Electronic Supplementary Material (ESI) for Lab on a Chip

On-chip microbial culture for the specific detection of very low levels of bacteria

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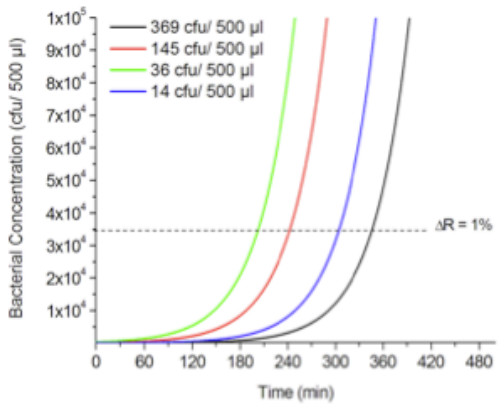
Supplementary Information

A



B

	Averaged SPR detection time for +1% reflectivity (min)	Theoritical detection time for +1% reflectivity (min)	Variance (%)
14 ufc/ml	351	347	0.04
36 ufc/ml	327	305	0.22
145 ufc/ml	242	243	0.01
359 ufc/ml	207	202	0.05

Figure S1. Theoretical modeling of the exponential bacterial growth and comparison to experimental data. (*A*) Based on an exponential growth model, theoretical modelling of the bacteria number increase can be plotted using the following equation: $C=C_t\ 2^{t}/\tau$ for each bacterial concentration used for sample spiking. τ (min) was experimentally calculated as described in the manuscript. (*B*) The comparison of the detection time calculated using this theoretical model with the experimental data confirmed the efficiency of the CCM method for following bacterial growth on a protein microarray.

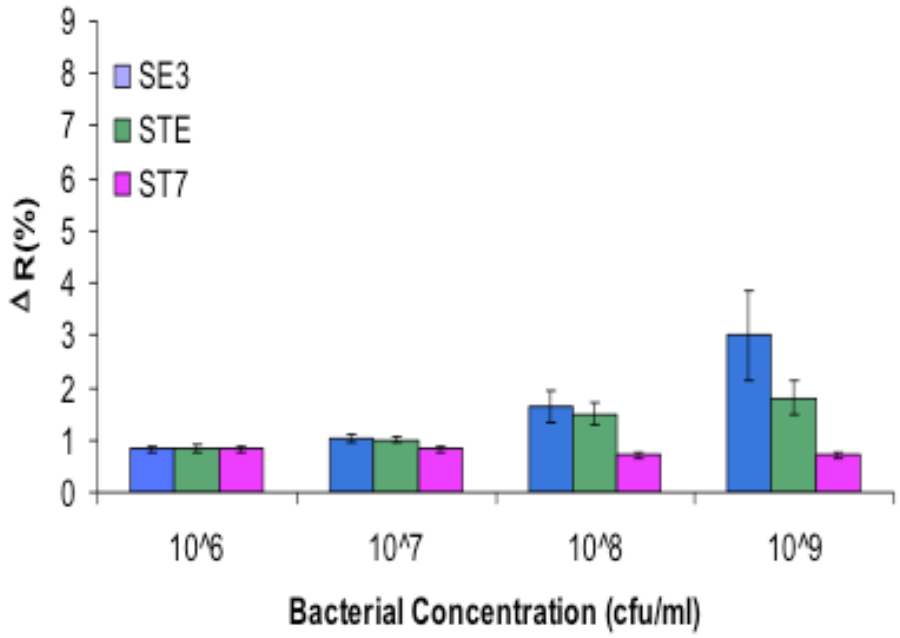


Figure S2. Detection of enriched solutions of *Salmonella* (growth-free detection). SPR imaging (SPRi) has been used to detect *Salmonella enterica* enteritidis present at high concentrations in pre-enriched media. The *S.* Enteritidis was cultured in Buffer Peptone Water media till reaching a mid-exponential phase. Then, bacterial solutions were washed, resuspended and diluted in PBS. Because it is deprived of nutrients, bacteria were not dividing in PBS during the SPR analysis. Diluted solutions from 10^6 to 10^9 cfu/ ml were prepared and tested on biochips for CCM. Biochips were grafted with triplicates of SE3, ST7, STE antibodies and control IgG. Triplicates of polypyrrole were also used as negative controls. Data are plotted according to the SPR signal shifts observed 100 minutes after sample loading on the biochip.

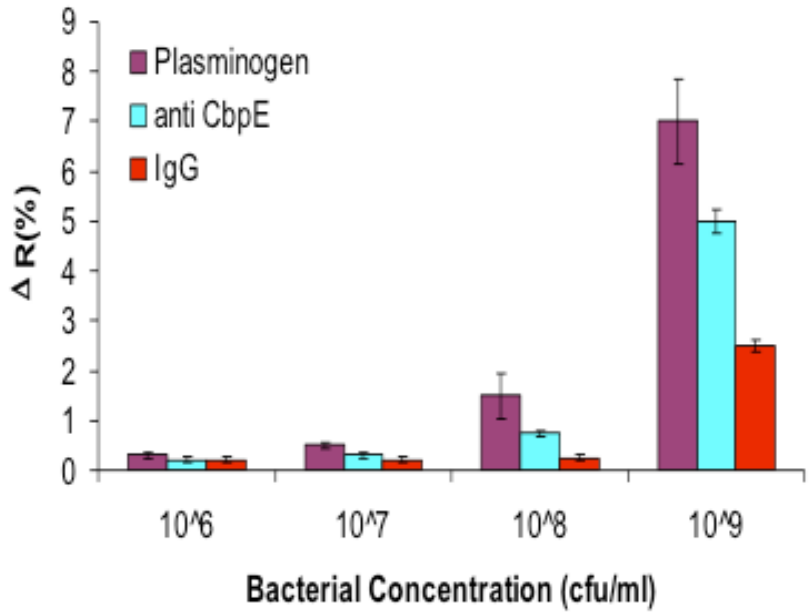


Figure S3. Detection of enriched solutions of *S. pneumoniae* (growth-free detection). The SPR imaging (SPRi) has been used to detect *S. pneumoniae* R6 strain present at high concentrations in pre-enriched media. The *S. pneumoniae* was cultured in Todd-Hewitt broth (TH) liquid medium till reaching a mid-exponential phase. Then, bacterial solutions were washed, resuspended and diluted in PBS. Because it is deprived of any nutrients, bacteria were not dividing in PBS during the SPR analysis. Diluted solutions from 10^6 to 10^9 cfu/ ml were prepared and tested on biochips for CCM. Biochips were grafted with triplicates of plasminogen and antibodies (against CbpE and irrelevant IgG), similarly to the experiment led in CCM with lower spiking solutions. Data are plotted according to the SPR signal shifts observed 100 minutes after sample loading on the biochip.

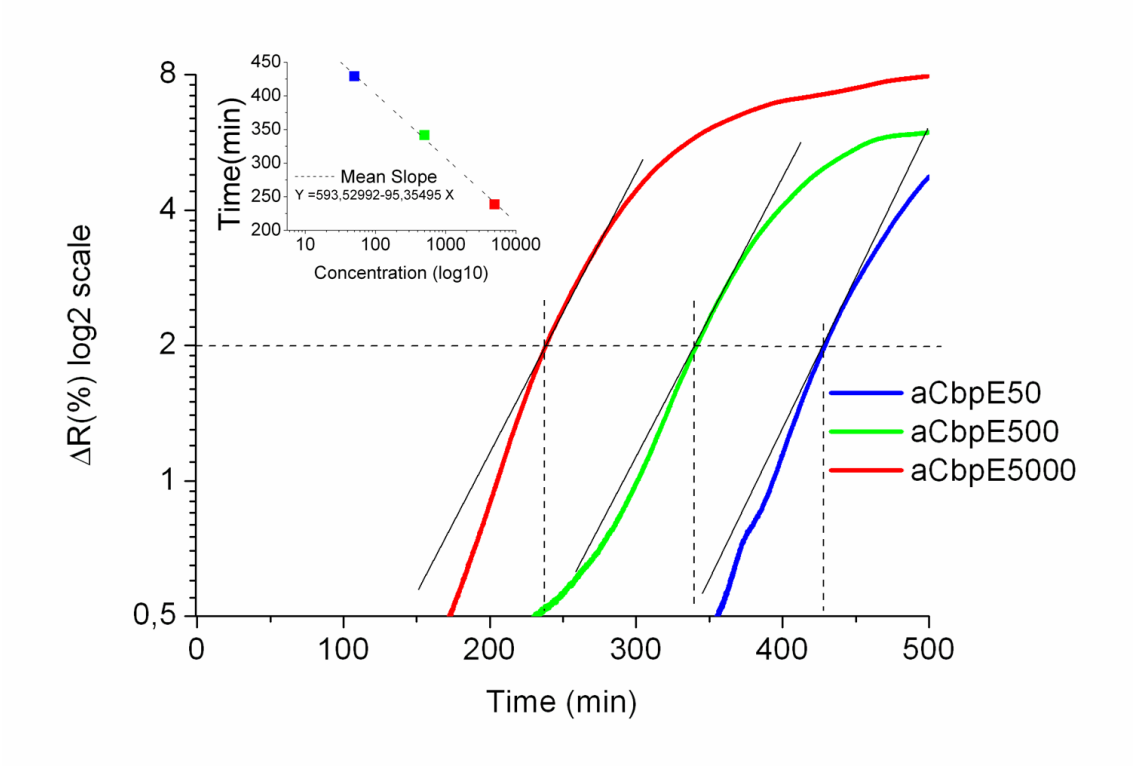


Figure S4. SPR data quantitative analysis for the detection of *S. pneumoniae.*

This graphic represents the SPR response increase (log 2) upon time for three independent experiments led with 50, 500 and 5,000 cfu/ ml. (inset) Due to the exponential bacterial growth, a calibration curve could be plotted and allowed the assessment of initial bacterial concentrations by reporting the time needed to reach a 2% reflectivity increase.

Supplementary information : Production of CbpE protein and purification of polyclonal IgG (anti-CbpE)

1) Production and purification of the recombinant protein CbpE

The coding region of the spr0831 locus (*cbpE* gene) was amplified from the genomic DNA of *S. pneumoniae* R6. Gene encoding CbpE was cloned into the pLIM vectors (PX Therapeutics SA, Grenoble, France) and the resulting constructions were fused to a His6 tag at the N terminus then overexpressed in Escherichia coli strain BL21-RIL (DE3) (from Novagen) using LB culture medium (10 g of NaCl, 10 g of Bacto-Tryptone, and 5 g of yeast extract/Litre). The transformed cells were grown at 37 °C in LB medium until the optical density (DO 600 nm) reached 0,8. Expression of the recombinant protein CbpE was then induced with 0.5 mM of isopropyl β-D-1-thiogalactopyranoside over night at 15 °C before harvesting. After the centrifugation of the culture at 4000 xg for 20 min, the recovered pellet containing cells was collected and resuspended in 40 ml of lysis buffer (50 mM Tris, 150 mM NaCl, 40 mM Imidazole). A Complete EDTA-Free Protease Inhibitor pastille is added to avoid the digestion of the produced protein.

After 2.5 min of sonication and centrifugation at 8000 × g for 20 min, the supernatant containing the target protein CbpE was collected and loaded onto a His Trap ® column using affinity chromatography. After loading of the supernatant on the column and washing with the the binding buffer (50 mM Tris, 150 mM NaCl, 40 mM Imidazole), an elution buffer (50 mM Tris, 150 mM NaCl, 150 mM Imidazole) was used to desorb the protein CbpE. Fractions containing the CbpE protein were combined, dialyzed against 50 mM Tris, 150 mM NaCl and concentrated. Purity of the protein was assessed using gel electrophoresis.

2) Anti-CbpE purification from rabbit serum

Rabbit sera against CbpE were a gift from A.M. Di guilmi. Antibodies were purified using the « CNBr activated sepharose 3B » (from GE HeathCare) affinity resin. The antibodies were eluted using the GE HealthCare recommendation notice.

An ELISA test was performed to check the anti-CbpE purified antibodies reactivity against the recombinant protein CbpE.