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Lab on a Chip

Electronic Supplementary Information (ESI)

Point-of-care testing system for digital single cell detection of MRSA directly from nasal swabs

Martin Schulz[a, *], Silvia Calabrese[a, *], Florian Hausladen[c], Holger Wurmc, Dominik Drossart[c], Karl Stock[c], Anna M. Sobieraj[d], Fritz Eichenseher[d], Martin J. Loessner[d], Mathias Schmelcher[d], Anja Gerhardts[e], Ulrike Goetz[e], Marina Handel[e], Annerose Serr[f], Georg Haecker[f], Jia Li[b], Mara Specht[a], Philip Koch[a], Martin Meyer[a], Philipp Tepper[a], Raimund Rother[a], Michael Jehle[a], Simon Wadle[a], Roland Zengerle[a, b], Felix von Stetten[a, b, ¥], Nils Paust[a, b, *] and Nadine Borst[a, b, *]

S1: Calculation of sample concentration by Poisson statistics

Under the assumption that the total amount of target molecules (m) is distributed throughout several partitions (n), the probability that a partition will contain k copies of the targets can be modelled by the Poisson-distribution [1]. The expectancy value equals to the mean occupancy rate (λ), which is the ratio of the number of target (m) molecules to the number of partitions (n).

$$\lambda = \frac{m}{n} \tag{1}$$

$$p_\lambda(k) = \frac{\lambda^k * e^{-\lambda}}{k!} \tag{2}$$

The probability for an unallocated partition is given by:

$$p_\lambda(0) = \frac{\lambda^0 * e^{-\lambda}}{0!} = e^{-\lambda} = N \tag{3}$$

Therefore, the mean occupancy rate (λ) can be calculated from the percentage of empty partitions (N):

$$\lambda = -\ln(N) = -\ln\left(1 - \frac{k}{n}\right) \tag{4}$$

By dividing the total amount of target molecules (m) by the reaction volume (V) the resulting concentration (c) can be calculated:

$$c = \frac{m}{V} = \frac{\lambda \cdot n}{V} \tag{5}$$

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S2: Statistic calculations

Data of the pre-study carried out by the University Hospital Freiburg (see Table S1). The probability was calculated with regard to a total number of 20,000 droplets and the assumption that all CoNS are methicillin resistant.

Table S 1: Data of the pre-study and calculated probability for false positive and positive MRSA results. *A bacterial range was measured. The displayed value represents the maximum value.

Nasal samples [#]	Bacterial count MRSA* [ml^{-1}]	Bacterial count MSSA* [ml^{-1}]	Bacterial count CoNS* [ml^{-1}]	Propability for a double signal in a droplet [%]	False positive MRSA signal [droplet number]	Positive MRSA signal [droplet number]
21	0	0	10,000			
4	0	10	1,000	0		
8	0	100	10,000	0		
15	0	1,000	100,000	0.015	3	
17	0	10,000	10,000	0.015	3	
11	0	100,000	10,000	0.146	29	
1	0	1,000,000	0			
1	100	0	1,000	0		2
1	10,000	0	10,000	0.015		248

S3: Detailed view on the cartridge

An overview of the fluidic cartridge design is displayed in Figure S1, geometry and material parameters are listed in Table S2.

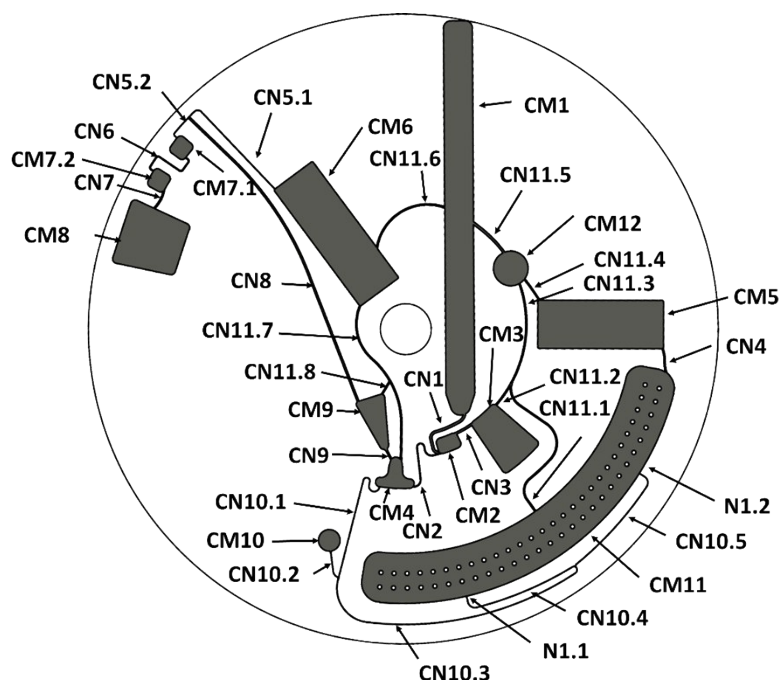

Fig. S 1: Schematic of the microfluidic cartridge with abbreviations corresponding to table E1.

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Table S 2: Description and designed values of the channels and chambers used in the microfluidic cartridge.

Abbreviation	Purpose	Width [µm]	Depth [µm]
CN1	Sample transport channel	500	450
CN2	Sample metering channel	175	150
CN3	Sample metering overflow channel	500	450
CN4	Oil supply channel	300	250
CN5.1- CN5.2	Rehydration buffer supply channel	106	80
CN6	Rehydration buffer resistance channel	200	150
CN7	Rehydration buffer metering overflow channel	300	250
CN8	Rehydration buffer inward pumping channel	400	350
CN9	Dry reagent transfer channel	500	500
CN10.1-CN10.5	Assay mix supply channel	125	125
CN11.1-CN11.7	Venting channel	300	250
N1.1 – N1.2	Droplet generation nozzle	90	60
CM1	Swab chamber		
CM2	Bacterial suspension metering chamber		
CM3	Bacterial suspension overflow chamber		
CM4	Mixing chamber		
CM5	Oil Stick-Pack chamber		
CM6	Rehydration buffer Stick-Pack chamber		
CM7.1 –CM7.2	Rehydration buffer metering chamber		
CM8	Rehydration buffer overflow and compression chamber		
CM9	Dry reagent pre-storage chamber		
CM10	Gas trapping chamber		
CM11	Droplet collection chamber		
CM12	DNA-Filter chamber		

S4: Used primer and probes in the digital RPA

The following primer and probe- sequences were used for the bi-plex RPA reaction:

- *vicK* forward primer: 5’CGTGGACGTATTCGTATCGTCAATGATATGGCACTC3’
- *vicK* reverse primer: 5’CACGTGCGATTAGACCTTCTTCTTCATTTAAATC3’
- *vicK* probe: 5’AGATGCTTGGTATGGCGAAAGAAGACATCATCGGATAT XT A X A FT GTTAAGTGTATTAAG—PH XT= BHQ1-DT, X=THF, FT = C2-DT-[FAM].
- *mecA* forward primer: 5’ATATCAATCTATTAACTGATGGTATGCAACAAGTCG3’
- *mecA* reverse primer: 5’CCAATTTGTCTGCCAGTTTCTCCTTGT3’
- *mecA* probe: 5’AGATCTTATGCAAACTTAATTGGCAAATCCGG XT X C FT GCAGAACTCAAAATGA—PH3’ XT = BHQ1 DT, X = THF, FT = C2-DT-[TAMRA].

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S5: Lysis efficiency

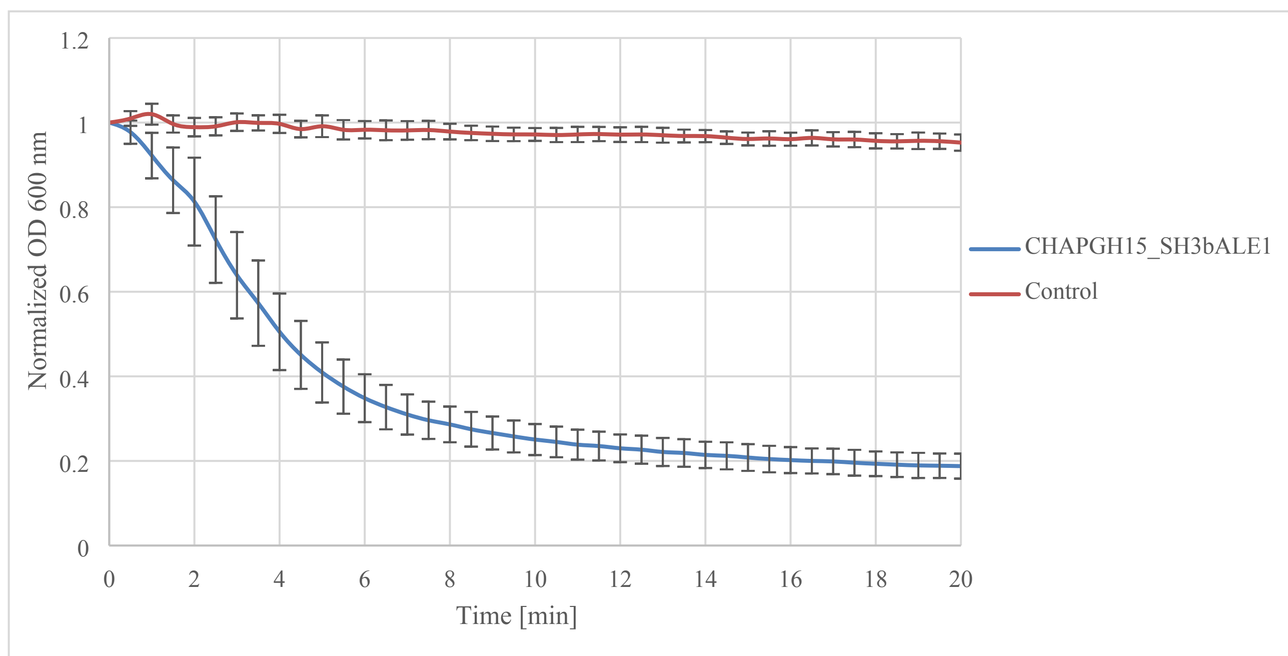

Fig. S 2: Enzyme activity of the recombinant CHAPGH15_SH3bALE1 enzyme. Lytic activity of the enzyme was demonstrated by measuring time dependent turbidity changes in a suspension of *S. aureus* (Newman) substrate cells at OD 600 nm (blue), compared to negative control (orange) where no enzyme was added to the substrate cells. Assays were performed in PBS using an enzyme concentration of 150 nM. Error bars represent die standard error of means of three independent experiment.

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S6: Optical design point-of-care testing (POCT) device

An overview of the optical design of the POCT device is displayed in Figure S2 and S3.

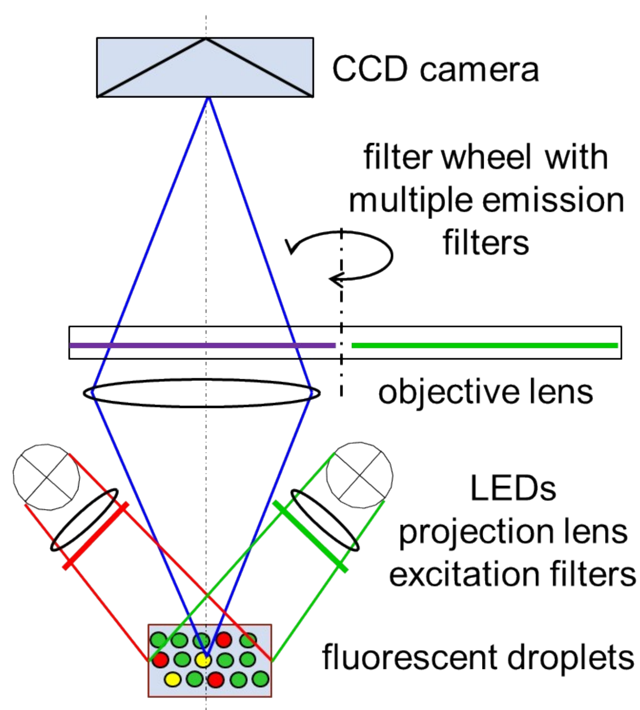

Fig. S 3: Schematic depiction of the optical setup used in the POCT device.

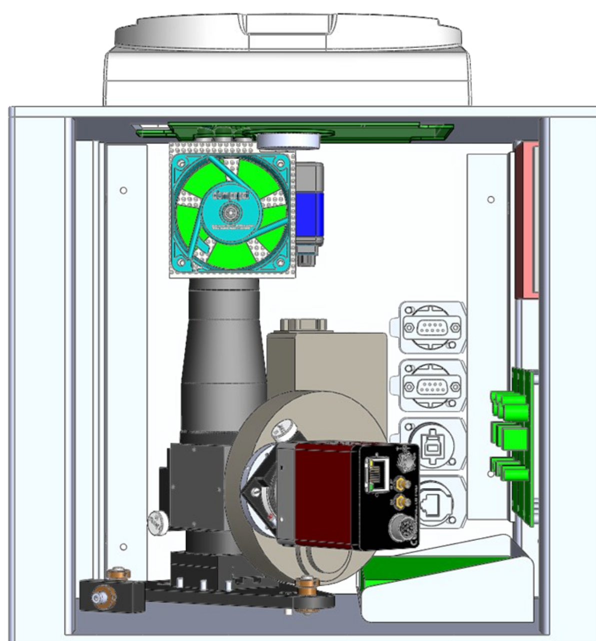

Fig. S 4: 3D-CAD inside view of the POCT device.

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References

1 Basu, Amar S. (2017): Digital Assays Part I: Partitioning Statistics and Digital PCR. In: *SLAS technology* 22 (4), S. 369–386. DOI: 10.1177/2472630317705680.

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