Foodborne Pathogen Detection and Identification Using VereFoodborne™ Chip Technology

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Introduction

Foodborne diseases are responsible for a wide range of illnesses and are a growing public health problem all over the world. Current methodology for foodborne identification requires much time and effort and this slows down the response of health authorities.

Here, we describe the technology of an integrated PCR-microarray platform, the VereFoodborne™ Chip. This platform is based on a miniaturized silicon chip that integrates a PCR reactor and DNA microarray. Hybridization of the multiplex-PCR products by DNA microarray enables multiple, accurate and highly sensitive detection of foodborne pathogens in a single test.

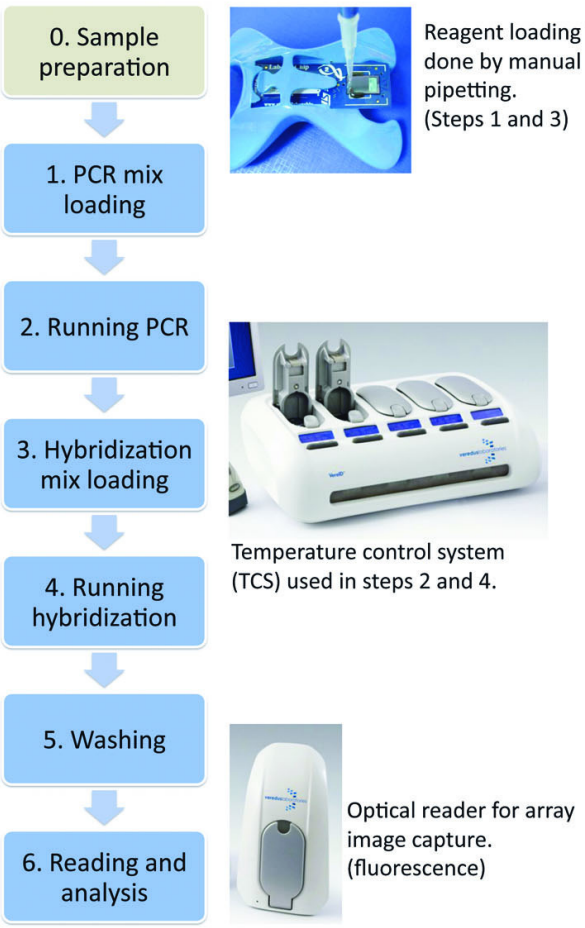


Figure 1 VereFoodborne™ assay process.
5 chips are processed in one TCS.
Assay time (steps 1 to 6): 2 hrs

#	pathogen	target gene		
1	Vibrio cholerae	Tu		
2	Vibrio parahaemolyticus	Tu		
3	Staphylococcus aureus	Tu		
4	Listeria monocytogenes	Tu		
5	Bacillus cereus	Tu		
6	Clostridium perfringens	Tu		
7	Campylobacter jejuni/coli/lari	Tu		
8	Escherichia coli	mutS	Stx 1A and 2A	STEC
9	Shigella flexneri/sonnei/ dysenteriae	mutS+sp		S. dysenteriae
10	Salmonella	mutS	ttr, invA	
11	Cronobacter sakazakii	sp		
12	Norovirus	pol-capsid		
	PCR control	Luciferase		

Table 3 VereFoodborne™ target pathogens (ver. 3.0).
Tu: elongation factor Tu; mutS: mismatch repair protein
sp: specific region found in our sequence analysis.

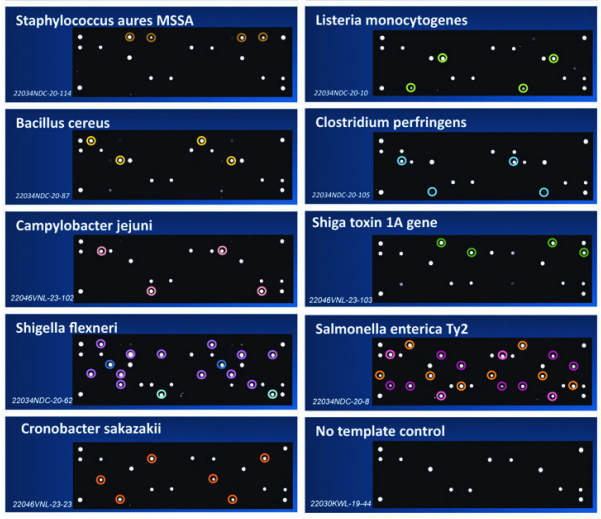
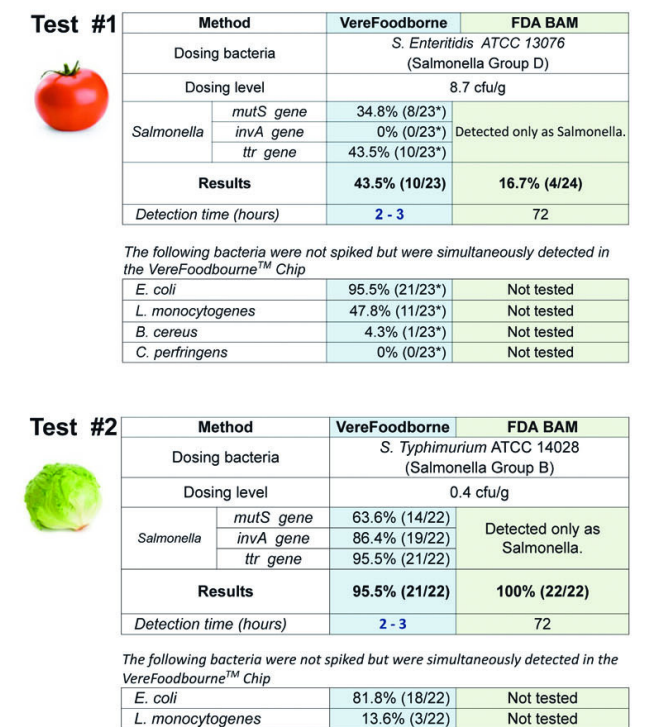


Figure 4 VereFoodborne™ chip result 1 (single target).
300-1,000 copies of genomic DNA or 100 copies of plasmid (stx1A) was tested as a template. Target signals are marked by color circles.



	Result	Sample information (showed after the test)
Test A	Positive for Vibrio parahaemolyticus, Camplybobacter and Salmonella.	Vibrio parahaemolyticus (50 CFU eq.), Campylobacter coli (50 CFU eq.), Salmonella spp. (5 CFU eq.).
Test B	Positive for Vibrio parahaemolyticus, Camplybobacter and Salmonella.	

Figure 5 VereFoodborne™ chip result 2 (mixed sample).
Mixed genome sample was given from National Environment Agency, Singapore (NEA). The sample was tested on VereFoodborne version 1.1 and 2.0.

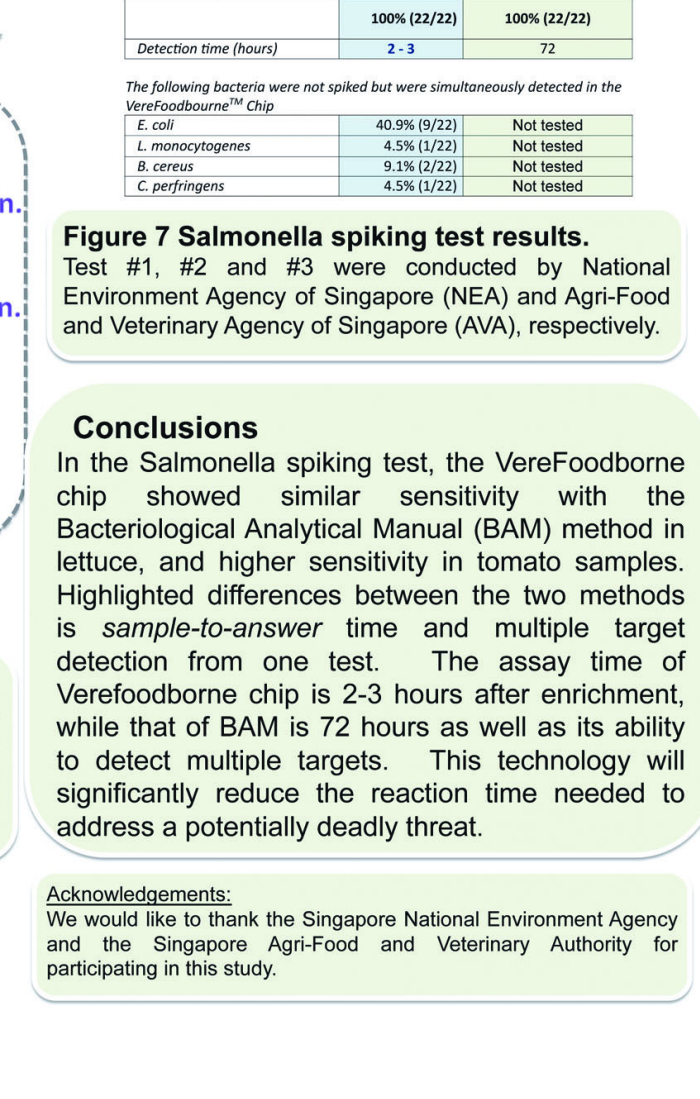


Figure 6 Experimental scheme of Salmonella spiking test.
Salmonella was inoculated onto separated tomato and lettuce samples, then enriched with universal pre-enrichment broth as suggested in Bacteriological Analytical Manual (BAM) procedure. Salmonella detection was performed by BAM and VereFoodborne chip.

Test #1

Method		VereFoodborne	FDA BAM
Dosing bacteria		*S. Enteritidis ATCC 13076* (Salmonella Group D)	
Dosing level		8.7 cfu/g	
Salmonella	*mutS gene*	34.8% (8/23*)	Detected only as Salmonella.
	invA gene	0% (0/23*)	
	ttr gene	43.5% (10/23*)	
Results		**43.5% (10/23)**	**16.7% (4/24)**
Detection time (hours)		**2 - 3**	72

The following bacteria were not spiked but were simultaneously detected in the VereFoodbourne™ Chip

E. coli	95.5% (21/23*)	Not tested
L. monocytogenes	47.8% (11/23*)	Not tested
B. cereus	4.3% (1/23*)	Not tested
C. perfringens	0% (0/23*)	Not tested

Test #2

Method		VereFoodborne	FDA BAM
Dosing bacteria		*S. Typhimurium* ATCC 14028 (Salmonella Group B)	
Dosing level		0.4 cfu/g	
Salmonella	*mutS gene*	63.6% (14/22)	Detected only as Salmonella.
	invA gene	86.4% (19/22)	
	ttr gene	95.5% (21/22)	
Results		**95.5% (21/22)**	**100% (22/22)**
Detection time (hours)		**2 - 3**	72

The following bacteria were not spiked but were simultaneously detected in the VereFoodbourne™ Chip

E. coli	81.8% (18/22)	Not tested
L. monocytogenes	13.6% (3/22)	Not tested
B. cereus	9.1% (2/22)	Not tested
C. perfringens	18.2% (4/22)	Not tested

Test #3

Method		VereFoodborne	FDA BAM
Dosing bacteria		*S. Typhimurium* (Salmonella Group B)	
Dosing level		0.4 cfu/g	
Salmonella	*mutS gene*	95.5% (21/22)	Detected only as Salmonella.
	invA gene	100% (22/22)	
	ttr gene	100% (22/22)	
Results		**100% (22/22)**	**100% (22/22)**
Detection time (hours)		**2 - 3**	72

The following bacteria were not spiked but were simultaneously detected in the VereFoodbourne™ Chip

E. coli	40.9% (9/22)	Not tested
L. monocytogenes	4.5% (1/22)	Not tested
B. cereus	9.1% (2/22)	Not tested
C. perfringens	4.5% (1/22)	Not tested

Figure 7 Salmonella spiking test results.
Test #1, #2 and #3 were conducted by National Environment Agency of Singapore (NEA) and Agri-Food and Veterinary Agency of Singapore (AVA), respectively.

VereFoodborne™ Panel 3.0

	1	2	3	4	5	6	7	8	9	10	11
1	AT683	BA_BC1s	SHX_2sd	ttr_2s	G1_157EC3s	BA_SA2s	G1STX_3s	BA_SA1s_SLD2	G2_VP2s	empty	AT809
2	AT730	G1_SE2s	LUC_2s	E_CJ1s	AT809	NLVG1_3s	STX2A_3	SAKA_4s	SHX_4sd	G1STX_2s	empty
3	empty	STX2A_1	BA_CP1s	G1_SHX1s	BA_BC3s	AT683	BA_LM4s	G2_VC2s	invA_4s	empty	empty
4	NLVG1_1s	LUC3_1s	NLVG1_2s	G2_VP1s_plus	SHX_3s	NLVG2_2s_plus	empty	G1_EC5s	LUC3_3s	AT809	empty
5	AT809	NLVG2_1s	G1_1257EC2s	invA_2s	G1_EC4s	LUC3_2s	SHX_4s	LUC_3s	ttr_4s	AT776	empty
6	AT683	STX2A_2	G2_VC1s	BA_LM2s	SAKA_2s	BA_CP2s	G1_SE3s	E_CJ2s	G1_157SHX 1s	empty	AT809

	12	13	14	15	16	17	18	19	20	21
1	empty	BA_BC1s	SHX_2sd	ttr_2s	G1_157EC3s	BA_SA2s	G1STX_3s	BA_SA1s_SLD2	G2_VP2s	AT683
2	AT730	G1_SE2s	LUC_2s	E_CJ1s	AT809	NLVG1_3s	STX2A_3	SAKA_4s	SHX_4sd	G1STX_2s
3	empty	STX2A_1	BA_CP1s	G1_SHX1s	BA_BC3s	AT683	BA_LM4s	G2_VC2s	invA_4s	empty
4	NLVG1_1s	LUC3_1s	NLVG1_2s	G2_VP1s_plus	SHX_3s	NLVG2_2s_plus	empty	G1_EC5s	LUC3_3s	AT809
5	AT809	NLVG2_1s	G1_1257EC2s	invA_2s	G1_EC4s	LUC3_2s	SHX_4s	LUC_3s	ttr_4s	AT776
6	empty	STX2A_2	G2_VC1s	BA_LM2s	SAKA_2s	BA_CP2s	G1_SE3s	E_CJ2s	G1_157SHX 1s	AT683

Vibrio cholerae
Vibrio parahaemolyticus
Staphylococcus aureus
Listeria monocytogenes
Bacillus cereus
Clostridium perfringens
Campylobacter (jejuni, coli, lari)
Escherichia coli spp.
Shigella spp.
Cronobacter sakazakii
NLV Geno Group I
NLV Geno Group II
Shiga toxin-producing Escherichia coli (e.g. E.coli O157)
Shiga toxin-producing Shigella (e.g. Shigella dysenteriae)
Salmonella spp.
RT-PCR control
Positive hybridization control
Negtive hybridization control

Figure 2 VereFoodborne™ probe layout (ver. 3.0).
Layout is in 6 x 21 format, which consists of:
84 capture probes (42 x 2 replicates)
10 hybridization control probes
10 PCR control probes
8 hybridization negative control probes

Conclusions

In the Salmonella spiking test, the VereFoodborne chip showed similar sensitivity with the Bacteriological Analytical Manual (BAM) method in lettuce, and higher sensitivity in tomato samples. Highlighted differences between the two methods is *sample-to-answer* time and multiple target detection from one test. The assay time of Verefoodborne chip is 2-3 hours after enrichment, while that of BAM is 72 hours as well as its ability to detect multiple targets. This technology will significantly reduce the reaction time needed to address a potentially deadly threat.

Acknowledgements:
We would like to thank the Singapore National Environment Agency and the Singapore Agri-Food and Veterinary Authority for participating in this study.