Electronic Supplementary Material (ESI) for Lab on a Chip.
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**Microfluidic device for single step measurement of protein C in plasma samples for sepsis prognosis**

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## S1 - Cost analysis of the device

Cost of a PET sheet 0.005" – 27" x 10ft – CAD $6

(McMaster Carr)

Area of sheet used per device - 1.5" x 1" per layer –3 layers

Cost of PET per device – CAD $0.0083

Cost of 3M tape – 600mm x 900mm - $9.5

Cost of 3M per device – 5 layers – CAD $0.009

Fluorescent dye used – qubit protein assay - $444 for 500 assays that use 3µL each

= $0.866 per assay

We use 15µL per run = CAD $4.33

https://www.thermofisher.com/order/catalog/product/Q33211#/Q33211

IMAC affinity beads – 25mL for CAD $485

25µL used per run = CAD $0.485

Barium chloride, citric acid, sodium phosphate dibasic, tris, hydrochloric acid - <$0.01 per device

Instrumentation cost

Cost of xurography using cricut explore - $350

Results

Setup cost = $350 for small scale production

Total cost per device = Cost of materials + Cost of reagents

= 0.0083 + 0.009 + 4.33 + 0.485

= CAD $4.83

## S2 Supplementary information 2 – Schematic of experimental setup

A schematic representation of the experimental setup used for the measurement of protein C is shown in fig. S1.

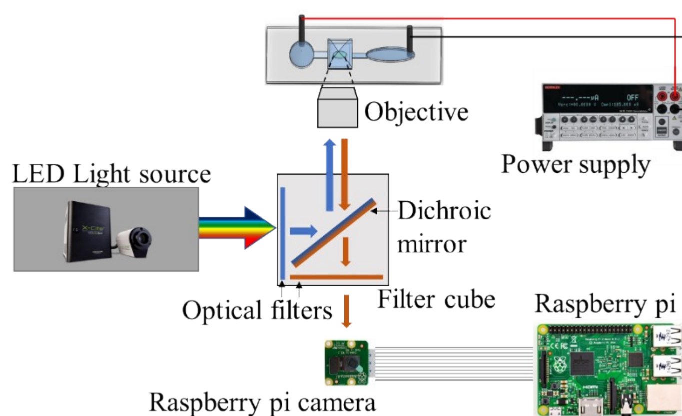


Figure S2-1 Experimental setup used for the capture of fluorescent images with a filter cube with an

## S3 Supplementary information 3 – Theoretical time for protein motion

In the absence of electroosmotic flow, the force balance on each protein is given by

$qED = fV$

Where q is the charge on the protein, theoretically predicted based on the functional groups on protein C. This was obtained from https://www.protpi.ch/Calculator/ProteinTool/

D is the diffusivity ratio which is the additional resistance to flow due to the structure of the gel and size of the protein and was approximated based on the size of protein C in agarose to be 0.85[1]

V is the velocity that is being calculated

f is the stokes drag acting on the particle obtained from

$f = 6\pi\eta r$

Where

Where η is the kinematic viscosity

r is the stokes radius of the protein, approximated to be 3nm[2]

When applied to protein C, with a theoretical pI of 5.9191, The theoretical velocity at a pH difference of 0.2 and 3.4 that corresponds to an ambient pH of 5 and 8.2 for protein C with a pI range of 4.4 - 4.8 are shown below.

| pH (pH difference) | charge (C) | stokes drag (N) | Location | Electric field | velocity (mm/s) |
|---|---|---|---|---|---|
| 6.2 (+ 0.2) | -3.9E-19 | 5.65E-11 | Isoelectric gate | 1.1 V/cm | 6.52E-03 |
| 9.3 (+ 3.4) | -6.8E-18 | 5.65E-11 | Central reservoir (pre membrane) | 0.5 V/cm | 0.06 |
| | | | Central reservoir (gel) | 0.25 V/cm | 0.0255 |
| | | | Sample reservoir | 0.35 V/cm | 0.042 |

Time needed to travel through

The sample reservoir = 14 mm / 0.042 mm/s = 333.3 s = 5.55 minutes

The isoelectric gate = 5 mm / 6.52E-03 mm/s = 766.87 s = 12.78 minutes

The central reservoir = 4 mm / 0.06 mm/s + 3mm / 0.0255mm/s = 360 s = 6 minutes

Total = 24.33 minutes in the absence of electroosmotic flow

References


[1] E.M. Johnson, D.A. Berk, R.K. Jain, W.M. Deen, Hindered diffusion in agarose gels: Test of effective medium model, Biophys. J. 70 (1996) 1017–1023. https://doi.org/10.1016/S0006-3495(96)79645-5.

[2] H.P. Erickson, Size and shape of protein molecules at the nanometer level determined by

sedimentation, gel filtration, and electron microscopy, Biol. Proced. Online. 11 (2009) 32–51. https://doi.org/10.1007/s12575-009-9008-x.

## S4 Supplementary information 4 – Fluorescent dye concentration

The independence of the measured fluorescent intensity with respect to fluorescent dye concentration when the dye was present in excess was tested by using samples with a protein C concentration of 4 µg/mL. The samples were prepared as described in section 3.1. Briefly, 8 µL of a 20 µg/mL Protein C sample was mixed with Tris-HCl buffer to a total volume of 400 µL. 6 samples were prepared, 3 samples each were mixed with 2 µL and 4 µL of Qubit protein assay's fluorescent dye. The protein concentration required for the saturation of 2 µL of fluorescent dye in the solution was 10 µL of 5 mg/mL as described in the manufacturer protocol. As a result, the solutions contained an excess of fluorescent dye. The measured intensity was 35.52± 0.65 with 2 µL of the dye compared to 34.91±1.66 with 4 µL of the dye. The intensities were not found to be significantly different showing that the dye concentration does not influence measured intensity