Performance evaluation of low cost microfluidic chips made using a digital craft cutter for point of care applications in nucleic acid tests

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***Abstract*—A point of care (POC) diagnostic system development for nucleic acid testing (NAT) for developing countries faces several challenges and barriers among which affordability is a very critical one [1,4]. Hence a study was made to evaluate the effectiveness of microfluidic chips made from a digital craft cutter to be used as a disposable cartridge. Low cost materials like double sided tapes, transparent sheets and connectors were used to realize the microfluidic chip [2]. An in-house IVD sample preparation kit for nucleic acid extraction was used as a representative assay. Modifications were made to the assay workflow considering the feature sizes, design and volume of the microfluidic chip made from the paper cutter and other POC system requirements like turnaround time (TAT). The workflow was optimized by reducing overall TAT from 50min to 15min, sample volume from 150 µL to 12.5 µL and reduced reagent volumes. The method was also optimized to work at an isothermal condition. The results showed good correlation and yield in terms of both quality and quantity when compared to results obtained from the established baseline protocol. Thus microfluidic chips made using a digital craft cutter can very well be a low cost alternative to manufacture disposable chips for POC applications in nucleic acid tests.**

I. Introduction

Point of care (POC) nucleic acid testing (NAT) for infectious diseases has a major role to play in better patient management and disease containment in developing countries where the disease burden is high [4,5]. Among several challenges and barriers identified for development and implementation of such tests, like rapid turnaround time, simplified workflow, infrastructure and performance requirements "affordability" of the tests is a major factor which determines the acceptance of a test [1]. Cost per test for a POC test is often more compared to a similar central lab test, if the central lab overhead costs are not considered [4]. In most practical cases there is a need for discounts and subsidies to be provided on the market price for making POC tests affordable for usage in developing countries.

A disposable cartridge is usually used in a POC system and one of the major costs in a typical cost break up of a cartridge is material cost [6]. Possible cost reduction methods need to be explored in the raw materials used and manufacturing process employed to make the cartridges. Hence we explored the use of a low cost method to fabricate and manufacture microfluidic chip commonly used as part of a cartridge. A low cost method for rapid prototyping of microfluidic devices using a desktop digital craft cutter, double sided tapes and transparency films was published by Yuen et al. in 2009 [2]. The ability to fabricate complex fluidic devices quickly by using inexpensive equipment and raw materials was reported. Since fabrication process and raw materials used were inexpensive, the cost per device would be much lower compared to the conventional manufacturing processes. A rough estimation of the manufacturing cost for microfluidic devices considering only the current list prices of the raw materials without including the instrument and labor costs is 0.2USD approximately. Thus a clear cost advantage is established by employing this procedure compared to similar microfluidic chips available in the market. However no functional evaluation of the microfluidic devices was reported to show its effectiveness to be used in a POC NAT system. Thus the aim of this article is to perform a functional evaluation of microfluidic devices manufactured by the above procedure.

An in-house, commercial extraction kit (VERSANT Sample Preparation 1.2 Reagent kit) was used for the functional evaluation of the devices fabricated from the craft cutter. The kit employs silica coated magnetic nanobeads coated with nanolayer of silica for DNA extraction [3] from whole blood. The kit allows manual DNA extraction procedure from whole blood which was modified to facilitate the evaluation of the chips. The modifications included reduction in reaction volumes to suit the microfluidic chip volumes, decreasing the turnaround time, and maintaining isothermal conditions for the whole extraction procedure for ease of use. A series of optimizations were made to the original workflow and the performance at each level was compared with the original protocol. These optimizations make the original manual SP 1.2 protocol more suitable to be realised on a point of care device. Finally this modified protocol was run on the microfluidic chips and the performances were compared to standard protocol. The DNA extracted from the chip was also subjected to a real time PCR to establish the quality and amount of DNA extracted compared to the manual workflow.

II. Materials & Methods

A. Microfluidic chip fabrication

Digital craft cutter called 'Cameo' was procured from Silhouette America along with cutting blade and cutting mat. 3M™ Double Coated Tape (Cat# 55257) made of polyester (PET) was procured of 25mm width and having a thickness of 0.142mm. Clear transparent OHP sheets were procured. A microfluidic chip sketch was made using SolidWorks design software as per the required design such that the total liquid

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978-1-4244-7929-0/14/$26.00 ©2014 IEEE

volume that the chip can accommodate is approximately 35 – 40 µL. The sketch was then opened through Silhouette Studio® software to be sent to the cutter. The double coated tape was pasted on the cutting mat with the aid of a metric ruler on the mat in the correct location where the sketch pattern had to be cut. The blade depth was set to the maximum to enable cutting of the PET tape material. The cutting mat with the tape was fed into the cutter and the software instruction was fed to the instrument to cut the sketch pattern.

After the cut was made the unwanted tape material was removed where fluidic channel would be present and the protection on the top adhesive layer was removed. Transparent OHP sheets were cut to a length of 75mm and a width of 25mm and pasted on the exposed adhesive on the tape in an orientation such that the transparent sheet's width orients itself with that of the tape. Another transparent sheet of the said dimensions was taken and two holes were punched at locations where the entry and exit of the fluidic channel would be present. The double coated sheet was removed from the cutting mat and the second transparent sheet was pasted on the second adhesive side of the tape. Care was taken to ensure the two punched holes on the transparent sheet align itself with the entry and exit of the fluidic channel. The fluidic pattern was cut on the double coated tape and the bottom and top sides were closed with transparent sheets, with the entry and exit ports cut into the top sheet. Thus, the microfluidic channel was fabricated. 20µL pipette tips were pasted on the entry and exit of the channels using an epoxy putty adhesive, to act as connectors. The length of the tip was cut as per requirement. Figure 1 shows the finished microfluidic chip cut using the above method.

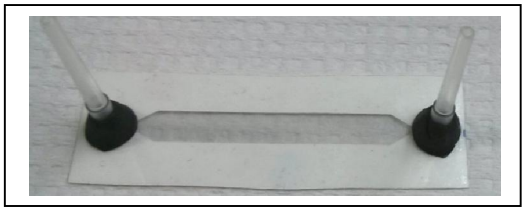

Figure 1. Figure shows the complete microfluidic chip fabricated using digital craft cutter.

The chips were then washed thoroughly by passing DNase, RNase free distilled water through the channel using tubes and syringe (10mL syringe from Hi-Tech Hospital & Healthcare Corp Ltd.) connected to the ports. The syringe was operated manually to transfer a certain volume of water through the chip. The chips were then dried in a hot air oven.

B. Blood sample collection

2mL of whole blood was collected from 3 healthy volunteers in K2 EDTA coated collection tubes (Cat# 367842, BD Vacutainer™). The samples have been anonymized after signing an informed consent, by a phlebotomist at the site clinic. These samples were stored in the Vacutainers at 4°C until extraction.

C. Operation of the microfluidic chips

Figure 2 shows the microfluidic chip manufactured using the digital craft cutter connected to an aspiration tube at the entry and a syringe at the exit. The syringe is manually operated back and forth to pull or push the liquid through the chip as per the requirement.

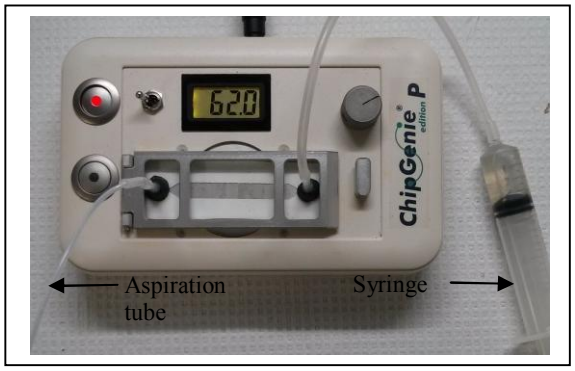

Figure 2. Figure shows microfluidic chip connected to sipper tube, syringe and mounted on ChipGenie(R) edition P instrument.

ChipGenie(R) edition P instrument was procured from Microfluidic-Chipshop GmbH. This is an on-chip sample preparation system which has a temperature controller and a linear moving permanent magnet below the section where chip is loaded. Figure 2 shows the ChipGenie(R) edition P instrument with the microfluidic chip loaded on it with the syringe and sipper tube to operate the chip.

D. Experimental plan and workflow optimization

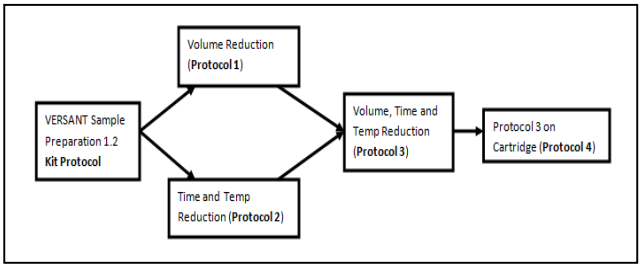

Figure 3. Figure explains the experimental plan.

For functional and performance evaluation of the microfluidic chip made from the digital craft cutter, VERSANT Sample Preparation 1.2 Reagent kit (Cat# 10629800 & 10629801) procured from Siemens Healthcare Diagnostics Ltd. was used. Figure 3 explains the experimental plan wherein a series of modifications were made to the kit protocol so that the assay could be tested on the chips fabricated. Table I details the kit protocol.

TABLE I. VERSANT SAMPLE PREPARATION 1.2 REAGENT KIT PROTOCOL

Workflow steps	*Volume (µL)*	*Temperature (°C)*	*Incubation Time (minutes)*	*Speed (RPM)*
Sample	150			
Pretreatment buffer	375			
Proteinase K	20	62	10	
Lysis buffer	825			
Magnetic particles	25	62	15	1100
Wash buffer 1	850			
Wash buffer 2	450			
Wash buffer 3	450			
Elution buffer	70	74	10	1100

To enable the functioning of the above protocol on the micro-fluidic cartridge, optimization and modification of the protocol was necessary. The performance of the optimized protocol was compared to the manual SP 1.2 protocol. The micro-fluidic cartridge fabricated above can accommodate for a maximum total volume of 35-40 µl at any given step. Accordingly, the starting blood sample volume was 12.5 µL or less and the elution buffer used was 100 µL to recover DNA eluted via this cartridge process. In order to achieve these optimizations, 4 protocols were tested:

The first modification made to the SP 1.2 protocol was to reduce the sample volume to 12.5µL (Protocol 1). The volumes of the buffers used were proportionately reduced and the elution volume was kept constant at 100µL. The next set of modifications was to make the method an isothermal process and to reduce the turnaround time of the whole experiment (Protocol 2 based on kit input 150). The elution conditions were hence altered to 62°C for 5 min., and the processing times for pre-treatment and lysis were reduced to 1 min., and 5 min., respectively. An additional method (Protocol 3), a combination of the previously modified protocols (1 and 2), was also tested, for direct translation to the cartridge. Protocol 4 was tested using the micro-fluidic cartridge, employing the same processing conditions as in Protocol 3. The above section explains the operation of the microfluidic chip. Figure 4 shows the microfluidic chip at the lysis step of the protocol.

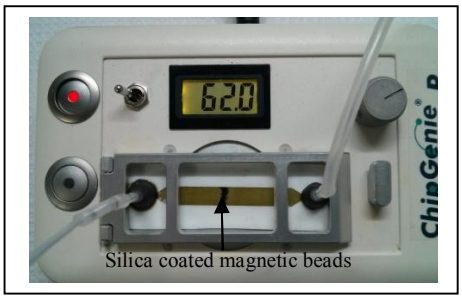

Figure 4. Figure shows microfluidic chip at the lysis stage of the protocol

All the workflow optimizations (Protocols 1, 2 & 3) were carried out manually. Dynamag^{TM} 2 magnet procured from Thermo Fisher Scientific Inc was used for magnetic bead separation. Thermomixer$^{(R)}$ comfort from Eppendorf AG was used to incubate the tubes at higher temperatures and also to shake the tubes at a required RPM. All experiments were performed on three different days using three different blood samples. Each blood sample was subjected to DNA extraction five times using each of the five protocols. The modified protocols (1, 2, and 3), and in turn, the cartridge protocol (4), were tested for quality and quantity of DNA extracted and assay repeatability. Four different quantitative, PCRs were run to analyze the DNA extracted from SP 1.2 Reagent Kit and protocols 1 to 4.

The samples extracted were assessed for the DNA amounts on Qubit® 2.0 Fluorometer (Q32866, Invitrogen™) using the dsDNA HS Assay Kit (Q32854, Invitrogen™). Extracts were also analyzed using quantitative PCR for the PAEP (Progesterone-Associated Endometrial Protein) gene constitutively present in the human genomic DNA. The KAPA SYBR® FAST qPCR Kit Master Mix (2X) (KR0390, KAPA BIOSYSTEMS) was used to run the PCR, with the forward primer 5'-CACAGAATGGACGCCATGAC-3' and reverse primer 5'-AAACCAGAGAGGCCACCCTAA-3' against the PAEP gene [7]. Experiments were performed on the Applied Biosystems® 7500 Real-Time PCR System (4351104, Life Technologies™) with an initial denaturation of 3 minutes at 95°C, Cycle denaturation for 10 secs, and annealing at 60°C for 30 seconds. The cycle denaturation and annealing were performed for 45 cycles. A 20 µl PCR reaction volume was used in this assay was 10µl of KAPA SYBR® FAST qPCR Kit Master Mix (2X); 10µM of forward and reverse primers were used at a final concentration of 200nM; template DNA was added at 12 ng/ 20 µl reaction volume.

III. Results & Discussions

Table II summarizes the yield of the DNA extracted for kit protocol, protocol 1, 2, 3 and 4 from samples 1, 2 and 3 and a negative sample control, which is water, respectively. The DNA yield reported in ng is the average of 5 replicates run for each sample under each protocol. Coefficient of variation (CV) percentage for each sample under each protocol is also reported in brackets. Table III summarizes the Ct values for the PCR performed on the extracted DNA from each of the above protocols for each of the 3 samples. The Ct (cycle threshold) values reported is the average of 5 replicates run for each sample under each protocol. Figure 5 shows a representative amplification plot for sample 1 where the normalized florescent signal intensity (ΔRn) vs. cycle number is shown for kit protocol, protocols 1, 2, 3 & 4. Negative sample is also shown for each of the protocols.

TABLE II. Results for Yield of DNA extracted ontained using Qubit

Protocol	*Sample1 Yield in ng (%CV)*	*Sample2 Yield in ng (%CV)*	*Sample3 Yield in ng (%CV)*	*Negative Yield in ng (%CV)*
Kit	246(8.5)	451.6(5.1)	285.6(6.1)	Too low
1	198(20.8)	391.2(5.4)	401.6(3.1)	Too low
2	421(7.8)	422.8(2.1)	539.2(1.6)	Too low
3	180.2(23.8)	346(6.1)	390(7.4)	Too low
4	64.8(13.5)	183.4(16.7)	260.4(6.7)	Too low

TABLE III. Results of quantitative PCR

Protocol	*Sample 1 Ct value (%CV)*	*Sample 2 Ct value (%CV)*	*Sample 3 Ct value (%CV)*	*Negative Ct value (%CV)*
Kit	18.4(3.2)	17.8(1.9)	19.1(1.6)	35.9(14)
1	21.2(1.4)	19.7(0.85)	20.6(0.8)	37.6(12)
2	19.4(1.5)	19.1(1.4)	19.7(1.7)	37(15)
3	21.6(3.4)	20.3(1.1)	20.6(0.8)	38(10)
4	23.1(2.4)	21.3(2.8)	20.9(1.3)	33(9.4)

The yield and Ct value obtained from the manual SP 1.2 kit protocol was considered as the reference with which the Ct values from the other modifications were evaluated. Samples 1, 2 & 3 showed the lowest Ct value when processed with the kit protocol. The Ct value from Protocol 1 was expected to increase compared to the kit protocol (as the starting blood sample volume is reduced). The results obtained are as per expectation. The results obtained from

Protocol 2 show that the Ct value obtained for the 3 samples are similar to or are better than the reference kit protocol results. This ensures that the modifications made to the protocol did not affect the yield both in terms of quality and quantity. The results obtained from Protocol 3 showed a comparable Ct value to that of the Protocol 1 but higher Ct compared to the kit protocol and Protocol 2, as expected since the sample volume is decreased. Protocol 4, where Protocol 3 was performed on the microfluidic device made using the digital craft cutter and the fluidics were actuated manually with a syringe, we observed that the DNA yield had dropped to almost 30-60% compared to that of Protocol 3 and was also lesser than Protocols 1, 2 and the kit protocol.

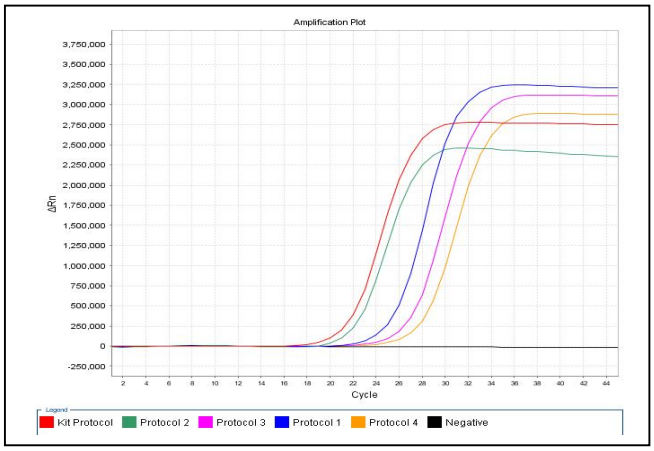

Figure 5. Amplification of PAEP gene in the whole blood using the kit protocol and 4 protocols on the microfluidic cartridge.

There are multiple factors that could have caused the decreased yield in the Protocol 4 pertaining to the fundamental limitations when manually operating the microfluidic device. Firstly, although the maximum volume the chip could accommodate was only 35 - 40 µL, 100 µL of volume was analyzed at any given time in these experiments. The reason for using this higher volume was to enable an ease of positioning of the liquid in the fluidic channel using manual actuation and also to account for bubbles formed during the fluidic movement and evaporation. While only 35 - 40 µL is in contact with the base of the ChipGenie instrument, the rest of the liquid did not experience the same incubation temperatures and hence would not be uniformly processed. Thus, out of 100 µL of total reaction volume only 35 - 40 µL was properly analyzed at the right incubation temperature and was in direct contact with the magnetic beads and the sample when the reaction occurs. By developing an automated liquid handling system, the total volume required for ease of handling can be brought much closer to the actual bed volume of the chip thereby increasing the effectiveness of analysis. Secondly, the mixing of the buffers with the sample in the cartridge was done using magnetic actuation and the speed of mixing was not the same as that obtained by vortex mixing employed during manual extraction of the sample.

IV. Conclusions

Using low cost materials like double sided tape, OHP transparent sheets and connectors, we have shown the ability to manufacture microfluidic devices using digital craft cutter. The microfluidic ships thus fabricated were subjected to a functional evaluation using VERSANT Sample Preparation 1.2 reagent kits employing silica-based nanobead technology. A series of modifications were made to the kit protocol like decreasing the sample volume, decreasing turnaround time and having an isothermal incubation temperature and tested on the chip fabricated. DNA yield and Ct values from PCR results were compared for three whole blood samples across all the modified protocols with the reference kit protocol. The microfluidic chip made from the craft cutter was established to be functional in providing an acceptable yield and high quality DNA as shown by encouraging Ct values from PCR. However, the yield and Ct values are not as high as the reference kit protocol and other protocols. The reason for low yields from the chip is thought to be the use of volumes higher than the bed volumes of the chip and insufficient mixing. Higher volumes were used since handling low volumes manually in a micro-fluidic chip was quite challenging. Hence, by appropriate automation of the fluidic actuation on the chip it is proposed that the yield can be significantly improved.

Acknowledgment

We would like to thank Gabriela Altmann, Jasmin Skorna and Dr. Guido Hennig for their guidance and support with the reference material and the VERSANT SP1.2 kits.

Author Contributions

All authors confirmed that they have contributed to the intellectual content of this paper and have met the following requirements (a) significant contributions to the conception and design (1, 2 and 3 only) (b) Acquisition of data (1 and 2 only) (c) analysis and interpretation of data (1, 2 and 3 only) and (d) drafting or revising the article for intellectual content (1, 2 and 3 only)

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