Supporting Information

Suspension Bead Array of the Single-Stranded Multiplex Polymerase Chain Reaction Amplicons for Enhanced Identification and Quantification of Multiple Pathogens

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■ MATERIALS and METHODS

Bacterial strains, Media and Cultures. Reference strains were used in the study for the establishment of the assay and to ensure the positive detection, including *Bacillus anthracis* ATCC 4229, *Bacillus anthracis* ATCC 14186, *Burkholderia mallei* ATCC 15310, *Burkholderia pseudomallei* ATCC 11668, *Brucella abortus* ATCC 4315, *Brucella melitensis* ATCC 23456, *Escherichia coli* O157:H7 ATCC 43895, *Francisella tularensis* ATCC 15482, *Salmonella typhi* ATCC 167, *Shigella dysenteriae* ATCC 11835, *Vibrio cholerae* ATCC 9458, *Yersinia pestis* ATCC 19428. Additional strains were analyzed to determine the specificity of the assay, including *Bacillus cereus* ATCC 11778, *Bacillus thuringiensis* ATCC 10792, *Bacillus circulans* ATCC 4513, *Haemophilus influenzae* ATCC 33533, *Haemophilus parainfluenzae* ATCC 7901, *Legionella pneumophila* ATCC 33153, *Listeria monocytogenes* ATCC 19111, *Pseudomonas aeruginosa* ATCC 27853, *Neisseria meningitides* ATCC 13077, *Staphylococcus aureus* ATCC 6538, *Streptococcus pneumonia* ATCC 6302, *Yersinia frederikenii* ATCC 29912. These bacterial strains were grown in brain-heart infusion agar (BHIA) at 37 ˚C for 20−48 h.

Somatic antigen suspension of *Coxiella burnetii* QNM strain was purchased from Life Science Research Israel (Israel).

Suspension Bead Array. (1) Preparation of probe-coupled microbeads: Briefly, five million microbeads were pelleted at 12,000 x *g* for 4 min and resuspended in 50 μL of 0.1 M

2−Morpholinoethane sulfonic acid (MES), pH 4.5 by vortexing and sonication for 30 s for each. 4 μL of 0.1 mM 5'−amino modified oligonucleotides was added into the suspension. Then 2.5 μL of 10 mg/ml 1−Ethyl−3−3−dimethylaminopropyl carbodiimide (EDC) (Thermo Fisher Scientific Inc., U.S.A.) was added into the mixture and the coupling reaction was incubated at room temperature in the dark for 30 min. A further addition of 2.5 μL of 10 mg/ml EDC and incubation was repeated once. 1 ml of 0.02% Tween−20 was added to the mixture and vortexed for 20 s. The microbeads were then pelleted by centrifugation at 12,000 x *g* for 2 min and resuspended in 1 ml of 0.1% SDS by vortexing. The microbeads were pelleted again and resuspended in 100 μL of Tris-EDTA (TE, pH 8.0) buffer and the concentrations of microbeads were determined with a hemocytometer. Finally the coupled microbeads were adjusted to a final concentration of 20,000 beads per μL with TE, pH 8.0 buffer and could be stored at 4 °C in the darkness for at least one year. (2) Hybridization: 17 μL of the ss-PCR product was analyzed in a 50 μL hybridization assay mixture containing 3 M tetramethyl ammonium chloride (Sigma, Denmark), 50 mM Tris−HCl (pH 8.0), 4 mM EDTA (pH 8.0), 0.1% Sarkosyl (Sigma), and 2,500 of probe-coupled microbeads from each set. The mixture was incubated for 10 min at 95 °C and 30 min at 50 °C. Then the mix was transferred to a 96-well filter plate (Millipore, Bedford, MA). The microbeads were washed once with 100 μL of hybridization buffer (3 M tetramethyl ammonium chloride, 50 mM Tris−HCl (pH 8.0), 4 mM EDTA (pH 8.0), and 0.1% Sarkosyl) followed by incubating with

100 μL of hybridization buffer containing 250 ng of PE-streptavidin (SAPE) (BD Pharmingen, NJ, U.S.A.) for 5 min. The hybridized microbeads were washed with 100 μL of hybridization buffer again and resuspended in 75 μL of hybridization buffer. (3) Flow Cytometry Analysis: The red laser (635 nm) identified the bead by its color coding and the green laser (532 nm) examined the SAPE fluorescent molecules binding to the hybridized PCR product on each bead. The reactions were analyzed with Bio-Plex 200 system (Bio-Rad) and Bio-Plex Manager (version 5.0) according to the manufacturer's instructions. At least one hundred beads from each bead set in each sample were analyzed and the results were reported as the median fluorescence intensity (MFI).

Lateral Flow Chromatography. The clinical isolate was tested by using a rapid lateral flow chromatography commercial kit, *Brucella* Smart Strip (New Horizons Diagnostics, Columbia, MD, U.S.A.) according to the manufacturer's instructions. It was applied to test the presence of *Brucella* antigen in the sample. Several colonies were picked with a sterile loop and resuspended in distilled water. 3 drops (100 μL) of the bacterial suspension were added into the sample well of the test device. After the suspension was absorbed into the sample well completely (about 3 min), 2 drops of chase buffer provided with the kit were added to the sample well. The results were read after 15 min but no later than 30 min. If only a red band was appeared at the control (C) line in the result window, the test was considered

negative. If distinct red bands were appeared on both control (C) and test (T) lines, the test was considered as positive. If there was no visually detectable band, the test was invalid.

Conventional PCR Analysis. The primers used to identify the *Brucella* species were F4 (5'-TCGAGCGCCCGCAAGGGG-3') and R2 (5'-AACCATAGTGTCTCCACTAA-3') according to the previous study (Journal of Clinical Microbiology 1995, 33, 615-7.). The expected size of the amplification product was 905 bp. PCR amplification was performed in a 25 μL reaction containing the following: 4 μL of template DNA, 0.5 μM of each primer and 1× AmpliTaq Gold PCR master mix (Applied Biosystems, U.S.A.). PCR was performed on an ABI 2720 thermal cycler by using the following cycling program: 95 °C for 10 min for enzyme activation, 35 cycles of 94 °C for 30 s, 50 °C for 1 min, and 72 °C for 30 s and 1 cycle of 72 °C for 7 min. The samples were analyzed by electrophoresis in a 2.0% agarose gel in TBE buffer (89 mM Tris−HCl, 89 mM boric acid, 2.0 mM EDTA, pH 8.0). PCR amplification products were visualized on the gel stained with GelRed (Biothium) under UV light.

■ FIGURES and TABLES

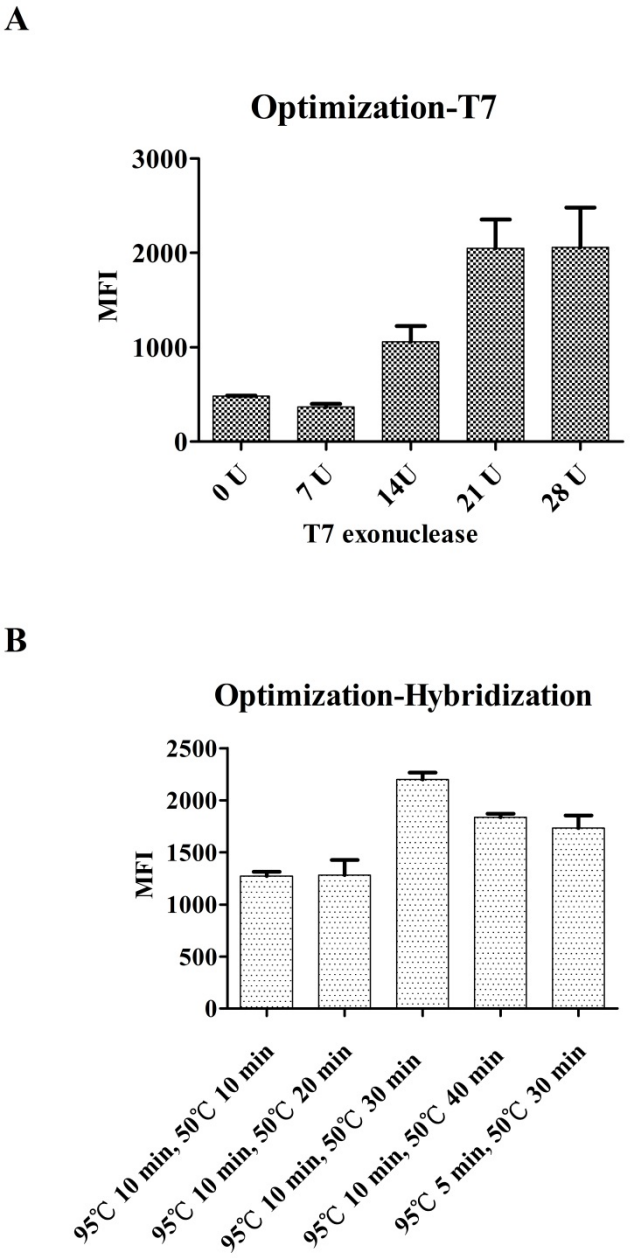
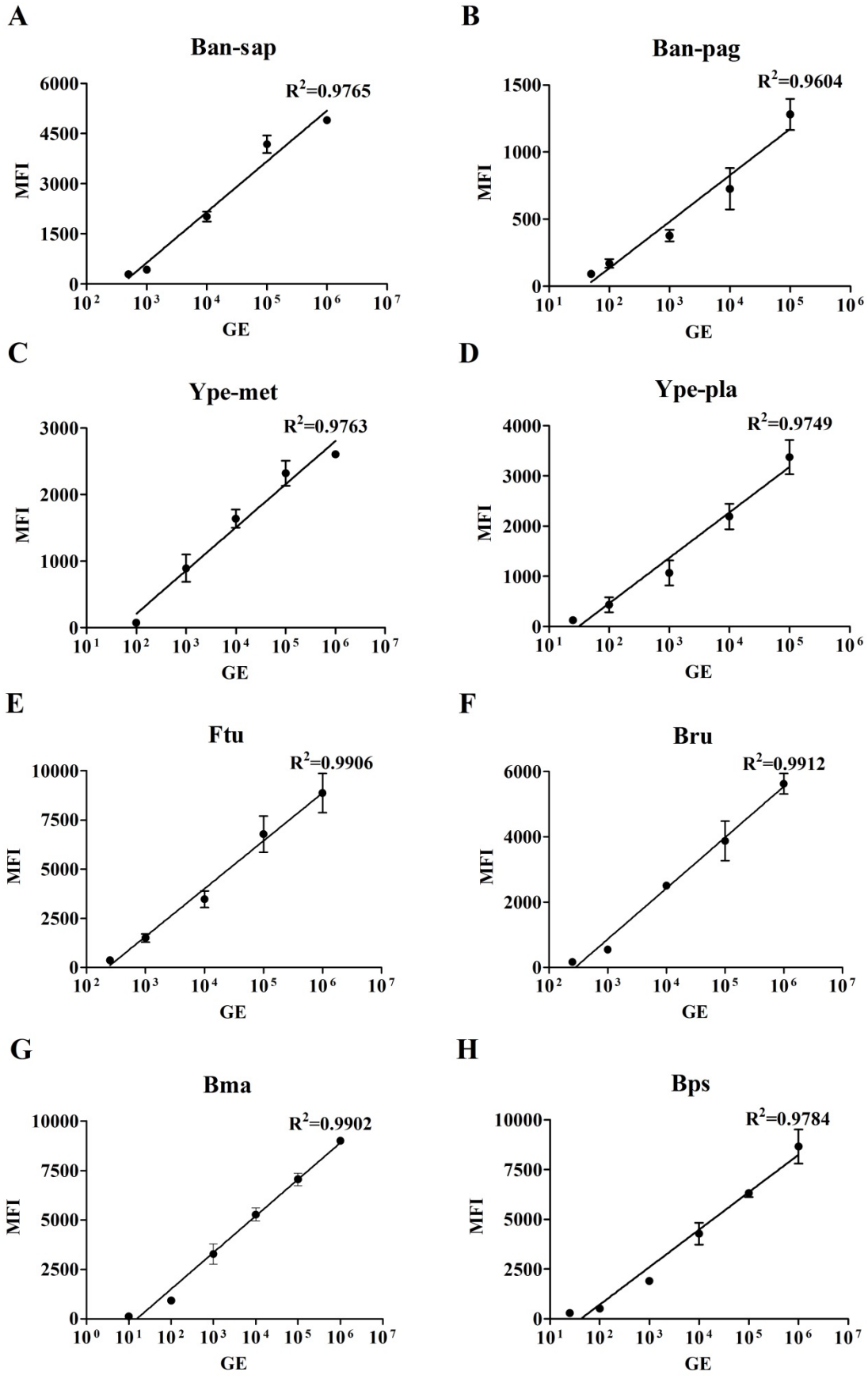


Figure S-1. Optimization of SSMP-SBA. (A) SSMP-SBA was performed by adding 0, 7, 14, 21 and 28 units of T7 exonuclease in the triplicate reaction with Ype DNA as template. The resulting MFI values (mean + SD) from YPE-met bead set are plotted against the amounts of added T7 exonuclease. (B) Optimization of the hybridization reaction was performed with several conditions with variations in the time of denaturation (95 °C) and hybridization (50 °C) with Ype DNA as template. The resulting MFI values (mean + SD) from YPE-met bead set are presented.


A
Ban-sap
R²=0.9765
MFI
GE
B
Ban-pag
R²=0.9604
MFI
GE
C
Ype-met
R²=0.9763
MFI
GE
D
Ype-pla
R²=0.9749
MFI
GE
E
Ftu
R²=0.9906
MFI
GE
F
Bru
R²=0.9912
MFI
GE
G
Bma
R²=0.9902
MFI
GE
H
Bps
R²=0.9784
MFI
GE

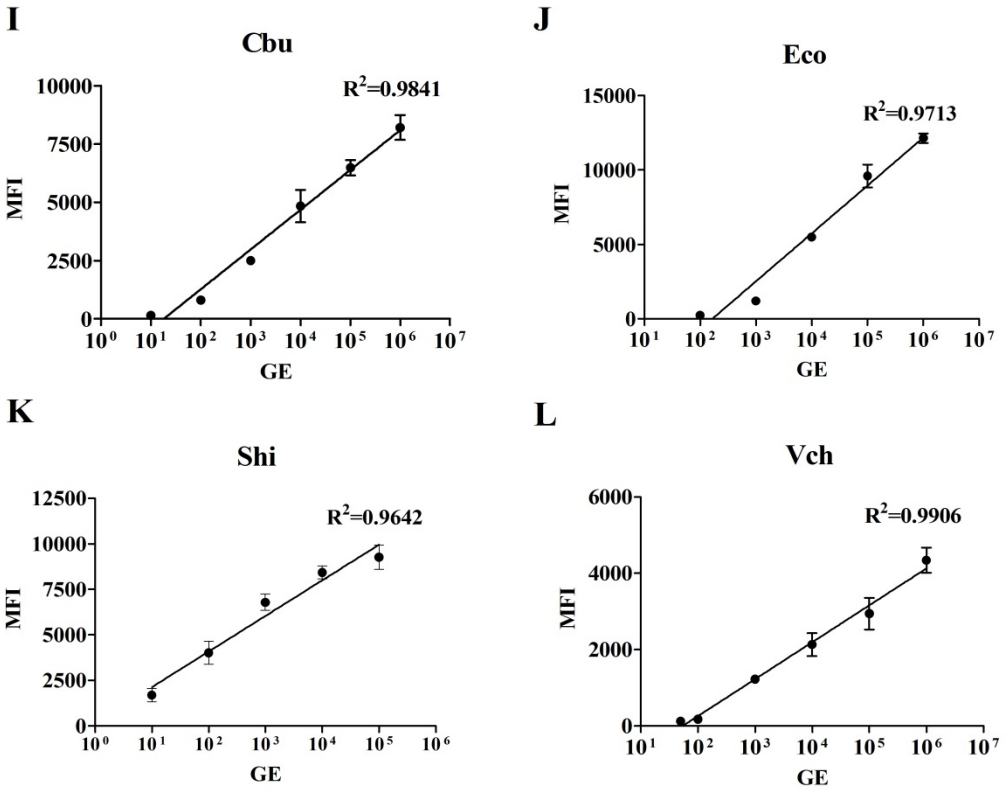


Figure S-2. Quantitative analysis of SSMP-SBA. The MFI values (mean ± SD) from specific bead set against the serial dilutions of DNA from reference strains are plotted. Each sample was tested in triplicate. (Ban-sap (A); Ban-pag (B); Ype-met (C); Ype-pla (D); Ftu (E); Bru (F); Bma (G); Bps (H); Cbu (I); Eco (J); Shi (K); Vch (L))

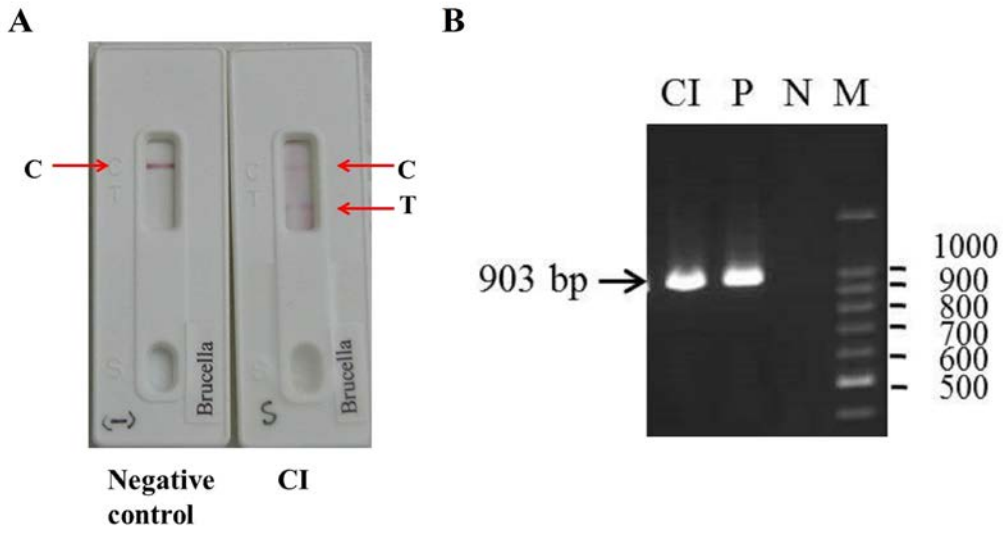


Figure S-3. Lateral flow chromatographic immunoassay and conventional PCR analysis. (A) The result of lateral flow chromatographic immunoassay of the clinical isolate (CI) and distilled water as negative control is presented. (B) The result of conventional PCR analysis with primers against the *Brucella* 16S rRNA gene is presented. The amplified fragment of 903 bp is indicated. (CI, the clinical isolate; P, *B. melitensis* as positive control; N, distilled water as non-template control; M, 100 bp DNA ladder.)

Table S-1. The Sequence and Location of Primers and Probes for Detection by SSMP-SBA

No.	Bacteria	Target gene	Primers & Probes			Accession No.
			Name	Sequence (5'→3')[1]	Position	
1	*B. anthracis*	*sap* (on chromo-some)	UF-Ban-sap-F	**CCATCCAGATTCACTCC**CGGTAAAGTAGTAGCTGAAAGTAAAGA	1104-1130	AE017225
			UR-Ban-sap-R	CACCTCGACCCACTCAACTGTCCAGTTAGAGATTGAAGCT	1184-1161	
			Ban-sap-probe	AGGTGCTGCAGTAGCTTCAATCT	1149-1171	
2	*B. anthracis*	*pag* (on pXO1)	UF-Ban-pag-F	**CCATCCAGATTCACTCC**CGACTAAACCGGATATGACATTAAAA	1634-1659	AE017336
			UR-Ban-pag-R	CACCTCGACCCACTCACGGTTATGTCTTTCCCTTGATATTG	1732-1708	
			Ban-pag-probe	GCCCTTAAAATAGCATTTGGATTT	1663-1686	
3	*B. anthracis*	*cap* (on pXO2)	UF-Ban-cap-F	**CCATCCAGATTCACTCC**ATAATGCATCGCTTGCTTTAGC	557-536	AE017335
			UR-Ban-cap-R	CACCTCGACCCACTCACGGATGAGCATTCAACATACCA	472-493	
			Ban-cap-probe	TTGGGATTGATGAGGAAACAGC	500-521	
4	*Y. pestis*	putative methyltrans-ferase (on chromo-some)	UF-Ype-met-F	**CCATCCAGATTCACTCC**GCAACAGCTCAACACCTTTGG	417-397	AL590842
			UR-Ype-met-R	CACCTCGACCCACTCAGCATTGGACGGCATCACGA	316-334	
			Ype-met-probe	AAACTTGGCAGCAGTTGGC	382-364	
5	*Y. pestis*	*pla* (on pPla)	UF-Ype-pla-F	**CCATCCAGATTCACTCC**CTATATGAGAGATCTTACTTTCCGTGAG	960-987	M27820
			UR-Ype-pla-R	CACCTCGACCCACTCAAGACTTTGGCATTAGGTGTGAC	1060-1039	
			Ype-pla-probe	GACATCCGGCTCACGTTATTATG	990-1012	
6	*F. tularensis*	*ISFtu2*	UF-Ftu-F	**CCATCCAGATTCACTCC**TGGTAGATCAGTTGGTGGGATAACC	141-165	EF059983
			UR-Ftu-R	CACCTCGACCCACTCATGAGTTTTACCTTCTGACAACAATATTTCT	236-207	
			Ftu-probe	TCCATGCTATGACTGATGCT	173-192	
7	*Brucella* species	*alkB*	UF-Bru-F	**CCATCCAGATTCACTCC**GCGGCTTTTCTATCACGGTATTCT	97-74	CP001489
			UR-Bru-R	CACCTCGACCCACTCACTAGAACGCCTTTCGGAAG	1-19	
			Bru-probe	CATTGAAGTCTGGCGAGCAT	71-52	
8	*B. mallei*	*IS407A-fliP*	UF-Bma-F	**CCATCCAGATTCACTCC**GGCAGGTCAACGAGCTTCAC	384-403	AM087437
			UR-Bma-R	CACCTCGACCCACTCAGCCCGACGAGCACCTGATT	483-465	
			Bma-probe	ATCATCGTCGTGCTGTCGC	408-426	
9	*B. pseudomallei*	*orf11*	UF-Bps-F	**CCATCCAGATTCACTCC**CGGGTTTCGGCGTTTTCG	4179-4196	AF074878
			UR-Bps-R	CACCTCGACCCACTCACAAATGGCCATCGTGATGTT	4276-4255	
			Bps-probe	CGCCGTTAATTTTCGCTCG	4199-4217	

Table S-1 (con't)

No.	Bacteria	Target gene	Primers & Probes			Accession No.
			Name	Sequence[1] (5'→3')	Position	
10	*C. burnetii*	*IS1111*	UF-Cbr-F	**CCATCCAGATTCACTCC**CGCAGGC GATAGCTGAAGC	369-387	M80806
			UR-Cbr-R	CACCTCGACCCACTCACCGTGCGGC TTTTGACTAAACGA	491-469	
			Cbu-probe	AACGGTGGAACAACAAGACG	424-443	
11	*E. coli* O157:H7	*rfb*	UF-Eco-F	**CCATCCAGATTCACTCC**TTCCATA ATCGGTTGGTGTGCTAA	604-627	CP001368
			UR-Eco-R	CACCTCGACCCACTCAATGCTGCCC ACAAAAATAATGTAAA	692-668	
			Eco- probe	TAAATACACGACGAACCGAGGA	635-656	
12	*Salmonella* species	*ttrC, ttrA*	UF-Sal-F	**CCATCCAGATTCACTCC**CCACCGA CGGCGAGACC	1321-1305	AY578070
			UR-Sal-R	CACCTCGACCCACTCACACCAGGAG ATTACAACATGGCTAAT	1252-1277	
			Sal-probe	TTTAGCCACTGACGACGGG	1300-1282	
13	*Shigella* species	*ipaH1.4*	UF-Shi-F	**CCATCCAGATTCACTCC**CTGTTGC TGCTGATGCCAC	1076-1094	AY206449
			UR-Shi-R	CACCTCGACCCACTCAGAGAGCAG AGCGCCGGTATCATTA	1205-1182	
			Shi-probe	GCAGAGACGGTATCGGAAA	1150-1169	
14	*V. cholerae*	*recA*	UF-Vch-F	**CCATCCAGATTCACTCC**TGAAATT CTACGCTTCTGTTCGTTTG	602-627	EU085357
			UR-Vch-R	CACCTCGACCCACTCAGGTTTCGTT ACCCACCACTTCTTC	684-661	
			Vch-probe	TGGCGCAATCAAAGAAGGC	642-660	
15			UF (forward unique primer)	**CCATCCAGATTCACTCC**		
			UR (reverse unique primer)	Biotin-C*A*C*C*TCGACCCACTCA (*: phosphorothioate linkage)		
			IC-probe (internal control probe)	TCGCATCTAGCGTCCATACG		
16			Internal control oligonucleotide (ICO)	ATTTCTAA**CCATCCAGATTCACTC C**TGCCATGGCATCGCATCTAGCGTC CATACGTGTGAGTGGGTCGAGGTGG TCATAT		

[1]The underlined sequences are forward (bold letters) and reverse unique tail sequences.

Table S-2. Specificity Analysis with SSMP-SBA

Bead / Template[1]	BAN-sap	BAN-pag	BAN-cap	YPE-met	YPE-pla	FTU	BRU	BMA	BPS	CBU	ECO	SAL	SHI	VCH	IC	Results
***Bacillus cereus* ATCC11778**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Bacillus thuringiensis* ATCC 10792**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Bacillus circulans* ATCC 4513**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Haemophilus influenzae* ATCC 33533**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Aggregatibacter aphrophilus* ATCC 7901**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Legionella pneumophila* ATCC 33153**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Listeria monocytogenes* ATCC 19111**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Pseudomonas aeruginosa* ATCC 27853**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Neisseria meningitidis* ATCC 13077**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Streptococcus pneumoniae* ATCC 6302**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Staphylococcus aureus* ATCC 6538**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Yersinia frederiksenii* ATCC 29912**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
***Yersinia enterocolitica* ATCC 9610**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−

[1]Each sample was tested in triplicate and the mean MFI greater than the cutoff value for the given microbead set was considered as positive. +, positive; −, negative.

Table S-3. Representative Data of Blind Samples and Clinical Specimen

No. \ Bead	BAN-sap	BAN-pag	BAN-cap	YPE-met	YPE-pla	FTU	BRU	BMA	BPS	CBU	ECO	SAL	SHI	VCH	IC	Result	Bacterial species
BT-01	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Aeromonas sobria* ATCC 9071
BT-02	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Acinetobacter junii* ATCC 17908
BT-03	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	*Bacillus anthracis*, pXO1+	*Bacillus anthracis* ATCC 14185 (pXO1+)
BT-04	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	*Vibrio cholerae*	*Vibrio cholerae* ATCC 9459
BT-05	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	*Burkholderia pseudomallei*	*Burkholderia pseudomallei* ATCC 11668
BT-06	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Citrobacter freundii* ATCC 8090
BT-07	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	*Brucella* spp.	*Brucella abortus* ATCC 7705
BT-08	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Haemophilus influenza* ATCC 33533
BT-09	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Listeria grayi* ATCC 19120
BT-10	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Pasteurella multocida* ATCC6530
BT-11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Staphylococcus aureus* ATCC 6538
BT-12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Yersinia enterocolitica* ATCC 9610
BT-13	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Bacillus cereus* ATCC 11778
BT-14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Bacillus subtilis* ATCC 6051
BT-15	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	*Brucella* spp.	*Brucella melitensis* ATCC 19396
BT-16	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Enterobacter cloacae* ATCC 23355
BT-17	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Escherichia coli* ATCC 23540
BT-18	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Haemophilus parainfluenzae* ATCC 7901
BT-19	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Legionella pneumophila* ATCC33153
BT-20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Neisseria meningitidis* ATCC13077

Table S-3. (con't)

No. \ Bead	BAN-sap	BAN-pag	BAN-cap	YPE-met	YPE-pla	FTU	BRU	BMA	BPS	CBU	ECO	SAL	SHI	VCH	IC	Result	Bacterial species
BT-21	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Pasteurella pneumotropica* ATCC 35149
BT-22	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	*Salmonella* spp.	*Salmonella typhi* ATCC 19430
BT-23	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	*Shigella* spp.	*Shigella boydii* ATCC 9207
BT-24	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Streptococcus pneumoniae* ATCC 6302
BT-25	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Bacillus mycoides* ATCC 6462
BT-26	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Corynebacterium bovis* ATCC 7715
BT-27	−	−	−	+	+	−	−	−	−	−	−	−	−	−	+	*Yersinia pestis*	*Yersinia pestis* ATCC 19428
BT-28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Citrobacter amalonaticus* ATCC 25405
BT-29	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Escherichia coli* ATCC 11775
BT-30	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Haemophilus parasuis* ATCC 19417
BT-31	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	*Brucella* spp.	*Brucella suis* ATCC 4312
BT-32	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Proteus vulgaris* ATCC 13315
BT-33	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	*Shigella* spp.	*Shigella flexneri* ATCC 11836
BT-34	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Staphylococcus epidermidis* ATCC 35984
BT-35	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	*Vibrio cholerae*	*Vibrio cholerae* ATCC 9458
BT-36	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Yersinia pseudotuberculosis* ATCC 29910
BT-37	+	−	+	−	−	−	−	−	−	−	−	−	−	−	+	*Bacillus anthracis*, pXO2+	*Bacillus anthracis* ATCC 4229 (pXO1-, pXO2+)
BT-38	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Bordetella bronchiseptica* ATCC 31124
BT-39	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	*Burkholderia mallei*	*Burkholderia mallei* ATCC 15310
BT-40	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Corynebacterium diphtheriae* ATCC 11952

Table S-3. (con't)

Bead / No.	BAN-sap	BAN-pag	BAN-cap	YPE-met	YPE-pla	FTU	BRU	BMA	BPS	CBU	ECO	SAL	SHI	VCH	IC	Result	Bacterial species
BT-41	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Enterobacter agglomerans* ATCC29904
BT-42	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	*E. coli.* O157:H7	*Escherichia coli* O157:H7 ATCC 43890
BT-43	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	*Francisella tularensis*	*Francisella tularensis* subsp. *novicida* ATCC 15482
BT-44	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Listeria monocytogenes* ATCC 19111
BT-45	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Neisseria meningitidis* ATCC 53044
BT-46	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Pseudomonas aeruginosa* ATCC 27853
BT-47	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	*Shigella* spp.	*Shigella dysenteriae* ATCC 11835
BT-48	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Streptococcus pneumonia* ATCC 6309
BT-49	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Bordetella parapertussis* ATCC 15311
BT-50	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	*Burkholderia pseudomallei*	*Burkholderia pseudomallei* ATCC 15682
BT-51	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Corynebacterium diphtheriae* ATCC 11913
BT-52	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	*E. coli.* O157:H7	*Escherichia coli* O157:H7 ATCC 35150
BT-53	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Staphylococcus saprophyticus* ATCC 15305
BT-54	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	undetectable	*Legionella pneumophila* subsp. *fraseri* ATCC 33156
BT-55	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	*Salmonella* spp.	*Salmonella enterica* subsp. *enterica* ATCC 167
BT-56	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	*Salmonella* spp.	*Salmonella typhimurium* ATCC 14028
BT-57	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	*Shigella* spp.	*Shigella sonnei* ATCC 25931
CI	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	*Brucella* spp.	*Brucella smart strip (+)*

500 pg DNA from each sample was tested in triplicate and the mean MFI greater than the cutoff value for the given microbead set was considered as positive. +, positive; −, negative.