SAMPLE-TO-ANSWER LABDISK FOR POINT-OF-CARE ANALYSIS OF TOTAL CHOLESTEROL FROM WHOLE BLOOD

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ABSTRACT

A centrifugal microfluidic LabDisk for total cholesterol [TC] determination from whole blood and a processing device have been developed. Centrifugal microfluidic sample-to-answer processing features direct sampling from the fingertip, aliquoting (for analysis of additional parameters), blood plasma separation, rehydration of prestored reagents, and real-time absorbance measurement of enzymatic reactions. Time to result for fluidic processing and readout is 7 min with room for optimization to less than 4 minutes. It was demonstrated that the TC assay can be integrated into LabDisk based multiparameter analysis of whole blood.

KEYWORDS: Centrifugal, Microfluidics, Cholesterol, Whole blood, Sample-to-answer, Point-of-Care

INTRODUCTION

Easy operability and a fast time-to-result with as little human interaction as possible are the main requirements by Point-of-Care tests. CLIAwaived.comTM [1] certified applications for determination of cholesterol like the LDX Analyzer system (Cholestech) allow the lipid screening (total cholesterol [TC], triglycerides [TG], high- respectively low-density lipoprotein [HDL/LDL-cholesterol]) from a 40 µL whole blood sample in approx. 6 min [2]. Another certified sample-to-answer application is the Piccolo® Xpress system (Abaxis), which requires a sample volume of approx. 100 µL and a time-to-result of approx. 10-12 min for determination of TC, TG, HDL/LDL-cholesterol [3]. Aim of this study is to demonstrate the integrability of TC testing in a centrifugal microfluidic LabDisk, aiming at one important parameter in multiparameter blood testing, a sample volume of 40 µL and a time-to-result of less than 7 minutes.

EXPERIMENT

A whole blood sample is taken from the fingertip applying a LabDisk integrated 40 µL capillary (figure 1). The LabDisk is placed into the processing device (see figure 2). The sample is aliquoted by centrifugal forces into two cavities (volumes, processing times & frequencies are listed in table 1), transferred to the next downstream chamber and separated into hematocrit and plasma via centrifugal sedimentation. Via a capillary siphon the plasma is transferred into a reaction chamber, where it rehydrates prestored enzymes. The mixture is transferred to LabDisk inserted micro cuvettes for analysis of a colour-change reaction via absorptiometry ($\lambda_{detection}$ = 632 nm).

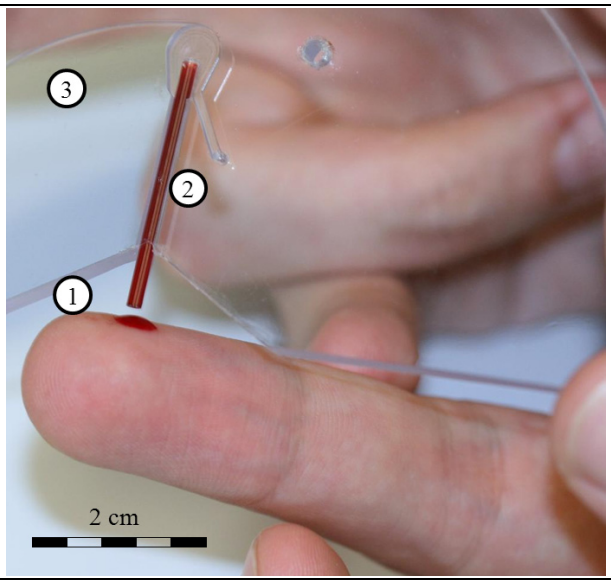

Figure 1: Image of the sample collection process from a puncture site on the fingertip (1) via an end-to-end capillary (2), which is integrated in the LabDisk containing the analysis network (3).

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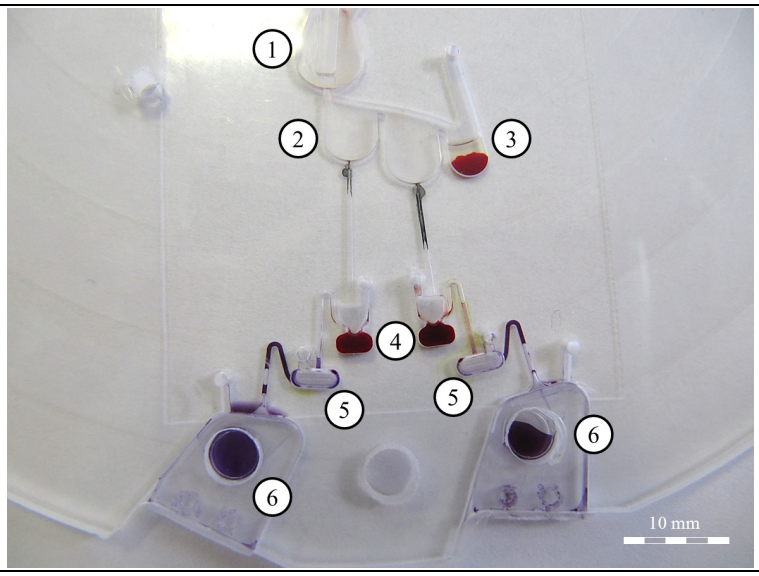

Figure 2: Image of the complete fluidic network of the LabDisk including a sample collection chamber (1), an aliquoting structure (2)for splitting the sample into 2 analytes, an overspill chamber (3) for collection of the residual whole blood, blood plasma separation structures (4), reaction chambers (5) with prestored reagents and integrated cuvettes for absorptiometric detection of the TC concentration (6).

Table 1. Unit operations integrated in the microfluidic network, processed volumes, processing time & rotational frequencies of each step. Frequency ranges indicate an increase of the spinning frequency over the given processing time.

Unit operation	**Volume [µL]**	**Time [s]**	**Rotational frequency [rpm]**
Sample collection	40	10	0
Aliquoting	40	30	480 – 720
Blood plasma separation	16	120	3000
Capillary transport	5	20	0 – 600
Rehydration & mixing reagents	5	45	± 500
Capillary transport	5	20	0 – 600
Reaction & detection	5	150 – 180	240

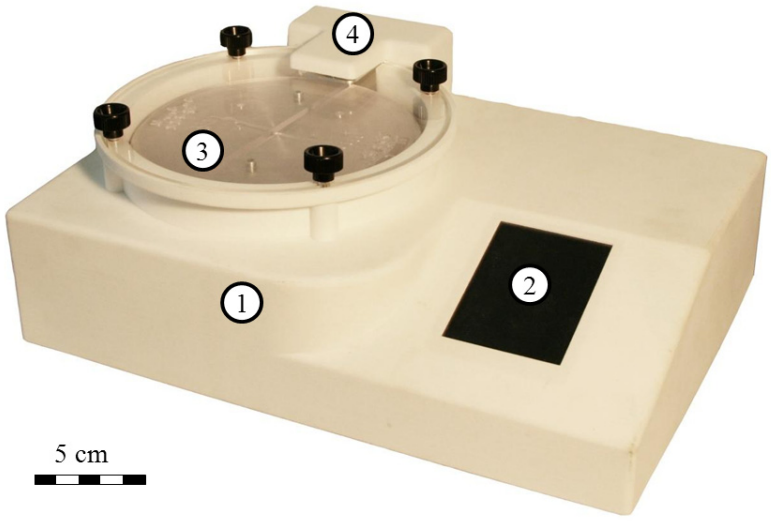

Figure 3: Image of the centrifugal processing device prototype (1) with an integrated touch-panel to operate the device and display results (2). After sample collection the LabDisk (3) is mounted onto the rotor, where the sample is processed following the frequency protocol displayed in table 1. The TC concentration is determined with an integrated absorptiometry unit (4).

RESULTS AND DISCUSSION

Three different samples have been processed on the LabDisk (figure 2) using the processing device shown in figure 3, whereby sample #3 has been repeated three times (3a-c). The same samples have been analyzed with a reference system (LDX) (figure 4). Samples #1 and #2 show comparable results to the LDX measurement (sample #1: 6.0 mM (c_{LDX} = 6.01 mM); sample #2 4.79 mM (c_{LDX} = 4.74 mM); sample #3 varies around the LDX result (c_{LDX} = 3.81 mM) with a CV of 6.3 %. Time to result is 7 minutes with further room for optimization.

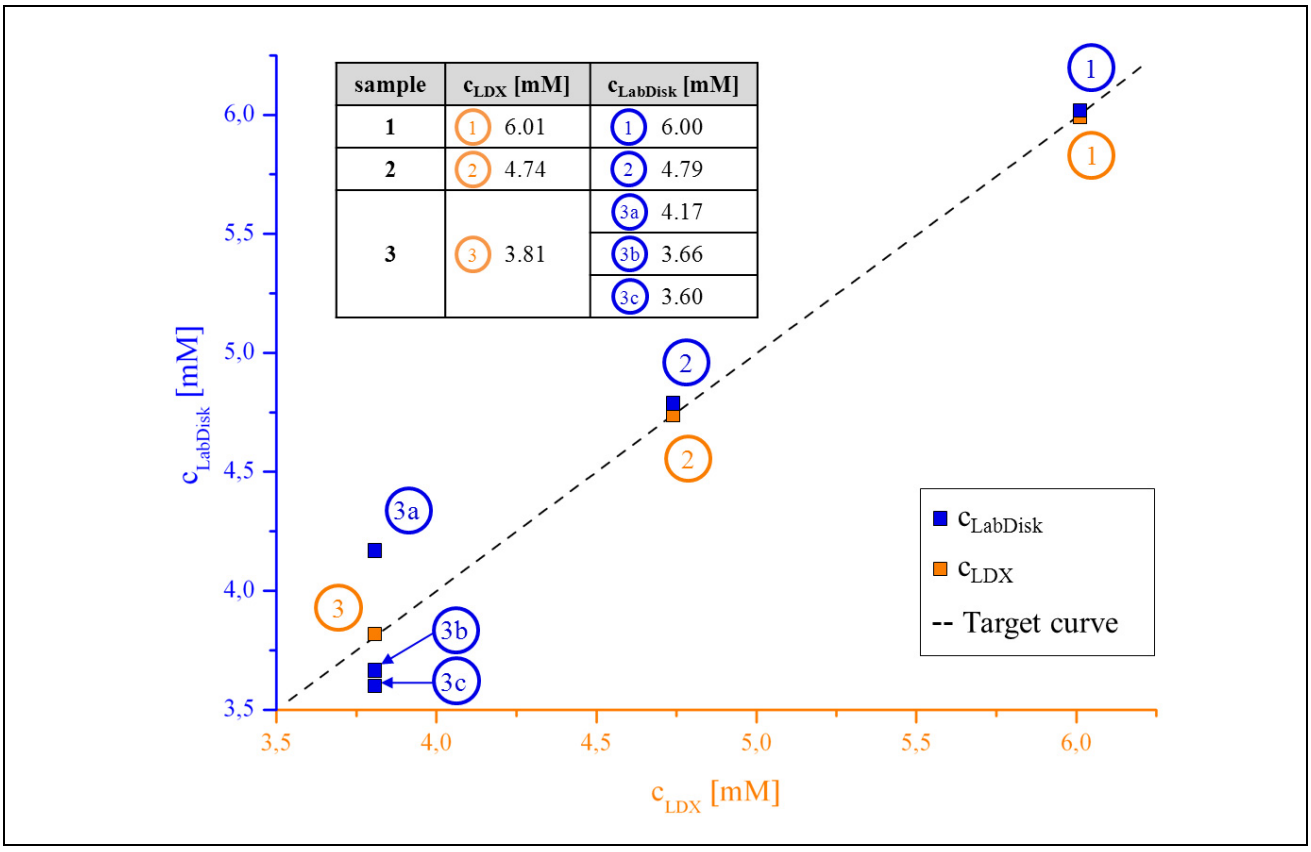

Figure 4: Variance comparison of the TC concentrations of the LabDisk to the TC concentrations determined with the LDX reference system using the same samples, whereas LDX results are shown on the X-axis, LabDisk results on the Y-axis. The dashed line shows the target curve for each concentration. Samples #1 & #2 show comparable results to the reference system, sample #3 varies around the reference result with a CV of 6.3 %.

CONCLUSION

Sample-to-answer determination of TC on a microfluidic LabDisk is demonstrated. Results are in line with common testing systems with just 40 µL sample volume. The time to result is 7 minutes and can be further optimized. Sample collection and application into the processing device is done via an integrated end-to-end capillary, which can be adapted to specific user requirements. The microfluidic network of the LabDisk is designed to be able to integrate multiparameter analysis like the detection of HDL-cholesterol and TG without affecting the time to result.

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REFERENCES

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