View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Old Dominion University

Old Dominion University
ODU Digital Commons

Bioelectrics Publications | Frank Reidy Research Center for Bioelectrics

3-2003

Comparison of Methods for DNA Isolation from Food Samples for Detection of Shiga Toxin-Producing Escherichia Coli by Real-Time PCR

Loree C. Heller
Old Dominion University, lheller@odu.edu

Carisa R. Davis

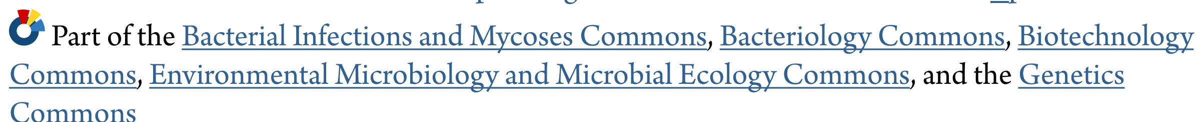
K. Kealy Peak

David Wingfield

Andrew C. Cannons

See next page for additional authors

Follow this and additional works at: https://digitalcommons.odu.edu/bioelectrics_pubs


Part of the Bacterial Infections and Mycoses Commons, Bacteriology Commons, Biotechnology Commons, Environmental Microbiology and Microbial Ecology Commons, and the Genetics Commons

Repository Citation

Heller, Loree C.; Davis, Carisa R.; Peak, K. Kealy; Wingfield, David; Cannons, Andrew C.; Amuso, Philip T.; and Cattani, Jacqueline, "Comparison of Methods for DNA Isolation from Food Samples for Detection of Shiga Toxin-Producing Escherichia Coli by Real-Time PCR" (2003). *Bioelectrics Publications*. 107.
https://digitalcommons.odu.edu/bioelectrics_pubs/107

Original Publication Citation

Heller, L. C., Davis, C. R., Peak, K. K., Wingfield, D., Cannons, A. C., Amuso, P. T., & Cattani, J. (2003). Comparison of methods for DNA isolation from food samples for detection of Shiga toxin-producing Escherichia coli by real-time PCR. *Applied and Environmental Microbiology, 69*(3), 1844-1846. doi:10.1128/AEM.69.3.1844-1846.2003

This Article is brought to you for free and open access by the Frank Reidy Research Center for Bioelectrics at ODU Digital Commons. It has been accepted for inclusion in Bioelectrics Publications by an authorized administrator of ODU Digital Commons. For more information, please contact digitalcommons@odu.edu.

Authors
Loree C. Heller, Carisa R. Davis, K. Kealy Peak, David Wingfield, Andrew C. Cannons, Philip T. Amuso, and Jacqueline Cattani

This article is available at ODU Digital Commons: https://digitalcommons.odu.edu/bioelectrics_pubs/107

Applied and Environmental Microbiology, Mar. 2003, p. 1844–1846 Vol. 69, No. 3
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.3.1844–1846.2003
Copyright © 2003, American Society for Microbiology. All Rights Reserved.

Comparison of Methods for DNA Isolation from Food Samples for Detection of Shiga Toxin-Producing *Escherichia coli* by Real-Time PCR

Loree C. Heller,[1]* Carisa R. Davis,[2] K. Kealy Peak,[2] David Wingfield,[3] Andrew C. Cannons,[2] Philip T. Amuso,[2,3] and Jacqueline Cattani[2]

Esoteric Testing, Department of Pathology, Tampa General Hospital, Tampa, Florida 33101,[1] and Center for Biological Defense, University of South Florida College of Public Health,[2] and Bureau of Laboratories—Tampa, Florida Department of Health,[3] Tampa, Florida 33612

Received 21 June 2002/Accepted 29 November 2002

In this study, food samples were intentionally contaminated with *Escherichia coli* O157:H7, and then DNA was isolated by using four commercial kits. The isolated DNA samples were compared by using real-time PCR detection of the Shiga toxin genes. The four kits tested worked similarly.

Enteric pathogens are classic potential agents of bioterrorism. For example, *Salmonella enterica* serovar Typhimurium was used to contaminate salad bars in an attempt to affect the outcome of a local election (7). In another instance, *Shigella dysenteriae* type 2 obtained from a laboratory was used by a medical technologist as an agent to sicken coworkers (3). Foods that are uncooked or undercooked after manufacture make feasible targets for intentional contamination. Conventional microbiological techniques for the detection of bacterial pathogens in food, including isolation on selective media and biochemical identification of the bacteria, are time-consuming and laborious. More-rapid alternatives, including the detection of pathogen DNA by real-time PCR, are available (1, 8). In this study, four commercial kits for isolation of DNA from food samples were compared by real-time PCR detection.

Bread slices, ground beef, commercially bagged salad greens, and salad dressing were purchased at local grocery stores. To confirm that none of these products already contained Shiga toxin-producing *Escherichia coli*, each product was tested for total coliform bacteria by using the Food and Drug Administration *Bacteriological Analytic Manual Online* (www.cfsan.fda.gov/~ebam/bam-toc.html) protocol. All enriched gram-negative bacilli were identified by classical microbiological techniques (5). Biochemical differentiations were made with the API 20E identification system for *Enterobacteriaceae* and other gram-negative rods (bioMerieux Vitek, Inc., Hazelwood, Mo.) with supplemental conventional biochemical tests as required. While both bread and salad dressing samples were negative for all coliforms, a variety of gram-negative organisms were isolated from the ground beef and salad greens after enrichment (Table 1). The *E. coli* strains isolated from the ground beef samples were tested per the manufacturer's instructions and were not identified as O157 or O157:H7 (SAS *E. coli* O157:H7 and *E. coli* O157 test; SA Scientific, San Antonio, Tex.).

Ten grams of each food product was spiked with *E. coli* O157:H7 (ATCC 700927) cells from Trypticase soy broth (TSB) and incubated for 16 h at 35°C. Assuming 100% efficiency in DNA isolation, an approximate amount of 1,000 CFU was included in each final PCR. Samples were diluted 1:10 (wt/vol) with TSB and homogenized for 1 min. One milliliter of homogenate was removed, and the DNA was isolated with four commercial kits (Table 2). Prepman Ultra (Applied Biosystems, Foster City, Calif.) and Bugs'n Beads (GenPoint, Oslo, Norway) were designed to extract the DNA from a wide variety of gram-positive and gram-negative bacteria in foods. The NucleoSpin food kit (Clontech, Palo Alto, Calif.) and the Wizard magnetic DNA purification system for food (Promega Madison, Wis.) were designed for detection of genetically modified organisms but were tested here for the additional isolation of bacterial genomic DNA. After DNA isolation per each manufacturer's instructions, sterile molecular-grade water was added to each DNA sample as necessary to achieve a final volume of 200 μl. Since in all kits except Bugs'n Beads the isolated DNA may contain food as well as bacterial genomic DNA, it was not possible to directly quantify the DNA yield.

To determine which DNA isolation method most effectively produced PCR-quality DNA, the DNA was subjected to TaqMan PCR using the primers and probes targeting the stx_1 and

TABLE 1. Coliform enrichment of foods before intentional contamination

Food	Sample no.	Total coliforms/g	Fecal coliforms/g	Organism(s) isolated
Bread slices	1	0	0	None
Ground beef	1	3.1	3.1	*Escherichia coli*
	2	24	0.36	*Escherichia coli*, *Citrobacter braakii*, *Acinetobacter calcoaceticus*, *Hafnia alvei*
Salad greens	1	21	0	*Enterobacter cloacae*, *Pseudomonas putida*, *Rahnella aquatilis*
	2	9.3	0	*Klebsiella planticola*, *Pseudomonas putida*, *Pseudomonas fluorescens*
Salad dressing	1	0	0	None

* Corresponding author. Mailing address: Tampa General Hospital, P.O. Box 1289, Tampa, FL 33101. Phone: (813) 844-4677. Fax: (813) 844-1312. E-mail: lheller@tgh.org.

TABLE 2. Commercial kits used for DNA isolation from foods

Kit	Method	Ease of use
Prepman Ultra	Proprietary solution	Very simple, minimum manipulation of sample
Bugs'n Beads	Magnetic beads	Simple, centrifuge not absolutely necessary
NucleoSpin food kit	Spin columns	Considerable hands-on time
Wizard magnetic DNA purification system for food	Magnetic beads	Considerable hands-on time, centrifuge not absolutely necessary

stx$_2$ genes (Table 3). Each 50-μl reaction mixture contained 1× TaqMan master mix, 45 pmol of each primer, 12.5 pmol of probe, and 5 μl of DNA. Thermal cycling and detection were performed by employing an ABI Prism 7700 sequence detection system using the associated version 1.7a application software as described by the manufacturer and by employing default parameters. To compare detection limits between the different kit brands, the threshold cycle (C_τ) value for each sample was divided by the log of the CFU used to spike the sample (Table 4). If a reaction reached cycle 40 without a significant increase in fluorescence, this reaction contained no DNA and was considered to be negative.

For both primer sets, the C_τ/log CFU values were grouped by food type. These values were found to be normal by using the Shapiro-Wilk test, and no significant effect due to the experiments being done on different days was found by a one-way analysis of variance. To test for differences between the four kits, a one-way analysis of variance was used, followed by a posteriori comparisons. In addition, to ensure that the experiment-wise error rate did not exceed 5%, we conducted the sequential Bonferroni procedure in which the alpha level is divided by the number of tests (in this case, five) and the resulting value is used to determine significance (6). Statistical analysis was conducted using the SAS system for Windows version 8.0 (SAS Institute, Cary, N.C.).

Overall significant differences were found with the results for both primer and probe sets for ground beef and salad greens, but only those for ground beef had significant contrasts. For both the primer and probe sets, the only two kits

TABLE 3. Oligonucleotides for detection of *stx*$_1$ and *stx*$_2$ genes[a]

Gene	Primer or probe	Sequence[b]
stx$_1$[c]	Forward primer	5′-TGTCACATATAAATTATTTCGTTC AACAA-3′
	Reverse primer	5′-GCAGTTGATGTCAGAGGGATAGA TC-3′
	TaqMan probe	5′-6-FAM-AAGCCGTAGATTATTAAAC CGCCCTTCCTCT-BHQ1-3′
stx$_2$[d]	Forward primer	5′-CAACGTGTCGCAGCGCT-3′
	Reverse primer	5′-AACGCCAGATATGATGAAACCA-3′
	TaqMan probe	5′-6-FAM-TCCGGAATGCAAATCAGT CGTCACTCA-BHQ1-3′

[a] All oligonucleotides were purchased from IDT DNA (Coralville, Iowa).
[b] 6-FAM, 6-carboxyfluorescein; BHQ, Black Hole Quencher.
[c] Corresponding to bp 2996678 to 2996764 of *E. coli* O157:H7 EDL933 (GenBank accession number NC_002655).
[d] Corresponding to bp 1352707 to 1352780 of *E. coli* O157:H7 EDL933 (GenBank accession number NC_002655).

TABLE 4. Mean Cτ/log CFU values and standard errors of three independent experiments each using Taqman PCR detection after intentional contamination of foods with *E. coli* O157:H7

Food	Target gene	C_τ/log CFU (mean ± SE) determined by: Prepman Ultra	Bugs'n Beads	Nucleospin food kit	Wizard magnetic DNA purification system for food
TSB	*stx*$_1$	10.2 ± 0.4	10.7 ± 0.4	9.8 ± 0.4	10.1 ± 0.3
	stx$_2$	10.4 ± 0.3	11.1 ± 0.4	10.3 ± 0.3	10.7 ± 0.3
Bread	*stx*$_1$	9.8 ± 0.2	11.0 ± 0.2	10.1 ± 0.5	9.8 ± 0.3
	stx$_2$	9.4 ± 0.3	10.9 ± 0.4	9.8 ± 0.4	9.7 ± 0.4
Ground beef	*stx*$_1$	10.3 ± 0.3	12.2 ± 0.2	11.1 ± 0.5	9.7 ± 0.3
	stx$_2$	10.3 ± 0.3	12.5 ± 0.4	11.6 ± 0.5	9.7 ± 0.2
Salad greens	*stx*$_1$	10.0 ± 0.1	10.9 ± 0.2	10.0 ± 0.0	10.1 ± 0.2
	stx$_2$	10.0 ± 0.2	10.9 ± 0.0	10.0 ± 0.2	10.4 ± 0.2
Salad dressing	*stx*$_1$	10.0 ± 0.3	10.3 ± 0.4	10.0 ± 0.8	9.4 ± 0.3
	stx$_2$	10.0 ± 0.3	10.3 ± 0.5	9.5 ± 0.3	10.1 ± 0.6

whose results were significantly different from each other were Bugs'n Beads and the Wizard magnetic DNA purification system for food. In this case, Bugs'n Beads was less sensitive.

Since Prepman Ultra was the easiest method to perform and its results did not differ significantly from the results of the other kits, this isolation method was further characterized (Fig. 1). Foods were intentionally contaminated with 10-fold dilutions of *E. coli* O157:H7. Since cell viability does not affect detection of DNA by PCR (2, 4), in this experiment stocks frozen at −80°C in TSB containing 15% glycerol were used. DNA was extracted as described above, PCR was performed,

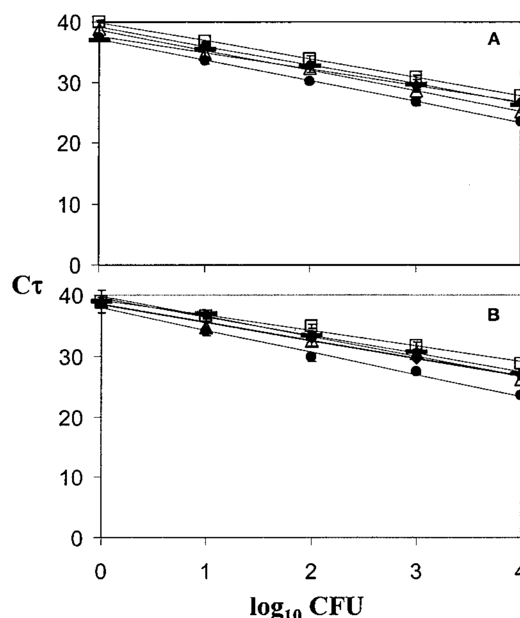


FIG. 1. Real-time PCR detection of *E. coli* O157:H7 from DNA isolated with the Prepman Ultra kit from various intentionally contaminated foods. C_τ values are plotted against the $\log_{10}$ CFU. (A) Detection of the *stx*$_1$ gene. ◆, salad greens (r^2 = 0.997); □, ground beef (r^2 = 1.00); △, salad dressing (r^2 = 0.97); –, bread (r^2 = 0.98); ●, TSB (r^2 = 0.999). (B) Detection of the *stx*$_2$ gene. ◆, salad greens (r^2 = 0.996); □, ground beef (r^2 = 0.99); △, salad dressing (r^2 = 0.97); –, bread (r^2 = 0.99); ●, TSB (r^2 = 0.99). The means and standard errors of the results of three independent experiments are indicated.

and a linear regression of C_T values on log CFU per PCR was used in order to determine the detection limit. Although the TSB control had significantly lower C_T values at the same number of CFU, no significant difference was observed between the detection limits for the foods and the TSB control (Fig. 1). The overall mean detection limit, with a 5-μl aliquot of the 200 μl of DNA isolated, was 13.35 CFU/PCR, which corresponded to 5,340 CFU per g of food. This value represents the practical detection limit of this method, although this limit would be theoretically lower if the entire DNA solution was used, for example, by concentrating the DNA before PCR.

The development of alternative assays for detection of pathogenic organisms commonly associated with food-borne diseases will allow early detection of food-related bioterrorism events and will also strengthen surveillance to provide timely detection of natural epidemics. Each kit tested efficiently isolated DNA from the four tested foods. Based simply on ease of use, the Prepman Ultra method was preferred.

This work was supported by Department of Defense contract number DAAD13-01-C-0043.

REFERENCES

1. **Jordan, J. A.** 2000. Real-time detection of PCR products and microbiology. New technologies for life sciences: a trends guide. **6:**61–66.
2. **Josephson, K. L., C. P. Gerba, and I. L. Pepper.** 1993. Polymerase chain reaction detection of nonviable bacterial pathogens. Appl. Environ. Microbiol. **59:**3513–3515.
3. **Kolavic, S. A., A. Kimura, S. L. Simons, L. Slutsker, S. Barth, and C. E. Haley.** 1997. An outbreak of *Shigella dysenteriae* type 2 among laboratory workers due to intentional food contamination. JAMA **278:**396–398.
4. **Masters, C. I., J. A. Shallcross, and B. M. Mackey.** 1994. Effect of stress treatments on the detection of *Listeria monocytogenes* and enterotoxigenic *Escherichia coli* by the polymerase chain reaction. J. Appl. Bacteriol. **77:**73–79.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1999. Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
6. **Rice, W. R.** 1989. Analyzing tables of statistical tests. Evolution **43:**223–225.
7. **Torok, T. J., R. V. Tauxe, R. P. Wise, J. R. Livengood, R. Sokolow, S. Mauvais, K. A. Birkness, M. R. Skeels, J. M. Horan, and L. R. Foster.** 1997. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. JAMA **278:**389–395.
8. **Wittwer, C. T., M. G. Herrmann, A. A. Moss, and R. P. Rasmussen.** 1997. Continuous fluorescence monitoring of rapid cycle DNA amplification. Biotechniques **22:**130–131, 134–138.