Rapid infectious diseases diagnostics using Smartphones

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Abstract: The "Smartphone" is an almost universal possession in high-income populations, and is rapidly becoming so in lower-income regions, particularly among urban populations, and serves social networking and a quest for information and knowledge. The field of infectious disease diagnostics is at a potential watershed moment, with the essential building blocks for the development of diagnostic assays being ever more available and affordable, which is leading to creative innovative approaches to developing much-needed accurate and simple point-of-care (POC) diagnostic tools for high disease burden, low-income settings. We review the importance and implications of a paper published in *Science Translational Medicine* on the development of a smartphone-powered and -controlled multiplex immunological assay that tests for HIV and syphilis simultaneously. This is reviewed in the context of other prototype smartphone-enabled/assisted diagnostic devices, and how such developments might shape the future of the POC diagnostics field.

Keywords: Smartphone; diagnostics; diagnosis; point-of-care (POC); HIV; helminth; TB; microscope; polymerase chain reaction (PCR)

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Introduction

Telemedicine is a field of medical research that is centred on the use of telephones and the Internet to improve public health services. The concept that telephones might have a role to play in diagnostics and health care provision was first noted in the 1950s with respect to the transmission of electrocardiograms (1,2). During the 1960s and 1970s, as telephones came in to routine use in homes and workplaces their uses extended to emergency calls, for monitoring therapy (3) and for bringing together physician and patient remotely, to establish a preliminary diagnosis (4,5) before referral or for management advice. Three decades later, many countries have centralized telephone-based diagnostic or triage services for non-life threatening health problems, such as National Health Service (NHS) 111 (formerly "NHS Direct") in the United Kingdom.

The rise of the "smartphone" (a mobile telephone with an integrated computer and an operating system, able to run software applications) has endowed an increasing number of people, with a small mobile computer, which when compared with a 1980s personal computer, has up to 500 times the processing power and 250,000 times as much Random Access Memory (RAM). With appropriate interfaces to sensors or other adapted or bespoke hardware, smartphones have phenomenal capacity to drive and control a range of medical devices, which is of particular interest to health systems in developing countries, where smartphone-driven monitoring and diagnostic devices might provide a cost-effective alternative to expensive dedicated stand-alone technology. We review several diagnostic devices developed in recent years, which use smartphones or components thereof, which have been developed specifically to address infectious disease diagnostic challenges in resource-poor settings.

The "Dongle"—a POC ELISA for HIV and syphilis

Laksanasopin and colleagues (6) describe the development and preliminary clinical evaluation of a point-of-care (POC) diagnostic "dongle" which is both controlled and powered by a 4th generation Apple iPod Touch. The dongle is unique, in that it is the first portable device designed for conducting an enzyme linked immunosorbant assay (ELISA), a common diagnostic test that is usually limited to well-equipped laboratories. The authors developed and evaluated the dongle for detection of antibodies to HIV and syphilis, although the technology can be readily adapted for detection of antibodies to numerous other microbial pathogens. This microfluidic device is largely mechanical in its operation, using a bulb-push to create negative pressure, which then pulls the reagents and sample through the system performing both the incubation, wash and detection components of the ELISA. The light emitting diode (LED), photodetector and microcontroller use very small amounts of power drawn through the audio jack on the iPod, which the microcontroller also uses to relay results back to the iPod, using frequency shift keying (FSK), where one high and one low audio frequency are assigned to 1 and 0 for binary coding of results. The assay run time is short at 15 min, so highly appropriate for POC settings where it may reduce waiting times for patients and family members.

The current standard of care for HIV testing across sub-Saharan Africa relies on rapid diagnostic tests (RDTs), typically dip-stick or lateral flow assays where a band or colour change appears to indicate a positive specimen. These assays are cheap to manufacture, have a long shelf life, but are less accurate than laboratory-based ELISAs. Currently the World Health Organisation (WHO) recommends a three-test strategy for HIV testing, where SD Bioline HIV 1/2 (Standard Diagnostics, Kyonggi-do, Korea) is used as the initial screen, with positives confirmed using the Determine HIV 1/2 test (Abbott Laboratories, Abbott Park, IL, US). Discordant samples are then tested by either the Uni-Gold HIV 1/2 (Trinity Biotech, Bray, Republic of Ireland) or HIV 1/2 STAT-PAK (Chembio Diagnostics, Medford, NY, US). Recent studies suggest poor specificity of some of these assays (7,8), and thus there is a need for alternative technologies that are more accurate. Furthermore there is a practical need for multiplex screening of several sexually transmitted diseases at the same time such as syphilis, which currently relies on lab-based RPR testing).

Laksanasopin and colleagues under a U.S-Rwanda collaboration evaluated the HIV-syphilis dongle ELISA against gold-standard standard lab-based ELISAs at an antenatal clinic in Rwanda. Accuracy for HIV diagnosis was comparable with the existing RDTs. It is not clear how the dongle could replace the currently recommended WHO three-test strategy, as the specificity of any one test on its own is insufficient and would result in large numbers of HIV-negative patients being started unnecessarily on anti-retroviral therapy. However, the multiplexing of syphilis testing with its high sensitivity is an attractive attribute of their test and could serve as the initial screen. Another smartphone application linked to serological assays is the use of the high definition camera for objective interpretation of leprosy IgG and IgM lateral flow assays, which might solve incorrect interpretation of faint bands by human operators (9). The software developed for this application, could be easily adaptable to solve read-out errors on a range of RDTs.

Smartphone microscopy

Several studies have explored the development of highly portable and affordable microscopes, combining mobile phones with various lens arrangements for classical bright-field and fluorescence microscopy. Phones have been combined with traditional microscope objectives (10), or a simple ball lens (11-13), but both strategies are confounded by limited field of view and image quality issues. Adding an inverted mobile phone lens has been shown to improve performance, mounting the lens, light source, specimen presentation, USB and even blue tooth connectivity, within a 3D-printed case that fits snugly onto the phone (14,15). Taking this further, one group has coupled such a clip-on device to compressive sensing algorithms to deliver wide-field fluorescent images with performance similar to a 10× objective on a conventional fluorescent microscope (16,17), a technology which is now being developed further as a basic flow cytometer, able to count fluorescently labelled cells passing through a microfluidic system (18). Preliminary field trials of smartphone microscopes have focussed on helminth (13,15,19) and *Giardia spp* infections (20), providing practical and logical applications since these infections are common in rural areas of developing countries where health centres are at their most basic.

Another strategy being explored is the use of imaging components of smartphones to develop novel diagnostic tools for low-income settings. Schistosomiasis is an important neglected tropical disease which affects hundreds of millions of people. The diagnosis requires an ELISA

test or microscopic examination of urine, stool or biopsy for schistosoma ova. ELISA analysis is the diagnostic strategy currently used in most schistosomiasis endemic countries (21). Recent innovations focus on the experimental use of the imaging sensors from several different smartphones, to develop "on-chip" imaging of schistosoma eggs (22). A recent study describes detection of schistosoma eggs on the sensor chip of a mobile phone with a computer vision algorithm to automatically read the image and detect the eggs. They achieved a sensitivity of 79%, and specificity approaching 100% compared with visual scoring. The technique shows promise for possible future development into a stand-alone device for use in remote locations, using either computer vision, or sending images via mobile data networks for scoring by trained personnel.

Nucleotide detection systems

The polymerase chain reaction (PCR) or hybridization assays (line probes and arrays) to detect pathogen DNA (or RNA) are important components of the diagnostics tool kit being developed for low-income countries. Quantitative or qualitative nucleotide-based assays are now preferable to CD4+ cell counts for HIV therapeutic monitoring (23). For Tuberculosis, the GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA, US), a molecular beacon PCR assay, has been extensively evaluated and rolled out for the diagnosis of pulmonary TB in both adults and children, and offers a 2-fold improvement in sensitivity over sputum smear microscopy, the standard of care in community health centres in developing countries (24,25). There are many different types of PCR reaction that have been developed but most require a thermocycler: a computer-controlled machine that can incubate a chemical reaction at different temperatures, repeating in a cyclic fashion. The one exception is LAMP (loop-mediated isothermal PCR), which is being developed by several groups for the diagnosis of important diseases of poverty in developing countries, such as tuberculosis (26,27) and trypanosomiasis (28).

Smartphone applications for nucleic acid detection are under development. Preliminary approaches include controlling a standard thermocycler using a smartphone via Bluetooth (29), or exploiting the imaging capabilities of smartphones to interpret reaction results (30,31). In one study researchers from the University of California subverted the central processing unit of a desk top PC, turning the heat sink into a thermocycling block, holding small capillaries containing the reaction mixture within the side ribbing. After the reaction is complete, the capillaries are then placed on a UV transilluminator, to excite the Sybr green dsDNA dye used in the reaction. A 520 nm filter was then attached to cover the lens of a Samsung Galaxy S, and the phones' camera was used to photograph the reaction capillaries in "night mode", with pixel intensities used as a proxy for quantity of PCR product (31).

Discussion

Over the coming decades, to what degree diagnostic technology development will be driven by localized innovation using off-the-shelf components, including smartphones and other hardware and software remains to be determined. As experts in the field of diagnostics development and evaluation, we are continually frustrated by the prior slow progress, and vast sums of capital required delivering desperately needed new diagnostic tools to markets in resource-poor developing regions. Despite colossal investment into research and development, these new diagnostic tools might reach the market with unanswered questions about assay accuracy, maybe in specific settings or patient groups, or their applicability to different presentations (32,33). Despite WHO-negotiated low pricing, costs might still be out of reach of most health systems, or poor planning could lead to procurement of expensive dedicated platforms which are at risk of becoming redundant, due to insufficient funds for consumables and maintenance, or when the field moves on and a subsequent technology is proven to be preferable due to accuracy or cost. Essentially, the needs of diagnostics development companies (a monopoly on a large market, with backing by global policy organisations for an expensive dedicated platform, locking the consumer into non-competitively priced consumables and maintenance) appear distinctly at odds with the needs of health systems in low-income countries (a range of competitively priced accurate POC diagnostic tests with minimal requirements for expensive hardware, electricity or skilled operators).

One can envisage a future diagnostic landscape, where a parallel diagnostics development sector emerges, with entrepreneurial development of low-cost diagnostic tools, maybe tailored to the specific needs of a given region. Such assays will not initially carry high levels of quality control and assurance, but the cheaper pricing may establish markets, with the quality of these "generic diagnostics" improving over time as profits increase, drawing obvious parallels to the generic drugs market.

Smartphones are getting smarter, but already possess more than enough processing power for the kinds of command and control functions associated with hi-tech diagnostics machines, as outlined above. One could point to next generation sequencing (NGS) as a growing corner of the diagnostics field for which considerably more processing power is required, but even in that lush corner of the meadow, the latest technology from Oxford Nanopore, the min-ION (34), is not much larger than a USB key, powered and controlled through a USB-3 connection on a top-end laptop. The massive growth of NGS researchers, and a blossoming marriage of traditional biomedical science researchers with bioinformaticians, is driving the development of more efficient sequence analysis algorithms (35).

Of all the approaches reviewed, the smartphone-powered and -controlled multiplex ELISA is probably the most impressive. With respect to the microscopy applications, second-hand light microscopes cost less than smartphones, are less likely to be stolen (a significant problem with highly desirable phones and laptops), and don't need any electricity to perform basic infectious disease diagnostics in resource-poor settings, for important infections such a TB, helminth infections and malaria. For rural health centres which lack a microscope, they probably also lack a trained technician and any reagents or consumables needed for sample preparation. They might also lack power and a reliable way to keep the mobile phone charged, whereas a simple light microscope can use a mirror to reflect the sun to back-light the specimen. This said, we anticipate an increasing number of publications describing the incorporation of smartphones into diagnostic tools, and are excited to see what other bright new innovations arise.

Many of the approaches for developing smartphones as RDTs for infectious diseases are promising but how these will eventually be adapted and incorporated into POC diagnostic tools for use in low-income settings requires to be determined. The important question is whether they will ever become widely applicable diagnostic tools at points of healthcare where there is the greatest need?

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Footnote

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