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

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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 ($\lambda$), which is the ratio of the number of target ($m$) molecules to the number of partitions ($n$).

$$\lambda = \frac{m}{n}$$  \hspace{1cm} (1)

$$p_{\lambda}(k) = \frac{\lambda^k * e^{-\lambda}}{k!}$$  \hspace{1cm} (2)

The probability for an unallocated partition is given by:

$$p_{\lambda}(0) = \frac{\lambda^0 * e^{-\lambda}}{0!} = e^{-\lambda} = N$$  \hspace{1cm} (3)

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

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

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

$$c = \frac{m}{V} = \frac{\lambda \cdot n}{V}$$  \hspace{1cm} (5)
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 S1: 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] | Probability 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                         |                                               |                                             |                                       |
| 8                 | 0                             | 100                           | 10,000                        |                                               |                                             |                                       |
| 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                         |                                               |                                             | 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. S1: Schematic of the microfluidic cartridge with abbreviations corresponding to table E1.
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’CGTGGACGTATTCGATACGATGATGGAACCTC3’
- **vicK reverse primer**: '5’CAGTGGACGTATTCGATACGATGATGGAACCTC3’
- **vicK probe**: '5’AGATCTTATGCGATACTCCTGCTGATATGATGATGATGGAACCTC3’
- **mecA forward primer**: '5’ATATCAATCTATTAACTGATGGTATGCAACAGCTCG3’
- **mecA reverse primer**: '5’CCAATTTGTCTGCGAATTTCCTTCTTCTTCTTCTTG3’
- **mecA probe**: '5’AGATCTTATGCGATACTCCTGCTGATATGATGATGATGGAACCTC3’
**S5: Lysis efficiency**

![Graph](image)

**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 \textit{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 the standard error of means of three independent experiments.
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.
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.