract N^{0} RIA2020EF-2918: Rapid diagnostics for COVID-19: manufacturable in Africa to increase affordability, improve epidemic preparedness and strengthen local resilience