Supporting information for

Direct droplet digital PCR (dddPCR) for species specific, accurate and precise quantification of bacteria in mixed samples

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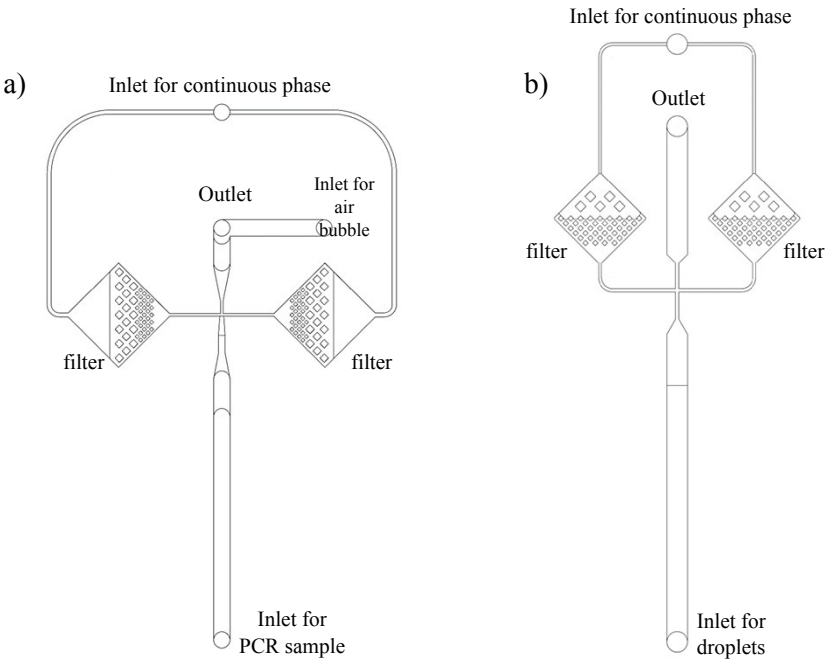

Figure S1. Schematics of a) a chip for droplets generation, b) a chip for droplets reading.

The dimensions of a chip for droplet generation are following:

- channel for disperse phase at inlet, width 800 µm x height 800 µm
- channel for continuous phase, width 200 µm x height 200 µm
- flow-focusing junction, width 100 µm x height 120 µm
- outlet channel, width 800 µm x height 800 µm

The dimensions of a chip for droplet reading are following:

- channel for droplets, width 1200 µm x height 1200 µm
- channel for continuous phase, width 114 µm x height 100 µm
- flow-focusing junction, width 124 µm x height 100 µm

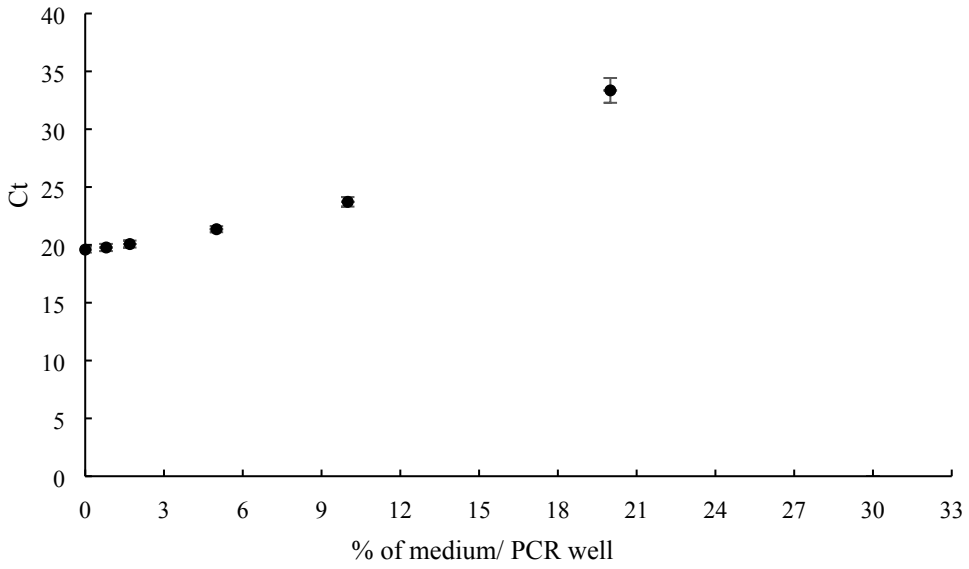

Figure S2. Bacterial growth medium strongly inhibits or completely hinders PCR. Graph shows percentage of growth medium in PCR sample as a function of PCR cycle threshold. The big advantage of dddPCR technology is lack of lysis and extraction of DNA form bacteria. The cells are directly introduced to PCR sample. Unfortunately, there can be a limitation connected with addition of bacterial growth medium. We prepared seven samples containing different amount of BHI (broth heart infusion) broth , from 0% to 30% v/v. The samples consist of PCR Supermix, primers, probe, *Staphylococcus aureus* purified DNA, growth medium and water were placed in Roche LightCycler thermocycler and real-time PCR was performed. The growth medium is a potent inhibitor. The concentration should be smaller than 1% v/v. Higher volume of broth inhibits or completely hinders amplification of DNA. Bacteria sample before analysis should be diluted or centrifuged and resuspended in non-inhibiting buffer, e.g. TRIS buffer.

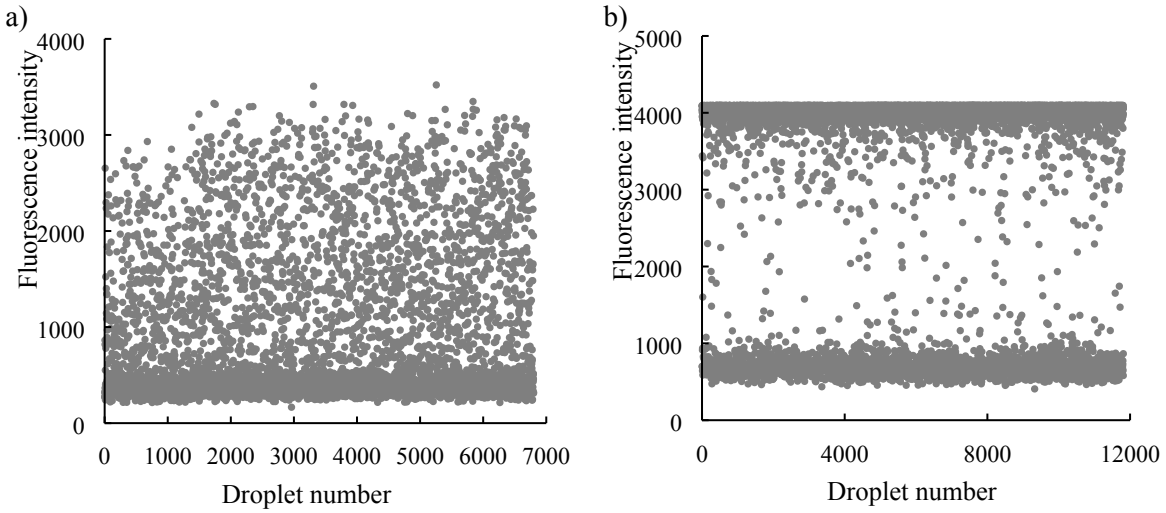

Figure S3. PCR temperature profile strongly influences the efficiency of PCR. Graphs show fluorescence intensity of all analyzed droplets after ddPCR performed with two different cycling conditions: a) 95°C for 10min, 40 cycles of 94°C for 30s, 60°C for 60s and final 37°C for 30s, b) 95°C for 10min, 60 cycles of 94°C for 60s, 60°C for 120s and final 37°C for 30s.

Determination of the concentration of DNA in the sample using droplet digital polymerase chain reaction does not require standard curve. It is based on the fraction of positive droplets and it is estimated according to Poisson distribution. The distribution of positive (containing target) and negative (no target present) populations of the droplets has to be clear and explicit. Therefore, when the ddPCR cycling program is not well established it provides many droplets with intermediate fluorescence intensity [1]. It hinders threshold setting and falsifies final results. We conducted an experiment showing an improvement of droplet separation due to ddPCR program modification. *Staphylococcus epidermidis* DNA, $1.5 \cdot 10^{-3}$ ng/µl, was encapsulated with PCR components into droplets using BioRad oil for probes. We prepared 6 samples containing 20 µl of droplets and about 20 µl of oil. The samples were divided into two groups and were exposed to two different PCR thermal conditions. The following temperature profiles were used: I) 95°C for 10min, 40 cycles of 94°C for 30s, 60°C for 60s and final 37°C for 30s, II) 95°C for 10min, 60 cycles of 94°C for 60s, 60°C for 120s and final 37°C for 30s. First shorter temperature profile provides 'rain' of the droplets, positive fraction is not clearly separated from negative population. Extended PCR program yields improved droplet distribution, both fractions are divided which significantly simplify threshold setting and data analysis.

		Concentration of bacteria [CFU/ml]								
Target	***S. capitis***	1.0E6	1.0E5	1.0E4	1.0E3	0	1.0E6	1.0E5	1.0E4	1.0E3
Background	*S. aureus* *S. epidermidis*	0				1.75E5				
Target	***S. aureus***	2.5E6	2.5E5	2.5E4	2.5E3	0	2.5E6	2.5E5	2.5E4	2.5E3
Background	*S. epidermidis* *S. capitis*	0				1.0E5				
Target	***S. epidermidis***	2.5E6	2.5E5	2.5E4	2.5E3	0	2.5E6	2.5E5	2.5E4	2.5E3
Background	*S. capitis* *S. aureus*	0				1.0E5				

Table S1. Concentrations of target and background bacteria in the samples analyzed by real-time PCR. Number of cells in PCR wells is estimated on basis of standard plate counting.

		Concentration of bacteria [CFU/ml]					
Target	***S. capitis***	5.0E5	5.0E4	5.0E3	5.0E5	5.0E4	5.0E3
Background	*S. aureus* *S. epidermidis*	0			1.0E7		
Target	***S. aureus***	2.0E6	2.0E5	2.0E4	2.0E6	2.0E5	2.0E4
Background	*S. epidermidis* *S. capitis*	0			1.7E7		
Target	***S. epidermidis***	5.0E4	5.0E3	5.0E2	5.0E4	5.0E3	5.0E2
Background	*S. capitis* *S. aureus*	0			1.0E7		

Table S2. Concentrations of target and background bacteria in the samples analyzed by dddPCR. Number of cells in PCR wells is estimated on basis of standard plate counting.

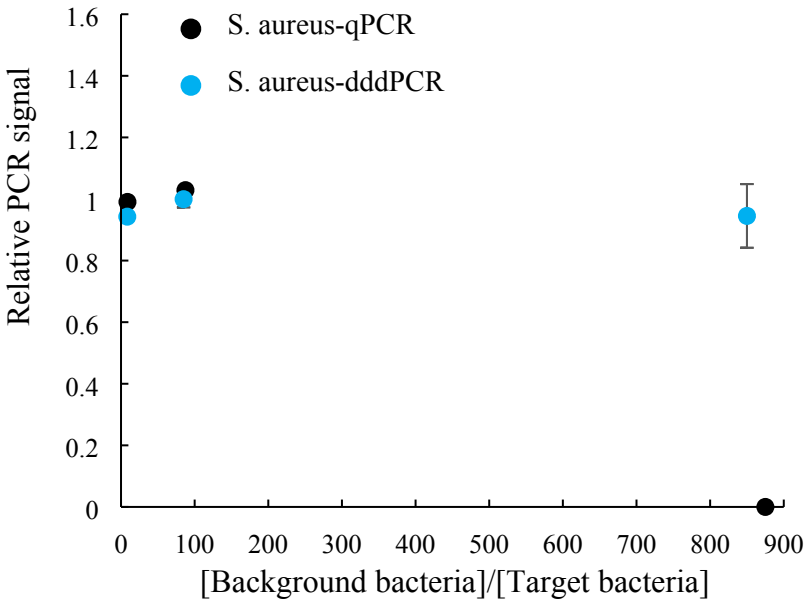

Figure S4. Comparison of qPCR and dddPCR assay. Graph shows a ratio of background bacteria concentration to target bacteria concentration as a function of relative PCR signal which is a ratio of a signal obtained in a sample containing target and background bacteria to signal obtained in a sample with just target cells.

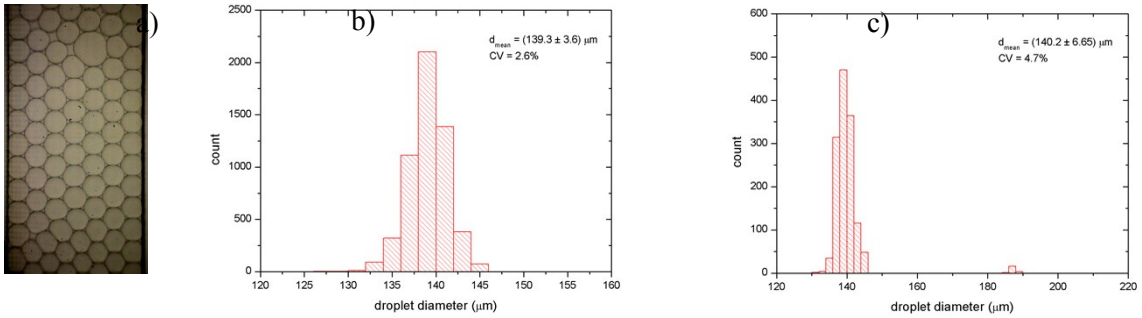

Figure S5. Distribution of the droplet size. a) snapshot of the droplets in the microdevice after re-injection, b) size distribution of the produced droplets and c) size distribution of the reinjected droplets (a small portion of the droplet coalesce during reinjection).