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Engineering Steps for Mobile Point-of-Care Diagnostic Devices

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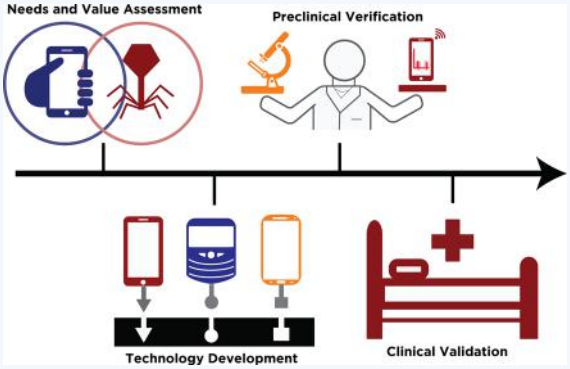
Supporting Information

CONSPECTUS: Mobile phone technology is a perfect companion for point-of-care diagnostics as they come equipped with advanced processors, high resolution cameras, and network connectivity. Despite several academic pursuits, only a few mobile phone diagnostics have been tested in the field, commercialized or achieved regulatory approval. This review will address the challenges associated with developing mobile diagnostics and suggest strategies to overcome them. We aim to provide a resource for researchers to accelerate the development of new diagnostics. Our Account includes an overview of published mobile phone diagnostics and highlights lessons learned from their approach to diagnostic development. Also, we have included recommendations from regulatory and public health agencies, such as the U.S. Food and Drug Administration and World Health Organization, to further guide researchers.



We believe that the development of mobile phone point-of-care diagnostics takes place in four distinct steps: (1) Needs and Value Assessment, (2) Technology Development, (3) Preclinical Verification, and (4) Clinical Validation and Field Trials. During each step, we outline developmental strategies to help researchers avoid potential challenges. (1) Researchers commonly develop devices to maximize technical parameters such as sensitivity and time which do not necessarily translate to increased clinical impact. Researchers must focus on assessing specific diagnostic needs and the value which a potential device would offer. (2) Often, researchers claim they have developed devices for feasible implementation at the point-of-care, yet they rely on laboratory resources. Researchers must develop equipment-free devices which are agnostic to any mobile phone. (3) Another challenge researchers face is decreased performance during field evaluations relative to initial laboratory verification. Researchers must ensure that they simulate the field conditions during laboratory verification to achieve successful translation. (4) Finally, proper field testing of devices must be performed in conditions which match that of the final intended use.

The future of mobile phone point-of-care diagnostic devices is bright and has the potential to radically change how patients are diagnosed. Before we reach this point, researchers must take a step backward and focus on the first-principles of basic research. The widespread adoption and rapid scaling of these devices can only be achieved once the fundamentals have been considered. The insights and strategies provided here will help researchers avoid pitfalls, streamline development and make better decisions during the development of new diagnostics. Further, we believe this Account can help push the field of mobile diagnostics toward increased productivity, leading to more approved devices and ultimately helping curb the burden of disease worldwide.

INTRODUCTION

Point-of-care diagnostics provide a clinically relevant output in a timely manner that allows a healthcare worker to make a clinical decision at the site of testing.[1,2] Their development has been encouraged by the World Health Organization (WHO) and other agencies[3,4] for use in developing nations which lack proper medical infrastructure. Ideally, these devices should be portable, rapid, cost-effective and not require the use of laboratory staff and facilities. Recently, researchers have strived to address these

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Table 1. List of Mobile Phone Diagnostic Studies and Their Application for Diagnosis of Different Diseases[a]

readout	disease	biomarker	setting	analytical sensitivity	clinical sensitivity	clinical specificity
colorimetric	measles, mumps, HSV-1/2	measles IgG, mumps IgG, HSV-1/2 IgG	laboratory	not discussed	100%, 99%, 100%, 100%	97%, 95%, 100%, 100%
	ovarian cancer	HE4	laboratory	20 ng/mL	89.5%	90%
	ebola	anti-IgG for SUDV GP_{1-649}, BDBV GP_{1-649}, and EBOV GP_{1-649}	field	200 ng/mL	100%	100%
	HIV	p24	laboratory	1 pg/mL	n/a	n/a
	UTI,	*E. coli, Neisseria gonorrhoeae*	laboratory	10 CFU/mL	n/a	n/a
	red blood cells	red blood cells	laboratory	50 mg/dL	n/a	n/a
	zika	NS-1	laboratory	0.05 ng/mL	n/a	n/a
	HIV, TB, malaria	HIV 1/2 IgG, TB IgG, *P. falciparum*	laboratory	not discussed	n/a	n/a
	dengue	dengue viral DNA	laboratory	5 nM	n/a	n/a
electrochemical	sepsis	IL-3	laboratory	22 pg/mL	91%	82%
	cervical cancer	HPV viral DNA	laboratory	10 amol	100%	92%
	HPV	HPV viral DNA	laboratory	50 amol	83−92%	90−100%
	HIV	p24, anti-p24	laboratory	48 ng/mL	100%	100%
	HIV, syphilis	HIV 1/2 and treponemal syphilis antibody	field	2 pg/mL	100%, 77%	91%, 89%
	malaria	PfHRP2	laboratory	20 ng/mL	n/a	n/a
	malaria	PfHRP2	laboratory	16 ng/mL	n/a	n/a
	HCV	hepatitis C core antibody	laboratory	12 pM	n/a	n/a
fluorescence	avian influenza	H5N1 nucleoprotein	laboratory	8×10^5 PFU/mL	96%	99%
	thrombin	thrombin	laboratory	18 NIH units/mL	n/a	n/a
	ebola	EBOV glycoprotein	laboratory	0.2 ng/mL	n/a	n/a
	zika	zika viral RNA and whole virus	laboratory	$3 \times x10^4$ PFU/mL	n/a	n/a
	CMV	HMCV	laboratory	1×10^3 PFU/mL	n/a	n/a
	HIV	p24	laboratory	17 pg/mL	n/a	n/a
	HIV, dengue	anti-HIV1-p17, anti-NS1	laboratory	100 pM	n/a	n/a
	E. coli	*E. coli* DNA	laboratory	300 copies/pL	n/a	n/a
	zika, dengue, chikungunya	ZIKV, DENV, and CHIKV viral RNA	laboratory	22 PFU/mL	n/a	n/a
	HSV-2	HSV-2 viral DNA	laboratory	−100 copies/pL	n/a	n/a
	HIV, HBV	HIV viral cDNA, HBV viral DNA	laboratory	1×10^3 copies/mL	n/a	n/a
microscopy	Loa loa filariasis	*L. loa* microfilariae	field	3×10^4 mf/mL	100%	94%
	schistosomiasis	*S. hematobium* ova	field	not discussed	56%	93%
	giardiasis	*G. lamblia* cysts	laboratory	1×10^6 cells/mL	n/a	n/a
	S. *aureus*	*S. aureus* cells	laboratory	50 cfu/mL	n/a	n/a
	malaria, tuberculosis	*P. falciparum smear*, M.	laboratory	n/a	n/a	n/a

[a]References are found in Supporting Information Table T1.

point-of-care diagnostics device characteristics by leveraging advances in mobile phone technology.[5−7] Mobile phones come equipped with large amounts of memory, high resolution cameras and sensors, advanced processors and network connectivity. These features allow them to achieve a wide spectrum of capabilities ranging from detection, processing to sending/receiving clinical information in real time.[8,9] Additionally, mobile phones have seen an exponential rise in adoption across the world including sub-Saharan Africa[10] making them a widely accessible technology to pair point-of-care diagnostics with. This wide-ranging adoption could also play a part in the broad utility of mobile phone diagnostics for personalized healthcare, especially in low-income countries. Researchers have started to detail the development of mobile phone diagnostic devices with peripheral accessories attaching to the phones,[11,12] accessory-free systems[13−16] or devices which use phones as a device interface.[12,17] These studies showcase diagnostic tools capable of detecting signals based on colorimetry, fluorescence, electrochemistry and microscopy (Table 1, Figures 1 and 2) at levels of clinically approved methods.

While the integration of mobile phones for point-of-care molecular diagnostics seems exciting and logical, only a few molecular-based devices mainly for urinalysis and glucometry have successfully achieved U.S. Food and Drug Administration (FDA) approval.[18−20] This is not a result of the FDA's regulatory pathway as they have recognized the potential of mobile phone diagnostics and have promoted them in their new *Digital Health Innovation Action Plan*.[21] In this Account, we present an engineering process for translating mobile diagnostic devices. This process can be divided into four steps: (1) Needs and Value Assessment, (2) Technology Development, (3) Preclinical Verification, and (4) Clinical Validation and Field Trials (Figure 3). We also outline steps and recommendations for researchers to overcome potential developmental challenges at each step. Additionally, we also assess the current landscape of mobile phone diagnostics by analyzing successful studies and how they have overcome these challenges. These guidelines will help researchers accelerate the development of mobile point-of-care diagnostics toward its ultimate end goal of curbing disease worldwide and potentially revolutionizing personalized healthcare.

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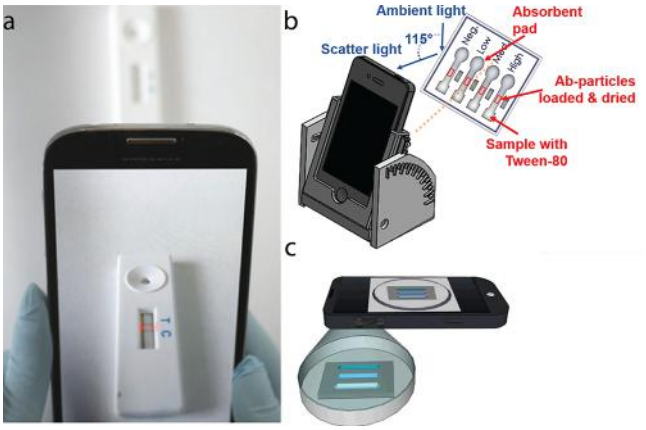

Figure 1. Mobile phone diagnostic devices capable of accessory-free image acquisition. (a) Serological point-of-care test for the detection of Ebola IgG antibodies imaged with a mobile phone. Reproduced from ref 14. Copyright 2018 American Chemical Society. (b) Sensitive μPAD detection of UTI and gonorrhea using a mobile phone. Reproduced from ref 15. [2015] Biosensors and Bioelectronics. (c) Detection of ovarian cancer HE4 biomarker through imaging of a microchip ELISA using a mobile phone. Reproduced from ref 16. [2011] Lab on a Chip.

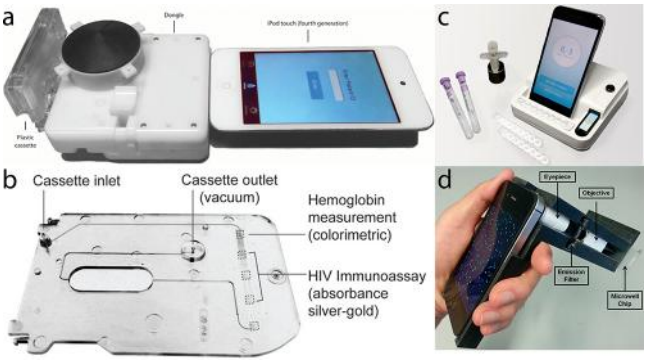

Figure 2. Mobile phone diagnostic devices which require external peripheries. (a) The smartphone dongle developed for the dual detection of both HIV and syphilis. Reproduced from ref 11. [2015] Science Translational Medicine. (b) Low-cost microfluidics platform for the detection of diseases in developing nations. This device is housed within the dongle presented in (a). Reproduced from ref 34. [2015] Lab on a Chip. (c) A biosensor which uses a mobile phone for the detection of sepsis. Reproduced from ref 29. Copyright 2018 American Chemical Society. (d) Mobile phone-based fluorescence reader for the readout of the quantum dot barcode assay. Reproduced from ref 39. 2015 American Chemical Society.

NEEDS AND VALUE ASSESSMENT

The first step in the development of mobile diagnostics is for researchers to identify the specific diagnostic target(s) and then assess how their device facilitates or expedites a clinical decision for these target pathogens or diseases more effectively than the current available diagnostics. To do this, they should attribute value judgements on two aspects of their devices: (1) the relevance of the specific molecular assay and (2) the impact of leveraging mobile phone technology.

To find a diagnostic problem to solve, we recommend that researchers collaborate with clinicians to gain expertise regarding a specific disease class. Finding clinicians which have experience on the ground in low-income nations can help researchers make more educated decisions about their target of choice. A more in-depth discussion about finding appropriate collaborators has been discussed by the Whitesides group.[22] Outside of collaboration, an important resource that researchers are encouraged to consult is the WHO's new list of *Essential In Vitro Diagnostics* which includes disease specific requirements such as recommended assay and specimen type, required facility levels as well as relevant regional incidence.[23]

Researchers should assess the value of their molecular test relative to the current gold standard diagnostic. Only molecular tests which facilitate or expedite clinical decisions more effectively are considered clinically useful.[2,24] Effectiveness of an assay is dependent on the specific diagnostic needs of a disease or target demographic. However, only 41% of the reviewed papers explicitly state the value of their diagnostic test relative to the current standard. The other 59% state the value in terms of increased sensitivity or turnaround time but these do not always lead to increased clinical impact. To illustrate, the same challenge has been found in the development of point-of-care devices for early detection of prostate cancer through detection of prostate specific antigen (PSA). Assays for PSA detection have become controversial in diagnostic oncology with many publications citing its misuse.[25,26] PSA assays provide quicker turnaround times than biopsy, but the marker has much lower diagnostic sensitivity as it often presents in noncancerous diseases. Even Richard Albin, its discoverer, stated that PSA screening is "inaccurate and a waste of money."[27] This does not discredit the development of PSA diagnostics as it remains a vital marker in a panel of analytes tested for prostate cancer confirmation.

Researchers must then consider the impact of integration of the molecular test with a mobile phone. Mobile phones have inherent advantages which can be used to develop more effective diagnostic devices; however, they are not initially designed with medical use in mind and therefore have limitations. Researchers should focus on the inherent benefits such as high adoption rates, network connectivity and processing speed to tailor their devices. High adoption rates reduce the cost of the potential point-of-care devices as most patients already own mobile phones,[10] network connectivity can be used to connect patients to treatment strategies[28] and the processing speed can be used to analyze results of tests in real time for prompt diagnoses.[29,30] These advantages are what will lead mobile phone diagnostics to increased clinical impact over their laboratory counterparts. Additionally, accessibility is often the most important parameter for the impact of a point-of-care diagnostic in what is called the "*rapid test paradox*".[31] An example of this paradox is that a point-of-care malaria diagnostic with 90% sensitivity and specificity with no laboratory requirements saves 22% more lives than a laboratory diagnostic with 95% sensitivity and specificity.[32] Mobile phones can drastically impact the accessibility of diagnostics due to their ubiquity.

Without a proper assessment of diagnostic need and the relevant analysis of the clinical impact for mobile phones, the utility of a diagnostic can never be unearthed. An example of a needs and value assessment is outlined here: the WHO reports that development of essential diagnostic technology for human immunodeficiency virus (HIV) should be prioritized for key populations such as men who have sex with men and pregnant women.[33] These demographics often go untested and untreated for which they cite accessibility as a factor for nontesting rather than improved sensitivity compared to the gold standard, outlining the diagnostic problem. Further, the WHO also insists that diagnostics for this purpose include dual testing for syphilis

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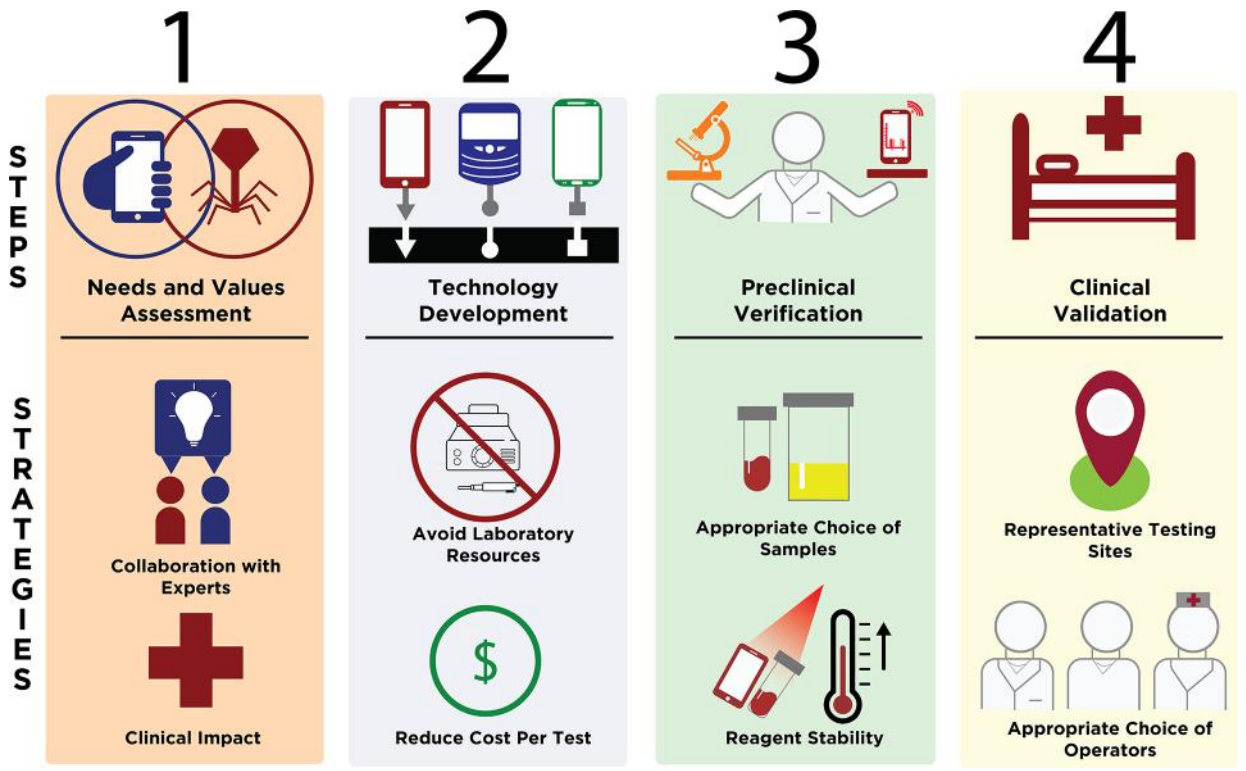

Figure 3. Depiction of the typical steps in the developmental pathway for engineering mobile point-of-care diagnostics.

and provide the capability to link patients to care after diagnosis.[28] The network connectivity of mobile phones can integrate seamlessly into a point-of-care test to provide a solution for this diagnostic need and result in high clinical impact. An elegant execution of this was demonstrated by the Sia group who developed a smartphone-based microfluidics technology to diagnose both HIV and syphilis[30,34] (Figure 2A,B). Their platform detects disease antibodies in under 15 minutes using a mobile phone to run and interpret the assay. Once results are analyzed, patients use the mobile phone to connect them to care in their area. Their technology is not more sensitive than the current gold standard, enzyme-linked immunosorbent assay (ELISA); however, they have shown that development of simple fluid handling and signal detection techniques drastically improves accessibility and as a result the impact of their platform.

TECHNOLOGY DEVELOPMENT

Once researchers have thoroughly assessed the value of a diagnostic, they can initiate formal technology development of their devices. During this phase, researchers must ensure that they are developing technology that matches the physical and infrastructural landscape of their intended target.[35] In our review, we have found that a proportion of published studies do not consider this landscape. Of the studies reviewed targeting infectious diseases (HIV, Malaria, etc.), 75% present results that rely on either trained users to interpret readouts, constant electricity or expensive laboratory resources (centrifuges, pipettes) to operate underlying molecular assays. However, these diseases often disproportionately affect developing nations,[35] which have poor medical infrastructure with densities of trained staff as low as 1/50 of a developed nation,[35,36] are electrified as low as 10% of the time,[37] and can lack a single accredited laboratory nationwide.[38] Therefore, the implementation of these devices becomes difficult and is a pitfall for researchers. To develop better mobile point-of-care devices, researchers should focus on developing technologies that operate without external resources and are agnostic to any mobile device.

To eliminate the need for external resources, researchers can leverage the integration of mobile and biotechnology to automate testing and readout. Researchers can consider alternatives such as automatic fluid handling in place of manual pipetting,[22,34] integration of vacuum pressure pumps to replace centrifuges required for sample extraction[1,30] and consider the use of isothermal amplification techniques to avoid thermocyclers.[39] These can be integrated into the development of a device or periphery which are controlled and powered through the connectivity and processing power of a mobile phone through Bluetooth or direct connection. Two examples of this were demonstrated by the Sia group's smartphone dongle[30] and the Erickson group's TINY device.[17] Researchers can consider a simpler approach by building a device with no external peripheries, using only the mobile phone camera for optical imaging. However, these devices often suffer from decreased performance and increased run to run variation due to a lack of control over sample position, exclusion of ambient lighting, and focal length.[40,41]

Existing studies detailing development of mobile diagnostics are taking steps forward, but the device hardware and software are typically tailored to a single version of a mobile phone. Development of devices and peripheries must account for the broad spectrum of available devices which possess inherent differences in hardware, firmware, and physical form factors.[10] A device's attachments and accessories for diagnostics must be either device-agnostic or come equipped to match every device. Researchers can consider designing modular peripherals with adjustable grooves for attachment or providing separate

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Table 2. World Health Organization REASSURED Criteria for the Development of Point-of-Care Diagnostics[a]

criteria	description
Real-time connectivity	tests are connected and/or a reader or mobile phone is used to power the reaction and/or read test results to provide required data to decision makers
Ease of specimen collection	tests should be designed for use with noninvasive specimens
Affordable	tests are affordable to end-users and the health system
Sensitive	avoid false negatives
Specific	avoid false positives
User-friendly	procedure of testing is simple; can be performed in a few steps, requiring minimum training
Rapid and robust	results are available to ensure treatment of patient at first visit (typically, this means results within 15 min to 2 h); the tests can survive the supply chain without requiring additional transport and storage condition such as refrigeration
Equipment free or simple **E**nvironmentally friendly	ideally the test does not require any special equipment or can be operated in very simple devices that use solar or batter power completes tests are easy to dispose and manufactured from recyclable materials
Deliverable to end-users	accessible to those who need the tests the most

[a]Reproduced from ref 44. [2018] Nature Microbiology.

connectors for wired connection of mobile phones. The Whitesides group demonstrated the Universal Mobile Electrochemical Detector (uMED)[42] which was made accessible to a range of devices. Their platform consists of a hand-held electrochemical sensor which connects to a mobile phone for analysis and readout via an audio cable. The reasoning behind this development is that a large range of mobile phones have a common audio port, and this allows the device to be used broadly. However, manufacturers have recently started to omit the audio port, highlighting the challenge of designing broadly accessible mobile phone diagnostics.

Additionally, researchers should keep the cost of materials as low as possible. As mobile phones are assumed to be fixed costs covered by patients, variable costs per test come from the cartridges and reagents used to eliminate external resources. As a benchmark, material costs for mobile point-of-care diagnostics on a per test basis range from roughly $0.50 to $1.00 USD for lateral flow and up to $10.00 USD for a molecular point-of-care test.[23,43] Optimizing for cost at an early stage is difficult but is key to ensuring costs do not become irreversibly high. Once device development is complete, a cost benefit analysis must be reported for all mobile phone diagnostic studies. This should include thorough analyses of salaries of healthcare workers and costs associated with quality assurance/control.

Another useful resource for researchers during technology development is the WHO's REASSURED framework[44] (Table 2), which is a set of guidelines regarding the practical and technical specifications for implementation of an ideal mobile point-of-care diagnostic. The framework was recently shifted from ASSURED (affordability, sensitivity, specificity, user friendliness, rapid and robust, equipment-free, and deliverable to end-users)[3] to REASSURED which incorporates *Real time connectivity* and *Ease of specimen collection* to ensure researchers compensate for the rapid adoption of mobile phones and mobile health. Researchers should keep in mind these guidelines are "ideal" and not absolute and should tailor their technology development for the specific landscape and relevant needs of the diseases they are targeting. The WHO has also partnered with MobileDiagnosis which aims to improve global health through the integration of mobile diagnosis. Researchers are also encouraged to consult information regarding mobile diagnostics included in the WHO's *Compedium of New and Emerging Health technologies*.[45]

PRECLINICAL VERIFICATION

Ultimately, the goal of mobile phone diagnostic development is to implement the technology for use in real-world settings. Preclinical verification is a step in the design process when researchers can verify the functionality of their systems, then iterate or move toward clinical validation. The FDA, WHO, and Centers for Disease Control and Prevention (CDC) cite two key parameters for diagnostics to progress toward successful field trials as (1) accuracy and (2) stability.[46−48] They recommend that preclinical verification should thoroughly test capabilities of a device by using appropriate sample types, comparing against gold standards and evaluating storage stability. Yet, of the 29 laboratory-based mobile diagnostic studies summarized in Table 1, only 7 (24%) include these verification tests. In this section, we will outline steps that researchers can take to design better preclinical verification studies for mobile phone diagnostics.

The accuracy of a diagnostic in a controlled laboratory can be drastically different than the accuracy when performed in the final intended setting.[2,22] As a result, researchers should match the testing conditions in the lab environment to those in the field to avoid challenges later in the developmental pathway. Appropriate choice of buffers is crucial for accuracy verification as the complex proteomic environment of samples such as whole blood, plasma, and serum can reduce the activity and function of enzymes and biologics found in point-of-care assays.[49] This phenomenon drastically affects the accuracy of mobile phone diagnostics with issues such as nonspecific adsorption of proteins decreasing the functionality of diagnostic probes and increasing background signals.[50] Thus, initial accuracy verifications using simple buffers rather than the intended sample type can mislead researchers about the true accuracy of their devices. To report precise accuracies and allow for optimization of mobile phone technology, the sample choice used in this phase should match that of the final use case. However, a proportion of the summarized studies[16,51−54] use nonrepresentative samples, such as simple buffers, diluted samples, or nonhuman samples to evaluate accuracy. Human diagnostics should always be evaluated with either human clinical samples or if clinical samples are unavailable, spiked human samples without dilution. A case example of proper buffer choice is demonstrated by the Lee group in the development of their mobile-phone-based biosensor for the detection of sepsis[29] (Figure 2C). Once the biosensor technology had been developed, they verified the use of their system with spiked buffer and spiked human serum, plasma, and whole blood. Their preclinical verification with nondiluted human samples allowed space for assay optimization, which led to a successful clinical validation. Although their study moved past preclinical verification, most mobile phone diagnostic studies typically end at this point.

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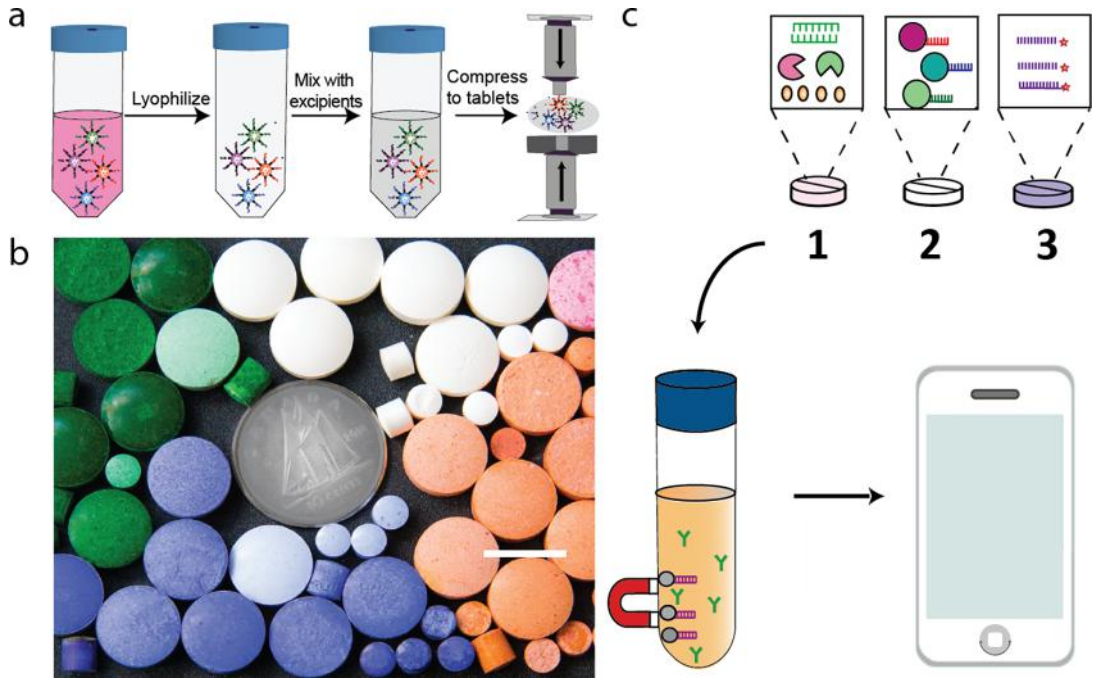

Figure 4. Tableting assay reagents can achieve increased reagent stability. (a) The tableting process is displayed where assay reagents are first freeze-dried and then mixed with excipients to be compressed into tablets. (b) Photograph of developed tablets (scale bar 1 cm) which can be customized into a range of colors and sizes for different purposes. (c) Tablets can be reconstituted into buffers and used in the quantum dot barcode assay buffer with analysis using a smartphone-based fluorescence reader. Images were reproduced from ref 57. Copyright 2017 American Chemical Society.

Verification of the accuracy of a device should at a minimum include metrics such as the limit of detection, analytical sensitivity and specificity, and repeatability.[47] More complex clinical analysis should be done to assess clinical sensitivity and specificity once an initial verification has been completed. These include assessments of positive and negative predictive values, and receiver operator characteristic curves. This analysis can be done during field testing if researchers plan to extend the work. To conduct these analyses on mobile phone diagnostics, samples need to be benchmarked in parallel with the current gold standard of their chosen target.[46] An example of benchmarking was performed by the Ozcan group in their development of a smartphone-based microplate reader.[40] Their device was developed to serve as a point-of-care alternative for ELISA measurements, thus avoiding the need for the standard but expensive clinical spectrophotometers. Therefore, their final reference comparisons were completed with an FDA approved clinical spectrophotometer to provide evidence of their device's performance. Proper benchmarking during initial analytical verification is critical as researchers will base their assessments of a device's capabilities on these results. Researchers will not iterate their devices before transitioning to clinical or field testing if results do not accurately reflect a device's true performance.

Reagent stability is often an underappreciated parameter in mobile phone diagnostic studies; however, it is vitally important. Mobile phone diagnostics are engineered to avoid the use of centralized laboratory equipment which means they do not have access to cold-chain storage. To further emphasize, the FDA recommends that reagent stability testing should be performed alongside analytical studies for in vitro diagnostics.[55,56] These guidelines are recommendations for a 510(k) clearance, which is the regulatory pathway under which previous mobile phone diagnostics have been cleared.[18] For example, we found that our in-house liquid quantum dot barcode assay reagents completely degrade after just 2 weeks at 37 °C.[57] We investigated a solution for improved reagent stability by compressing premeasured quantities of assay reagents into solid color-coded tablets in a high-throughput manner. We were able to maintain reagent stability for testing over 12 weeks. Readout of the quantum dot barcode assay was done using a custom smartphone apparatus which included microscope lenses and lasers for fluorescence readout and was able to detect all three of the hepatitis B infected patient samples tested (Figures 2D and 4).

CLINICAL VALIDATION

Clinical validation is the final stage of mobile phone-based diagnostic development. Here, proof-of concept studies can begin testing with clinical samples and studies that were preclinically verified with clinical samples can extend their technology to field testing. Researchers must ensure that they comprehensively evaluate not only the clinical parameters of their device but also its clinical utility. Simply testing the clinical accuracy of a device does not necessitate the utility for an end-stage user. To accurately assess the clinical utility of a test, researchers can follow FDA recommendations for field testing of in vitro diagnostics.[47] Researchers should consider three parameters: (1) representative testing sites and sample populations, (2) comparisons to clinical reference standards, and (3) testing with representative operators. These three considerations ensure that a diagnostic is validated under conditions that match its final intended use.

Representative testing sites are of critical importance as sites where devices will be deployed suffer from widely variable workspace conditions and climate conditions. To illustrate, the GeneXpert (Cepheid Inc., Sunnyvale, CA), a highly sensitive and specific tuberculosis diagnostic device, had high failure rates when taken into the field in tropical countries.[58,59] Failures were

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attributed to warmer climates, accumulation of dust, and unpredictable power supplies. It is imperative that the environment, climate, and logistical setting during clinical validation matches that of the final intended test setting as these are all factors which can affect the utility of mobile phone diagnostics. Further, representative sample populations should be considered as there is little benefit in evaluating a technology that will not affect or cannot be afforded by the intended study population. An example of a well-conducted field study was demonstrated by the Fletcher group for the analysis of *L. loa microfilarie* patient samples in Cameroon using their smartphone-based video microscope.[12] *L. loa* is endemic in Cameroon with healthcare workers currently using microscopy for detection. Their pilot study included analysis of finger prick blood samples by local healthcare workers and readout using the smartphone device. Their technology diagnoses an endemic disease and integrates seamlessly into the existing diagnostic workflow to provide improved performance and high clinical impact.

Clinical validation is not complete until results are compared to an appropriate reference standard. Unlike in preclinical verification, the goal of using an appropriate standard here is to validate the clinical accuracy rather than assess capabilities for further iteration. The importance is underlined by the WHO as they emphasize evaluation relative to reference standards as one of the four main components of developments of a field trial and as part of their minimum standards.[60,61] Without an appropriate standard, validation of new diagnostics can lead to flawed and biased results.[47] We recommend that researchers match the reference standard to the format of the developed test. For example, serological assays should not be compared with microscopy results, which directly detect a microorganism. Rather, these can be validated with ELISA, nucleic acid tests with PCR, and smartphone-based microscopes with standard microscopy. Further, consideration must be made during field trials of the availability of a reference standard. Often, mobile phone point-of-care studies are attempting to improve on the availability of current reference standards, which may leave their accessibility a challenge to overcome.

An additional consideration to be made during clinical validation is the choice of operators. Operator choice should revolve around which operators the mobile diagnostic is intended for. Levels of complexity must be accounted for as populations of healthcare workers will have more experience and knowledge than others. There are separate FDA approval guidelines for mobile diagnostics intended for home use, which emphasize the complexity and difficulty of a test,[46] rather than stringent evaluations relative to other approved diagnostics.

CONCLUSIONS AND FUTURE DIRECTIONS

Integration of mobile technology into the current point-of-care diagnostic landscape provides promise for the development of more cost-effective and rapid diagnostics. Despite the efforts of several academic groups over the past decade, only a few devices have advanced past a proof-of-concept phase and are actively being used at the point-of-care. Pitfalls can be avoided along the developmental path if researchers consider the challenges emphasized in this review. Instead of jumping onto optimizing and verifying the capabilities of a new technology, researchers must assess the needs, demographics, costs, and relevant geography. The preclinical testing phase should not compromise on comparisons with gold standards, relevant sample types and environmental conditions. Finally, researchers must clinically validate their device in the final intended setting ensuring proper choice of operators and comparisons with clinical reference standards to fully evaluate the performance and clinical utility of their device.

During our review of the current literature, we found that some groups show great diligence in some of these areas but show little to none in other areas. We found that of the reviewed studies only 41% explicitly stated how their device could improve clinical outcomes compared to current laboratory tests. This is problematic as devices that do not provide any added value relative to the gold standard will not be taken up by end users.[22] We also found that very few studies developed diagnostics which could be used by more than one type of mobile phone. Additionally, 75% of the studies intending to design equipment-free diagnostic devices relied on laboratory resources for operation. To successfully transition to field testing, researchers must test the accuracy and stability of their developed devices. However, only 25% of the reviewed studies had analyses which included proper testing for accuracy and reagent stability. We believe that researchers that are aware of these pitfalls can use this review as a guide to develop more thorough and comprehensive studies to push the field of mobile phone diagnostics toward its ultimate end goal and potential.

Mobile point-of-care technology has the potential to disrupt the current diagnostics landscape by decentralizing and democratizing access to testing. Diagnostics will no longer require lengthy turnaround times from resource intensive centralized laboratories. Further, the real-time connectivity of smartphones can increase patient participation by sending results and allowing patients to receive "next-steps" through their devices. Devices could also be integrated into home use and personalized testing. This will require development of personalized home testing kits that are easy to use and mobile-phone-agnostic. The widespread adoption of mobile phones will allow researchers to globally database diseases rapidly and in real-time. Machine learning techniques can be leveraged to analyze these databases to better predict disease onset and progression. These efforts will help lead the next frontier of public health and epidemiological analysis to provide opportunities to curb the progression of disease epidemics. Security of collected patient data and privacy laws are challenges that researchers will need to comply with as the field advances.[29,62] Before the field can reach these heights, researchers must be able to produce objective academic studies which conduct thorough scientific analyses on the capabilities and performances of their devices. Once completed, then we believe the productivity in the field will be greatly accelerated.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.accounts.9b00200.

List of mobile phone diagnostic studies and their application for diagnosis of different diseases (PDF)

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