

Comparison of the results of the microbiologic quality of an untreated water sample using conventional culture media and a DNA chip for simultaneous detection of microorganisms

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INTRODUCTION

Water quality assessment
(drinking and recreational)



 **Public Health** 

↓
Legislation / EU Directives
98/83/CE and 2006/7/EC



**Presence of pathogenic
organisms**



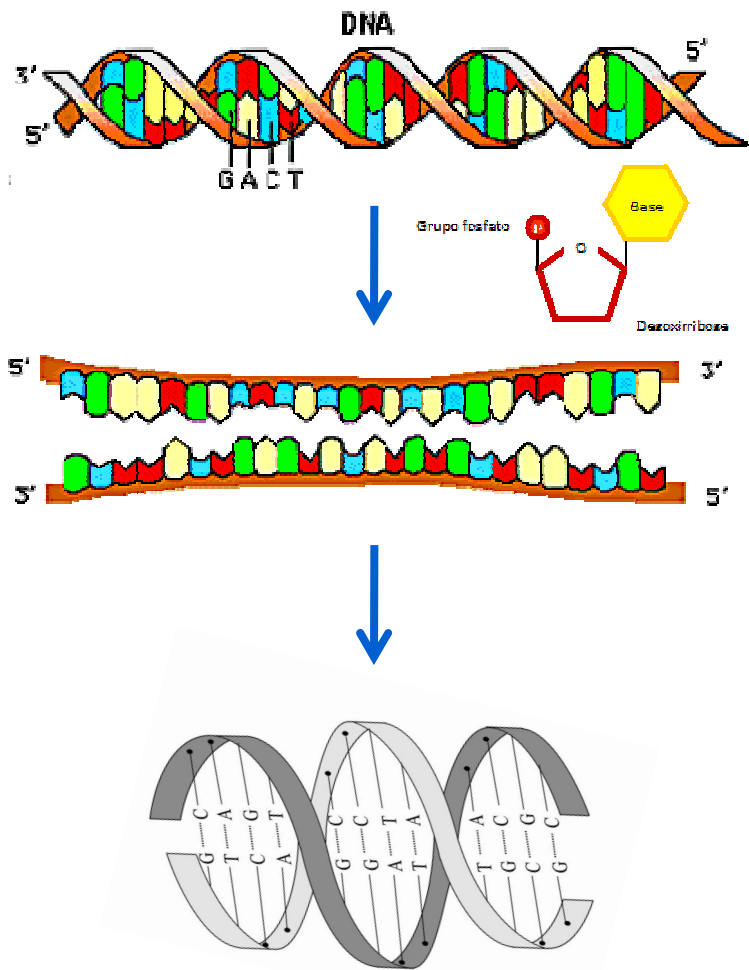
**Conventional
microbiological methods**

Time consuming, indicator bacteria
limitations...

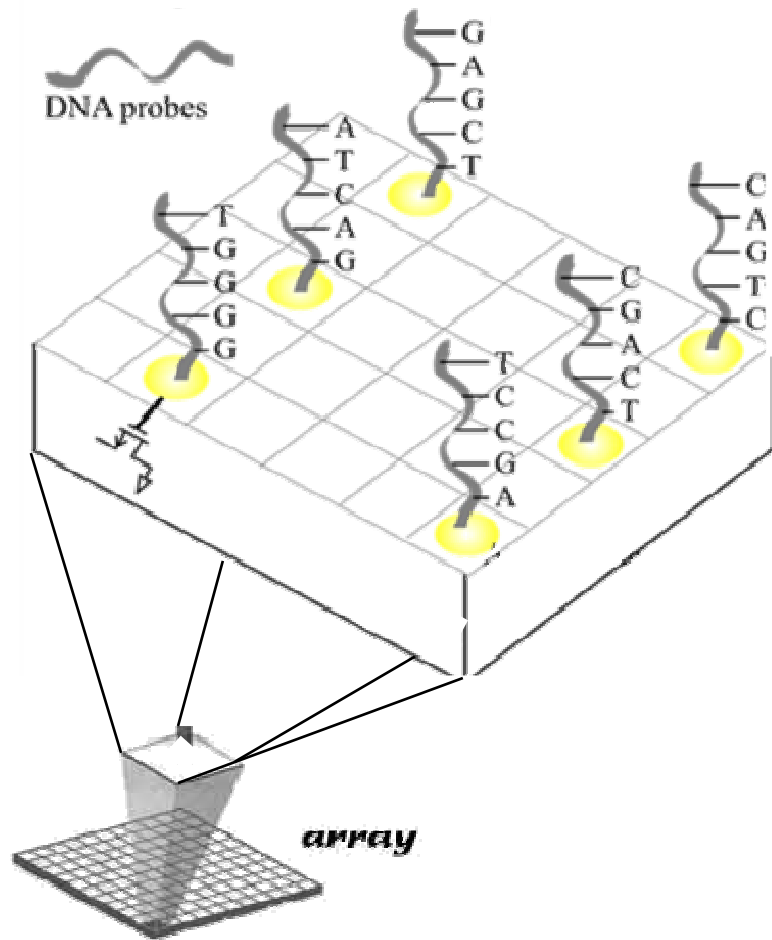
Goal of AQUACHIP project

Development of a rapid method for simultaneous detection of several
microorganisms in water samples by the use of a DNA chip

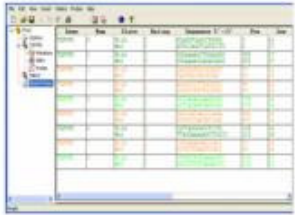
General concepts



Chip de DNA



AQUACHIP



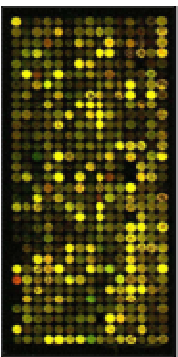
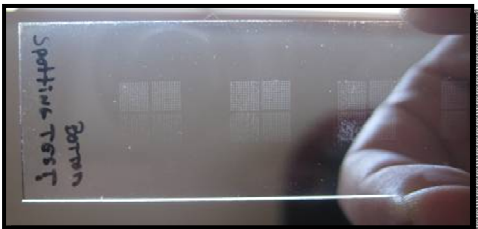
Probes designed by the software



Synthetic Oligo Manufacture



Microarray Spotter



Microarray Data for Analysis



Microarray Scan



Hybridization Process

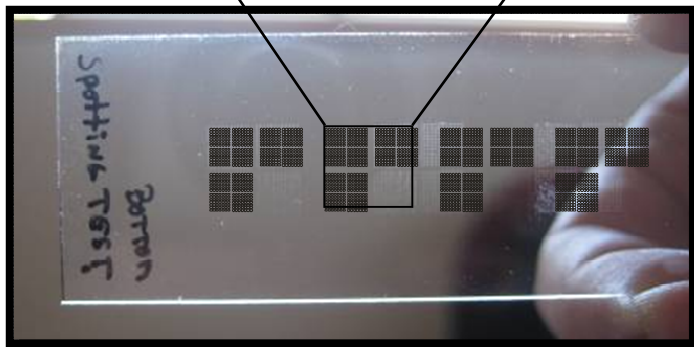
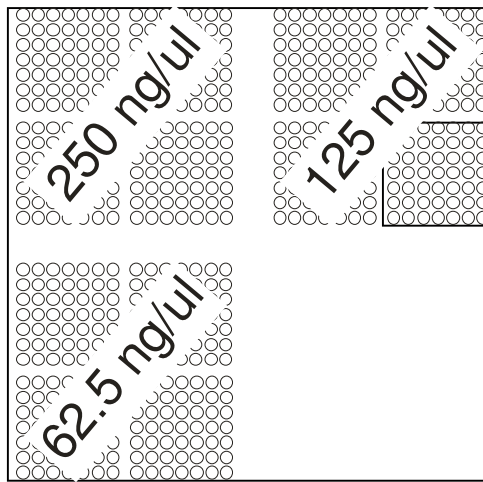


AQUACHIP

	Probe	Microorganism	Sequence	bp
Microorganisms	1	Total coliforms	16S rRNA	147
	2	<i>E. faecalis</i>	23S rRNA	140
	3	<i>E. faecium</i>	23S rRNA	161
	4	<i>E. coli</i>	putative toxin of gyrase inhibiting toxin-antitoxin system (locus_tag "ECP_0049")	162
	5	<i>E. coli</i>	<i>YeeR</i>	186
	6	<i>C. perfringens</i>	<i>spoVD</i>	184
	7	<i>E. coli</i>	<i>uidA</i>	139
	8	<i>E. coli</i>	<i>lacZ</i>	187
	9	<i>S. aureus</i>	GAP (glyceraldehyde-3-phosphate dehydrogenase, type I)	128
	10	<i>L. pneumophila</i>	<i>dotA</i>	128
	11	<i>E. coli</i> O157	putative_enterotoxin (ECs3855)	152
	12	<i>C. jejuni</i>	<i>pebA</i>	107
	13	<i>C. coli</i>	<i>gyrB</i>	134
	15	<i>Shigella</i> spp.	<i>ipaH_1</i>	111
	16	Faecal coliforms	<i>hha</i>	164
	17	<i>Salmonella</i> spp.	<i>aroG</i>	110
	18	<i>P. aeruginosa</i>	<i>oprL</i>	141
	Control	C1	Random sequence 1	Negative control
C2		Random sequence 2	Positive control	148

AQUACHIP

Random distribution
 3 different probe concentration
 Each probe spotted 8 times at each concentration



Mandatory species/groups

- 1 - Total coliforms
- 2 - *Enterococcus* spp.
- 3 - *Enterococcus* spp.
- 4 - *E. coli*
- 5 - *E. coli*
- 6 - *C. perfringens*
- 7 - *E. coli*
- 8 - *E. coli*

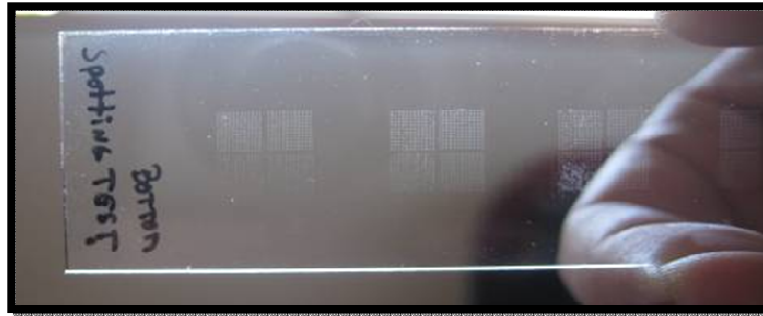
Non-mandatory species/groups

- 9 - *S. aureus*
- 10 - *L. pneumophila*
- 11 - *E. coli* O157
- 12 - *Campylobacter* spp.
- 13 - *Campylobacter* spp.
- 15 - *Shigella* spp.
- 16 - Faecal coliforms
- 17 - *Salmonella* spp.
- 18 - *P. aeruginosa*

Controls

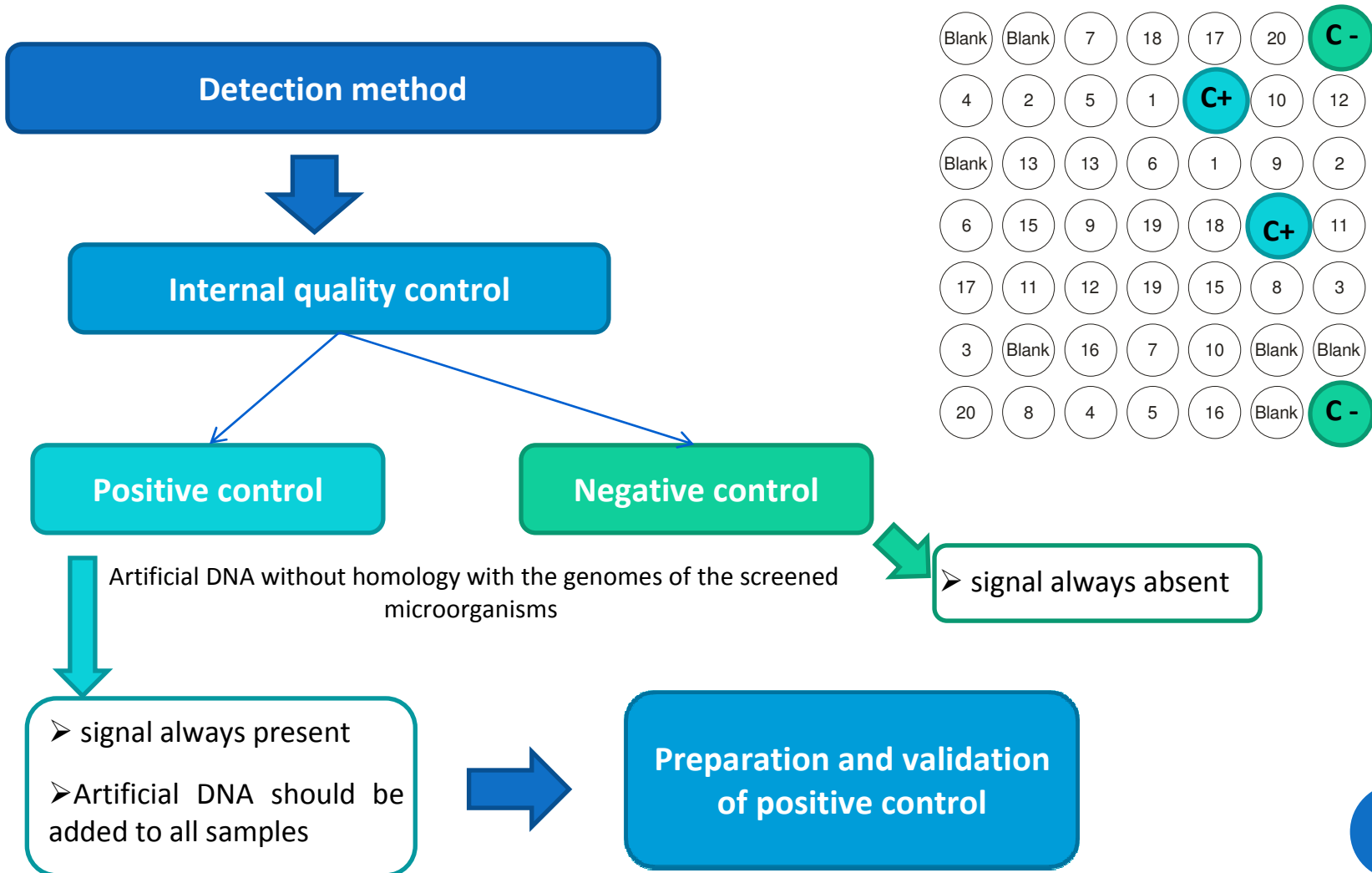
- C1 - Negative control
- C2 - Positive control

Probe and chip fabrication

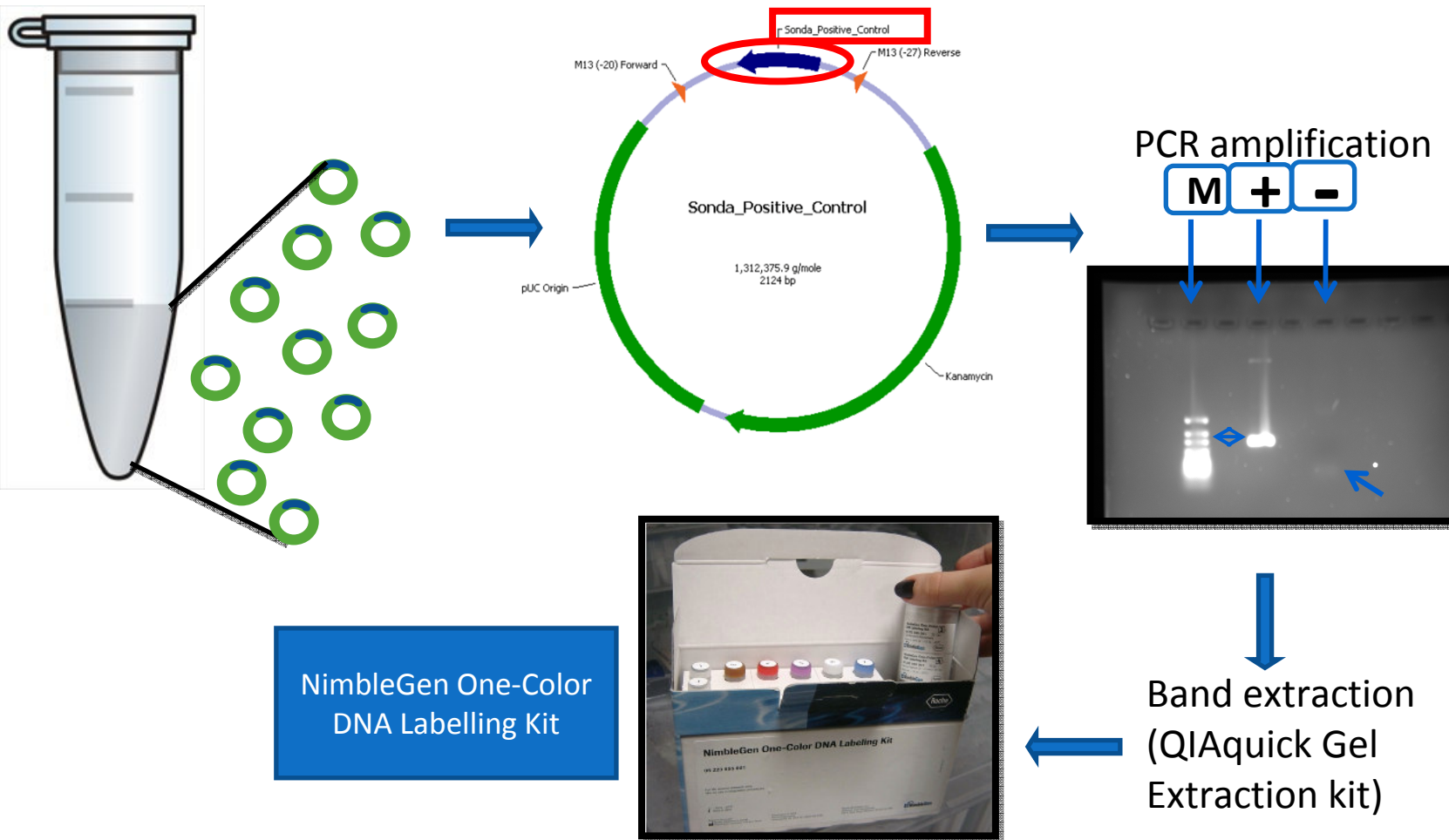


- Single-strand probe synthesis using 3' modified primers with a C6 spacer, pretreatment of Codelink[®] slides (Surmodics, Eden Prairie, MN, USA).
- Probe spotting, covalent binding of probes to slides and slide washing were all done at the external contractor facilities (Biocant, Portugal).

1. Positive control validation



1. Positive control preparation



NimbleGen One-Color
DNA Labelling Kit

Band extraction
(QIAquick Gel
Extraction kit)

two control probes with randomly generated sequences
(<http://baderlab.bme.jhu.edu/cgi-bin/gd/gdRandDNA.cgi>)

1. Positive control hybridization

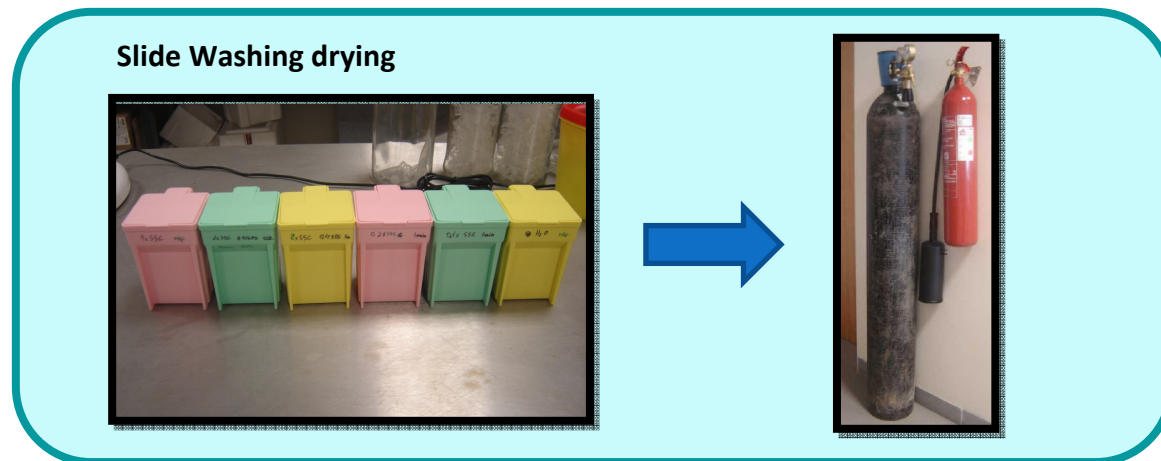
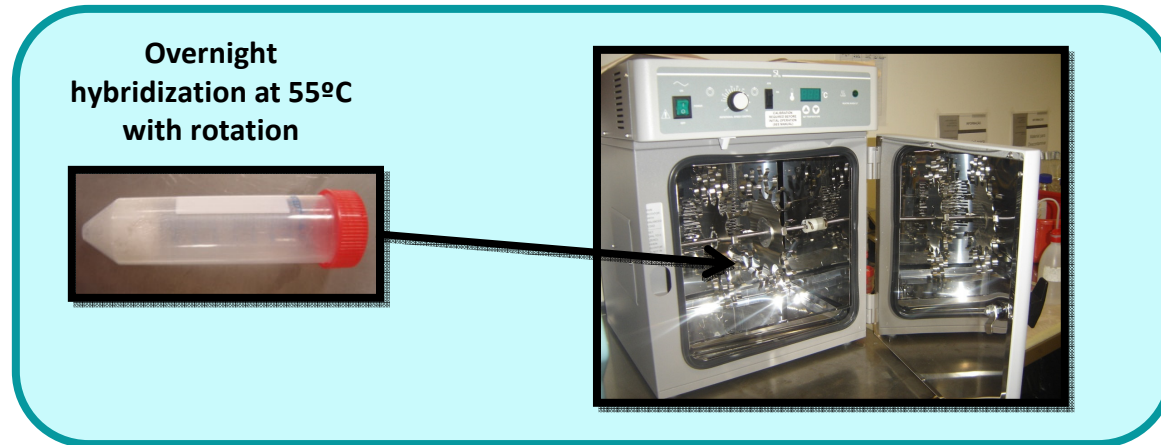
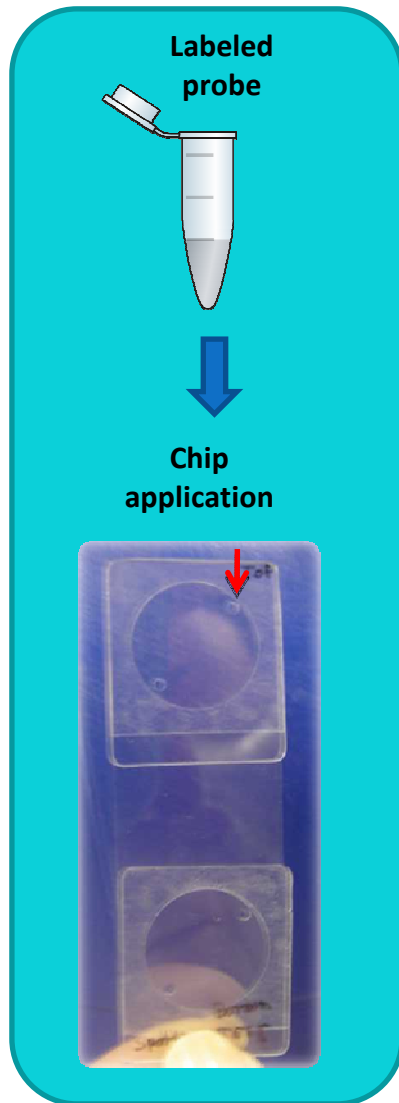
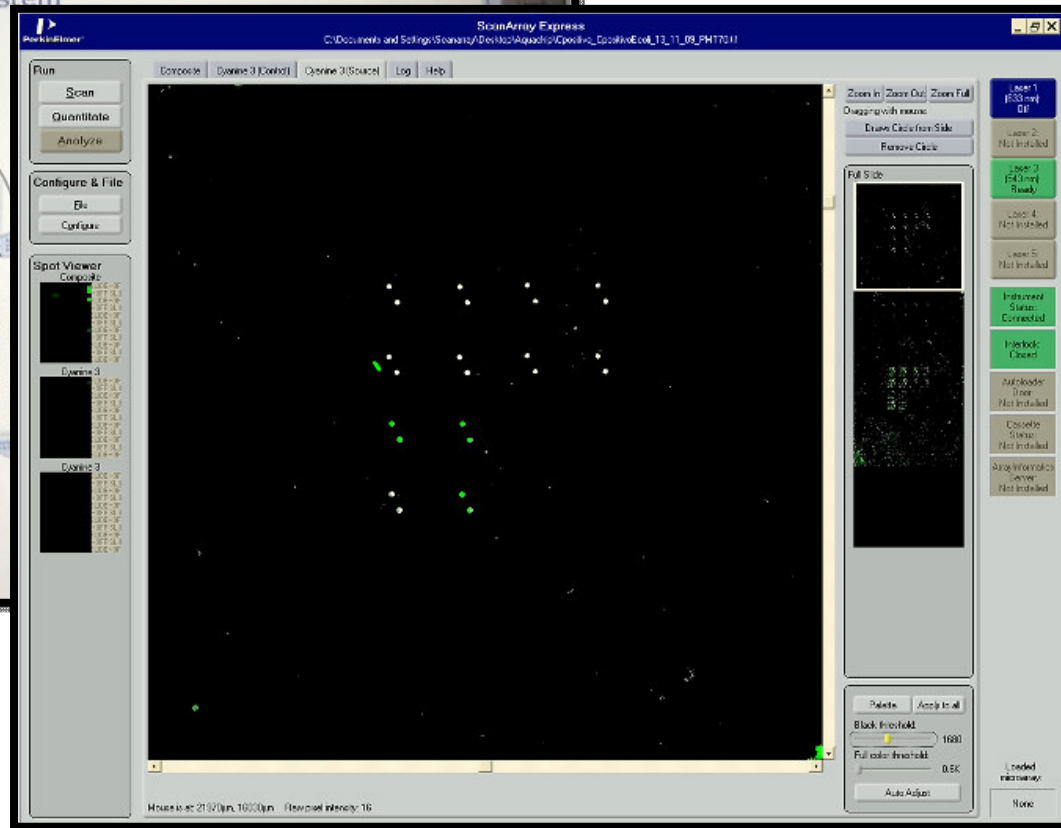
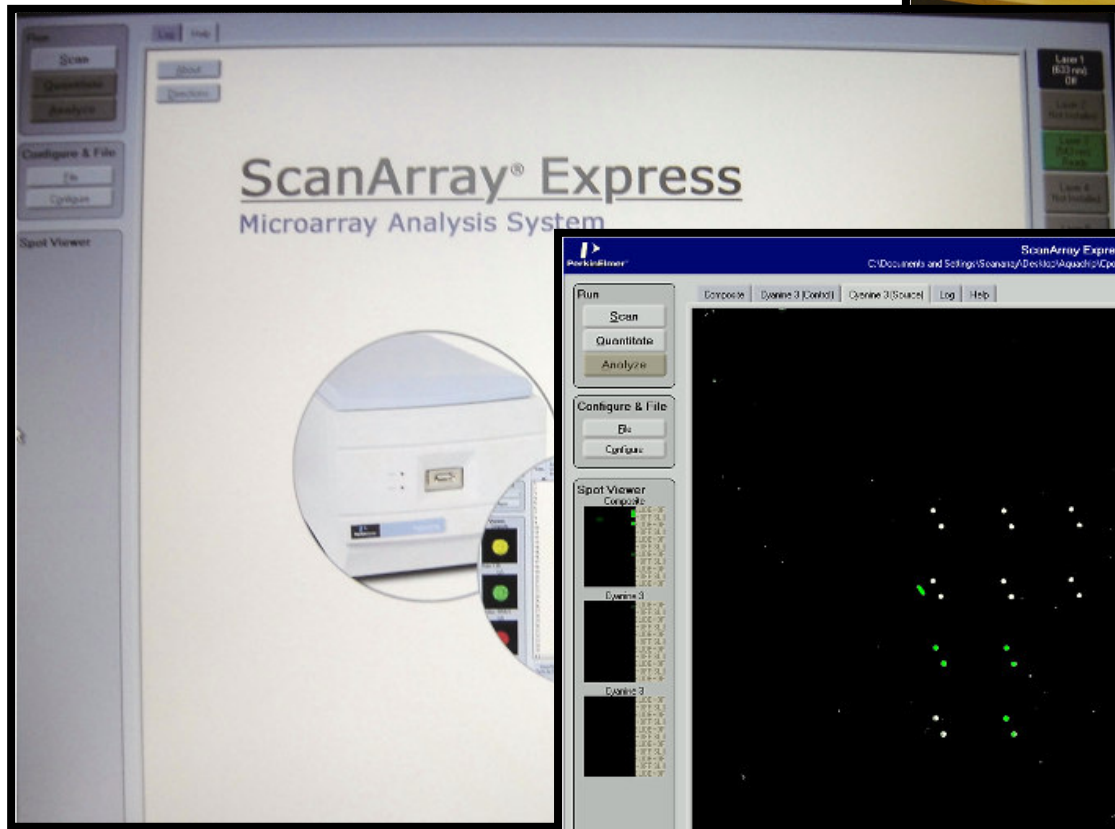


Image acquisition and data analysis

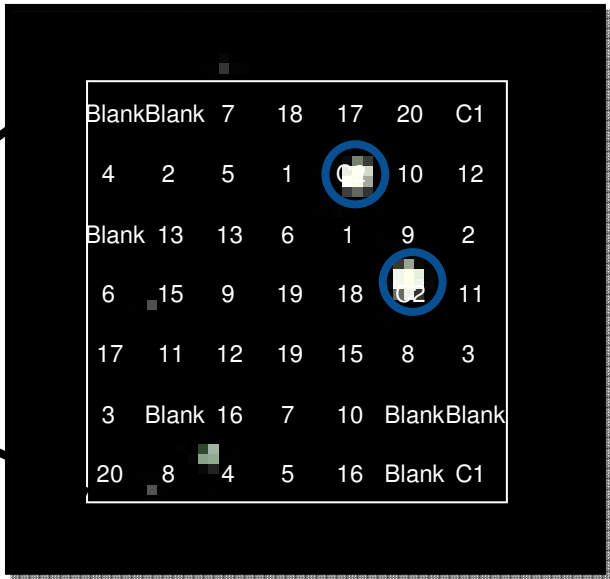
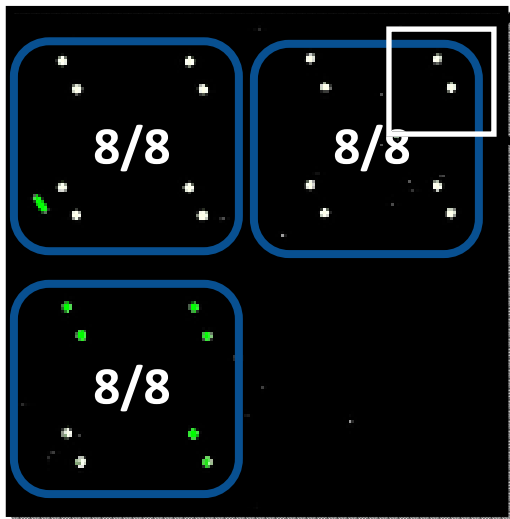
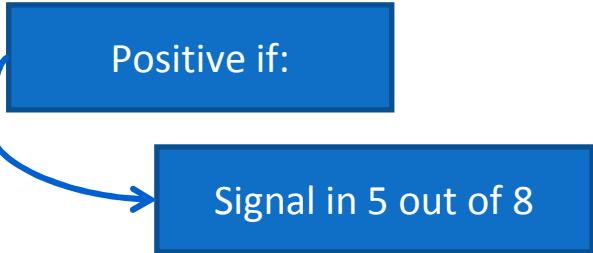
1. Positive control validation

Image acquisition



1. Positive control validation

Data analysis

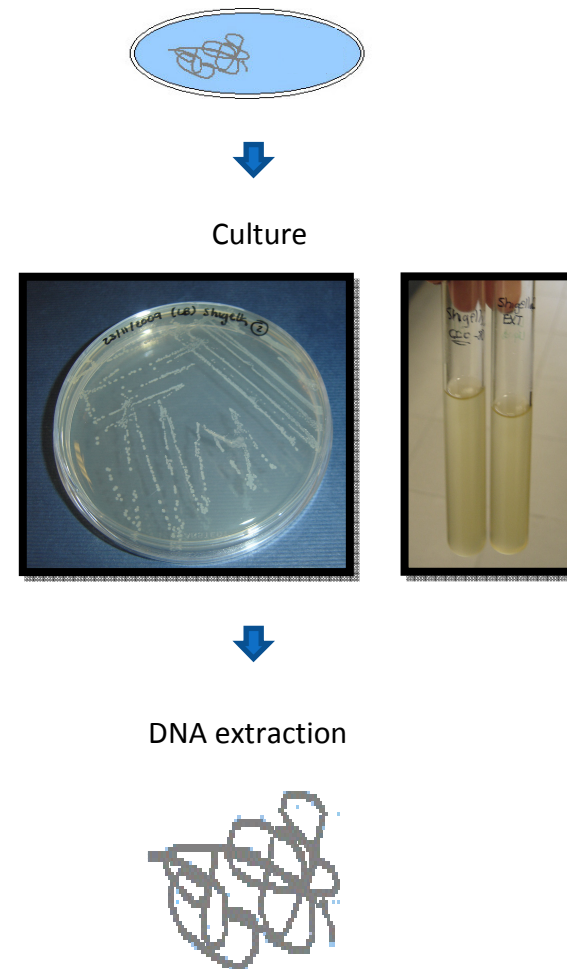
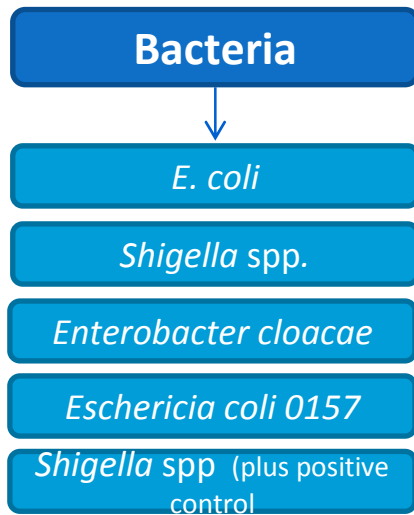


Controls
C1 – Negative control
C2 – Positive control

Positive control validated

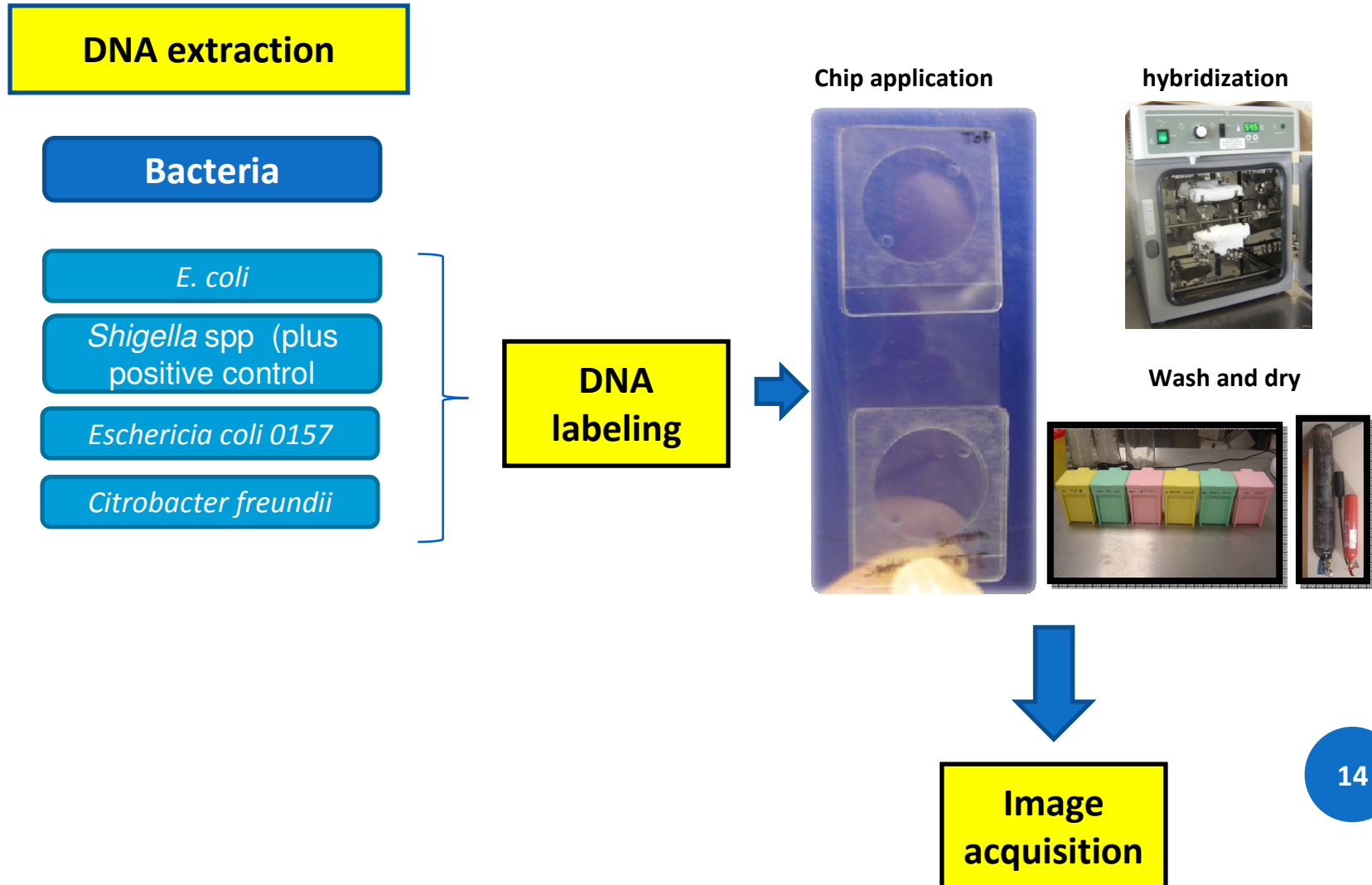
2. Validation of other probes

Sample preparation



2. Validation of other probes: some examples

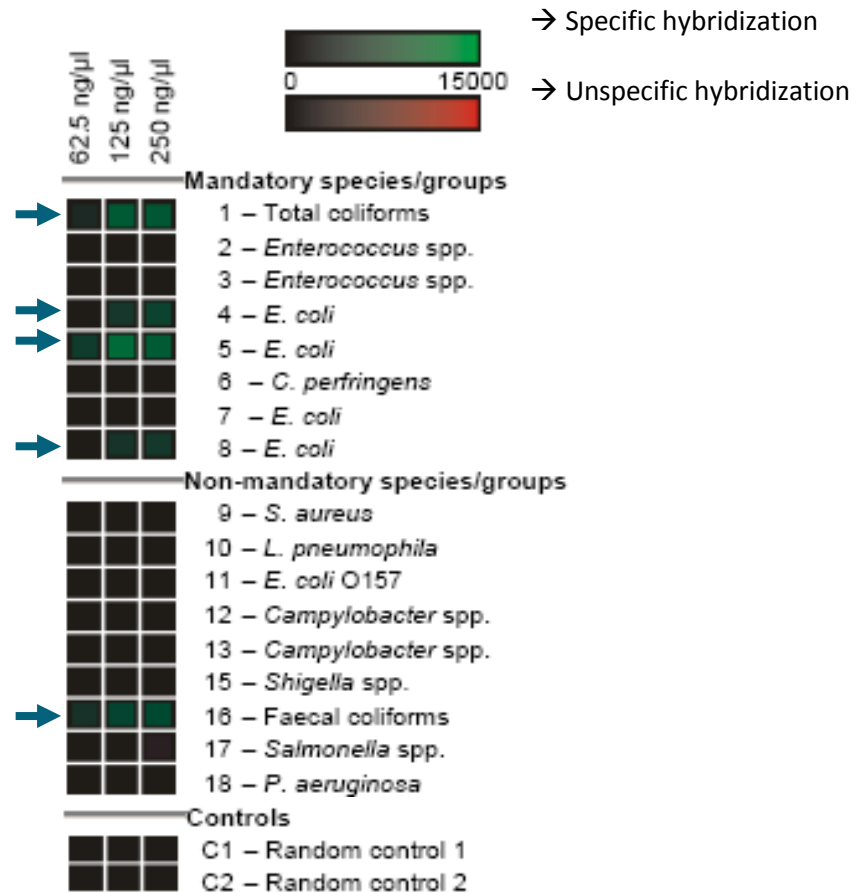
Labelling and hybridization



Probes for *E. coli* : validation

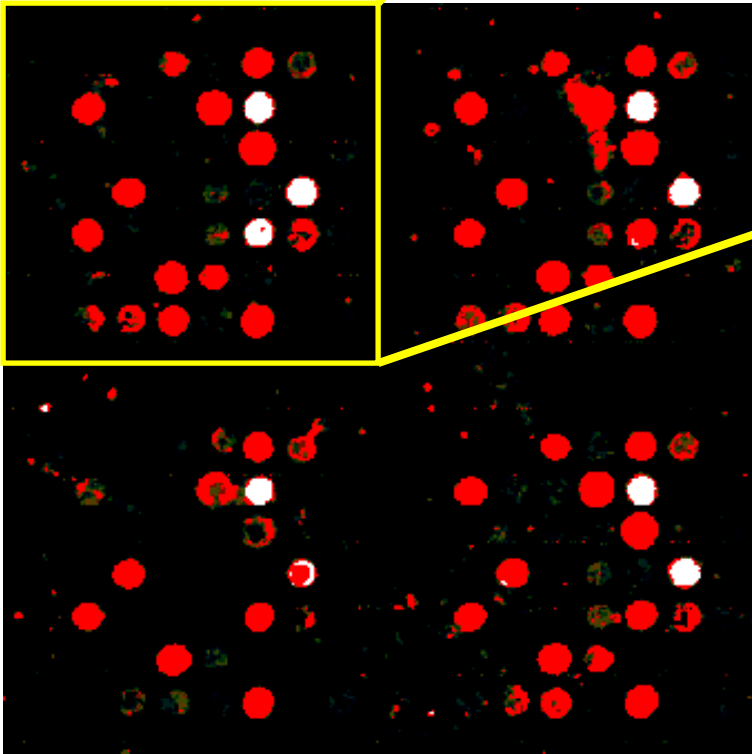
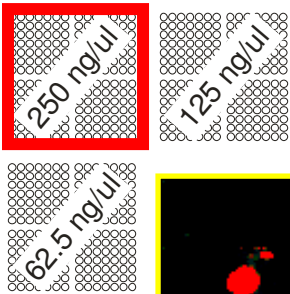
➤ Test different probes

➤ *Escherichia coli*



Probe 4 weak signal
Probe 7 no signal

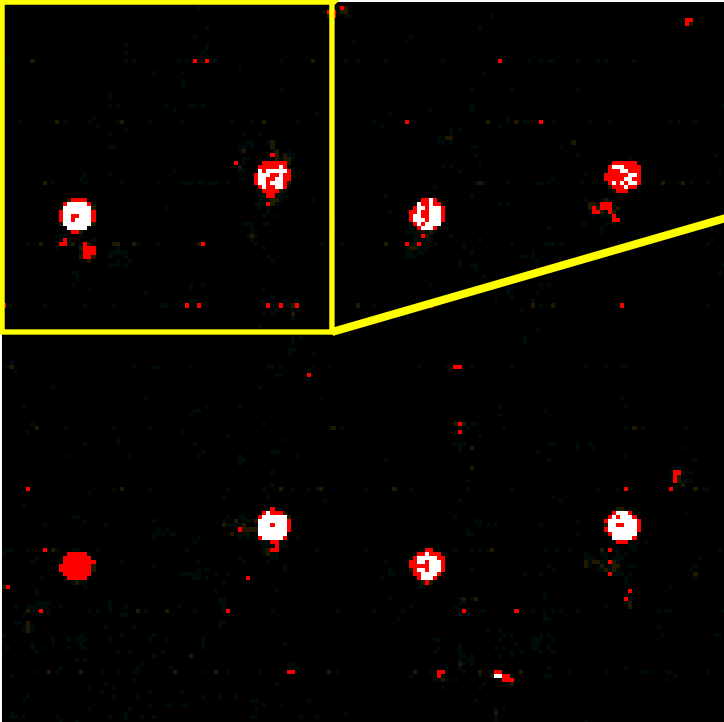
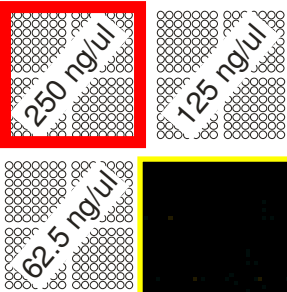
Shigella spp (plus positive control) : validation



Results

- 1 – Total coliform
- 16 – Faecal coliform
- 15 - *Shigella*
- C+ - positive control
- 4,7,8 – *E. coli* X
- 17 – *Salmonella* X

Eschericia coli 0157

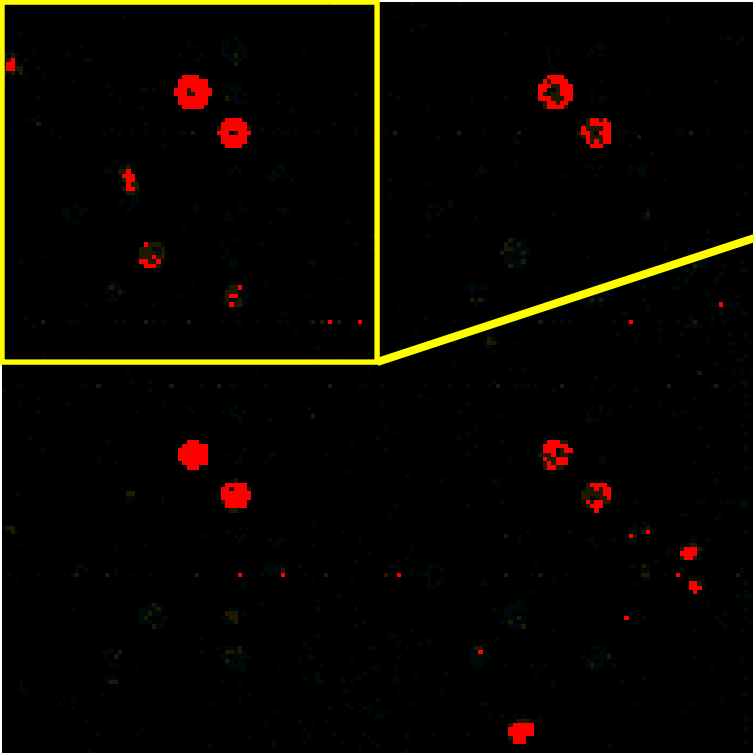
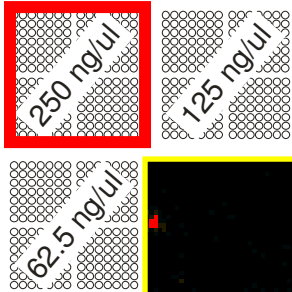


Results

11 – *E. coli* 0157

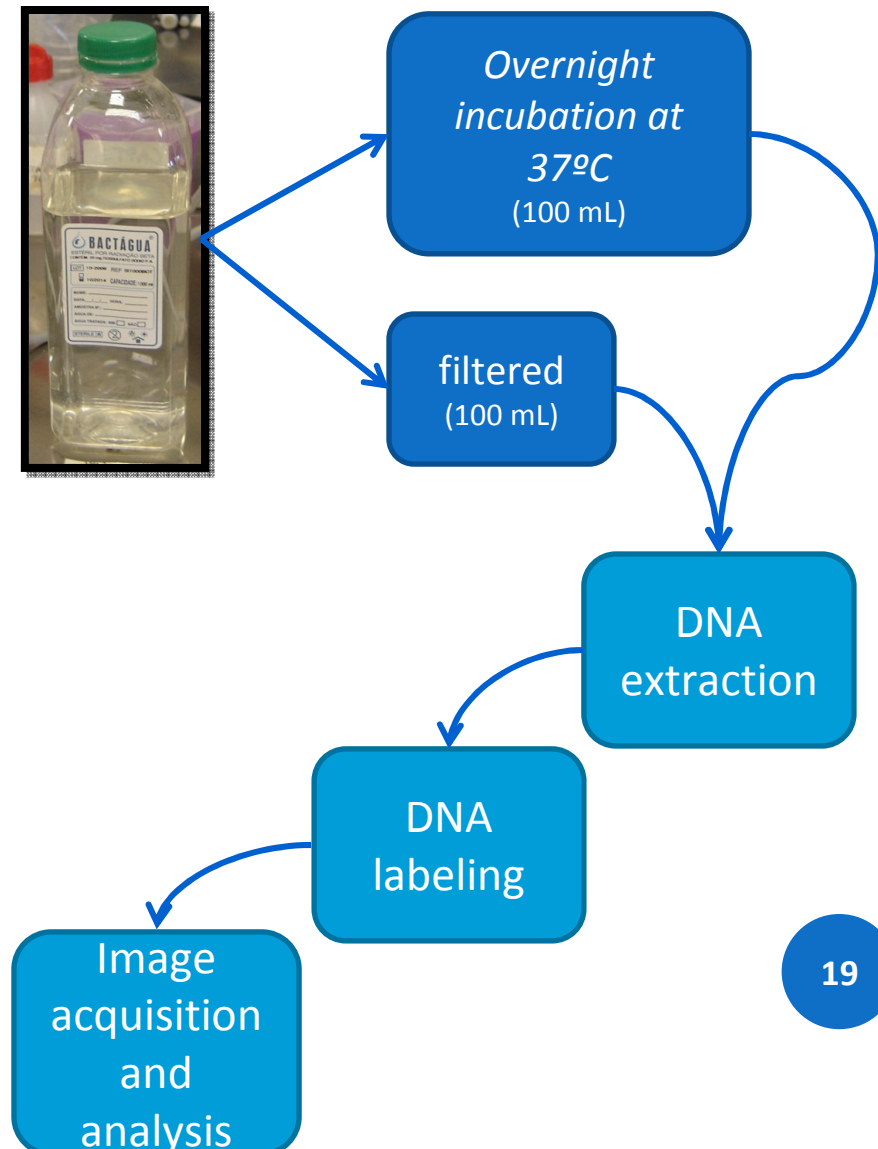
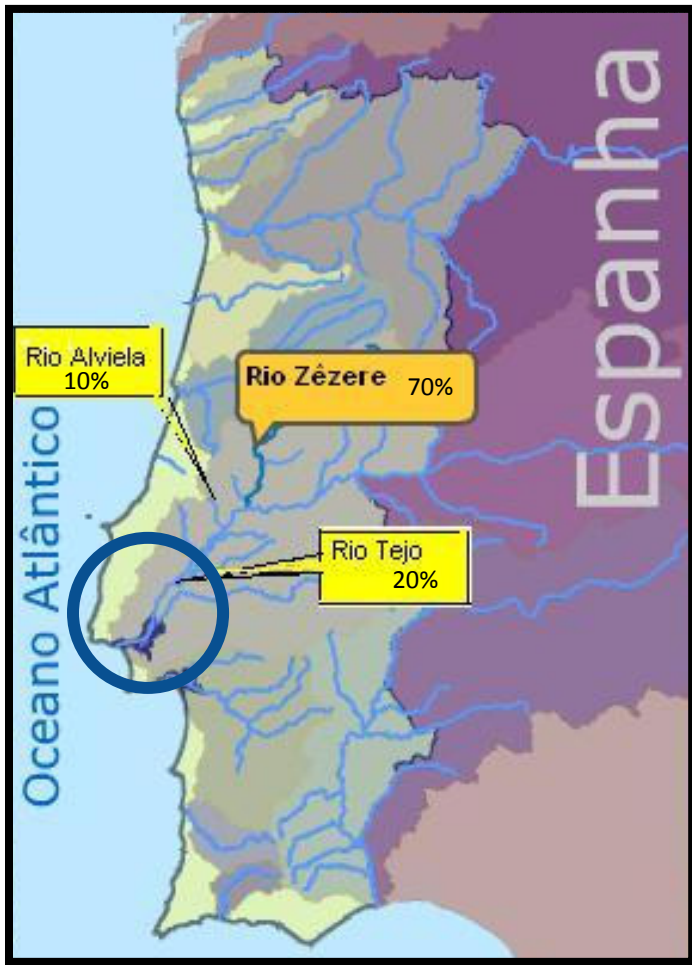
Without hybridization with other *E. coli* probes

Citrobacter freundii



Results
1 – Total coliform

3. Real water test: Tagus river captation of EPAL (untreated water!)



Water filtration...



DNA extraction
from water
sample

DNA labelling
and
hybridization

result

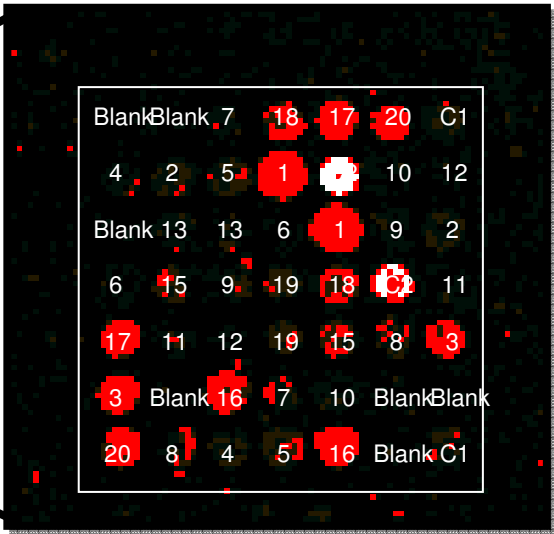
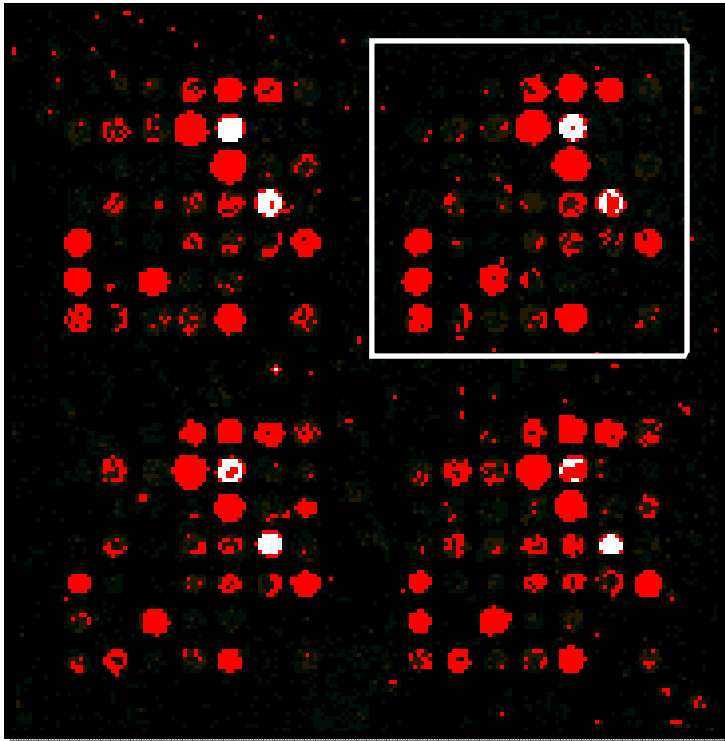
3. Real water test: Tagus river captation of EPAL (untreated water!)

CHIP RESULTS



Culture Tagus River + positive control

250 ng/μL



- C2 – Controlo Positivo
- 1 – Coliforme Total
- 2 – *Enterococcus* spp.
- 3 - *Enterococcus* spp.
- 5 – *E.coli*
- 8 - *E.coli*
- 15 – *Shigella* spp.
- 16 – Coliforme Fecal
- 17 – *Salmonella* spp.
- 18 – *P.aeruginosa*

Chip vs. Conventional results

	Probe	Microorganism	Chip positive		ufc/100ml	Conventional method
Microorganisms	1	Total coliforms	✓		2282	Total coliforms
	2	<i>E. faecalis</i>	✓			Enterococcus
	3	<i>E. faecium</i>	✓		68	
	4	<i>E. coli</i>			268	<i>E. coli</i>
	5	<i>E. coli</i>	✓			
	6	<i>C. perfringens</i>				
	7	<i>E. coli</i>				
	8	<i>E. coli</i>	✓			
	9	<i>S. aureus</i>				
	10	<i>L. pneumophila</i>				
	11	<i>E. coli</i> O157				
	12	<i>C. jejuni</i>				
	13	<i>C. coli</i>				
	15	<i>Shigella</i> spp.	✓			
	16	Faecal coliforms	✓		282	Faecal coliforms
	17	<i>Salmonella</i> spp.	✓		Absence 1L	<i>Salmonella</i> spp.
	18	<i>P. aeruginosa</i>	✓		0	<i>P. aeruginosa</i>
	Control	C1	Random sequence 1			
C2		Random sequence 2	✓			

Chip vs. Conventional results

	Probe	Microorganism	Chip positive	ufc/100ml	Conventional method
Microorganisms	1	Total coliforms	✓	2282	Total coliforms
	2	<i>E. faecalis</i>	✓		Enterococcus
	3	<i>E. faecium</i>	✓	68	
	4	<i>E. coli</i>	X	268	<i>E. coli</i>
	5	<i>E. coli</i>	✓		
	6	<i>C. perfringens</i>			
	7	<i>E. coli</i>	X		
	8	<i>E. coli</i>	✓		
	9	<i>S. aureus</i>			
	10	<i>L. pneumophila</i>			
	11	<i>E. coli</i> O157			
	12	<i>C. jejuni</i>			
	13	<i>C. coli</i>			
	15	<i>Shigella</i> spp.	✓		
	16	Faecal coliforms	✓	282	Faecal coliforms
	17	<i>Salmonella</i> spp.	✓	Absence 1L	<i>Salmonella</i> spp.
	18	<i>P. aeruginosa</i>	✓	0	<i>P. aeruginosa</i>
	Control	C1	Random sequence 1		
C2		Random sequence 2	✓		

CONCLUSION

- ❖ Positive control validation
 - ❖ Correct hybridization
 - ❖ Helpful for array alignment and identification of positive spots in samples of unknown composition

- ❖ *E. coli* (principal indicator bacteria) with 4 different probes on chip
 - ❖ Two of these probes give expected results (5 and 8) and the other two (4 and 7) should not be considered

- ❖ *E. coli* O157; *Citrobacter freundii*
 - ❖ Correct hybridization

- ❖ Shigella probe
 - ❖ DNA from *Shigella* hybridizes with probes for *Shigella* and *E. coli*
 - ❖ DNA from *E. coli* do not hybridize with probes for *Shigella*
 - ❖ Great homology between *Shigella* spp. and *E. coli* genomes

CONCLUSION

- ❖ Real water: untreated water from Tagus river captation of EPAL
 - ❖ pre culture step
 - ❖ increase the sample volume of filtered water
 - ❖ discordant results for *Pseudomonas* spp. and *Salmonella* spp. (chip positive only)
 - ❖ *Salmonella* probe, which has 95% homology to *E. coli* DNA, according to BLAST [4] (E-value 3E-28), may justify a positive signal
 - ❖ Stressed bacteria may be unable to grow in selective mediums
 - ❖ general agreement of results between Aquachip and conventional methods
 - ❖ reinforces the utility and the proof-of-concept of the DNA chip

ACKNOWLEDGMENTS

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