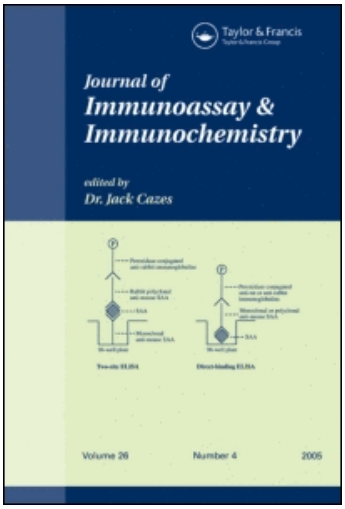

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Antibody-Based Detection of Acid-Shocked, Acid-Adapted, and Apple Juice-Incubated *Escherichia coli* O157:H7

Cynthia Nyquist-Battie[a]; Leigh Mathias[a]; Laura E. Freeman[a]; Deanna Lund[a]; Daniel V. Lim[b]
[a] Department of Public Health, University of North Florida, Jacksonville, Florida, USA [b] Department of Biology and Center for Biological Defense, University of South Florida, Tampa, Florida, USA

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Antibody-Based Detection of Acid-Shocked, Acid-Adapted, and Apple Juice-Incubated *Escherichia coli* O157:H7

Cynthia Nyquist-Battie, Leigh Mathias, Laura E. Freeman, and Deanna Lund
Department of Public Health, University of North Florida, Jacksonville, Florida, USA

Daniel V. Lim
Department of Biology and Center for Biological Defense, University of South Florida, Tampa, Florida, USA

Abstract: Sandwich enzyme-linked immunosorbent assays (sELISA) allow for rapid detection of *Escherichia coli* (*E. coli*) O157:H7. Acidic conditions similar to those in certain foods and juices may reduce the ability to detect *E. coli* O157:H7. Growth of *E. coli* O157:H7 at pH 4 compared to pH 5–7 reduced fluorescent signal at the lower bacterial concentrations without altering the range of detection. Both acid-adaptation and a subsequent pH 7 incubation reversed sensitivity. Incubation in apple juice was not deleterious to sELISA detection. Exposure to acidic conditions can cause a small reduction in sELISA sensitivity used to detect *E. coli* O157:H7.

Keywords: *E. coli* O157:H7, Immunoassay, Acid stress, Acid adaptation, Apple juice, Food protection

INTRODUCTION

E. coli O157:H7, a food-borne pathogen, has the potential to cause significant morbidity and mortality,[1–3] and thus it is necessary to develop detection methods for its presence in a variety of foods including acidic foods and juices such as apple and orange juice, which have been implicated in outbreaks.[4–6]

Address correspondence to Cynthia Nyquist-Battie, Ph.D., Department of Public Health, University of North Florida, 4569 St. Johns Bluff Road, Jacksonville, FL 32224, USA. E-mail: cnyquist@unf.edu

The current USDA Food Safety and Inspection Service protocol for *E. coli* O157:H7 detection employs selective enrichment and culturing steps followed by biochemical tests that require several days.[7] Recently, more rapid antibody-based methods including those for juices such as immunomagnetic separation coupled with PCR[8,9] or selective plating,[10] time-resolved immunoassay,[11] flow cytometry,[12] and solid phase fluid capillary[13] have been developed. Most promising is the use of sELISAs coupled to portable evanescent wave fiber optic biosensors for near real time detection of *E. coli* O157:H7 directly in juices and foods without enrichment steps.[14–17]

Although antibody-based techniques offer great promise for detection of *E. coli* O157:H7-contaminated food and juices, little is known regarding the effect of environmental stressors on the ability to detect this pathogen. Acidic conditions found in certain foods and juices are considered environmental stressors for *E. coli* O157:H7.[18] Acidic conditions can lead to changes in gene expression and surface characteristics,[19] and, thus, could alter the expression of surface antigens necessary for robust antibody-based detection. *E. coli* O157:H7 survives in acidic environments,[19–21] including apple juice.[22–24] Other acidic foods such as yogurt,[25] fermented foods[19,26] and mayonnaise[27] are possible vehicles for transmission of *E. coli* O157:H7. Additionally, weak acids may be introduced during food processing of meat and animal feeds.[26] Acidification is an important measure commonly employed to control growth and survival of pathogenic bacteria in foods.[28] Various acidic foods such as apple juice, mayonnaise and yogurt have been implicated in outbreaks of *E. coli* O157:H7.[29] Prolonged survival of acid-adapted organisms in acid and fermented food products has been demonstrated.[20]

The premise underlying this paper is that surface properties necessary for antibody-based detection vary as a function of acidic environments, thereby reducing assay sensitivity with an increased number of bacteria needed to exceed the minimal limit of detection. To test this premise, the effects of acid-stressing and acid-adapting of *E. coli* O157:H7 on the ability of *E. coli* O157:H7 to be detected was tested using a polyclonal sELISA. Acid adaptation results in enhanced resistance to severe acid challenge after exposure to a sub-lethal acid[29,30] and is inadvertently possible in food processing.[20,29] Additionally, the ability to detect *E. coli* O157:H7 after prolonged incubation in an acidic juice (apple juice) was determined, because survival in apple juice has been observed.[31]

EXPERIMENTAL

Bacterial Strains and Incubation Conditions

The *E. coli* O157:H7 isolates used in this study were an Odwalla apple juice outbreak[5] strain, obtained from Dr. Harvey George (Massachusetts

Department of Public Health State Laboratory Institute, Jamaica Plain, MA), and ATCC strain 43894 (American Type Culture Collection (Manassas, VA). All incubations were performed at 37°C at 250 RPM unless specified. Bacteria, maintained on tryptic soy agar (TSA, BD Diagnostic Systems, Sparks, MD) plates at 4°C, were revived by incubating an isolated colony in TSB (BD Diagnostic Systems, Sparks, MD) overnight for use in the experiments described below.

Acid-Shock and Acid-Adaptation

Aliquots of overnight cultures were incubated for 4 h at a final OD_{600} (optical density) of 0.1 in TSB, adjusted with 1 N HCl, 2 M lactic acid, or 2 M malic acid to pH 4–7, to determine the effects of an acute acid shock on sELISA detection. To determine if there is recovery from the 4 h acid incubation, a subset of duplicate broth cultures were centrifuged at 3000 × g for 5 min at 4°C, washed, and resuspended in TSB pH 7.0 for overnight incubation prior to harvesting. The effects of acid adaptation versus acid shock were studied using a modified protocol adapted from Leenanon and Drake.[18] For acid adaptation, bacteria were grown for 18 h in TSB with 1% glucose. This condition reduces pH slowly to approximately 4.8 by the end of the 18 h period. For acid shock, bacteria were grown for 16 h in TSB, and then the medium was adjusted to pH 4 by the addition of 2 M lactic acid or 2 M acetic acid. Cultures were incubated for an additional 2 h. Control cultures were grown in TSB for 18 h. Broth cultures were centrifuged as described above at the end of the 18 h incubation, and then bacteria were washed to remove broth and resuspended in TSB at either pH 7 or pH 4 for 4 h. After this final 4 h-incubation, broth cultures were centrifuged as described above. Cells were re-suspended to an OD_{600} of 0.8 in sterile 0.01 M PBS pH 7.4, and then serially diluted at a ratio of 1 : 10 in PBS.

Apple Juice Incubation

For these experiments, overnight cultures of *E. coli* O157:H7 were pelleted and washed as described above prior to re-suspension in apple juice, PBS or TSB to an OD_{600} of 0.3. Aliquots were incubated at 23–25°C for up to 7 days without shaking. Commercially-available pasteurized apple juice with no additives was used for all experiments. Prior to seeding, samples of apple juice were tested for the presence of viable *E. coli* O157:H7 organisms by plating 100 μL aliquots of non-diluted apple juice in triplicate on BCM™ O157:H7 agar (Biosynth, Naperville, IL) plates. Apple juice was free of bacterial contamination.

Cell Enumeration using Viable Plate Counts

Serial-diluted samples in PBS were used for viable counts on BCM™ O157:H7 selective agar plates. Plates were examined for the presence of sorbitol-negative colonies after 18–20 h at 37°C.

Cell Enumeration using LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit

Total bacterial number and percent live bacteria were determined using a *Bac*light™ Kit (Molecular Probes, Eugene OR) using a method modified from Braux et al.[32] Aliquots (100 μL) of the serial diluted cells ($\sim 10^3$ cells) were incubated in the dark for 15 min in the stain solution prepared per kit instructions and then captured on 0.2 μm black filters. Subsequently, filters were mounted on glass slides. Filters were observed at X1,000 using an oil immersion lens with a Zeiss epifluorescence microscope. Live (green fluorescent) and dead (red fluorescent) bacteria were viewed separately with fluorescein and Texas Red bandpass filter sets, respectively. Enumerations were performed visually by examination of 20 fields that were randomly selected.

Sandwich ELISA

Lyophilized affinity-purified anti-*E. coli* O157:H7 polyclonal antibody conjugated with horseradish peroxidase (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was rehydrated according to manufacturer's instructions. Optimal capture and detection antibody concentrations were determined by checkerboard titration. The sELISA protocol was as follows. Capture antibody (2 μg/ml KPL) in PBS was used to coat 96-well NUNC Maxisorp® microplates (Nalge Nunc International, Rochester, NY) overnight at 4°C. Following overnight incubation, wells were washed once with PBS using a microplate washer and then incubated at 23°C for 1 h with PBS containing 0.2% casein hydrosylate, and 0.2% IgG-free bovine serum albumin. Following washing once with PBS, wells were incubated with 100 μL of the serially-diluted *E. coli* O157:H7 in either apple juice or PBS. Non-spiked PBS or apple juice was added to negative control wells. After incubation at 37°C for 1 h and washing three times with PBS, the wells were incubated with 100 μL of the primary detection antibody (1 μg/mL). After incubation, wells were washed 3X for 5 min with 0.05%(v/v) Tween-20 in PBS, and incubated for 30 min with QuantaBlu substrate (Pierce, Rockford, IL) stabilized with hydrogen peroxide. The reaction was stopped by the addition of 100 μL of QuantaBlu stop solution. Fluorescence intensity was measured using a FL_x800 BioTek fluorescence microplate reader (Winooski, VT). Net fluorescence intensity was calculated

by subtracting the background values (wells without antigen). Assay precision was determined by calculating the coefficient of variation. Only those assays with a coefficient of variation $<10\%$ were included in the results.

Statistical Analysis

Statistical significance was determined by Analysis of Variance followed by Dunnett Multiple Comparison tests, using GraphPad Instat™ software. Values are means $\pm$ standard error of the mean (SEM), $n = 4-5$.

RESULTS AND DISCUSSION

Short Term Incubation in Acidified TSB

Bacteria were grown for 4 h in TSB at various pHs and prepared as described in Experimental to determine if incubation in acid reduces the ability to detect *E. coli* O157:H7. Figure 1 illustrates that the pH 4 incubation did not markedly shift the linear range of detection, which was 10^3-10^5 colony units (CFU)/ml for both *E. coli* O157:H7 isolates. However, the pH 4 medium reduced the fluorescence signal at 10^5 CFU/mL by approximately 60% compared to the other pHs ($p < 0.001$). Net fluorescent intensities at pH 4 for the MA and ATTC #43894 isolates were 5498 ± 233 and 5874 ± 192, respectively, compared to 13465 ± 191 and 15787 ± 667 at pH 5. The pH 5 mean net fluorescent intensity was not significantly different from that of pH 6 and 7. When lactic acid or acetic acid were used to acidify TSB, results were similar to those reported above for the HCl-acidified medium (data not shown). Although pellets were re-suspended to the same OD prior to plating, the CFU/mL were reduced for the pH 4 cultures. Additionally, the percent live cells determined by direct cell counts was reduced significantly ($p < 0.01$) at pH 4 compared to the other pH media. The percent live cells were as follows: 49.3 ± 7 (pH 4), 62 ± 8 (pH 5), 78 ± 10 (pH 6) and 97 ± 4 (pH 7). When the pH 4-incubated bacteria were centrifuged, washed and the pellet re-suspended in TSB pH 7 and grown overnight, sELISA results and viable counts returned to normal (data not shown).

Acid-Adaptation

Bacteria were acid-adapted according to the protocol of Leenanon and Drake[18] to determine the effect of acid adaptation on sELISA detection. Figure 2 illustrates the results of acid-adaptation compared to normal incubation and brief acid shock for the MA isolate. After treatment, cells were pelleted, washed and resuspended for a 4 h incubation in either pH 7 or

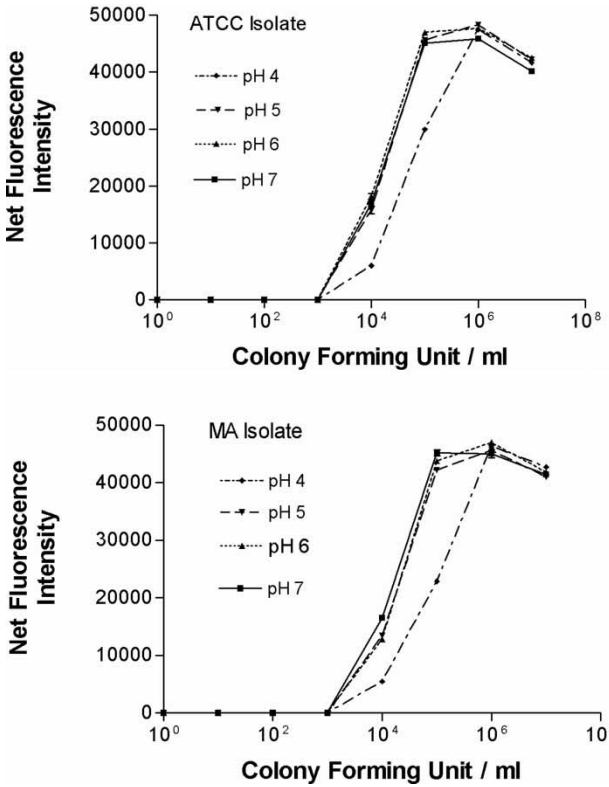

Figure 1. Detection of *E. coli* O157:H7 after growth in tryptic soy broth with various pHs. ATCC isolate #43894 (upper panel) and the MA (Odwalla) isolate (lower panel) were used for this experiment. After incubation bacteria were pelleted, washed and serial diluted in phosphate-buffered saline. Results show net fluorescence intensity as a function of colony forming units per ml. Net fluorescence intensity was measured by sandwich ELISA using the KPL polyclonal antibody for both capture and detection elements. Net fluorescence intensity was determined by subtracting the fluorescence intensity for samples without bacterial spiking. Each value represents the mean $\pm$ SEM (n = 4).

4 TSB. When incubated in the TSB pH 7, acid-adapted and control bacteria had a similar linear range of detection. However, the acid-shocked bacteria had a slight shift in range of detection similar to what was found previously. When the final growth was at pH 4, both control and acid-shocked bacteria exhibited a less favorable range of detection than acid-adapted.

Apple Juice

E. coli O157:H7 (MA isolate) was incubated in apple juice, TSB and PBS for a one week period. Sampling was performed on Days 0, 3 and 7. Results are

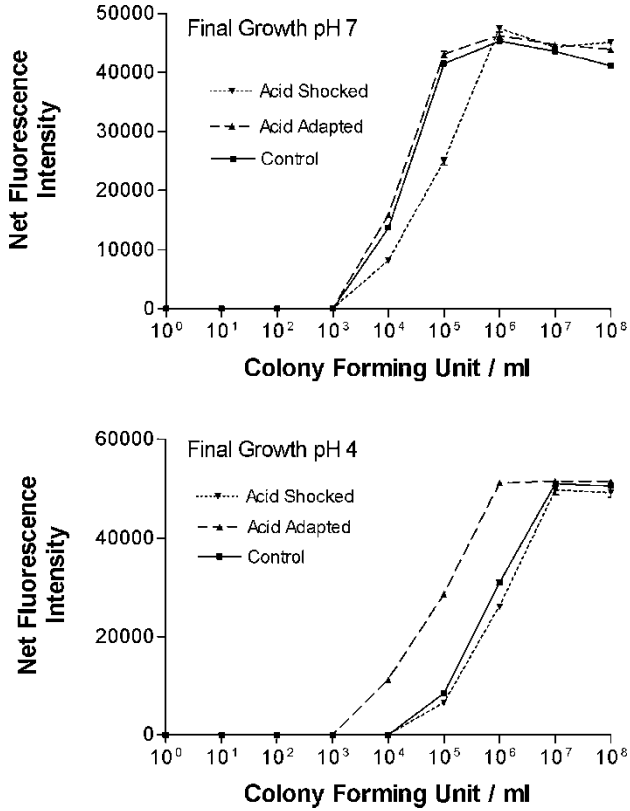

Figure 2. Detection of *E. coli* O157:H7 (MA isolate) after acid-adaptation, acid-shocking and growth at pH 7 followed by growth in TSB at pH 7 (upper panel) or pH 4 (lower panel). After incubation, bacteria were pelleted, washed and serial diluted in phosphate-buffered saline. Results show net fluorescent intensity as a function of colony forming units per ml. Fluorescent intensity was determined by sandwich ELISA using the KPL polyclonal antibody for capture and detection elements. Net fluorescence intensity was determined by subtracting the fluorescence intensity for samples without bacterial spiking. Each value represents the mean $\pm$ SEM (n = 4).

shown in Figures 3 and 4. Bacteria in the apple juice and PBS did not increase in number, determined by optical density at 600 nm, over the 7-day period while there was an increase in bacteria grown in TSB (Figure 3). After centrifugation and serial dilution in PBS and plating on selective agar, CFU/mL at the -7 dilution was reduced as a function of time for all three sets of bacteria. Since the CFU/mL was reduced to zero for apple juice but not cell number (Figure 3), sELISA net fluorescent intensity was expressed as a function of bacteria number determined by the direct count method. As shown in Figure 4, there was no change in range of detection, determined by sELISA for any of the samples. However, at 10^5 cells/mL there was an increased net

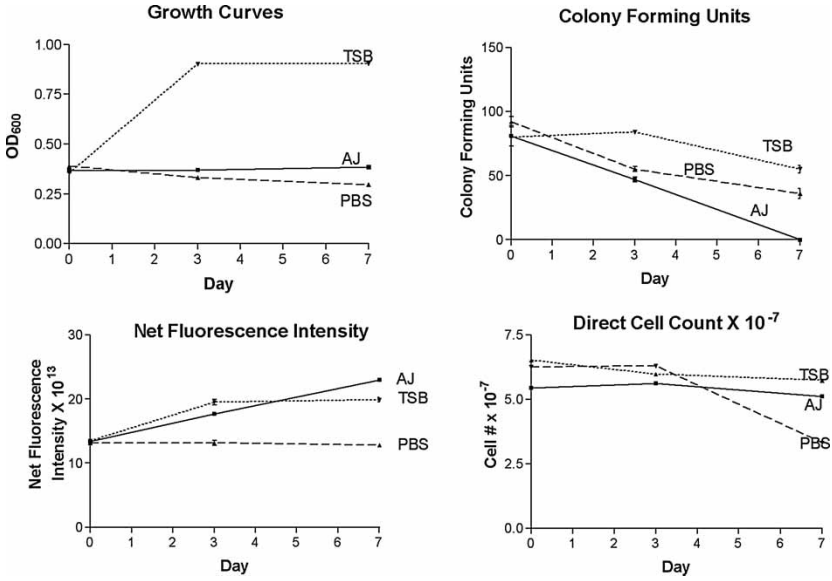

Figure 3. The effect of incubation in apple juice, TSB and PBS for 3 and 7 days on optical density at 600 nm (top left), colony forming units per optical density unit (top right), net fluorescent intensity for 10^5 cell number/mL (bottom left) and bacterial cell number per optical density unit (bottom right). Optical density was measured in aliquots from each incubation while colony forming units, net fluorescence intensity (sELISA), direct cell counts were determined after bacteria were centrifuged, washed, resuspended to Optical Density of 0.8 at 600 nm and serial diluted in PBS. Colony forming units and direct cell counts were for the −7 dilution. Net fluorescent intensity are values for 10^5 cells/ml. Each value represents the mean ± SEM (n = 4).

fluorescent intensity for apple juice and TSB at Days 3 and 7 (Figure 3; $p < 0.01$), but this change was not apparent for the cells incubated in PBS.

Outbreaks of *E. coli* O157:H7 infections associated with unpasteurized apple juice and apple cider[4–6] and the threat of the contamination of other acidic foods with this pathogen emphasize the need for rapid and sensitive tests to detect the presence of *E. coli* O157:H7 in acidic foods and juices. Since surface characteristics are necessary for detection of *E. coli* O157:H7 by immunoassays, alterations in surface antigenicity as a function of different growth conditions merits concern when developing immunoassays. The premise underlying the present work is that the number of available binding sites will vary as a function of growth conditions. For example, differences in the protein composition of the cell envelope as a function of acid stressed and acid tolerated conditions even for short times have been observed.[19]

The present study investigated the effect of exposure to acid on the ability to detect *E. coli* O157:H7 using a sandwich ELISA with a commercially

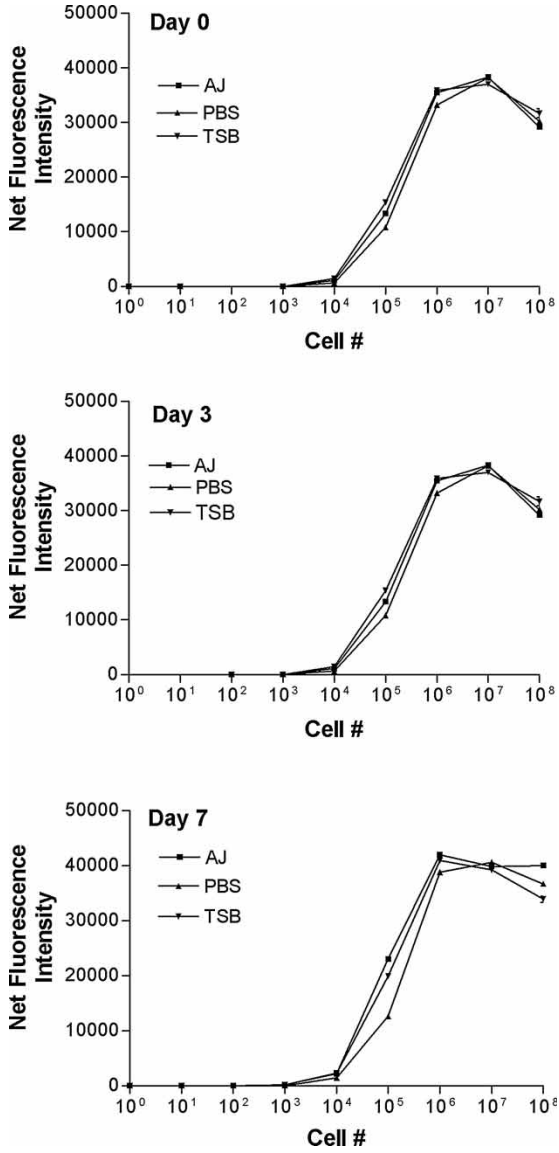

Figure 4. Detection of *E. coli* O157:H7 (MA isolate) at Day 0 (upper panel), Day 3 (middle panel), and Day 7 (lower panel). After incubation in tyrptic soy broth (TSB), phosphate buffered saline (PBS) and apple juice (AJ), bacteria were pelleted, washed and serial diluted in phosphate-buffered saline (PBS). Results show net fluorescent intensity as a function of cell number per ml, determined by direct cell counts after staining with *Bac*light live/dead kit. Fluorescent intensity was determined by sandwich ELISA using the KPL polyclonal antibody for capture and detection elements. Net fluorescence intensity was determined by subtracting the fluorescence intensity for samples without bacterial spiking. Each value represents the mean $\pm$ SEM (n = 4).

available antibody. Regardless of the acidulant (HCl, lactic acid or malic acid), brief incubations at ~pH 4 caused a slight reduction in the ability to detect this bacterium when data was expressed per number of viable cells indicated by colony forming units. Given the fact that the results were the same regardless of the acidulant, there is possibility that there is a slight reduction in assay sensitivity if sampling were to be done directly from a wide variety of acidic foods and juices. Since the pH 4 incubation reduced the number of live cells and the number of colony-forming cells, the actual number of cells was under-represented by the colony-forming unit measurement. Since more cells were present than indicated by the viable method, the sensitivity for pH 4 incubation would be worse than pictured in our graphs. This work does not allow us to determine if there is a difference in antibody binding per dead versus live cells. The change in detection was not due to the effect of acid on the assay itself because cells were washed and resuspended in PBS prior to sELISA. Overnight incubation at normal pH reversed this change suggesting that growth in enrichment broths prior to sELISA would negate deleterious changes in epitopes necessary for antibody-based detection. The results also showed that acid-adaptation prior to growth in acidic conditions protect sELISA detection. Acid adaptation has been shown to increase resistance to acid conditions as well as heat and freeze-thaw conditions,[18] and is a complex biological phenomenon including *de novo* protein synthesis and changes in fatty acid composition of the cell membrane.[33]

Although acidic conditions may deleteriously effect detection, incubation in an acidic juice, apple juice, for up to 7 days did not alter the ability to detect *E. coli* O157:H7, although the colony forming units were reduced to zero, similar to what has been reported previously.[31] Again, cells were harvested, washed, and resuspended in PBS prior to assay, because our earlier work showed that the presence of apple juice in the first step of the sELISA alters assay performance.[34] Only one brand of apple juice was used for this study, and the results could vary as a function of apple juice brand.[35] Moreover, the results showed that incubation in starvation conditions (PBS) for 7 days also did not alter sELISA detection.

The findings of this study suggest that incubation in acidic conditions can adversely affect surface antigens of *E. coli* O157:H7 that are necessary for antibody binding, but not in all conditions and most importantly not in apple juice even after 7 days. Limitations of this study include the use of only one polyclonal antibody as both capture and detection elements. Other work has indicated that there is a loss of O antigenicity, measured by agglutination of *E. coli* O157:H7 when grown in starvation conditions.[36] In contrast to our short-term study, the change in O antigenicity during starvation took months. Outer membrane proteins of *E. coli* O157:H7 do change during incubation under adverse conditions.[37] Composition of culture broth may affect immunomagnetic separation[38] and ELISA performance.[39] In this regard, the inclusion of acriflavine and casmino acid in enrichment broth increased

ELISA sensitivity but inclusion of EDTA resulted in no ELISA signal.[39] However, cold stressing of bacteria did not alter automated-enzyme-linked florescent immunoassay in cheese.[40] Moreover, freeze-thawing spiked ground beef did not reduce sensitivity of a solid phase fluorescent immunoassay.[13]

CONCLUSIONS

The present results indicate that some caution should be used when directly sampling acidic foods and juices for *E. coli* O157:H7 contamination using immunoassays, although the detection of this bacterium using a polyclonal sandwich ELISA was not greatly altered and, if present, was easily reversed by further growth at normal pH.

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REFERENCES

1. Coia, J.E. Clinical, microbiological, and epidemiological aspects of *Escherichia coli* 0157:H7 infection. FEMS Immunol. Med. Mic. **1998**, *20*, 1–9.
2. MacDonald, K.L.; Osterholm, M.T. The emergence of *Escherichia coli* 0157:H7 infection in the United States. JAMA **1993**, *269*, 2264–2266.
3. Griffin, P.M.; Tauxe, R.V. The epidemiology of infections caused by *Escherichia coli* 0157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. **1991**, *13*, 60–67.
4. Besser, R.E.; Lett, S.M.; Weber, J.T.; Doyle, M.P.; Barret, T.J.; Wells, J.G.; Griffin, P.M. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* 0157:H7 in fresh pressed apple cider. JAMA **1993**, *269*, 2217–2220.
5. Centers for Disease Control and Prevention, Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice-British Columbia, California, Colorado, and Washington, MMWR, October 1996, *45*, 975.
6. Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infection and Cryptosporidiosis associated with drinking unpasteurized apple cider-Connecticut and New York, MMWR, 1997, October 1996, *46*, 5–8.
7. Dey, B.P.; Lattuada, C.P. *Microbiology Laboratory Guidebook*, 3rd Ed.; United States Department of Agriculture, Food Safety and Inspection Service Office of Public Health and Science, Microbiology Division. U.S. Department of Agriculture: Washington, D.C., 1998; Vol. 1.

8. Ogunjimi, A.A.; Choudary, P.V. Adsorption of endogenous polyphenols relieves the inhibition by fruit juices and fresh produce of immuno-PCR detection of *Escherichia coli* O157:H7. FEMS Immunol. Med. Mic. **1999**, *23*, 213–220.
9. Tortorello, M.L.; Reinke, K.F.; Stewart, D.S.; Rayborn, R.B. Comparison of methods for determining the presence of *Escherichia coli* O157:H7 in apple juice. J. Food Protect. **1998**, *61*, 1425–1430.
10. Ogden, I.D.; Hepburn, N.F.; MacRae, M. The optimization of isolation media used in immunomagnetic separation methods for the detection of *E. coli* 0157:H7 in foods. J. Appl. Microbiol. **2001**, *91*, 373–379.
11. Yu, L.S.L.; Reed, S.A.; Golden, M.H. Time-resolved fluorescence (TRFIA) for the detection of *Escherichia coli* 0157:H7 in apple cider. Microbiol. Meth. **2002**, *49*, 63–68.
12. Seo, K.H.; Brackett, R.E.; Frank, J.F. Rapid detection of *Escherichia coli* 0157:H7 using immunomagnetic flow cytometry in ground beef, apple juice, and milk. Int. J. Food Microbiol. **1998**, *44*, 115–123.
13. Czajka, J.; Batt, C.A. A solid phase fluorescent capillary immunossay for the detection of *Escherichia coli* 0157:H7 in ground beef and apple cider. J. Appl. Bacteriol. **1996**, *81*, 601–607.
14. DeMarco, D.R.; Saaski, E.W.; McCrae, D.A.; Lim, D.V. Rapid detection of *Escherichia coli* 0157:H7 in ground beef using a fiber optic biosensor. J. Food Prot. **1999**, *62*, 711–716.
15. DeMarco, D.R.; Lim, D.V. Direct detection of *Escherichia coli* 0157:H7 in unpastuerized apple juice with an evanescent wave biosensor. J. Rapid Meth. Aut. Mic. **2001**, *9*, 241–257.
16. Lim, D.V. Detection of microorganisms and toxins with evanescent wave, fiber optic biosensors. Proc. IEEE **2001**, *18*, 13–17.
17. Lim, D.V. Detection of microorganisms and toxins with evanescent wave, fiber optic biosensors. Proc. IEEE **2003**, *91*, 902–907.
18. Leenanon, B.; Drake, M.A. Acid stress, starvation, and cold stress affect post-stress behavior of *Escherichia coli* 0157:H7 and nonpathogenic *Escherichia coli*. J. Food Prot. **2001**, *64*, 970–974.
19. Jordan, K.N.; Oxford, L.; O'Byrne, C.P. Survival of low-pH stress by *Escherichia coli* 0157:H7: Correlation between alterations in cell envelope and increased acid tolerance. Appl. Environ. Microbiol. **1999**, *65*, 3048–3055.
20. Cheng, H.-Y.; Yang, H.-Y.; Chou, C.-C. Influence of acid adaptation on the tolerance of *Escherichia coli* 0157:H7 to some subsequent stresses. J Food Prot. **2002**, *65*, 260–265.
21. Yokoigawa, K.; Takikawa, A.; Okubo, Y.; Umesako, S. Acid tolerance and *gad* mRNA levels of *Escherichia coli* 0157:H7 grown in foods. Int. J. Food Microbiol. **2003**, *82*, 203–211.
22. Miller, L.; Kaspar, C.W. *Escherichia coli* 0157:H7 acid tolerance and survival in apple cider. J. Food Prot. **1994**, *57*, 460–464.
23. Roering, A.M.; Luchansky, J.B.; Ihnot, A.M.; Ansay, S.E.; Kaspar, C.W.; Ingham, S.C. Comparative survival of *Salmonella typhimurium* DT 104 *Escherichia coli* 0157:H7 in preservative-free apple cider and simulated gastric fluid. Int. J. Food Microbiol. **1999**, *46*, 263–269.
24. Zhao, T.; Doyle, M.P.; Besser, R.E. Fate of enterohemorrhagic *Escherichia coli* 0157:H7 in apple cider with and without preservatives. Appl. Environ. Microbiol. **1993**, *59*, 2526–2530.

25. Morgan, C.; Newman, C.P.; Hutchinson, D.N.; Walker, A.M.; Rowe, B.; Majid, F. Verotoxin producing *Escherichia coli* 0157:H7 infections associated with the consumption of yogurt. Epidemiol. Infect. **1983**, *111*, 181–187.
26. Lin, J.; Smith, M.P.; Chapin, K.D.; Baik, H.S.; Bennett, G.N.; Foster, J.W. Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. Appl. Environ. Microbiol. **1996**, *62*, 3094–3100.
27. Weagant, S.D.; Bryant, J.L.; Bark, D.H. Survival of *Escherichia coli* 0157:H7 in mayonnaise-based sauces at room and refrigerated temperatures. J. Food Prot. **1994**, *57*, 629–631.
28. Brown, M.H.; Booth, I.R. Acidulants and low pH. In *Food Preservatives*; Russell, N.J., Gould, G.W., Eds.; Blackie: Glasgow, Scotland, 1991, 22–43.
29. Cheng, H.-Y.; Chou, C.-C. Acid adaptation and temperature effect on the survival of *E. coli* 0157:H7 in acidic fruit juice and lactic fermented milk product. Intl. J. Food Microbiol. **2001**, *70*, 189–195.
30. Leyer, G.J.; Wang, L.-L.; Johnson, E.A. Acid adaptation of *Escherichia coli* 0157:H7 increases survival in acidic foods. Appl. Environ. Microbiol. **1995**, *61*, 3752–3755.
31. Duffy, S.; Schaffner, D.W. Modeling the survival of *Escherichia coli* 0157:H7 in apple cider using probability distribution functions for quantitative risk assessment. J. Food Prot. **2001**, *64*, 599–605.
32. Braux, A.-S.; Inet, J.; Tamanai-Sharcoori, Z.; Riou, G.; Cormier, M. Direct enumeration of injured *Escherichia coli* cells harvested onto membrane filters. J. Microbiol. Meth. **1997**, *31*, 1–8.
33. Koutsoumanis, K.P.; Sofos, J.N. Comparative acid stress response of *Listeria monocytogenes, Escherichia coli* 0157:H7 and *Salmonella* Typhimurium after habituation at different pH conditions. Lett. Appl. Microbiol. **2004**, *38*, 321–327.
34. Nyquist-Battie, C.; Frank, L.E.; Lund, D.; Lim, D.V. Optimization of a fluorescence sandwich enzyme-linked immunosorbent assay for detection of *Escherichia coli* 0157:H7 in apple juice. J. Food Prot. **2005**, *67*, 2756–2759.
35. Ryu, J.-R.; Beuchat, L.R. Influence of acid tolerance responses on survival, growth and thermal cross-protection of *Escherichia coli* 0157:H7 in acidified media and fruit juices. Int. J. Food Microbiol. **1998**, *45*, 185–193.
36. Hara-Kudo, Y.; Miyaharo, M.; Kumagai, S. Loss of 0157 0 antigenicity of verotoxin-producing *Escherichia coli* 0157:H7 surviving under starvation conditions. Appl. Environ. Microbiol. **2000**, *66*, 5540–5543.
37. Wand, G.; Doyle, M.P. Survival of enterohemorrhagic *Escherichia coli* 0157:H7 in water. J. Food Prot. **1998**, *61*, 662–667.
38. Shelton, D.R.; Karns, J.S. Quantitative detection of *Escherichia coli* 0157 in surface waters by using immunomagnetic electrochemiluminescence. Appl. Environ. Microbiol. **2001**, *67*, 2908–2915.
39. Padhye, N.V.; Doyle, M.P. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* 0157:H7 in food. Appl. Environ. Microbiol. **1991**, *57*, 2693–2698.
40. Cohen, A.E.; Kerdahi, K.F. Evaluation of a rapid and automated enzyme-linked fluorescent immunoassay for detecting *Escherichia coli* serogroup 0157 in cheeses. J. A. O. A. C. Intl. **1996**, *79*, 858–860.

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