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Perspective Piece

Molecular Detection of Neglected Tropical Diseases: The Case for Automated Near–Point-of-Care Diagnosis of Leishmaniasis

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Abstract. Neglected tropical diseases affect those in poorer nations disproportionately across the globe. One example of these, leishmaniasis, is a debilitating and potentially fatal parasitic infection. Molecular detection of this disease can provide accurate and fast diagnosis, and with near point-of-care technologies, detection can be provided in many health-care settings. Traditionally, the perceived limitations to such detection methods have hindered their provision to resource-limited nations, but new technologies and techniques are helping to overcome these perceptions. The current pandemic offers an opportunity to maintain and develop further advances, ensuring molecular diagnostics are accessible to all.

The 20 neglected tropical diseases (NTDs) place huge health, social, and economic burdens on 1 billion people globally. The availability of effective, standardized, and affordable diagnostics may help ameliorate morbidity, and lower intervention program costs and achieve WHO elimination targets.[1] Of the 19 infectious NTDs, leishmaniasis—both the visceral and cutaneous forms—are associated with an estimated 50,000 to 90,000 new cases of visceral leishmaniasis (VL) and 600,000 to 1 million new cases of cutaneous leishmaniasis each year.[2] Early diagnosis was attributed to the success of the 2005 intervention program, targeted to eliminate VL in India, Nepal, and Bangladesh. This program reduced VL cases in these regions from 2,220 per 10,000 inhabitants per year to 254 per 10,000 inhabitants per year between 2003 and 2017.[3] The development and approval of nucleic acid-based tests to overcome the limitations of the current antigen-based testing has been encouraged to achieve elimination targets in these regions.

Concurrently, major advances in the detection of leishmaniasis and other infectious NTDs have occurred during the past few decades. However, these sensitive and specific molecular methods have often been deemed inappropriate for the geographical regions that need them most.[4–7] Real-time polymerase chain reaction (PCR), and the nucleic acid extraction preceding it, is being performed increasingly by automated platforms in the laboratory in many parts of the globe. Such platforms aim to free researchers and technicians from manual processes and increase accuracy, reproducibility, and throughput of results. Here, we discuss—with a focus on leishmaniasis—that the oft-disregarded molecular detection assays and the automated platforms that can perform them have become more relevant in resource-limited settings. The automation of manual molecular techniques has increased in reach and performance in the form of near–point-of-care (NPOC) testing. These are device-based or low–equipment-based technologies enabling onsite, decentralized testing. Now, more than ever before, in the setting of the SARS-CoV-2 global pandemic, is an opportune time for automation to be applied to the detection of these NTDs using NPOC testing.[8]

In recent years, molecular diagnostic technologies have improved in terms of accuracy and meeting user needs on a global scale. This includes the automation of nucleic acid extraction or the PCR master mix setup to sample-to-result function (including onboard nucleic acid extraction, amplification, and analysis). Automation can increase reproducibility, and reduce the risk of laboratory contamination and human error, such as sample mix-ups and laboratory-acquired infection. It is acknowledged that such processing could improve throughput, speed, and sensitivity of *Leishmania* detection. For the detection of a related species, such as *Trypanosoma cruzi*, automated methods achieved the same performance as an in-house, manual method.[9] Furthermore, that study concluded that the broader use of real-time PCR methods could help to standardize methods across different laboratories.[9,10] Table 1 lists common automated nucleic acid extraction liquid-handling systems, highlighting the range of throughput, speed, and processing capabilities and the area (or "footprint") the instrument requires in a laboratory.[11]

The implementation of diagnostic tests differs at varying levels of national health-care systems, depending on their affordability, accessibility, and accuracy (Figure 1).[12–14] This tiered system relates to the provision of services (tests, staffing, communication infrastructure, equipment, turnaround times, and surveillance networks) at each level. Tier 0 is characterized by community health centers or outreach programs serving outpatients performing point-of-care (POC) tests and refers further tests to tiers 2 or 3. Tier 1 includes primary care/health center laboratories serving mostly outpatients and performing POC/single-use tests, and refers tests to tiers 2 or 3. Tier 2 laboratories are within district hospitals, serving inpatients, and receives referrals from tiers 0 and 1, performing a limited number of routine tests. Tier 3 laboratories are within regional hospitals, serving inpatients; receives referrals from tiers 0, 1, and 2; and performs multidisciplinary routine testing. Tier 4 laboratories in national or teaching hospitals serve inpatients and receive referrals from tiers 0, 1, 2, and 3. They perform routine tests and highly specialized tests, and provide education/training for all tiers. Although staffing may be relatively fixed within each tier, the diagnostic technologies and their increasing accessibility are being adapted to suit the lower, less-resourced tiers. It is in

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TABLE 1
Automated liquid handling platforms

Instrument	Manufacturer	No. of samples	Runtime, min	Sample input volume, μL	Elution volume, μL	Dimensions, cm; width × height × depth
m2000sp	Abbott	24–96	90–250	400–4000	15–190	145 × 217.5 × 79.4
EasyMag*	Biomerieux	1–24	40–60	10–1,000	25–110	100 × 53 × 65
GS-mini*	Genetic Signatures Ltd.	1–12	40–75	100–2,000	50–400	56 × 59 × 51
chemagic Prime 8 Instrument	PerkinElmer	1–192	55–75	10–10,000	Various	86.6 × 194 × 228.5
Maxwell RSC 48*	Promega	1–48	30–70	100–300	30–100	53.3 × 35.6 × 53.3
EZ1 Advanced XL*	Qiagen	1–14	20–50	200–400	50–100	51 × 57 × 51
QIAsymphonySP	Qiagen	1–96	90–290	> 200	30–500	128 × 103 × 73
Magnapure 96	Roche	1–96	50–170	50–4,000	50–200	136 × 100 × 81.5

* Suitable for near–point-of-care testing.

tier 2, tier 3, and tiers 0 and 1 (when serviced by mobile laboratories) where the implementation of automated molecular detection could have the most impact in low- and middle-income countries (LMICs).[15,16] The challenge is to bring these technologies down the tiers; however, this requires changing the perspectives and assumptions of key stakeholders.[17] This implementation is important as part of routine testing schedules and in outbreak scenarios, when the ability to upscale is imperative—when the demand on the health-care system increases.

In a 2002 report,[18] scientific experts identified "Modified molecular technologies for affordable, simple diagnosis of infectious diseases" as the major biotechnology that could improve health in developing countries. The authors found that many assumptions made regarding the lack of usefulness and cost of molecular diagnostics in controlling infectious diseases in poorer nations were not supported by evidence. Since then, the continued view that infectious diseases diagnostics are not accessible to these developing countries has led to the ASSURED/REASSURED criteria. These criteria emphasize the ideal characteristics of a diagnostic test across all health-care levels, encompassing affordability, accessibility, and accuracy (Figure 2).[19,20] POC tests, generally accepted as those tests performed and analyzed at the place of patient care, have broad and fluid definitions, with many derivatives still requiring a laboratory infrastructure.[21,22] Although promising and fulfilling many of the ASSURED/REASSURED criteria, POC tests can also be limited in sensitivity and specificity in *Leishmania* diagnostics, and molecular-based POC tests can be prohibitively expensive.[23,24] It is important to view the move from large-scale centralized laboratory testing to "true" POC tests as a continuum where varying testing modes overlap in technology and usefulness in situations in which they are used.[25] NPOC tests (Table 2) can be placed along this continuum, decentralizing testing by eliminating or reducing the need for sample transport and reducing turnaround time. Furthermore, NPOC tests have the flexibility of interchangeable assays and can retain the greater throughput that is lost in true POC tests. This becomes increasingly critical during times of outbreak, which occur frequently for both forms of leishmaniasis.[26–28] Tiers 0 and 1 settings with no or minimal infrastructure, including locations with no or intermittent electricity or no assigned laboratory space, may continue to be a challenge for the molecular diagnosis of *Leishmania* and other NTDs.[29] However, well-designed NPOC tests—including their automation—could find a place in most regions of the world in tier 2 and tier 3 laboratories (and mobile laboratories).

The major challenge in providing molecular testing and automation to resource-limited settings is that they have traditionally been considered an expensive diagnostic method. However, automation reduces direct and indirect staffing costs, and the miniaturization of PCR platforms and reagent

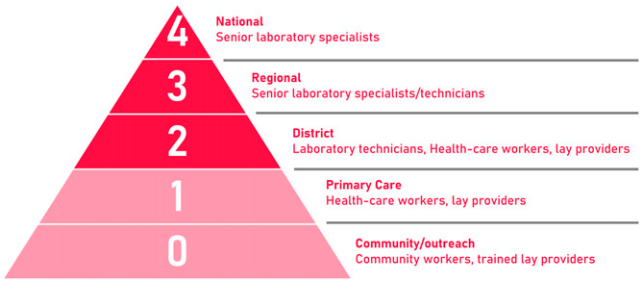

FIGURE 1. The different levels of healthcare and staffing requirements associated with these, adapted from ref.[16]

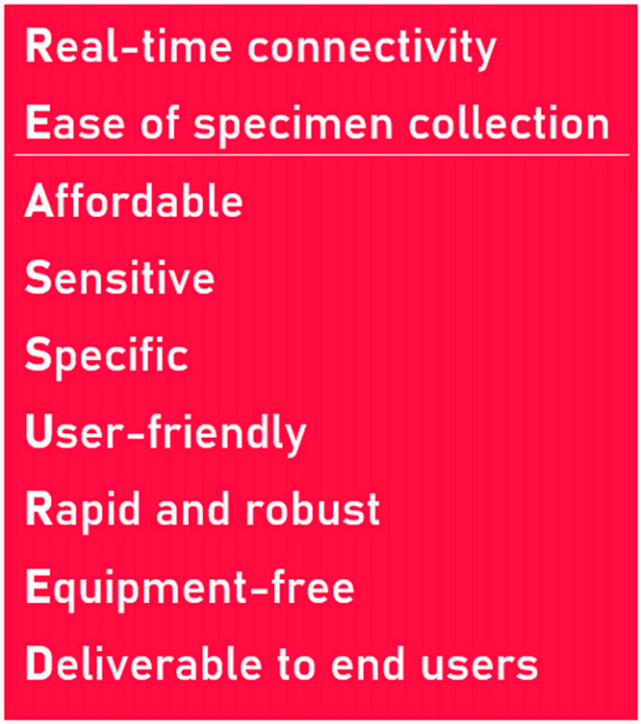

FIGURE 2. The ASSURED/REASSURED criteria for the ideal characteristics of a diagnostic test, adapted from ref.[23,24]

production methods has been accompanied by a further reduction in cost for these technologies.[10,30] It is predicted in LMICs that, as staffing costs increase over time, there will be a greater need to drive down the cost per test through automation.[17] Furthermore, the broad and interchangeable diagnostic panels and their capability for assay multiplexing (the detection of multiple organisms simultaneously) can offset the initial equipment costs, increasing impact and cost-effectiveness in the appropriate contexts.

Automation decreases the level of interaction between the user and the test; therefore, the risks of human error, laboratory-based accidents, and cross-contamination of samples are minimized. This is particularly apparent in settings where staff lack sufficient specialist training and educational background in manual molecular techniques.[31] In molecular diagnostics, real-time PCR with lyophilized reagents has also greatly reduced the risk of contamination that may be introduced to a laboratory.[32] For instance, sample-to-result systems eliminate manual preanalytical sample processing and postamplification analysis steps through premeasured, cartridge-based, and lyophilized reagents.[31] Large, complex, and high-throughput automated equipment share drawbacks in terms of instrument errors and breakdowns that require complex troubleshooting performed by specialist technicians.[33] The simplified nature of NPOC devices and platforms (e.g., cartridge-contained reagents) are less prone to such errors.

Endemic areas serviced by laboratories that currently lack the required physical infrastructure, including access to refrigerated transport or storage, sterile workspaces, or permanent laboratories, are considered unsuitable to perform molecular testing.[34] Freeze-dried PCR reagents were developed in the late 1990s and were found to be stable for up to 12 months at ambient temperatures, allowing reagents to be cold-chain independent.[35] DNA-free areas are less critical when reactions can be fully enclosed in an instrument, and DNA-free water can be provided with testing kits.[36] The advent of small, automated systems now allows for flexibility in laboratory location and may be incorporated into mobile laboratories or even a mobile suitcase laboratory (developed for pathogen detection such as *Leishmania* in the field).[15,37,38] Automation in other NTD detection is being seen in diverse technologies such as microscopy, loop-mediated isothermal amplification, and DNA extraction.[39–41]

The preanalytical phase of diagnostic testing can present challenges to retain sample quality when decentralized. Staff training and expertise, sample collection methods, containers, and handling all affect specimen quality. Although cutaneous leishmaniasis specimens are collected increasingly by relatively simple methods such as tape strips, skin scrapings, or exudate, VL specimen collection often requires invasive sampling methods, including spleen, lymph, and bone marrow biopsies.[42–45] These sampling methods may have to be performed using ultrasound guidance, and in the case of splenic aspirates, face the risk of patient death if performed improperly. Recently, the WHO has prioritized less-invasive, highly specific tests to measure parasite levels for VL to reach elimination targets for the disease.[1] Less-invasive sampling methods for PCR detection of *Leishmania* in visceral cases, such as peripheral blood collection, although not yet recommended, are being investigated with increasingly improving detection limits.[46,47] When applied to real time-PCR, the potential for quantification of parasitic load in the blood is possible. Monitoring parasitic load during and after treatment can give an indication of relapse, as validated in blood samples.[48] However, collection of blood specimens is not yet designed for POC, in terms of sample collection and prevention of diagnostic errors.[49] Clinical sample referral and transport needs to be avoided to keep the testing near to the patient. Thus, the challenge remains that the simplicity of specimen collection must be in line with the resources and limitations of the laboratory tier in which they are collected. For VL, sensitive detection of *Leishmania* DNA in the urine was possible, and its depletion correlated with treatment.[45] Adapting novel sample types for molecular detection of VL and monitoring of parasite load to NPOC testing could present a solution for specific WHO elimination priorities.

The global pandemic experience exemplifies that NPOC testing can and has been implemented across most healthcare levels. This challenges the assumptions of the lack of appropriateness of molecular technologies in LMICs and resource-limited settings. Many LMICs have had increased opportunities to develop infrastructure, logistic, administrative, and workforce systems skilled in testing procedures suitable for mass diagnosis and screening programs. Concurrently, manufacturers of the assays and their associated diagnostic platforms have scaled up capacity for product production and

TABLE 2
Characteristics of the "true" POC test vs. the near-POC test

Characteristic	True POC test	Near-POC test
Turnaround time	Minutes	Hours
Throughput	Single test, predetermined target	12 Samples per run, flexible target selection
Infrastructure	No need for electricity or air conditioning	Constant electricity, computer interface, and some degree of temperature control
Staff expertise	Nonlaboratory training required	Basic laboratory training required
Cost	More than conventional	Can be reduced to conventional depending on the type of device used, the type of test run, and where the device is placed
Quality	Decentralized quality control, equipment maintenance, supply chain and waste management	Centralized quality control, equipment maintenance, supply chain and waste management
Example test	CL Detect Rapid Test™ (Inbios International Inc., Seattle, WA)	GeneXpert (Cepheid, Sunnyvale, CA), GS-mini (Genetic Signatures Ltd., New South Wales, Australia)

POC: point-of-care

provision of expertise to these settings. If the momentum we are seeing in the diagnostic development and delivery capabilities for SARS-CoV-2 is not sustained and applied further to NTDs such as leishmaniasis in a postpandemic environment, it could be a missed opportunity to achieve important global public health gains in the fight against NTDs.

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