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RESEARCH PAPER

Optimization of DNA Microarray Biosensors Enables Rapid and Sensitive Detection

Byeong Hee Hwang, Hwa Hui Shin, and Hyung Joon Cha

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Abstract DNA microarray biosensors are essential tools for analyzing transcriptome expression levels and single nucleotide polymorphisms in disease pathology and diagnostics. Since rapid and quantitative detection is necessary for these applications, optimization of the experimental conditions is essential. Here, experimental conditions for DNA microarray biosensors were optimized using an artificial target strategy without reaction or purification bias. Most importantly, hybridization time was reduced to one hour for rapid and homogeneous detection of target DNA. High and low concentrations of capture probe are appropriate for optimizing the limit of detection and dynamic range, respectively. Bleaching effects can be minimized by measuring fluorescence intensity at night. These conditions enable quantitative and precise detection of target DNA and offer experimental guidelines for genobiosensors in general.

Keywords: DNA microarray, biosensor, detection, pathogen, optimization, quantification

1. Introduction

DNA microarrays are essential genomic tools that can be used to determine transcriptome expression levels. Because DNA microarrays are high-throughput and quantitative tools, they can be applied broadly in biology, medical science, and diagnostics. Because oligonucleotide microarrays can detect single nucleotide polymorphisms (SNPs) with high sensitivity and in a high-throughput manner, various microarrays have been developed as genobiosensors to diagnose various diseases, analyze sequences, and detect pathogens [1-7]. For purposes of disease prevention, genobiosensors should be able to detect target genes rapidly, accurately, sensitively, and quantitatively. Therefore, there is a need to optimize the experimental conditions for DNA microarray biosensors for rapid and quantitative detection.

Several reports have described the optimization of different platforms or approaches for detection of DNA using microarrays [8-12]. For microbial identification studies, oligonucleotide microchips have been optimized using a non-equilibrium dissociation approach [8], or by adjusting the labeling method and the parameters of probe length, spacer length, and hybridization temperature [9]. Another study reported an optimization strategy based on hybridization temperature, solution composition, and spacer length of capture probes to reduce false-positive and false-negative signals [10]. Optimization of PCR primer design has been used to reduce PCR bias and cross-hybridization in clinically validated microarrays for virus detection [11]. In another study, a microfluidic microarray device using centrifugal flow method was optimized for rapid and sensitive SNP detection by optimization of the flow channel depth, and temperature [12]. However, some important parameters remain to be optimized for rapid and quantitative detection.

Here, the optimal experimental conditions for DNA microarray biosensors were investigated using an artificial standard probe strategy, which utilizes synthetic target DNA and can be used for quantitative analysis without reaction or purification bias [13]. We found that hybridization time, which constitutes a major component of the detection time, could be reduced for rapid detection. We varied the

Byeong Hee Hwang, Hwa Hui Shin, Hyung Joon Cha*
Department of Chemical Engineering, Pohang University of Science and Technology, Pohang 790-784, Korea
Tel: +82-54-279-2280; Fax: +82-54-279-2699
E-mail: hjcha@postech.ac.kr

Byeong Hee Hwang
Division of Bioengineering, Incheon National University, Incheon 406-772, Korea

concentrations of specific capture probe and target DNA to optimize the limit of detection, dynamic range, and quantitative linearity for quantitative detection of target DNA. Finally, the effects of photobleaching and light intensity were investigated by comparing experimental results obtained at daytime and nighttime.

2. Materials and Methods

2.1. Probe design and fabrication

Capture probes and target DNA were prepared as described previously [13,14]. The designed specific capture probe and synthetic *Vibrio vulnificus* target DNA were obtained from Integrated DNA Technologies, USA (Table 1). The designed artificial standard capture probe and artificial target DNA were obtained from the same company (Table 1). Capture probes were chemically synthesized with a 5′ amino linker modification and a 6-atom spacer composed of ethylene glycol units between the oligonucleotide and the amine. The target DNAs were complementary to the capture probes, and were chemically synthesized with a 3′ Alexa Fluor 647 (Cy5 substitute) modification.

2.2. Design of microarray

The design of the DNA microarray is shown in Fig. 1. 7 × 5 replicates of the artificial standard capture probes were arranged on the dark gray spots, using a concentration of 20 μM. 6 × 4 replicates of the specific capture probes were arranged on the light gray spots. Each specific capture probe was surrounded by four artificial standard capture probes in a rectangular arrangement. The interval between spots of the same kind was 800 μm, sufficient to minimize crosstalk. The overall dimensions of the spotted area were 4.8 × 3.2 mm, and each slide contained four replicates of the whole array set with an interval of 28.3 mm for statistical analysis.

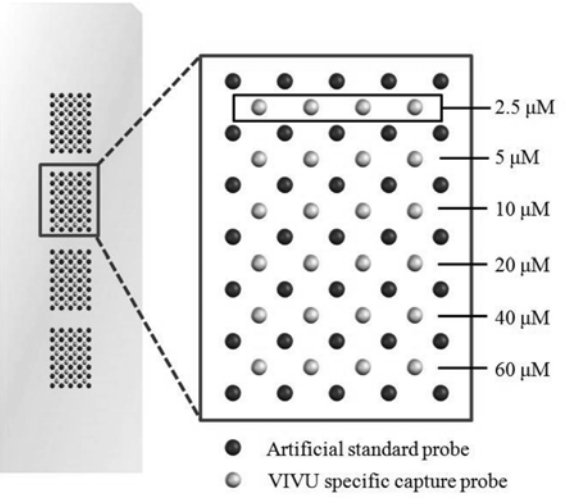

Fig. 1. Schematic diagram of newly designed array formats. A row containing four replicates of *V. vulnificus* (VIVU) specific capture probes at a given concentration is surrounded by rows of five replicates of artificial standard probes. Each slide contains four array repeats.

2.3. Fabrication of microarray

To determine the effect of probe concentration, the 1 × 4 arrays were printed using the amine-modified specific capture probe at concentrations of 2.5, 5, 10, 20, 40, and 60 μM in series from the top (Fig. 1). In brief, the array was spotted onto aldehyde-coated slides (Super Aldehyde; Telechem International, USA) using the Microsys 5100 microarrayer (Cartesian Technologies, USA) with the Chip Maker 2 pin (Telechem International) at 74% humidity. The spotting solution was prepared with 30 μL of 3× saline-sodium citrate (SSC) and 1.5 M *N*,*N*,*N*-trimethylglycine (betaine; Sigma, USA) at pH 6.6 with different capture

Table 1. Oligonucleotide sequences employed as capture probes and targets

No.	Capture probe	Sequence (5'→ 3')	Length (bp)	Ref.
1	Artificial standard capture probe	CCCAAGGGAACCCAAGGGAAA-C6-Amine	21	[13]
2	*Vibrio vulnificus* capture probe	AAACAAGTTTCTCTGTGCTGCCGC	24	[4]
3	Artificial standard target DNA	TTTCCCTTGGGTTCCCTTGGG-Alexa Fluor 647	21	[13]
4	*V. vulnificus* target DNA	Cy5- TGGCTCAGATTGAACGCTGGCGGCAGGCCTAACA CATGCAAGTCGAGCGGCAGCACAGAGAAACTTGT TTCTCGGGTGGCGAGCGGCGGACGGGTGAGTAA	101	[4]

No.	Melting temperature[a] (°C)	Probe quality[b]	16S rRNA accession no.
1	66.8	85	
2	66.9	91	X76333
3	66.8	85	
4			X76333

[a]Calculated in Primer Premier 5.
[b]Probe quality ratings were calculated in Primer Premier 5. A high rating corresponds to low stability of hairpins, dimers, falsely-primed DNA, and cross-dimers.

probe concentrations in a 386-well plate. After overnight incubation in a drying chamber, slides were reacted with a solution of 1.3 g $NaBH_4$ in 375 mL phosphate-buffered saline (PBS; pH 7.4) and 125 mL ethanol for 5 min. The slides were then washed twice in 0.2% sodium dodecyl sulfate (SDS) for 1 min each, and twice with ddH_2O. Slides were dried by centrifugation at 1,500 rpm for 3 min and stored at room temperature in a vacuum chamber until further use.

2.4. Investigation of experimental conditions

To optimize the experimental parameters for speed, sensitivity, and accuracy, the following experimental conditions were tested: (1) hybridization times of 1, 2, 3, 4, 6, and 9 h, (2) target DNA concentrations of 1.67, 5, 15, and 45 μM, (3) capture probe concentrations of 2.5, 5, 10, 20, 40, and 60 μM, and (4) washing and scanning at 9 am or 9 pm, to test the effect of partial exposure to sunlight. In summary, the fabricated array was preincubated for 30 min at 45°C in 50 mL 3× SSC containing 10 mg/mL bovine serum albumin (BSA; Sigma, USA) and 0.1% SDS. Hybridization was achieved by incubation of the array with a mixture of 1 μM Alexa Fluor 647-labeled artificial standard target and various concentrations of the Alexa Fluor 647-labeled *V. vulnificus* target in fresh hybridization buffer (100 μL of 3X SSC, 0.4 mg/mL BSA, and 0.1% SDS) under a supported coverslip at 45°C for various lengths of time. After hybridization, the array was washed three times, with the first solution (1× SSC, 0.2% SDS) for 2 min, the second solution (0.1× SSC, 0.2% SDS) for 2 min, and the third solution (0.1× SSC) for 1.5 min. Finally, the array was dried by centrifugation at 1,500 rpm for 3 min.

2.5. Fluorescence intensity scanning and data analysis

Images of the DNA arrays were acquired using a commercial confocal laser scanner (ScanArray Lite, GSI Lumonics, USA), and the data were analyzed quantitatively using QuantArray software (GSI Lumonics, USA). For fluorescence intensity analyses, spot intensities were calculated by subtraction of background intensities from observed intensities. The intensity values of target spots were normalized against the intensity values of the artificial standard spots.

3. Results and Discussion

3.1. Hybridization time

First, we tested the effect of hybridization time on the fluorescence intensity of artificial standard spots to determine the optimal conditions for rapid, homogeneous, and reliable detection using DNA microarray biosensors. The fluorescence intensity of the artificial standard spots was highest when a hybridization time of 1 h was used (Fig. 2A). As the hybridization time increased, the fluorescence intensity decreased rapidly at first, then plateaued. These results imply that slow degradation of the fluorophores occurred during the hybridization period; indeed, significant degradation of fluorescent dyes by ozone exposure has been reported previously [15]. Thus, short hybridization times have many advantages, including rapid detection, high intensity, and high sensitivity. In addition, the relative standard deviation of the fluorescence intensity of the artificial standard spots was similar for all hybridization times (Fig. 2B). The artificial standard probe strategy is useful in positive controls and assessment of hybridization homogeneity [13]. Here, this strategy was used to show that a hybridization time of 1 h is sufficient for homogeneous hybridization for every spot. Consequently, a hybridization time of 1 h was chosen for rapid and sensitive detection in this system.

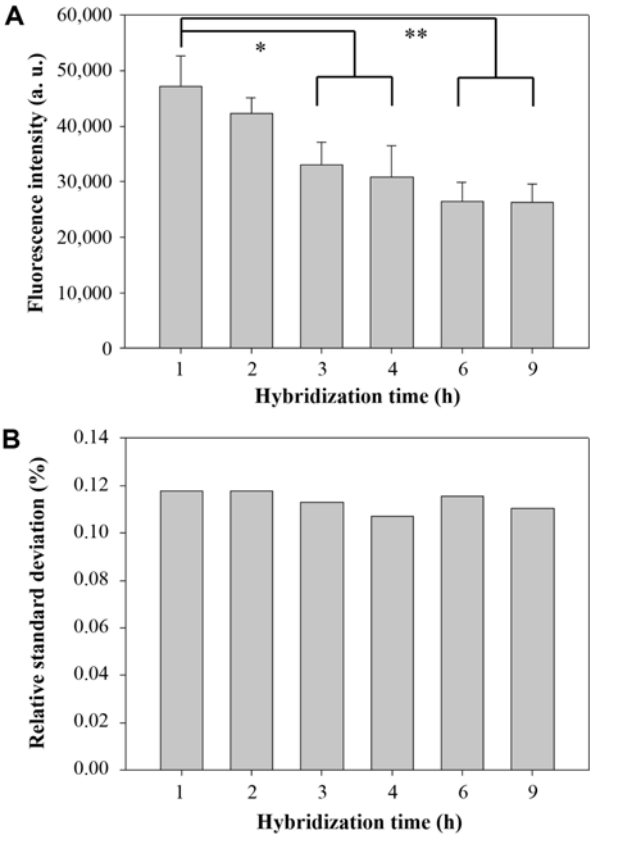

Fig. 2. Effect of hybridization time on fluorescence intensity using artificial standard spots. Hybridization times of 1, 2, 3, 4, 6, and 9 h were used, while other experimental conditions were held constant. Data represent (A) mean ± s.d. and (B) s.d. from four replicates. *$p < 0.05$, **$p < 0.01$ by *t*-test.

For general DNA microarray biosensors, both fluorescence intensity and hybridization homogeneity should be considered in determining the optimal hybridization time. The diffusion rate of the target is inversely proportional to the square root

of its molecular weight, according to Graham's law, and its diffusion coefficient in solution is approximately proportional to absolute temperature, according to the Stokes-Einstein equation. Therefore, as temperature increases or the molecular weight of the target decreases, the diffusion rate increases, and accordingly, the time required for homogeneous hybridization decreases. In this system, the length of the artificial standard target was 21 bp and the hybridization temperature was 45°C. The relatively short target length could be the main factor in the short time required for homogeneous hybridization. If the target length increases to several hundreds or thousands of base pairs, the time required for homogeneous hybridization will increase. Therefore, the hybridization time depends on the length of the target DNA and should be optimized for a given target to maximize fluorescence intensity and homogeneity of hybridization.

3.2. Capture probe concentration

We next tested the effect of capture probe concentration on the dynamic range of target DNA concentration using *V. vulnificus* target DNA, to determine the optimal conditions for reliable, sensitive, and quantitative biosensor analysis. Synthesized *V. vulnificus* target DNA was chosen because it showed the best detection limit among several target DNAs in the previous report [4]. The fluorescence intensity increased as the capture probe concentration increased, and the slope of the data increased as the target concentration increased (Fig. 3A). The slope of the fluorescence intensity versus target DNA concentration was similar for all capture probe concentrations tested (Fig. 3B). The dynamic range of target DNA concentrations using 60 mM capture probe was 3 ~ 100 μM (Fig. 3B), because the fluorescence intensity had an upper detection limit of 50,000 arbitrary units. On the other hand, a capture probe concentration of 2.5 mM could be used to detect high target concentrations up to 1,000 μM.

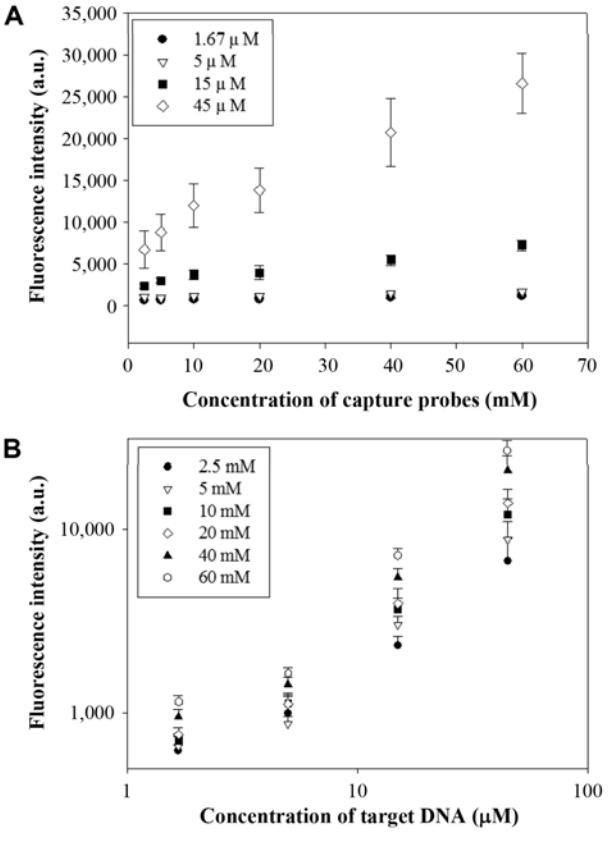

Fig. 3. Effect of target concentration and capture probe concentration on detection of a *V. vulnificus* model target. (A) Relationship between capture probe concentration and fluorescence intensity at various target DNA concentrations. (B) Relationship between target DNA concentration and fluorescence intensity at various capture probe concentrations. Data represent mean ± s.d. from four replicates.

The concentration of capture probe can be varied depending on the purpose of quantitative detection. For sensitive detection, a high concentration of capture probe is preferable. For wide dynamic range and for measuring high target concentrations, a low concentration of capture probe should be chosen. For example [16], because the infectious dose of *Shigella dysenteriae* is on the scale of several cells [16], a capture probe concentration of 60 mM could be used for sensitive *Shigella* detection. In the case of *Vibrio cholerae*, with an infectious dose of tens of thousands of cells [16], a capture probe concentration of 2.5 ~ 10 mM could be used for detection with a broad dynamic range. Although similar tendencies would be observed with other array-type biosensors, because quantitative values could be different depending on other experimental conditions, the optimal concentration of capture probe should be determined experimentally.

3.3. Experimental timing

Because fluorescent dyes can degrade upon light exposure, the effect of light exposure was investigated by comparing results obtained from daytime and nighttime experiments. Higher fluorescence intensities were observed during nighttime experiments than during daytime experiments (Fig. 4). Although light exposure was minimized by performing most of the experiments in dark room conditions or with the materials wrapped in aluminum foil, light exposure during the washing and scanning steps reduced the observed fluorescence intensity up to 50%. Therefore, detection of DNA using fluorescent dyes should be performed at night.

The timing of the experiment is important to avoid interference caused by exposure of the fluorescent dyes to light. As is well known, fluorescence intensity can easily be affected by light exposure; therefore, light exposure should be minimized during the experimental procedure. For microarray experiments, the dye can be exposed to light during the DNA labeling, purification, and washing

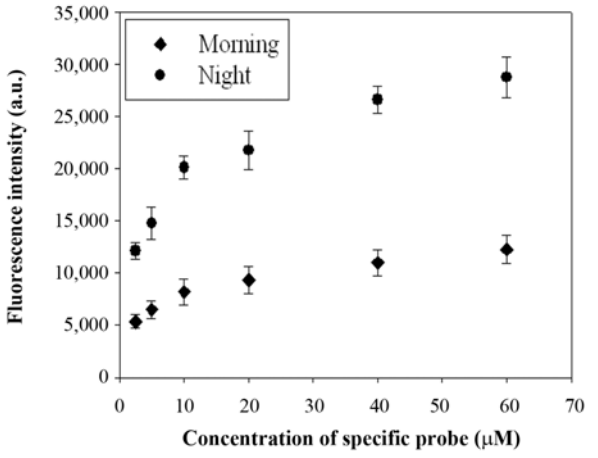

Fig. 4. Effect of light exposure during washing and scanning steps on detection of *V. vulnificus* target DNA. The relationship between capture probe concentration and fluorescence intensity is shown for experiments performed in the morning and at night. Data represent mean ± s.d. from four replicates.

steps after hybridization. Experiments could be performed in two possible ways: (1) washing and scanning during the day after DNA labeling and hybridization at night, or (2) washing and scanning at night after DNA labeling and hybridization during the day. Our results show that light exposure during the washing and scanning steps has a greater effect on fluorescence intensity than light exposure during the DNA labeling and purification steps. Therefore, we suggest that the washing and scanning steps are performed at night or in a dark room without any sunlight.

4. Conclusion

In summary, for DNA microarray biosensors designed for pathogen detection, a hybridization time of 1 h yielded the highest fluorescence intensities with short targets. High concentrations of capture probe were appropriate for sensitive detection, and low concentrations of capture probe were appropriate for detection with a wide dynamic range. Nighttime experiments were confirmed to yield results superior to those of daytime experiments for the detection of DNA using fluorescent dyes. These conditions enable quantitative and precise detection of target DNA, and could provide useful guidelines for DNA microarray biosensors and other types of genobiosensors.

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References

1. Wang, J. (2000) From DNA biosensors to gene chips. *Nucleic Acids Res*. 28: 3011-3016.
2. Alizadeh, A. A., M. B. Eisen, R. E. Davis, C. Ma, I. S. Lossos, A. Rosenwald, J. C. Boldrick, H. Sabet, T. Tran, X. Yu, J. I. Powell, L. Yang, G. E. Marti, T. Moore, J. Hudson, Jr., L. Lu, D. B. Lewis, R. Tibshirani, G. Sherlock, W. C. Chan, T. C. Greiner, D. D. Weisenburger, J. O. Armitage, R. Warnke, R. Levy, W. Wilson, M. R. Grever, J. C. Byrd, D. Botstein, P. O. Brown, and L. M. Staudt (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403: 503-511.
3. Wang, D., A. Urisman, Y. T. Liu, M. Springer, T. G. Ksiazek, D. D. Erdman, E. R. Mardis, M. Hickenbotham, V. Magrini, J. Eldred, J. P. Latreille, R. K. Wilson, D. Ganem, and J. L. DeRisi (2003) Viral discovery and sequence recovery using DNA microarrays. *PLoS Biol*. 1: E2.
4. Eom, H. S., B. H. Hwang, D. H. Kim, I. B. Lee, Y. H. Kim, and H. J. Cha (2007) Multiple detection of food-borne pathogenic bacteria using a novel 16S rDNA-based oligonucleotide signature chip. *Biosens. Bioelectron*. 22: 845-853.
5. Hwang, B. H., H. H. Shin, J. H. Seo, and H. J. Cha (2012) Specific multiplex analysis of pathogens using a direct 16S rRNA hybridization in microarray system. *Anal. Chem*. 84: 4873-4879.
6. Shin, H. H., B. H. Hwang, J. H. Seo, and H. J. Cha (2014) Specific discrimination of three pathogenic *Salmonella enterica* subsp. *enterica* serotypes by carB-based oligonucleotide microarray. *Appl. Environ. Microbiol*. 80: 366-373.
7. Shin, H. H., J. H. Seo, C. S. Kim, B. H. Hwang, and H. J. Cha (2016) Hybrid microarray based on double biomolecular markers of DNA and carbohydrate for simultaneous genotypic and phenotypic detection of cholera toxin-producing *Vibrio cholerae*. *Biosens. Bioelectron*. 79: 398-405.
8. Liu, W. T., A. D. Mirzabekov, and D. A. Stahl (2001) Optimization of an oligonucleotide microchip for microbial identification studies: a non-equilibrium dissociation approach. *Environ. Microbiol*. 3: 619-629.
9. Relogio, A., C. Schwager, A. Richter, W. Ansorge, and J. Valcarcel (2002) Optimization of oligonucleotide-based DNA microarrays. *Nucleic Acids Res*. 30: e51.
10. Peplies, J., F. O. Glockner, and R. Amann (2003) Optimization strategies for DNA microarray-based detection of bacteria with 16S rRNA-targeting oligonucleotide probes. *Appl. Environ. Microbiol*. 69: 1397-1407.
11. Wong, C. W., C. L. Heng, L. Wan Yee, S. W. Soh, C. B. Kartasasmita, E. A. Simoes, M. L. Hibberd, W. K. Sung, and L. D. Miller (2007) Optimization and clinical validation of a pathogen detection microarray. *Genome Biol*. 8: R93.
12. Wang, L. and P. C. Li (2010) Optimization of a microfluidic microarray device for the fast discrimination of fungal pathogenic DNA. *Anal. Biochem*. 400: 282-288.
13. Hwang, B. H. and H. J. Cha (2008) Quantitative oligonucleotide microarray data analysis with an artificial standard probe strategy. *Biosens. Bioelectron*. 23: 1738-1744.
14. Hwang, B. H. and H. J. Cha (2010) Pattern-mapped multiple detection of 11 pathogenic bacteria using a 16s rDNA-based oligonucleotide microarray. *Biotechnol. Bioeng*. 106: 183-192.
15. Fare, T. L., E. M. Coffey, H. Dai, Y. D. He, D. A. Kessler, K. A. Kilian, J. E. Koch, E. LeProust, M. J. Marton, M. R. Meyer, R. B. Stoughton, G. Y. Tokiwa, and Y. Wang (2003) Effects of atmospheric ozone on microarray data quality. *Anal. Chem*. 75: 4672-4675.
16. Kothary, M. H. and U. S. Babu (2001) Infective dose of foodborne pathogens in volunteers: A review. *J. Food Safety* 21: 49-68.