Performance characterization of Inito Device for quantification of Lateral Flow test strips.

by

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<u>**Scope of the Study Conducted at IIT Delhi**</u>

This report discusses the study conducted at IIT Delhi for the characterization and validation of Fertility Monitor "Inito" using standard Lateral Flow Reader. The device "Inito" is a novel mobile based diagnostics testing system developed by Samplytics Technologies Pvt. Ltd. Bangalore. The Inito reader uses an optical waveguide coupled to the camera of a smartphone. Optical densities, representative of the analyte concentrations, are obtained by processing the images of the chemically impregnated tests strips captured using smartphone reader. The study performed at Laser Applications and Holography Laboratory, IIT Delhi, compares the performance of the Inito device against a lab grade lateral flow assay reader.

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Abstract

The study compares a novel mobile based diagnostics testing system, Inito reader against a lab grade lateral flow assay reader. The aim of the study is to characterize the quantification performance of the Inito reader. The Inito reader uses an optical waveguide coupled to the camera of a smartphone. Test strips were inserted into the reader and images were captured by the Inito app and processed to obtain optical densities representative of the analyte concentrations. Comparison of Inito with a desktop reader (ESEQuant Lateral Flow Reader) for lateral flow assays was performed. Further, the variation of Inito across different phones were measured.

The Inito reader and software was found to agree with the ESE Quant reader with an $R^2 > 0.99$ indicating a high degree of similarity in measurement. The performance across mobile phones from different manufacturers also showed similar agreement of $R^2 > 0.99$. The Inito reader can be an effective alternate to standard lateral flow assay readers while offering the portability of a mobile device. Further, a fertility tracking application of the Inito reader has been discussed and the found that Inito is capable of tracking 2 hormones and providing 6 fertile days.

Introduction

Point of Care (POC) has seen increase in demand at the test site owing to quick diagnosis and testing near the patient site. However, to enable an effective POC system, it needs to be portable, easy to use, offer quick results and not expensive. Mobile diagnostics has been gaining more traction in diverse applications via bypassing the use of bulky instrumentation based tests. Smartphones have been enhancing several features such as processing power, improved sensors and better connectivity. The ability to run tests on smartphones in the convenience of home can overcome the reluctance of taking tests and increases privacy. Several applications (apps) have emerged to track movement, steps, track food intake, reader sleep etc. using inbuilt sensors such as accelerometers and GPS sensors. However, in order to test analytes in blood, urine and saliva, additional devices connected with the phone are required. Patrick et al. reviewed the application of smartphone in healthcare respectively.

To enable a ubiquitous platform that enables testing of the most number of tests at low costs, the mobile POC should be capable of testing multiple assay formats. The following formats cover a range of tests.

Lateral flow assays are a mature technology and perform measurements at low cost. Typical tests that the lateral flow format offers is Vitamin-D, Thyroid (T3,T4 and TSH), Cortisol, infectious diseases such as Dengue, Malaria, Chikungunya, sexually transmitted diseases such as HIV, Herpes, Chlamydia etc.
Vertical flow assays are smaller in form factor but provide results quicker and are used for tests such as Glucose, Cholesterol (LDL, HDL), Hemoglobin etc. Urine dipsticks offer a quick estimation of several analytes in urine such as Ketones, Leukocyotes, microalbumin and so on.
Several mobile phone add-on devices have been developed which can read vertical flow assays. However, a single reader to measure all the three types of strips in a small form factor is still elusive. Hence, Inito can become a single device that functions as a platform to test the three formats of assays.

The Inito Reader

Inito uses a patent pending optical waveguide that captures images of the test strips in an enclosed casing to prevent external illumination from affecting the quantification results. Illumination of the test strip is done by light sources configured for uniform illumination. The waveguide transfers the image of the test strip internally and feeds it to the viewport of the device. The viewport is coupled to the smartphone's camera. In this manner, the camera captures the image of the test strip in controlled lighting. The device consists of calibration charts to ensure illumination and color compensation across different phones. The optic allows to capture a large area in a small form factor thereby enabling all three assay formats to be measured in the same device, namely lateral flow, vertical flow assays and urine dip sticks.

Figure 1: The Inito System applied to a smartphone

Quantification Principle

The image captured by the phone is sent to a remote processing server to obtain quantified results of analyte concentrations

The image processing pipelines can be detailed in the following steps

1. The strip is located using template matching based on Normalized Cross-Correlation(NCC) provided with a threshold of 0.8 for a very high matching accuracy and to avoid false match.

$$R(x,y) = \frac{\Sigma x', y' \left(T(x',y').I(x+x',y+y')\right)}{\sqrt{\Sigma x', y'\, T(x',y')^2 . \Sigma x', y'\, I(x+x',y+y')^2}}$$

   where T is Template Image and I is input source Image.

   NCC is used commonly used to evaluate the degree of similarity between template and source image. The main advantage of normalized cross correlation over other method is that it is less sensitive to changing intensity and template size. [10]

2. Following detection of location of the strips, illumination correction, and background light level is estimated by using a low-pass filtering with a large kernel. The background is then subtracted from the input image to compensate the illumination. The corrected image $g(x,y)$ is obtained from the input image $f(x,y)$ by:

$$g(x,y) = f(x,y) – LPF\left(f(x,y)\right) + mean\left(LPF\left(f(x,y)\right)\right)$$

where $LPF\left(f(x,y)\right)$ is the low-pass filtering of image f (x, y), and $mean$ is the average value of the low pass image.

3. The optical density of strip test lines (OD) is computed from the intensity difference with respect to background. In-order to obtain analyte concentration from OD, we are using 4PL Curve calibration against Elisa Standards or similarly obtained reference curves.

$$y = d + \frac{(a-d)}{(1+ (x/c)^b)}$$

a = response at low concentration
b = absolute value of slope at point of inflection
c = value of x at point of inflection
d = response at higher concentration
x = concentration
y = Optical Density (OD)

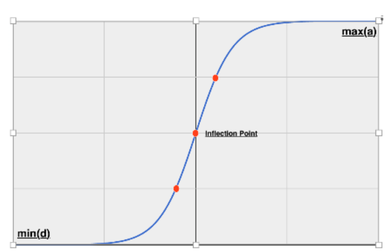

Figure 2: 4PL curve for concentration estimation

The INITO Reader applied to fertility tracking

The Inito Reader is used for ovulation tracking device to helps identify up to 6 fertile days in a cycle. Planning a sexual contact in these days increases chance of getting pregnant faster. The Inito Fertility app along with the reader make an “over-the counter quantitative” urine test to estimate the female fertile window in her ovulation cycle. Detection and quantification of Luteinizing Hormone (LH) and Estrogen (Estradiol-E3G) can be obtained by estimating their levels based on the developed line intensity in strips.

A comparative study was under taken to verify with respect to Strip Characterization Values using Qaigen Output by measuring R-square. This study was undertaken to determine the R-square using Qaigen Readouts and estimated concentration calculated by our INITO device after quantitative processing.

A test strip for measurement of 2 hormones in urine. The Inito fertility reader consists of a compact, mobile connected reader and disposable test strips. The test strips detect LH and E3G in urine. The LH assay is a sandwich assay and the E3G is a competitive assay. The reader is coupled to the smartphone and measures the intensity of the LH and E3G lines and use calibration data to determine the concentration of analytes. Ovulation normally occurs 3 to 4 days after increased estrogen level and 24 to 36 hours after the LH surge. This is the reason why these two hormones are excellent predictor for peak fertility and define the exact science behind tracking ovulation. In the days leading up to ovulation, Female body produces a hormone called estrogen, which causes the lining of uterus to thicken and helps create a sperm friendly environment. The high estrogen levels activate a surge of another hormone called LH (Luteinizing hormone). This LH surge causes the release of the mature egg from the ovary.

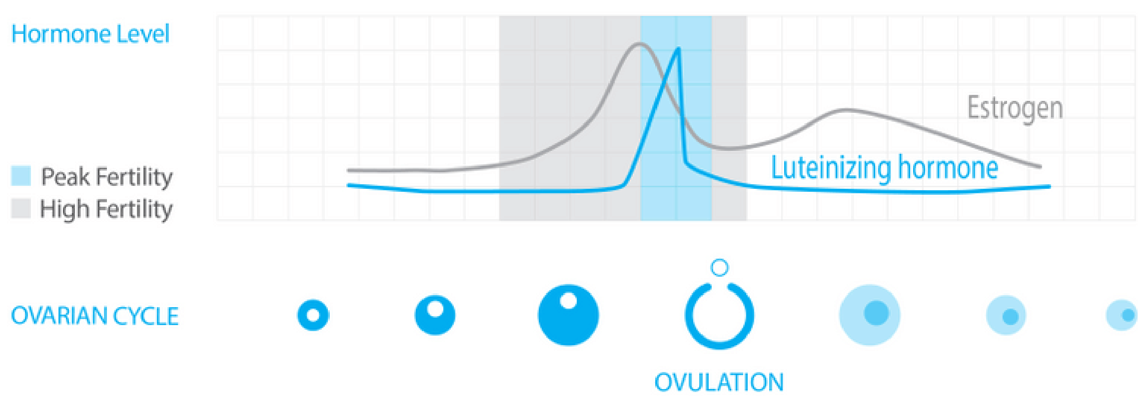

Figure 3: Trends of Luteinizing hormone and Estrogen on days close to ovulation.

Using the trends of LH and E3G, Inito outputs three fertility levels, namely, Low, High and Peak. When the reader outputs Low fertility, it indicates that the user is not in her fertile window and may need to take additional tests on later days. When the reader outputs high fertility, the user is requested to have intercourse every alternate day in this period. Low fertility will be indicated on all days until E3G is seen to rise above the baseline levels. The change from Low to High fertility occurs when a rise in E3G concentration is measured. When LH surge occurs (greater than 10 mIU/ml), the reader declares peak fertility.

For Concentration from OD, we are using 4PL Curve calibration against Elisa Standards from DRG LH-Urine ELISA for LH and ARBOR E3G KIT for E3G. Similar technique of standard curve generation for IgG1 was generated using ELISA and immunoglobulin concentrations (μg/ml) were determined from OD values [9].

Qaigen Reader (ESEQuant Lateral Flow Reader):

Qaigen Lateral Flow Reader is a testing device enabling point of care tests. It detects either fluorescence markers or metal colloids and colored particles. The Qiagen ESE Quant Lateral flow reader can also detect multiple strip lines of different reagents like gold colloids, latex beads, and fluorescence dye. The ESE Quant reader is equipped with a PC-based Lateral Flow Studio Software to interpret inferences from flow assays. Calibration curves can be generated either automatically or manually using its curve-fit functions. The Qiagen reader has been used for DNA Test for HIV Detection at Rice University USA who designed an assay to amplifies qRPA markers in blood. The assay was used with ESEQuant Tube Scanner and the study was published in Analytical Chemistry journal on May 26,2014, [1]. Similarly, a novel qualitative RPA Assay is developed to detect infectious agent causing tularemia for Francisella Tularensis detection and the assay was used with ESEQuant Tube Scanner and study was published in Journal of Clinical Microbiology [2]. The same ESEQuant reader is used to develop assays of polyclonal antibodies to detect beverages of soy and nuts contaminated with allergens modified during manufacturing published in Journal of Food Protection, Vol 79,No 9,2016 [3]. Further, the ESEQuant LR3 Reader have also been used to develop a test enabling quantitative detection of HPV antibodies for Oral Cancer caused by Human Papillomaviruses (HPV) colonizing the oral cavity [4].

Figure 4: The ESEQuant Lateral Flow Reader

Considering the existing studies and usage of ESEQuant Scanner for different Assays Development and testing, the scanner was a good candidate to characterize the quantification performance of Inito reader.

Experimental Methodology for the Characterization:

To characterize the performance of Inito reader with reference to ESEQuant reader, a set of sample strips, representing six different concentration levels (0, 10, 20, 40, 100 and 200 mIU/ml) of the LH sample were used to perform the experiments. These strips were scanned for all the six concentrations using Qiagen Reader. The experiments were repeated thrice to study the variability. Similar study was performed for E3G samples, wherein each set contains the strips with six concentration levels i.e. 0, 6.25, 12.5, 25, 50, 100 ng/ml.

a. Experimental Observations for LH Samples

Two valleys were observed in case of each LH sample. Peak intensity data was extracted for both the valleys. Peak intensity values corresponding to six different concentrations were extracted and respective calibration curves were plotted separately. Logarithmic curve fitting was used to get the calibration curve. These calibrations curves yield very high correlation coefficient (~0.99). This exercise was repeated for both the valleys.

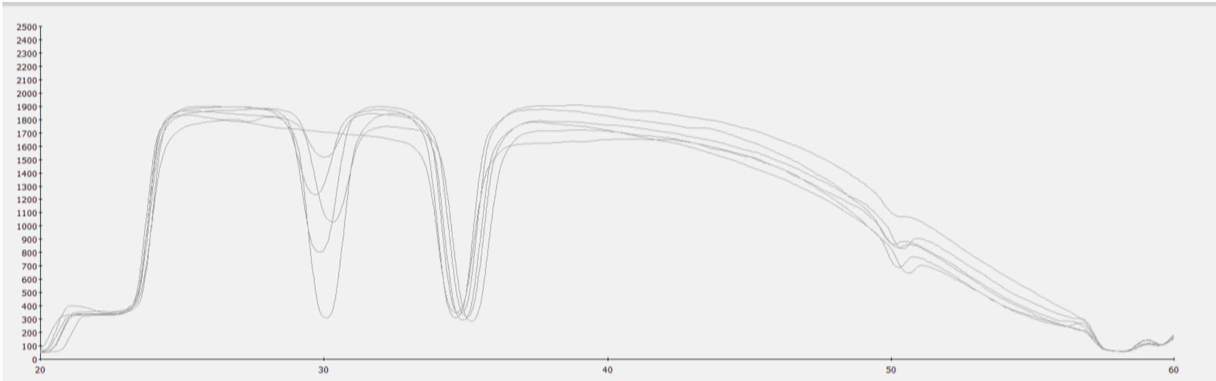

Figure 4 (a): LH samples scanning for Valley 1

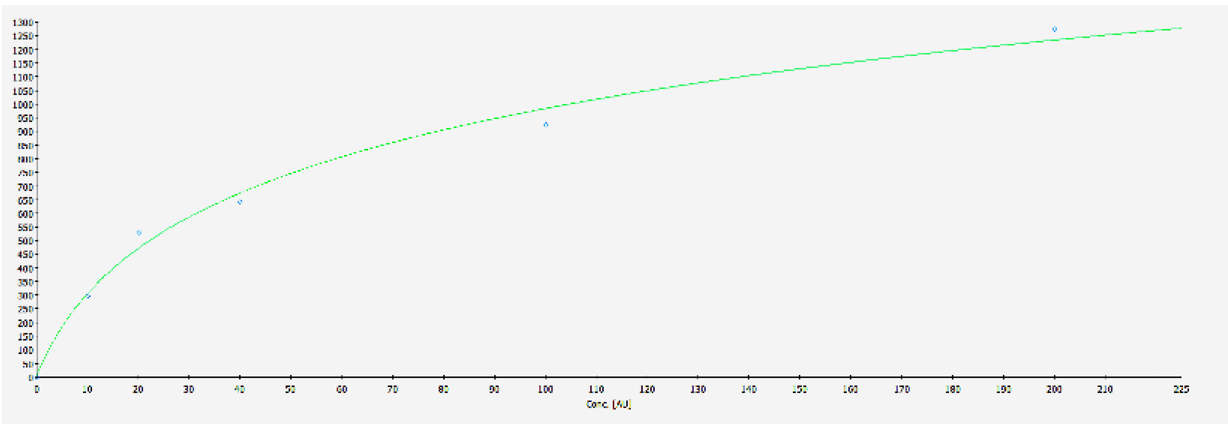

Figure 4 (b): LH samples calibration for Valley 1

Reliability and repeatability of the performance of the reader for LH tests was ensured by repeating the entire set of experiments thrice. The read out data points for these three experiments, depicting the optical density (OD) values at six concentrations are provided in Tables 1. The coefficient of variation was less than 1 for all the six concentration values.

Sl. No.	Concentration	Read out (OD)					
		Experiment 1	Experiment 2	Experiment 3	Average	Standard Deviation	CV (%)
1	0	0	0	0	0		
2	10	297.87	297.18	298.89	298.0	0.9	0.3
3	20	530.83	531.58	536.09	532.8	2.8	0.5
4	40	644.94	677.32	676.39	666.2	18.4	2.8
5	100	925	920.36	935.83	927.1	7.9	0.9
6	200	1275.71	1278.06	1285.26	1279.7	5.0	0.4

Table 1: Optical read out Data at different concentration values and its repeatability analysis for LH Tests

b. Experimental Observations for E3G Samples

Procedure used for LH test was followed for the screening of E3G samples. Contrary to LH samples, only one valley was observed in this case. Calibration curve was derived from the scanned results using Rod bard curve fitting method. The correlation coefficient was found to be > 0.99 for all the experiments.

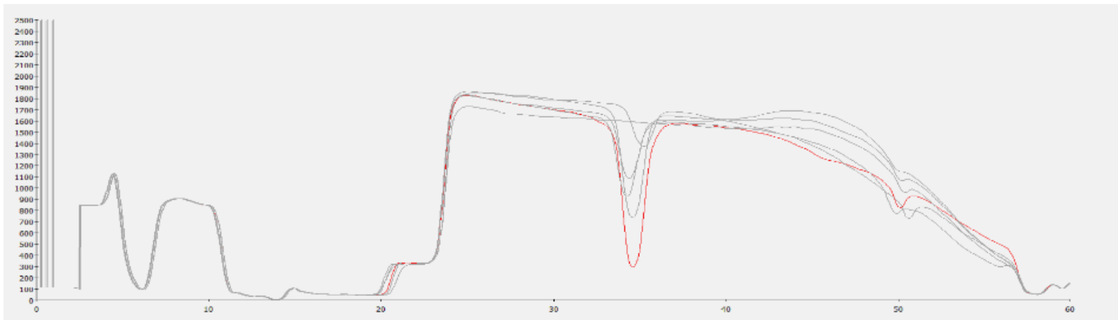

Figure5 (a): E3G sample scanning

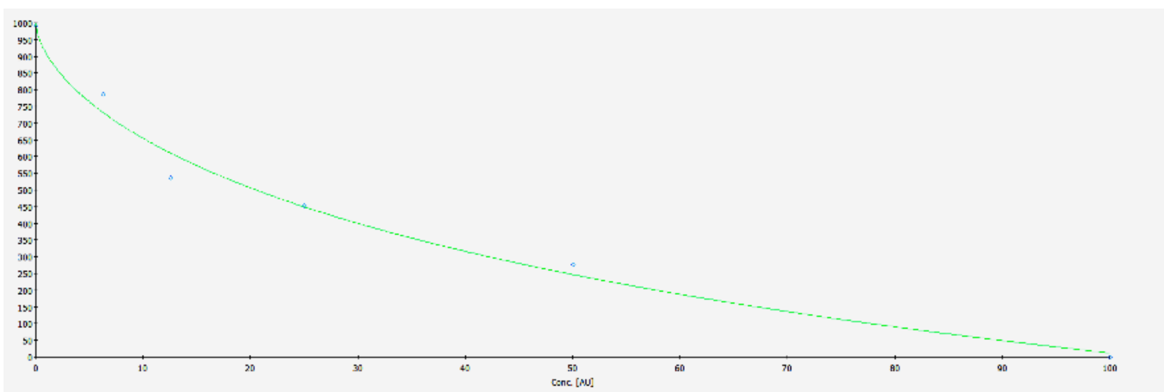

Figure 5 (b): E3G samples calibration curve

Similar to the LH tests, repeatability of the Qiagen reader was evaluated by repeating the entire set of experiments thrice. The read out data points for these experiments are summarized in Table 2. The variation was well within the acceptable limit at all the concentration levels.

Sl. No.	Concentration	Read out (OD)					
		Experiment 1	Experiment 2	Experiment 3	Average	Standard Deviation	CV (%)
1	0	993.47	1035.71	995.31	1008.2	23.9	2.4
2	6.25	787.37	801.1	804.11	797.5	8.9	1.1
3	12.5	537.62	581.39	566.35	561.8	22.2	4.0
4	25	454.04	470.02	443.83	456.0	13.2	2.9
5	50	277.6	290.81	279.72	282.7	7.1	2.5
6	100	0	0	0	0	0	0

Table 2: Optical read out Data at different concentration values and its repeatability analysis for E3G Tests

c. Characterization using Inito Device

Both, LH and E3G tests were performed for the same set of sample strips using Inito device. Here, the sample cartridge was inserted into the strip reader. Four different mobile phones namely LetV, HTC, Nexus and Samsung were used as strip reader and the data was recorded for each type of the phone using Inito device. Each mobile phone shows the status whether the scan results are accepted or not depending upon the success of the tests as shown in Table 3. In case the scans are accepted, the device gives the intensity values corresponding to different concentration levels can be extracted from the device from the back end data corresponding to all the six sample concentrations.

LH Tests using Inito Device

The LH tests were performed using six different sample strips, representing different concentration values 0,10,20,40, 100 and 200. First, HTC mobile phone was plugged into the Inito device and the scan was performed. The same sample strips were used in the Qiagen reader to perform the LH tests. Both the findings were compared to study the correlation between the two methods. These experiments were also repeated thrice to ensure the stability and repeatability of the Inito device. A curve fit model was used to fit the OD data to a sigmoid curve. The R^2 value was found > 0.99 in these studies. This demonstrates a very good correlation between the studies performed using Qiagen reader and the Inito device using HTC mobile as the reader. These experiments were repeated using other three mobile phones and a correlation map was established with respect to Qiagen reader in each case. All of the experimental results using the Qiagen reader and the Inito device exhibit a very high correlation with R^2 values. This has been summarized in Fig. 6 (a) and Table 3 (a).

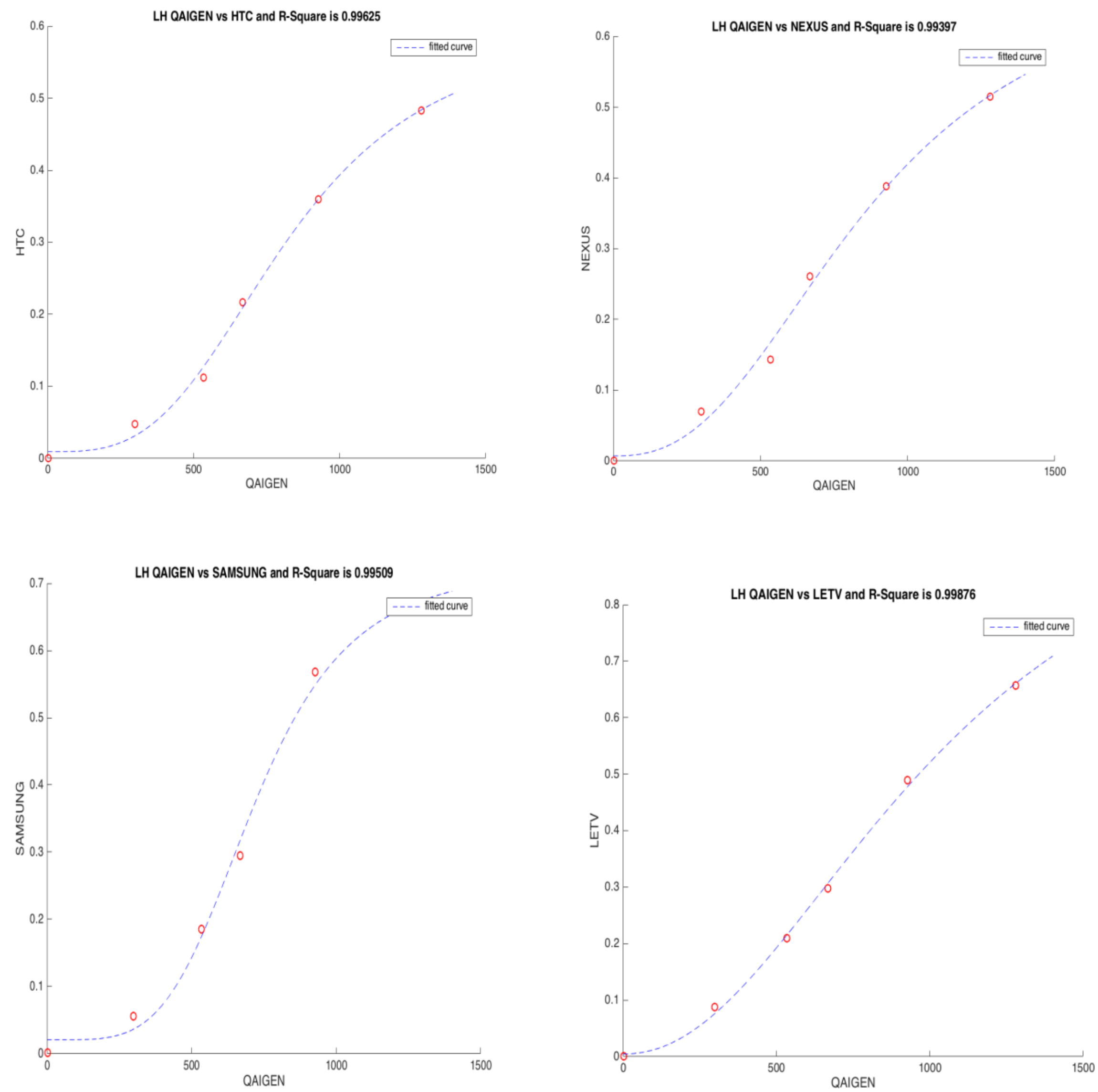

Figure 6 (a): Performance evaluation of the Inito devices for LH test using four different mobile phones as the Reader with reference to the Qiagen Reader

Sl. No.	Read outs from Qiagen Reader	Read outs from Inito Device using different mobile phones as Reader fitted by a 4PL curve			
		HTC	Samsung	Nexus	Let V
1	0	0	0.001	0	0
2	298.0	0.048	0.056	0.070	0.087
3	532.8	0.112	0.185	0.143	0.209
4	666.2	0.217	0.295	0.261	0.298
5	927.1	0.360	0.568	0.388	0.489
6	1279.7	0.483	0.663	0.515	0.657
R^2 values (Reference: Qiagen Reader)		**0.996**	**0.995**	**0.994**	**0.999**

Table 3 (a): Study of the performance of Inito Device using different mobile readers with reference to HTC

A comparison was made between the performances of the Inito devices when they were plugged in with different mobile phones. For e. g. the LH scan data sets using HTC and Nexus readers on the same Inito Device were extracted and compared. Similar comparison was also performed for Samsung vs Nexus and LetV vs Nexus. Very high correlation was found in all the cases with R^2 values being greater than 0.99 in all the three comparisons as shown in Fig. 6 (a).

Sl. No.	**Read outs from HTC Mobile in the Inito Device**	**Read outs from different mobile readers when plugged into the Inito Device**		
		Nexus	**Samsung**	**Let V**
1	0	0	0.001	0
2	0.048	0.070	0.056	0.087
3	0.112	0.143	0.185	0.209
4	0.217	0.261	0.295	0.298
5	0.360	0.388	0.568	0.489
6	0.483	0.515	0.663	0.657
R^2 values (Reference: HTC Mobile in Inito Reader)		**0.996**	**0.988**	**0.993**

Table 3 (b): Comparison of the performance of Inito Device using different mobile phones as Reader with reference to the HTC mobile as the Reader when plugged into the Inito Device

E3G Tests using Inito Device

The procedure similar to the LH tests, was followed for the E3G tests using Inito device to characterize its performance. The E3G tests performed using Qiagen reader and the Inito device using different phones are summarized in Fig. 6 (b). A curve fit model was used to fit the OD data to a sigmoid curve. Very good correlation (R^2>0.99) was observed between the performances of the two devices i.e. the Qiagen reader and the Inito Device.

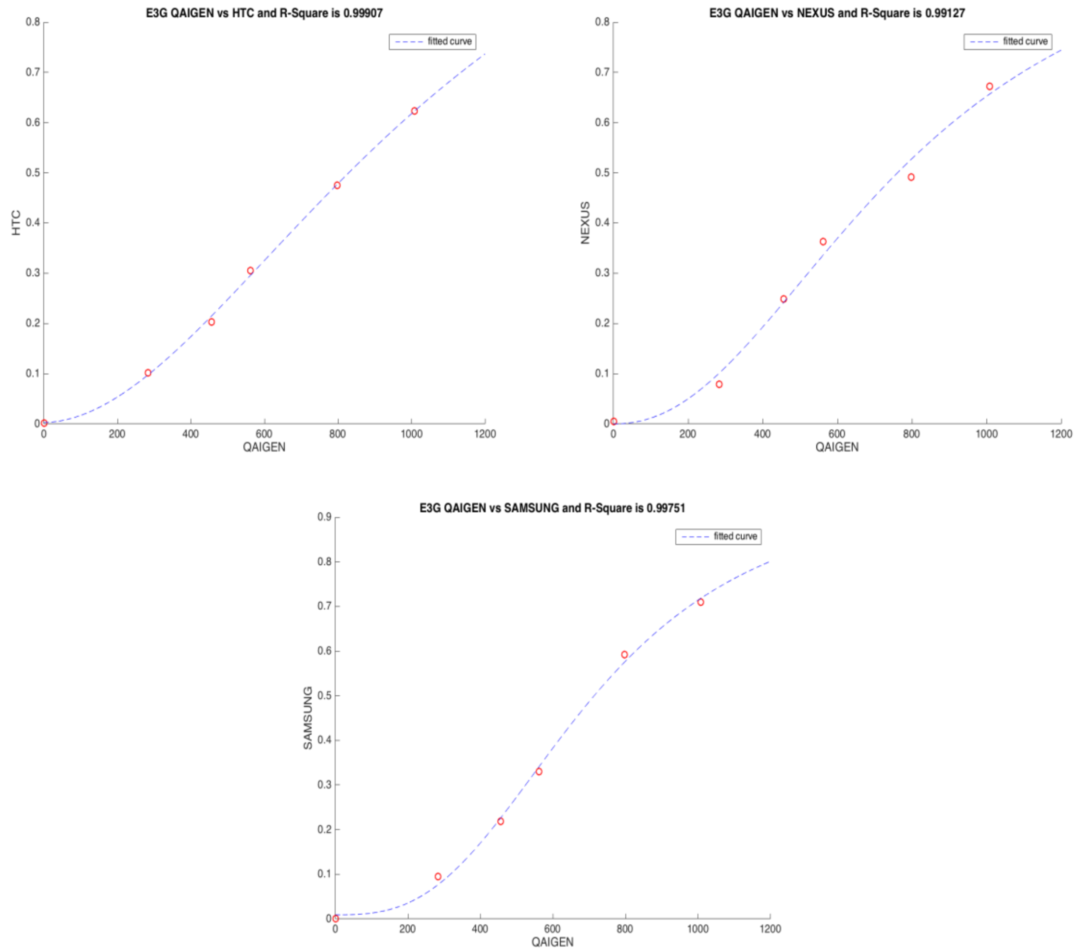

Figure 6 (b): Performance evaluation of the Inito devices for E3G test using different mobile phones as the Reader with reference to the Qiagen Reader

The performance of the Inito device for different mobile phones was also studied and compared to understand the feasibility of different mobile phone readers in the Inito device. A good correlation was observed amongst the performances of different mobile phone readers when plugged into the Inito device.

Conclusion

Performance of the Inito reader has been found to be in line with that of the Qiagen Reader with very high correlation between the two devices. This study was performed using four different mobile phones as the reader, when plugged into the Inito device. It was observed that the performance of the Inito device had very high correlation with that of the Qiagen reader irrespective of the mobile phones used. Therefore, it can be concluded that the Inito device when plugged in with any of the four mobile phones described above can be used for the LH and E3G tests.

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