

AN INTEGRATED MICROFLUIDIC LAB-ON-A-CHIP PLATFORM AND ANALYZER FOR RAPID AND WIDE DYNAMIC RANGE DETECTION OF QUANTITATIVE β -hCG IN EMERGENCY MEDICINE

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ABSTRACT

To provide a point-of-care testing platform for quantitative β -hCG measurement, we have developed an integrated microfluidic lab-on-a-chip capable of detecting β -hCG from ~ 5 to 100,000 mIU/ml rapidly (< 20 min) can be used in a wide variety of emergency management to improve workflow and OB/GYN settings to increase quality of care.

KEYWORDS: Point-of-care Testing (POCT) platform, Lab-on-a-chip (LOC), Wide dynamic range detection, Quantitative β -hCG immunoassay

INTRODUCTION

Quantitative determinations of hCG are used to predict complications especially in early pregnancy. Ectopic pregnancy is a major cause of morbidity and mortality in reproductive-aged women, accounting for 9% of pregnancy-related deaths in the first trimester. During the first six weeks of pregnancy, serum hCG concentrations rise linearly and rapidly with a doubling time of 1.3 to 2 days [1]. The current approach for quantitative β -hCG testing use a 96 well platform which typically takes ~ 4 -8 hours in central lab. We propose a microfluidic lab-on-a-chip platform using a chemiluminescence based sandwich immunoassay for high sensitivity detection of protein biomarkers.

DESIGN AND FABRICATION

As shown in Figure 1, the lab-on-a-chip consists of multiple liquid reagent reservoirs connected on one end to a series of detection chambers where microbeads coated with the capture antibody [2], and on the other end to Siloam's proprietary on-chip pressurized gas generator module [3]. The capture antibodies targeted towards the beta chain of the hCG molecule are differentiated by their affinity towards β -hCG.

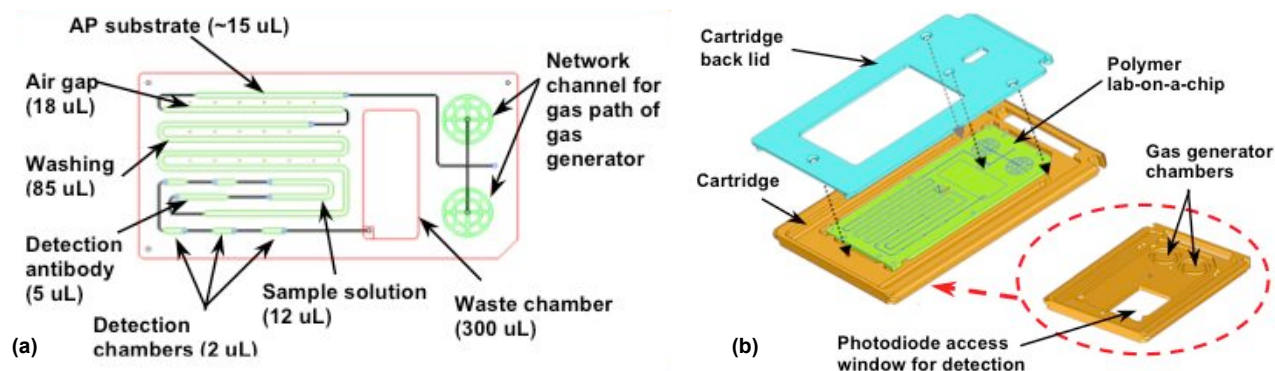


Figure 1: Integrated microfluidic lab-on-a-chip platform for POC testing in ED and OB/GYN setting; (a) Schematic diagram of the developed lab-on-a-chip (76mm \times 38 mm) and (b) Assembly method of microfluidic LOC with cartridge

After loading all reagents, the microfluidic lab-on-a-chip is assembled with an interface cartridge as shown in Figure 1(b). The sample is injected using a standardized interface port. The packaged cartridge is loaded in the analyzer that initiates further sequencing as shown in Figure 2(a) and (b). The patient sample is then pushed to the detection chamber using Siloam's proprietary pressurized gas generators (completing the sandwich assay). After the labeled detection antibody is injected, the detection chambers are then washed with on-chip stored buffer and finally the chemiluminescence substrate is injected.

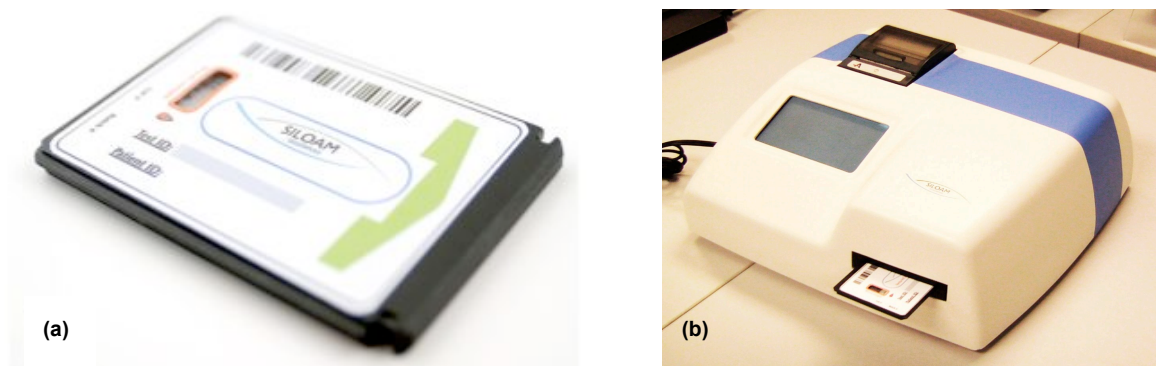


Figure 2: (a) Assembled and integrated microfluidic LOC platform and (b) Developed analyzer system with the front-loading mechanism and touch screen.

RESULTS AND DISCUSSION

The wide dynamic range detection was accomplished in this design by using an array of detection chambers some of which contain capture antibodies with increasing affinity to the target analyte. The capture antibodies were chosen such that in instances when the analyte concentration is very low, only the highest affinity capture antibody can capture sufficient number of analyte molecules to generate a detectable response as shown in Figure 3.

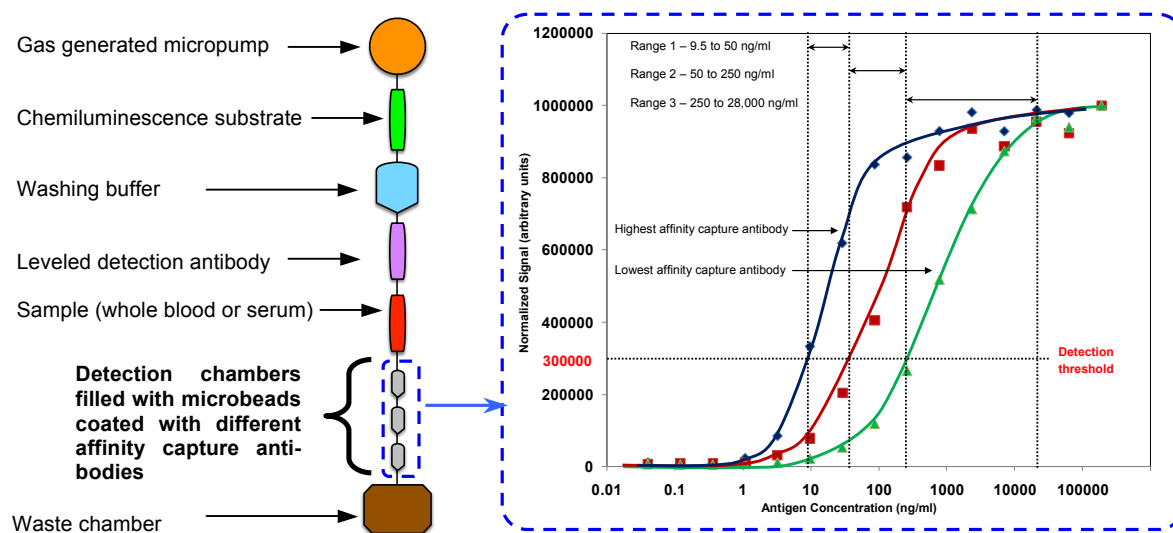


Figure 3: The wide dynamic range is accomplished in this design by using an array of detection chambers some of which contain capture antibodies with increasing affinity to the target analyte.

The optical signal for β -hCG was measured in 20 min by a customized photo-detection unit of the developed analyzer system shown in Figure 2(b). A calibration curve was developed for low and high affinity antibodies as shown in Figure 4. The threshold for low and high affinity antibodies were set to 0.5 and 0.44 units, respectively. The detection range for β -hCG was defined as $\sim 15 - 100,000$ ng/mL (in the clinically relevant range for β -hCG from $\sim 5-100,000$ mIU/mL).

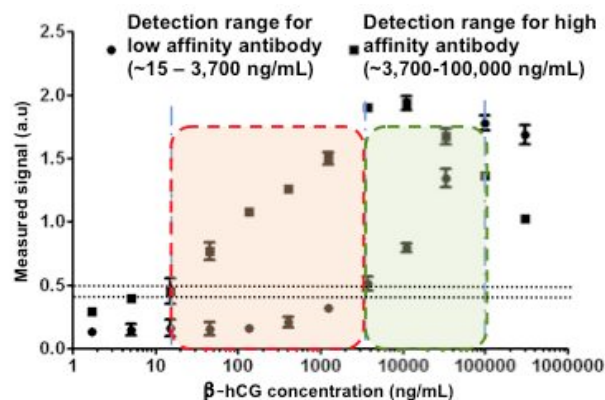


Figure 4: Calibration curve measured for the low & high affinity antibodies using the proposed LOC platform.

Table 1: Interpretation rule for unknown β -hCG concentration

Signal from Low affinity Ab	Signal from High affinity Ab	Interpretation
< 0.44 units	< 0.44 units	Analyte concentration lower than detection threshold.
< 0.5 units	> 0.44 units	Use signal from High affinity Ab only to calculate unknown analyte concentration.
> 0.5 units	NA	Use signal from Low affinity Ab only to calculate unknown analyte concentration.
> 1.78 units	NA	Analyte concentration is higher than detection limit.

CONCLUSION

The proposed β -hCG POCT can have the greatest impact as a preliminary diagnostic tool for ectopic pregnancy and monitoring high-risk pregnancies. Each detection chamber is “tuned” to operate in a different concentration range with a $\sim 100x$ assay functional range. The proposed POCT platform represents the key innovation allowing for quantitative detection of β -hCG across the complete desired range ($\sim 5-100,000$ mIU/ml) in 20 min.

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