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Analytical validation of an ultra low-cost mobile phone microplate reader for infectious diseases

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Abstract

Most mobile health (mHealth) diagnostic devices for laboratory tests only analyzed one sample at one time that is inconvenience in performing large volume of serology tests, especially at low-resource settings with shortage of health professionals. In this study, we developed an ultra-low-cost clinical-accurate mobile phone microplate reader (mReader) for laboratory serology tests, and clinical validated this optical device for 12 infectious diseases. The mReader optically reads 96 samples on a microplate at one time. A total of 771 de-identified patient samples in 12 serology assays for bacterial/viral infections were tested. The mReader and the clinical instrument blindly read and analyzed all tests in parallel. The analytical accuracy and the diagnostic performance of the mReader both demonstrated across the clinical reportable categories by comparison with clinical laboratorial testing results. The mReader exhibited 97.59~99.90% analytical accuracy. The positive percent agreement (PPA) in all 12 tests achieved 100%, negative percent agreement (NPA) was higher than 83% except for one test (42.86%), and overall percent agreement (OPA) from 89.33 ~ 100%. We envision the mReader can benefit underserved areas/populations and low-resource settings in rural clinics/hospitals at a low cost (~\$50 USD) with clinical-level analytical quality. It has the potential to improve health access, speed up healthcare delivery, reduce health disparities, and contribute to educating optical sensing science.

Keywords

Mobile health diagnostic device; microplate reader; infectious diseases; Analytical validation; Optical device; Serology tests

1 Introduction

Every year, millions of serology tests are performed in well-equipped central laboratories in the United States, especially for the infectious disease diagnosis in high burden areas. Slow turnaround time and long sample waiting time in line make infection ineffectively control

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and not timely prevent spreading, especially in rural underserved areas and densely populated cities [1]. Decentralizing infectious disease serology testing, rapid tests on site and immediately sharing data on line are the promising actions to control and prevent infection transmission emergently [2,3,4].

To meet the multifunctional needs of analytical sensing and digital health management, mobile health (mHealth) technology supported by mobile devices is rapidly developing, including using mobile communication devices for health information, data collection, diagnostics, etc [5,6,7]. Currently, there are two major streams in mHealth. One is the mHealth applications (Apps) and the other is the mHealth diagnostic/monitoring devices [8,9]. The number of mHealth applications (apps) is increasing rapidly [10,11]. Until 2016, mHealth apps listed on major app stores have grown to 259,000 to help people improve health conditions, such as weight/diet managements, improving sleep quality, emergency first aid/treatment guide, or monitoring vital signs (glucose, heart rate, etc.) [12,13,14]. However, few reported mHealth diagnostic devices in clinical trials for further medical translation [15]. Limited researchers investigated in developing clinical-level diagnostic devices for laboratory tests. One challenge is to tailor the design of mHealth diagnostic devices to the complex diagnostic needs of various diseases and high-throughput diagnosing large-volume samples at one time. Strong clinical, medical translation and engineering capabilities are needed to research, develop, and deploy mHealth diagnostic devices. Due to these challenges, most reported mHealth diagnostic devices remain at academic developing stage. Very few mHealth devices proceed to the clinical translation stage [16].

In this study, we developed and clinically validated an ultra-low-cost mobile phone microplate reader (mReader) using 12 human infectious diseases serology tests. The detection modality of this mReader is colorimetric designed to achieve one-time optical sensing of 96 samples in the microtiter plate. The major technological challenge is unmatched field-of-view (FOV) between the mobile phone camera and the 96-well plates. To address these obstacles, we invented a unique microprism array using our novel low-cost hybrid manufacturing process and integrated it with mobile phones [17]. The FOV of the first prototype was only able to detect 64 samples at one time and just tested at academia stage. In this study, we scaled up the microprism array to enlarge the FOV to the entire microtiter plate for clinical usage. At the same time, we redesigned the power supply system for longer and more stable power supplying. Most important, mReader was evaluated the analytical accuracy and diagnostic performance using a wide spectrum of 12 types of human infectious serology tests with a total number of 771 patient samples towards further clinical transition stage. The results demonstrate the potential of the mReader as a point-of-care microplate reader in rural clinics/hospitals.

2 Material and methods

2.1. Design of the mReader

Figure 1 illustrates the 3D model and the assembled setup of the mReader constructed with the total size of 172 mm x 156 mm x 126 mm. The detecting light source in the mReader is illuminating by 3 identical LED backlight panels in a series circuit (each one is 45 mm x 87 mm x 3.6 mm, 3V DC). The main body of the mReader is a holder for a 96-well microplate,

top and bottom cover lids, two black-painted aperture arrays, a microprism array, one LED backlight panel, and a battery and switch holder. We 3D printed (uPrint SE Plus, Stratasys, Eden Prairie, MN, USA) the main holder and the battery holder using opaque acrylonitrile-butadiene-styrene (ABSplus-P430, Stratasys, Eden Prairie, MN, USA). Two 9V batteries and a DC voltage regulator were used to provide a constant 9V voltage to the backlight panels. A microprism array of 96 prisms was designed and fabricated using the same method introduced in ref [17]. The microprism array precisely aligned above the top aperture array and pressed tightly by a top coverlid. The mobile phone was placed above the prism array at a distance about 81mm.

2.2. Image acquisition, optimization and data processing

The optical sensing parameters, such as focus, ISO, shutter speed and white balance, must fix and are agnostic to the diseases/tests for clinical use. To optimize the optical parameter settings, we took the following two steps. First, we utilized a DC voltage regulator to keep the input voltage constant to provide stable illumination light. Secondly, we developed an iPhone mobile application (App) to control image capture parameters. The size of the App is 31 MB. This self-built App can take pictures with desired parameters to avoid unwanted and inconsistent data errors. Moreover, this App can align the mobile phone camera with the 96 sample wells. Three main channels (color models) that are recorded by the camera are red (R), green (G), and blue (B). Each of these channels covers a range of wavelengths that may vary depending on the type of the camera sensor. The depth of each color channel is 8 bits. The image size of a microplate obtained by an iPhoneSE mobile phone is 2 MB on average.

To explore the fixed optical parameters for enzyme-linked immunosorbent assays (ELISA), we tested two sets of imaging parameters, including ISO 50, ISO 75, ISO 100 and blue gains of 2.0, 2.5, 3.0, and 3.5. All the other parameters fixed as exposure time 0.01 seconds, red gain 1.6, and green gain 1.0. After comparing and analyzing the captured images, the optimized optical imaging parameters confirmed. In this study, we kept the parameters fixed at ISO 50, blue gains 3.0, red gain 1.6, green gain 1.0, and the exposure time 0.01 seconds for all tests.

The self-built App combined with the data processing system. This system can connect with a cloud-based server in the future. At the beginning stage of the mReader, we deployed a MATLAB (Mathworks, MA, USA) Graphical User Interface (GUI) program instead of the cloud-based server. Every sample was taken 5 pictures continuously by the App. Our self-coded MATLAB program analyzed light intensities of a circular area located in the center of each well (a radius of 30 pixels). The average light intensity was calculated and converted to optical density. To correct the non-uniform illumination caused error, we measured optical intensity of each individual well by using blank samples as the reference baseline. The illumination variation across 96 wells is present in Table S1. We found that most variation was between wells located on the center of the backlight panel and wells located on the edges of the backlight panel.

2.3. Testing the mReader with standard samples

To test the mReader, we measured two salivary cytokines, IL-8 and IL-1 β . We purchased the commercial human IL-8 and IL-1 β ELISA kits from Invitrogen (Carlsbad, CA, USA). We followed standard procedures to measure human IL-8 and IL-1 β level in spiked human saliva purchased from Pickering Laboratories (Mountain View, CA). The concentration of serial dilute IL-8 standards and IL-1 β standards spiked in human saliva ranges from 15 to 1000 pg/mL and from 375 to 5 pg/mL, respectively. The mReader in blue channel and a laboratory microplate reader at 450 nm wavelength (Tecan Safire2, Männedorf, Switzerland) measured all samples for two replicates. The readouts were fitted by a sigmoidal dose-response curve governed by Equation (S1).

2.4. Clinical Validation

12 infectious disease serology tests conducted for 771 patients using FDA-approved ELISA kits. All ELISA plates were analyzed in parallel by the mReader and a FDA-approved laboratory instrument (DSX ELISA instrument, Dynex, Chantilly, VA 20151–1621 USA) in the CLIA certified laboratory at the Hospital of the University of Pennsylvania (HUP). The analytical validity of the mReader was evaluated by comparing to the central laboratorial testing results. 10 viral infection serology tests included (1) Varicella zoster virus IgG (VZV), a virus of the etiologic agent of Chicken Pox (Varicella) and Shingles (herpes zoster), (2) Cytomegalovirus IgG (CMV), a ubiquitous human viral pathogen belonging to the family of herpes viruses, (3) Measles virus IgG, (4) Mumps virus IgG, (5) Herpes simplex virus type 1 IgG (HSV-1), (6) Herpes simplex virus type 2 IgG (HSV-2), and (7) the Epstein Barr virus (EBV) four antibody test panel— viral capsid antigen IgM and IgG; Epstein-Barr nuclear antigen (EBNA IgG) and early antigen (EAD IgG) [18,19,20,21,22, 23, 24, 25, 26]. In addition, 2 bacterial infection serology tests include: Lyme disease (*Borrelia burgdorferi*) and *Helicobacter pylori* [27, 28].

All tests were de-identified. All patient samples and in-house controls were 1:21 diluted in sample buffers, except EBV antibody tests, which were 1:101, diluted. All ELISAs proceeded according to the standard operating procedure in the Immunology Laboratory at HUP. All ELISA plates incorporated calibrator/standards, kit-derived positive/negative and in-house controls prepared from patient sera. Both the mReader and the DSX instrument read the optical density (O.D.) at 450 nm. A presumptive immune status ratio (I.S.R.) calculated for each test specimen and control by Equation (1) in the Immunology Laboratory at HUP.

$$ISR = \frac{\text{specimen } O.D.}{\text{correction factor} \times \text{mean calibrator } O.D.} \quad 1$$

The correction factor determined by the manufacturer was labelled on the calibrator vial. For every clinical assay, a medical technologist inspected the wells and checked the quality control values. This study exempted from Institutional Review Board (IRB) review at University of Pennsylvania.

3 Results and discussion

3.1. Proof-of-concept for the mReader

In Figure S1-S6, we tested IL-8 and IL1 β spiked in artificial human saliva, using ELISA kits for proof-of-concept. The good-of-fitness achieved 99.71% for quantifying salivary IL-8 level and 99.07% for quantifying salivary IL-1 β level by the mReader. It showed comparable quantification capability compared with the lab reader for salivary IL-8 level (99.61%) and salivary IL-1 β level (99.38%). The readouts of both instruments showed strong correlation up to 99.61% agreement in salivary IL-8 level reading and 99.81% agreement in salivary IL-1 β level reading. These results demonstrate the reliable quantification capability of our mReader compared with a lab reader.

3.2. Clinical validation of the mReader

In the clinical validation study, we blindly read 919 clinical samples including 771 de-identified patient samples and 148 controls/calibrators covering 12 types of human infectious diseases in the Immunology Laboratory at HUP. Among these, 697 clinical samples, including 597 patient samples and 100 standards and controls, were for human viral exposure testing due to either infection or immunization. A total of 222 samples, including 174 patient samples and 48 calibrators and controls, were for human bacterial infection. We assessed the analytical accuracy of the mReader *via* examining the optical sensing capability in a wide concentration range. To cover the whole clinically required measuring range, we respectively examined minimum and maximum detectable signals by reading kit-derived negative controls, kit-derived positive controls, and in-house controls made from pooled patient positive sera of 12 types of human infectious diseases.

Figure 2 (a)-(f) are present across clinical reportable range, including kit-derived and in-house control, respectively. The readout of VZV (total 142 clinical samples), CMV (total 124 clinical samples), Measles virus (total 87 clinical samples), Mumps virus (total 87 clinical samples), HSV-1 (total 38 clinical samples), and HSV-2 (total 30 clinical samples) obtained by the mReader achieved 98.44%, 98.63%, 97.72%, 98.33%, 99.38%, and 99.90% agreement to O.D. value reported by the DSX instrument, respectively.

Figure 3 (a)-(d) shows the EBV panels of testing 4 antibodies. EBV-CA IgG with a total of 37 clinical samples, EBV-CA IgM with a total of 78 clinical samples, EBV-EBNA IgG and EBV-EAD with a total of 37 clinical samples read by the mReader achieved 99.58%, 98.60%, 98.77%, and 99.42% agreement to O.D. value obtained by the DSX instrument, respectively, across clinical reportable range. The human bacterial infection serology test in Figure 4 included 179 clinical samples tested for Lyme diseases and 43 clinical samples tested for *H. pylori*. The mReader achieved 97.59% and 99.09% agreement with O.D. obtained by the DSX instrument. Data in Figures 2–4 demonstrated the clinical-level analytical accuracy and the reliability across the clinical reportable range with the wide spectrum of human infectious diseases.

Next, we evaluated the diagnostic performance of the mReader by analyzing the diagnostic accuracy, referring to the extent of agreement between the clinically reported categories of the mReader and the FDA-approved instrument. The patient's I.S.R. values for different

human infection diseases were interpreted according to the standard protocols of the HUP Immunology laboratory. The trichotomous outcomes in $ISR < 0.9$, $ISR > 1.1$, and $0.9 \leq ISR \leq 1.09$ are interpreted as negative, positive, and equivocal, respectively for VZV, CMV, Measles, Mumps, HSV-1, HSV-2, and *H. pylori* tests. The EBV panel results are reported negative, positive and borderline corresponding to $ISR < 0.80$, $ISR > 1.1$, and $0.8 \leq ISR \leq 1.1$, respectively. To interpret the results of Lyme diseases, $ISR < 1.0$, $ISR > 1.19$, and $1.0 \leq ISR \leq 1.19$ reported as negative, positive, and indeterminate, respectively.

Table 1 summarized the diagnostic accuracy in a 3×3 table for all 12 types of infectious disease serology testing. Positive percent agreement (PPA), negative percent agreement (NPA), equivocal percent agreement (EPA), and overall percent agreement (OPA) were calculated according to equation (S2)–(S5) in Table S2. The positive percent agreements (PPA) were 100% in all 12 types of bacterial and viral infectious diseases. It clearly illustrates that the mReader is capable to identify 100% of positive clinical samples from 771 patient samples (total 441 positive results). The negative percent agreement (NPA) achieved 100% in testing 7 types of infections, including *H. pylori* bacterium, VZV IgG, HSV-1 IgG, HSV-2 IgG, EBV-CA IgM, EBV-CA IgG, and EBV-EAD IgG. The NPA in assessing Lyme disease, CMV, Measles virus, Mumps virus, EBV-EBNA IgG are 98.57% (138/140), 97.01% (65/67), 83.33% (5/6), 42.86% (3/7), and 90.00% (9/10), respectively.

We further examined the samples reported as equivocal/indeterminate/borderline by the Immunology Laboratory at the HUP. Equivocal/indeterminate/borderline results may indicate possible early infection or potential interference. A new specimen may be collected for repeat testing. The EPA in Lyme disease, *H. pylori* bacteria, Mumps virus, HSV-1, EBV-CA IgM, and EBV-EBNA IgG are 33.33% (1/3), 0% (0/1), 0% (0/4), 0% (0/2), 100% (1/1), and 100% (1/1), respectively. The equivocal cutoff criteria depend on different instruments and ELISA kits. The customized equivocal cutoff criteria for the mReader need future study with a statistically large number of clinical samples. In summary, the overall percent agreement (OPA) for 12 types of infectious diseases ranging from 89.33% to 100% indicated that the mReader can identify 89.33% ~100% of patient samples with the same reported results provided by the Immunology Laboratory at the HUP.

4 Conclusions

In this clinical validation study, we clinically validated the analytical accuracy of a mobile phone microplate reader (mReader) across the clinical reportable range and categories of 12 infectious disease serology tests using 771 patient samples. The mReader attained 97.59% ~ 99.90% of analytical accuracy across the clinical reportable range with a high degree of agreement to the FDA-approved instrument. The diagnostic performance of the mReader was 100% of PPA, 42.86~100% of NPA, and 89.33 ~ 100% of OPA compared with the HUP Immunology laboratory results according to HUP criteria settings. This high diagnostic performance of the mReader demonstrated its capability as the mHealth diagnostic device for clinical use.

The mReader can be massive produced at an affordable cost by combining 3D printing and vacuum forming to fabricate the optical components, and assembling an LED backlight

panel. The estimated total cost for the mReader prototype is ~ \$50 USD. The mReader has the potential to improve healthcare access, especially benefiting the low-resource settings and underserved areas. This ultra low-cost mReader could also have an impact in secondary schools and colleges' education throughout the world.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

(a) The 3D model and (b) the assembled setup of the mReader.

Fig. 2.

The linear correlation between O.D. read by the mReader and the DSX instrument for viral infections: (a) Varicella-zoster virus (VZV) with 142 samples, (b) Cytomegalovirus (CMV) with 124 samples, (c) Measles virus using 87 samples, (d) Mumps virus with 87 samples, (e) Herpes simplex virus type 1 (HSV-1) using 38 samples, and (f) Herpes simplex virus type 2 (HSV-2) with 30 samples, all including kit-derived and in-house control across clinical reportable range, respectively.

Fig. 3.

The linear correlation between O.D. read by the DSX instrument and mReader for the Epstein-Barr virus (EBV) panel: (a) IgM viral capsid antigen (CA) read 37 samples, (b) IgG CA read 78 samples, (c) IgG Epstein Barr nuclear antigen (EBNA) with 37 samples, and (d) IgG early antigen (EAD) using 37 samples, all including kit-derived and in-house control across clinical reportable range, respectively.

Fig. 4. The linear correlation between O.D. read by the DSX instrument and mReader for bacterial infections: (a) Lyme disease with 179 samples, and (b) *Helicobacter pylori* infection with 43 samples, including kit-derived and in-house control across a wide concentration range, respectively.

Table 1.

Diagnostic performance of the mReader by a 3×3 table for 12 human infectious disease serology tests

Bacterial Infectious Diseases							
Test	Criteria ISR	mReader	Positive	Equivocal	Negative	Total	Diagnostic Performance
Lyme IgG/M (n _{patient} =155)	>1.19	Positive	12	0	0	12	PPA=100% (12/12); EPA=33.33% (1/3); NPA=98.57% (138/140); OPA=97.42% (151/155)
	1.0–1.19	Indeterminate	0	1	2	3	
	<1.0	Negative	0	2	138	140	
		Total	12	3	140	155	
H. pylori IgG (n _{patient} =19)	>1.10	Positive	4	1	0	5	PPA=100% (4/4); EPA=0% (0/1); NPA=100% (14/14); OPA=94.74% (18/19)
	0.91–1.09	Equivocal	0	0	0	0	
	<0.9	Negative	0	0	14	14	
		Total	4	1	14	19	
Viral Infectious Diseases							
Test	Criteria ISR	mReader	Positive	Equivocal	Negative	Total	Diagnostic Performance
VZV IgG (n _{patient} =124)	>1.10	Positive	117	0	0	117	PPA=100% (117/117); EPA=N/A (0/0); NPA=100% (7/7); OPA=100% (124/124)
	0.91–1.09	Equivocal	0	0	0	0	
	<0.9	Negative	0	0	7	7	
		Total	117	0	7	124	
CMV IgG (n _{patient} =106)	>1.10	Positive	39	0	1	40	PPA=100% (39/39); EPA=N/A (0/0); NPA=97.01% (65/67); OPA=98.11% (104/106)
	0.91–1.09	Equivocal	0	0	1	1	
	<0.9	Negative	0	0	65	65	
		Total	39	0	67	106	
Measles IgG (n _{patient} =75)	>1.10	Positive	69	0	0	69	PPA=100% (69/69); EPA=N/A (0/0); NPA=83.33% (5/6); OPA=98.67% (74/75)
	0.91–1.09	Equivocal	0	0	1	1	
	<0.9	Negative	0	0	5	5	
		Total	69	0	6	75	
Mumps IgG (n _{patient} =75)	>1.10	Positive	64	4	0	68	PPA=100% (64/64); EPA=0% (0/4); NPA=42.86% (3/7); OPA=89.33% (67/75)
	0.91–1.09	Equivocal	0	0	4	4	
	<0.9	Negative	0	0	3	3	
		Total	64	4	7	75	
HSV-1 IgG (n _{patient} =30)	>1.10	Positive	17	0	0	17	PPA=100% (17/17); EPA=0% (0/2); NPA=100% (11/11); OPA=93.33% (28/30)
	0.91–1.09	Equivocal	0	0	0	0	
	<0.9	Negative	0	2	11	13	
		Total	17	2	11	30	
HSV-2 IgG (n _{patient} =22)	>1.10	Positive	3	0	0	3	PPA=100% (3/3); EPA=N/A (0/0); NPA=100% (19/19); OPA=100% (22/22)
	0.91–1.09	Equivocal	0	0	0	0	
	<0.9	Negative	0	0	19	19	
		Total	3	0	19	22	
EBV-CA IgM (n _{patient} =31)	>1.10	Positive	1	0	0	1	PPA=100% (1/1); EPA=100% (1/1); NPA=100% (29/29); OPA=100% (31/31)
	0.8–1.1	Borderline	0	1	0	1	
	<0.8	Negative	0	0	29	29	

			Total	1	1	29	31	
EBV-CA IgG (n _{patient} =72)	>1.10	Positive	63	0	0	0	63	PPA=100% (63/63); EPA=N/A (0/0); NPA=100% (9/9); OPA=100% (72/72)
	0.8–1.1	Borderline	0	0	0	0	0	
	<0.8	Negative	0	0	9	9	9	
		Total	63	0	9	9	72	
EBV-EBNA IgG (n _{patient} =31)	>1.10	Positive	20	0	0	0	20	PPA=100% (20/20); EPA=100% (1/1); NPA=90.00% (9/10); OPA=96.77% (30/31)
	0.8–1.1	Borderline	0	1	1	1	2	
	<0.8	Negative	0	0	9	9	9	
		Total	20	1	10	10	31	
EBV-EAD IgG (n _{patient} =31)	>1.10	Positive	5	0	0	0	5	PPA=100% (5/5); EPA=N/A (0/0); NPA=100% (26/26); OPA=100% (31/31)
	0.8–1.1	Borderline	0	0	0	0	0	
	<0.8	Negative	0	0	26	26	26	
		Total	5	0	26	26	31	