Supporting information

An Innovative Silicon-chip for Sensitive Real Time PCR Improvement in **Pathogens Detection.**

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ESI 1- CDC Chip Architecture



Figure ESI1 – CDC Chip Architecture Flow Chart

ESI 2 - CDC-chip disk-reader



Figure ESI-2: a) CDC-Chip; b) plastic holder for CDC-Chip; c) CDC-Chip reader.

The *chip reader* is an instrument prototype developed by STMicroelectronics (Size: length 30cm, 15cm heigh and width 17cm; weight: 600 g; power requirements: 12 V DC) (Fig. SI-2 c). It has been properly designed to manage two main technological modules:

a) Thermal module. It consists of electronic boards driving the temperature sensors and heaters of the chip to reach the following temperature performances: temperature resolution of 0.1°C, temperature accuracy of \pm 0.2°C and a heating rate of 15°C/s. The cooling step is guaranteed by a fan installed on bottom and achieving a cooling rate of 10°C/s.

b) Optical module. It is composed by 2 optical channels, for multiple Fluorescent reporters (FAM, VIC[®]) [1] ⁱ. The two light sources (LED) are centered at wavelength value of 470, 530 nm. The optical detector is a standard CMOS camera that contains a high-pass filter at 520 nm \pm 20 nm and band-pass filter centered at 556. Finally, the thermal module is able to drive the temperature sensor and heater integrates on CDC-The reader is provided by a custom software package to manage both the PCR process and carried out the data analysis. To guarantee the best signal-to-noise ratio, the automatic setting of Ct were performed for each experiment by software.

For the using, the CDC chip is insert in a polycarbonate holder designed to be compatible with Chip reader (Fig. ESI-2-1 b). The plastic holder is designed to accommodate four independent CDC-chips for the thermal and optical reading. It is has been manufactured by 3D milling technology (Roland MDX-40A).

Reference

[1] Marco Bianchessi and Alessandro Cossi, Patent US9016936B2

ESI 3- CDC Chip Architecture and Characterization



Figure ESI-3 – Thermal and Optical Characterization CDC-chip versus Single Cylinder Chip: (a) initial PCR thermal ramp performances; (b) PCR cycle thermal profiling performances; (c) Optical signals versus light power; (d) representative fluorescence images @5nM of FAM (LED power 11).

Figure ESI-4 "Thermal Characterization" illustrates the heating thermal profile for the initial heating ramp (Fig. a) and PCR cycles (Fig. b) for both the CDC-chip (3x replicates) and the single cylinder chip (red line). Data clearly indicate that the CDC-chip best performs in terms of heating/cooling rate respect to the version at single cylinder, exhibiting a faster heating ramps (about 10-20s for CDC-Chip vs 150s for single cylinder Chip) and cooling (about 15s for CDC-Chip vs 60s for single cylinder Chip).

Figure ESI-4 "Optical Characterization" show experiments carried out by exciting at various light power (from 1-11 a.u.) a solution containing 5nM of FAM-dye highlight that CDC chip is able to substantially enhance the signal up to 4 times respect to SCC-chip version (See Figure ESI-4 c) and d)).

The experiments were done according to the procedure 2.3 *RT-PCR procedure on CDC-chip* described in the Expertimental Section of the manuscript.



Figure ESI-4 Fig. RT-PCR experiments for Gram positive and Gram negative discrimination testing: Ct of *S. aureus* genome amount versus Ct of A. baumannii genome amount.

Oligonucleotide type		Name	5'->3' sequence	Lenght (bp)	Tm (°C)
primer		16Sfor	5'-GCAACGCGAAGAACCTTACC-3'	20	58.5
primer		16Srev	5'-TGCGGGACTTAACCCAACAT-3'	20	58.9
probe MGB	taqman	G+ probe	5'-FAM-ACAACCATGCACCACC-MGB-NFQ3'	16	68
probe MGB	taqman	G- probe	5'-VIC-CGACAGCCATGCAGCA-MGB-NFQ3'	16	71

Table ESI-1. Primers and probes sequence, Length and melting temperatures of 16s rRNA Real-Time assay.

Table ESI-2. Representative sequences for Gram-positive and Gram-negative bacterial species retrieved from GeneBank with the corresponding accession number. In red are indicated the bacterial genomic DNA used for 16s assay specificity.

Gram-Negative bacteria	Gram-Positive bacteria		
Escherichia coli (EU014689)	Staphylococcus aureus (BA000017)		
Klesbiella pnuemoniae (AY043391)	<i>Staphylococcus</i> saprophyticus (KC503908)		
Klesbiella oxytoca (AB353048)	Streptococcus pneumoniae (AF003930)		
Pseudomonas aerouginosa	Enterococcus faecalis (KC150142)		
(EU090892)			
Acinetobacter baumannii (EU221389)	Enterococcus faecium (AB326300)		
Stenotrophomonas maltophilia	Listeria monocytogenes (KR012147)		
(SMDB11_RS21915)			
Salmonella enterica (Gene ID:	Bacillus thuringiensis (KC153529)		
17778976)			
Haemophilus influenzae (AF076035)	Staphylococcus epidermidis (AM157417)		
Neisseria meningitidis (AJ239312)	Staphylococcus haemolyticus (EF522132)		
Proteus mirabilis (EU287466)	Bacillus anthracis (KF973291)		
Serratia marcescens (EU233275)	Enterococcus avium (LC269274)		
S. dysenteriae (KF928763)	C. diphtheriae (KF418791)		
N. gonorrhoae (CP016017)	<i>C. difficile</i> (JX073560)		
E. aerogenes (AM933751)	C. botulinum (L37593)		
<i>E. cloacae</i> (AM778415)	S. pyogenes (AB002521)		
P. vulgaris (AJ301683)	S. salivarius (NR042776)		
Y. enterocolitica (KT266804)	B. subtilis (KY088048)		
M. catarrhailis (MCR_RS08425)	M. tuberculosis (KY810766)		
E. sakazakii (GU122216)	<i>M. abscessus</i> (LC149865)		
C. freundii (AF025365)	L. pneumophila (FR799695)		