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# FOOD PROTECTION TRENDS

SCIENCE AND NEWS FROM THE INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION

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# A NOTE FROM THE *FPT* SCIENTIFIC EDITOR...

## EDMUND A. ZOTTOLA

*Food Protection Trends* is designed to serve as the primary source of information for members of the International Association for Food Protection. It should provide to members up-to-date material and serve as a mechanism to convey to all members of IAFP current information that will assist them in their chosen endeavor. The Journal contains information that should be useful in daily activities, such as, peer-reviewed research papers, food safety news, association news, industry products, career opportunities and other information pertinent to members daily activities.

I assumed the position of Scientific Editor in May of 2004. It is an interesting and challenging opportunity. John Cerveny served as Interim Scientific Editor for several months. He did an outstanding job filling in and we should all thank him for the excellent job he did while Editor. Thank you, John!

During this past year, 2004, 33 manuscripts have been submitted to IAFP for possible publication in Volume 24 of *Food Protection Trends.* Of these 33 manuscripts 12 have been published, 4 have been rejected, 5 accepted but not yet published, 5 returned to authors for final revision and 7 are still in the review process. One of the goals of the *FPT* Journal Management Committee is to obtain and publish manuscripts that appeal to our membership. The manuscripts are reviewed by at least 2 members of the Editorial Board and with some manuscripts 3 reviewers are used. The review process does take time but your Editor is attempting to reduce the time it takes for a manuscript to get from receipt to publication.

The Editorial Board for *FPT* is composed of 50 members that are involved in the review process. It takes time for them to review these manuscripts and we should all give them a big thank you for their timely assistance. If you are interested in serving on the Editorial Board contact the Scientific Editor.

As your new Editor I would like to know if there are changes that could be made to the Journal that would better serve the membership. If you have an idea on what could be done to make *FPT* more user friendly, please contact me at 218.666.0272 or E-mail: lansibay@cpinternet.com.

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The International Association for Food Protection (IAFP) Foundation Fund was established in the 1970s to support the mission of IAFP – "To provide food safety professionals worldwide with a forum to exchange information on protecting the food supply."

We live in a global economy and the way food is grown, processed, and handled can impact people around the globe. From a public health perspective, it often provides unique challenges to the food safety professional. Combine these issues with the complexity of protecting the food supply from food security threats and the challenges seem overwhelming. However, with your support the Foundation can make an impact on these issues. Funds from the Foundation could help to sponsor travel for deserving scientists from developing countries to our Annual Meeting, sponsor international workshops, and support the future of food scientists through scholarships for students or funding for students to attend IAFP Annual Meetings.

The Foundation is currently funded through contributions from corporations and individuals. A large portion of the support is provided from the Sustaining Members of IAFP. The Sustaining Membership program is a unique way for organizations to partner with the Association. Contact the Association office if you are interested in this program.

Support from individuals is also crucial in the growth of the Foundation Fund. Contributions, big or small, make an impact on the programs supported by the IAFP Foundation. Programs currently supported by the Foundation include the following:

- Ivan Parkin Lecture
- Travel support for exceptional speakers at the Annual Meeting
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- Shipment of volumes of surplus *JFP* and *FPT* journals to developing countries through FAO in Rome

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It is the goal of the Association to grow the Foundation to a self-sustaining level of greater than $1.0 million over the next 10 years. This would allow the Foundation to provide additional programs in pursuit of our goal of *Advancing Food Safety Worldwide*®!

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# "A VIEW FROM WISCONSIN"

As we turn the calendar to 2005, the IAFP staff and Board are ready to tackle another year with enthusiasm. January is a busy month for IAFP with technical abstracts for the annual meeting due January 12. The Program Committee meets in Baltimore on January 21–22 to review the abstracts for acceptance, make the final determination on submitted symposia, and try to piece together the educational sessions to avoid overlap of speakers and audience. It is a complicated task to coordinate over 500 presentations for the three-day conference and to produce a balanced program. Then, the work load shifts back to the symposia organizers to confirm invitations to speakers and to the IAFP staff to contact the technical session presenters and begin work on the program book. I have heard comments from some fellow researchers that this year's eight-month lag between submission of abstracts and presentation is excessive. We will work to improve this issue for the 2006 meeting. However, while electronic submissions have helped their work load significantly, we are still working with the same number of office staff as when we had only half the number of presentations. As a small association with limited resources, the staff needs to be able to start on the program early in order to balance all their other work responsibilities during the year.

By KATHLEEN A. GLASS
PRESIDENT

***"Our primary objective is to provide both science and practical solutions to an international audience composed of industry, regulators, and academic food protection professions"***

Certainly, we are ecstatic about the steady annual increase in conference attendance, exhibits, sponsorship, and submitted technical abstracts and symposia. On the other hand, our meeting is quickly reaching a size that we may be victims of our own success. While the vast majority of the returned surveys from the 2004 Phoenix meeting were very favorable, I paid particular attention to comments from members who felt overwhelmed by having too many concurrent sessions or too many technical presentations. To give you a "behind the scenes" view, we recently added more sessions to our schedule, such as the early Tuesday afternoon short sessions, to accommodate requests from members who need educational sessions on diverse topics. The IAFP meeting is the only meeting that many attend during the year; therefore, these members need a comprehensive meeting to address all their needs. But, we don't want to resolve one issue at the expense of spreading ourselves too thin. One of the items that will be added to the agenda for this year's Program Committee meeting is to make a rigorous evaluation of our current design for developing the educational program. I also invite your suggestions for unique alternatives that are feasible to implement. Keep in mind that our primary objective is to provide both science and practical solutions to an international audience composed of industry, regulators, and academic food protection professions. Many of these attendees have responsibilities related to both microbiological and toxicological food safety issues, and as well as issues related to multiple commodities. We want to build on our current success and maintain our status as the premier food safety association.

Our association also continues its work on the IAFP Strategic Plan for 2010 and needs your assistance in fulfilling our objectives related to publications. As you may recall from my October column, our goals for enhancing our publications included increasing accessibility to publications by adding back volumes of *JFP* online and archiving *FPT* articles online after one year, developing applied food safety booklets, and developing "white papers" on important food safety issues. The addition of articles online will go forward as budget permits. But, we are still looking for ideas for topics for booklets and the white papers, and are always in need of practical food protection manuscripts for publication in *FPT*.

During the past year, the Outreach Education Professional Development Group (PDG) came forward to revise two booklets, *Food Safety at Temporary Events* and *Before Disaster Strikes…A Guide to Food Safety in the Home*, including a Spanish language version. Both of these booklets are available for purchase through the IAFP Web site. If you have responsibilities for the food safety education of consumers, you will find these booklets very useful. This summer the Executive Board endorsed proposals by two of our committees to develop new booklets. The 3-A Committee on Sanitary Procedures will develop a booklet on sanitary equipment design, and the Committee on the Control of Foodborne Illness will revise a 6th edition of the manual outlining *Procedures to Investigate Foodborne Illness*. We know that there is a strong need for applied food safety publications. Please send me an E-mail with your ideas for topics or if you are willing to help in the development of a booklet. In light of our overly successful call for symposia for the annual meeting, the development of booklets and other resources for food safety professionals may be an excellent alternate activity for PDGs.

We are also soliciting ideas for white papers. So far, we have received proposals for authoritative papers on redefining pasteurization, and the controversy regarding *Mycobacterium avium paratuberculosis* and Crohn's Disease. Once again, if you have suggestions, send me an E-mail so that we can add it to our list for consideration and appoint an appropriate task force to develop the paper.

Lastly, we continue our call for *FPT* manuscripts on practical, applied food protection research that can be readily put into practice by field inspectors, retail managers, product developers, or quality assurance departments. I would like to repeat my appeal to researchers, professors and students to consider submitting manuscripts that provide viable solutions to our food safety problems. In addition, we are encouraging submissions in the area of applied food toxicology as it pertains to current food safety questions, as well as microbial food safety and quality research.

As always, I welcome your comments. Please E-mail me at kglass@wisc.edu with your ideas for enhancing our annual meeting, booklet topics, and white papers. I look forward to hearing your view.


# Call for Abstracts

IAFP 2005 abstract submission deadline is January 12, 2005. Abstracts submitted will be evaluated for acceptance by the Program Committee.

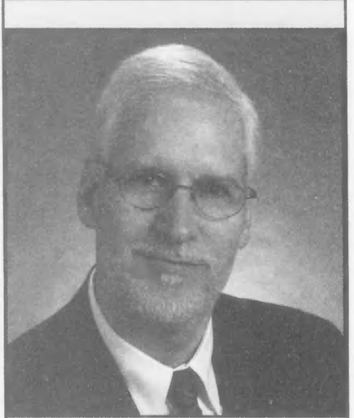
Please return completed abstracts through one of the following methods:

1. Online: Use the online abstract submission form located at www.foodprotection.org. You will receive an E-mail confirming receipt of your submission.
2. E-mail: Submit via E-mail as an attached text or MS Word document to abstracts@foodprotection.org.

For further information on abstract submission, visit our Web site at

**www.foodprotection.org**

# "COMMENTARY"
## FROM THE EXECUTIVE DIRECTOR



By **DAVID W. THARP, CAE**
EXECUTIVE DIRECTOR

***"As you know, we had a great year last year and we are looking forward to an even better 2005"***

This month we start the New Year, 2005 and we are now halfway through the first decade of the twenty-first century. It doesn't seem all that long ago that we turned the calendar from 1999 to 2000, but five years seems like it has gone fast! Thinking back to January of 2000, it was an exciting time as the Association name had just been changed to the International Association for Food Protection, thus ending 34 years of being known as IAMFES. I know that some Members still call the Association IAMFES and that will always be! There is nothing wrong with that, it is just that our new name is so much more descriptive of what our Members do – protect the food supply.

Preparations for IAFP 2005 in Baltimore are well underway. Abstract submissions are due January 12. If you have interest in being included in the technical or educational program, see page 11 for additional details. The Program Committee will meet toward the end of January to review all submissions in addition to approving the symposium proposals and then set the program for IAFP 2005. Those who are on the Committee or who have served on the Program Committee in the past know that there is a lot of work involved and some very long hours. Of course the satisfaction of knowing the job was completed efficiently and that the end result provided great program content are some of the elements that keep Committee Members' interest high.

We are fortunate to have a large number of Members who express interest in serving on the Program Committee. If you are interested in serving on Committees or our Professional Development Groups (PDGs), please contact our office or the Committee Chairperson to express your interest. PDGs are open to everyone (Members or nonmembers), while the Standing Committee Members are appointed with specified terms. Special Committees are open to interested Members and most do not carry term appointments.

Watch your mail early in February for the Secretary ballot. We have two outstanding candidates who are willing to serve the Association and both would make excellent Board Members. Stan Bailey and LeeAnne Jackson have agreed to be candidates for the 2005-2006 Secretary. Your completed ballot is due back to the IAFP office by March 18. Our newly elected Secretary will be announced in the May issue of *Food Protection Trends* and takes office upon the conclusion of IAFP 2005.

Some other items of interest are that the registration form for IAFP 2005 appears on page 77. Additional information, including the program, will become available in the coming months. Watch your *Food Protection Trends* and check the IAFP Web site for current information. You may also make reservations at the hotel now as the room block is open. Just so you are aware, there will be a need for IAFP to use a second hotel property as the host hotel will not be able to accommodate our entire group – so make your reservation early! Also, if you are interested in exhibiting, the same goes – get your booth space reservation in now! We do not have space for as many exhibitors as we were able to take in 2004. Don't get turned away – call today!

As you know, we had a great year last year and we are looking forward to an even better 2005. Best wishes to all IAFP Members for a happy, healthy, and prosperous New Year!

# Sponsorship Opportunities Available for IAFP 2005

Sponsor an event to promote your company as a supporter of IAFP!

contact Dave Larson
at 515.440.2810
E-mail: larson6@earthlink.net

## Is Your Program Crumbine Material? Put it to the Test!

**The Samuel J. Crumbine Consumer Protection Award for Excellence in Food Protection at the Local Level is seeking submissions for its 2005 program.** The Crumbine Award is given for excellence and continual improvement in a comprehensive program of food protection at the local level. Achievement is measured by:

- Sustained improvements and excellence over the preceding four to six years;
- Innovative and effective use of program methods and problem solving to identify and reduce risk factors that are known to cause foodborne illness;
- Demonstrated improvements in planning, managing, and evaluating a comprehensive program; and
- Providing targeted outreach; forming partnerships; and fostering communication and information exchange among regulators, industry and consumer representatives.

All local environmental health jurisdictions in the U.S. and Canada are encouraged to apply, regardless of size, whether "small," "medium" or "large."

The Award is sponsored by the Conference for Food Protection, in cooperation with the American Academy of Sanitarians, American Public Health Association, Association of Food and Drug Officials, Foodservice & Packaging Institute, Inc., International Association for Food Protection, International Food Safety Council, National Association of County & City Health Officials, National Environmental Health Association, NSF International, and Underwriters Laboratories, Inc.

For more information on the Crumbine Award program, and to download the 2005 criteria and previous winning entries, please go to www.fpi.org or call the Foodservice & Packaging Institute at (703) 538-2800. **Deadline for entries is March 15, 2005**.

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International Association for Food Protection.

# Single and Sequential Treatment of Beef Tissue with Lactic Acid, Ammonium Hydroxide, Sodium Metasilicate, and Acidic and Basic Oxidized Water to Reduce Numbers of Inoculated *Escherichia coli* O157:H7 and *Salmonella* Typhimurium

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## SUMMARY

This study was conducted to determine if several potential decontamination intervention solutions, applied either singly or sequentially onto beef tissue, could effectively reduce numbers of inoculated *Escherichia coli* O157:H7 and *Salmonella* Typhimurium. Samples (5×2.5×1 cm) of boneless beef plates were inoculated (ca. $10^5$ CFU/cm$^2$) with four-strain composites of *E. coli* O157:H7 or *S.* Typhimurium and dipped (5 pieces in 1.5 l for 30 s at 23°C, unless otherwise indicated), either singly or sequentially into all possible combinations of two of the following solutions: acidic oxidative water (AOW; 0.005%, pH 2.67); basic oxidative water (BOW; pH 11.21); lactic acid (LA; 2.5%, pH 2.12 at 55°C); ammonium hydroxide (AH; 0.1%, pH 10.89); sodium metasilicate (SM; 4%, pH 12.35 at 82°C); or distilled water (W, pH 7.01). In phase II, an approach incorporating sequential treatments that could be applied in commercial beef harvesting plant multiple-hurdles systems was evaluated. That system included sequential dipping in 1% SM (82°C), hot (82°C) water, and 5% LA (55°C), followed by 5% LA (55°C) after 48 h (at -3°C for 10 h and 1°C for 38 h) of simulated carcass spray-chilling (by overhead misting of the inoculated product surface every 30 min for the first 10 h by use of a handheld sprayer). Additional systems ranged from no dipping steps to four sequential dipping steps using combinations of 1% SM (82°C), 5% LA (55°C), warm (55°C) or hot (82°C) water. Treatments, individual or in combinations, W, AOW, BOW or AH resulted in minimal decontamination (0.1–0.4 log CFU/cm$^2$) compared with treatments using 2.5% LA at 55°C and 4% SM at 82°C. In general, pathogen reductions via the multiple-hurdles approach were separated into two groups with respect to efficacy: those treatments with one or more hot (82°C) application or a single 5% LA application were less effective than those combining two 5% LA applications or at least one hot (82°C) and one 5% LA application.

A peer-reviewed article

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TABLE 1. Treatment systems indicating sequence of four possible dipping applications that simulate decontamination of beef at a pre-evisceration stage (Application #1), followed 20 min later by a post-evisceration stage (Application #2), followed 10 min later by a pre-chill organic acid rinse stage (Application #3), and lastly 48 h later* by a post-chill organic acid rinse stage (Application #4)

| Treatment | Pre-chill Dipping | | | | Post-chill Dipping |
|---|---|---|---|---|---|
| Systems | Application #1 | Application #2 | Application #3 | Spray-chilling* | Application #4 |
| 1 | Undipped | Undipped | Undipped | 4°C W | Undipped |
| 2 | Undipped | 82°C W | Undipped | 4°C W | Undipped |
| 3 | 82°C W | 82°C W | 55°C W | 4°C W | 55°C W |
| 4 | 82°C SM | 82°C W | 55°C LA | 4°C W | 55°C LA |
| 5 | Undipped | 82°C W | 55°C LA | 4°C W | 55°C LA |
| 6 | 82°C SM | Undipped | 55°C LA | 4°C W | 55°C LA |
| 7 | 82°C SM | 82°C W | Undipped | 4°C W | 55°C LA |
| 8 | 82°C SM | 82°C W | 55°C LA | 4°C W | Undipped |
| 9 | 82°C W | 82°C W | 55°C LA | 4°C W | 55°C LA |
| 10 | 82°C SM | 82°C W | 55°C W | 4°C W | 55°C W |
| 11 | Undipped | Undipped | 55°C LA | 4°C W | Undipped |
| 12 | Undipped | Undipped | Undipped | 4°C W | 55°C LA |
| 13 | Undipped | Undipped | 55°C LA | 4°C W | 55°C LA |
| 14 | Undipped | 82°C W | Undipped | 4°C W | 55°C LA |

*Spray-chilling involving storage at -3°C for 10 h, with intermittent (every 30 min) spraying using water cooled to 4°C, followed by storage for 38 h at 1°C

LA: 5% lactic acid

SM: 4% sodium metasilicate

W: water

## INTRODUCTION

In the conversion of beef cattle into carcasses and raw beef products, underlying and essentially sterile carcass surface tissue may become contaminated with bacterial flora from the environment; such contamination may include pathogenic bacteria, posing a public health risk. Carcasses can become contaminated from various sources, including via fecal material derived from either the gastrointestinal tract or the hide, or through cross-contamination introduced from worker's clothes, hands or personal equipment, from other carcasses, or from plant equipment *(15)*. Contamination of raw beef products entering the food supply may have adverse economic implications for the industry and, more importantly, result in illness and even death among consumers *(18)*. Thus, reduction of pathogen prevalence on surfaces of beef carcasses has been a constant challenge to the meat processing industry. In order to improve the microbiological status of fresh beef, the United States Department of Agriculture Food Safety and Inspection Services (USDA-FSIS) established the Pathogen Reduction and Hazard Analysis Critical Control Point (HACCP) Systems Final Rule *(36)*. As part of efforts to comply with the new regulations, beef packers worked diligently to implement new decontamination technologies that would reduce risks associated with pathogen contamination. Today, approved technologies are applied sequentially to carcasses as they are processed, a system that has been coined the "multiple hurdles" approach. With respect to beef carcass decontamination, the principle of hurdle technology *(22)* implies that if the initial microbial load is significantly reduced by one or more decontamination procedures, the surviving microorganisms should be better inhibited in subsequent processing steps. This principle has been demonstrated experimentally *(3, 17)* and in applied plant settings *(1)*.

Interventions previously shown to result in reduction of contamination on beef have included: (i) spraying with hot or cold water *(2, 3, 26, 29, 34)*; (ii) steam pasteurization *(25, 28)*; (iii) hot water/steam vacuuming *(16, 28)*; and (iv) sanitizing with solutions such as organic acids *(5, 6, 9, 10, 13, 14, 19, 30)*, acidified sodium chlorite *(4, 13, 30)*, peroxyacetic acid *(13, 30)*, and cetylpyridinium chloride *(7, 30)*. Acidic and basic electrolyzed water are GRAS compounds for beef and have been reported as effective against pathogenic bacteria in cell suspensions *(12, 20, 37)*, on various food contact surfaces *(26, 31, 38)*, on seeds and produce *(8, 21, 33, 35)*, and on poultry *(12, 27)*. Although there is no published scientific research suggesting that sodium metasilicate might be an effective decontamination compound, it is approved for use on raw beef carcasses, subprimals and trimmings at a maximum concentration of 4% (FSIS Directive 7120.1, Amendment 1).

This study was conducted to: (i) evaluate the effectiveness of applying (either singly or in combination) acidic and basic electrolyzed oxidative water, lactic acid, ammonium hydroxide, sodium metasilicate, or water in decontaminating beef tissue inoculated with *E. coli* O157:H7 and *S.* Typhimurium; and (ii) evaluate efficacy of simulated multiple-hurdle systems that

incorporate sodium metasilicate, lactic acid, or water as treatments applied to beef tissue inoculated with *E. coli* O157:H7 and *S.* Typhimurium.

## MATERIALS AND METHODS

### Preparation and inoculation of beef tissue

Fresh boneless beef plates were obtained from a local commercial beef processing plant, stored at 4°C, and used within 72 h postmortem. Beef samples were portioned into 5×2.5×1 cm (total surface area of 40 $cm^2$) pieces. The inoculum used in this study was a composite of either *E. coli* O157:H7 strains ATCC43895, ATCC43894, ATCC43890 and ATCC43889, or *S.* Typhimurium DT104 strains ATCC 700408, ATCC14028, *S.* Typhimurium DT104 var. Copenhagen (isolated from beef animal hides) and UK1 (isolated from horse wound). These strains were available as frozen cultures (-70°C; tryptic soy broth [TSB; Difco, Becton Dickinson Co., Sparks, MD] with 25% glycerol) and were activated by streaking onto tryptic soy agar (TSA) (Difco) and incubating at 35°C for 48 h. After incubation, a single colony of each strain was picked and subsequently subcultured (35°C, 24 h) by inoculating 10 ml of TSB (containing 0.25% glucose) with 100 µl of the activated stock cultures. The overnight cultures then were mixed to form a 40-ml composite culture, which was centrifuged at 4,628 × g (Eppendorf, model 5810 R; Brinkmann Instruments, Inc., Westbury, NY) for 15 min at 4°C. The resulting pellets were washed in sterile phosphate-buffered saline (PBS, pH 7.4, Sigma Chemical Company, St. Louis, MO), centrifuged a second time, and resuspended in 20 or 40 ml PBS for *E. coli* O157:H7 and *Salmonella*, respectively, for further use in the experiment. Different volumes for re-suspension were used based on preliminary observations that indicated higher yields of *Salmonella* than of *E. coli* O157:H7 cells after overnight incubation. Individual meat samples were inoculated (*ca.* $10^7$ CFU/ml) with 0.2 ml of each composite culture, which was subsequently spread over all surfaces of the sample. Samples were kept at 25°C for 30 min to allow for bacterial attachment.

### Preparation and application of decontamination solutions

Lactic acid (2.5 and 5%, LA, pH 2.12 and 2.04, respectively) solutions were prepared with 88% lactic acid (Purac® FCC 88; Purac America, Lincolnshire, IL) and completed to a final volume of 30 l with distilled water. Ammonium hydroxide (0.1%, AH, pH 10.89) solutions were prepared using a 28–30% ammonium hydroxide solution (Mallinckrodt AR® [ACS]; Mallinckrodt and Baker, Inc., Paris, KY) and completed to 30 l with sterile distilled water. Sodium metasilicate (1 and 4% wt/wt, SM, pH 12.27 and 12.35, respectively) solutions were prepared with anhydrous sodium metasilicate (Avgard® XP, Rhodia Inc.; Rhodia Food Ingredients, Cranbury, NJ). Acidified oxidized water (AOW) was generated using an electrolyzed oxidized (EO) water generator (Primacide® P-5000) instrument (Electric Aquagenics Unlimited, Inc., Lindon, UT) that yielded 0.005% AOW (pH 2.67/oxidation-reduction potential [ORP] 1107 mV) and, as a byproduct, basic oxidized water (BOW, pH 11.21/ORP -805 mV). For water (W) treatments, distilled water (pH 7.01) was used. Properties (pH and ORP readings) of the treatment solutions were measured immediately after preparation by use of a dual mode digital pH meter (*Ultra*Basic, UB-10; Denver Instrument, Denver, CO) with a glass pH/ORP electrode (pH/ATC Electrode #300729.1; Denver Instrument). Residual chlorine concentrations in AOW were determined with a chlorine test kit (Hach Co., Ames, IA).

### Experimental design

In phase I, single treatments (five samples in each of two replicates for each pathogen) included: (1) untreated control (UT); (2) W (23°C); (3) 2.5% LA (pH 2.12, 55°C); (4) 0.1% AH (pH 10.89, 23°C); (5) 0.005% AOW (pH 2.67, 23°C); (6) BOW (pH 11.21, 23°C); (7) 4% SM (pH 12.35, 82°C). Sequential treatments (combinations of two) were applied by combining each of the above-mentioned single treatments with each of the remaining treatments. Effects of temperature (4, 23, 55 or 82°C) and concentration (1 or 4% wt/wt) of SM also were evaluated on populations of *E. coli* O157:H7 attached to beef tissue. The following treatments (five observations per treatment) were applied to evaluate effects of temperature on pathogen reductions: (1) untreated control; (2) SM (4%) at 4°C; (3) SM (4%) at 23°C; (4) SM (4%) at 55°C and, (5) SM (4%) at 82°C. In addition, the following treatments (with n = 5 per treatment) were applied to determine effects of concentration-temperature gradients for sodium metasilicate on pathogen reductions: (1) untreated control; (2) SM (1%) at 82°C; (3) SM (4%) at 82°C; (4) SM (1%) at 55°C; and (5) SM (4%) at 55°C.

A second phase (phase II) was designed to simulate commercial multiple-hurdles systems that could be applied in the beef packing industry to decontaminate carcasses. The sequence of applications was chosen to simulate a packing-house scenario in which samples were dipped (30 s) in sodium metasilicate (1% at 82°C) first (representing a pre-evisceration application), then in hot (82°C) water after 20 min (representing a post-evisceration application), and then in 5% lactic acid (55°C) after 10 min, but before samples were subjected to simulated spray-chilling (at -3°C for 10 h, with intermittent [every 30 min] spraying with water cooled to 4°C, followed by storage for 38 h at 1°C) for a total of 48 h (representing a pre-chill application) and, lastly, application of 5% lactic acid (55°C) (representing a post-chill application). Treatment of beef tissue during the chilling process was achieved by applying overhead (20 cm from tissue surface) spray (simulated misting) on samples suspended on a wire mesh, using a 1-l Envirokind® All-purpose Sprayer (Delta Industries, N. Hollywood, CA). Additional treatment systems studied consisted of up to four sequential dipping steps combining 1% SM (82°C), 5% LA (55°C), warm (55°C) or hot (82°C) water (Table 1). Inoculated samples (five observations for each of two replicates for each organism) were left undipped or were dipped (30 s in 1.5 l) in solutions according to the treatment systems outlined in Table 1. After exposure, samples were placed into an 18-oz Whirl-Pak® filter sterile plastic bag (Nasco, Fort Atkinson, Wisconsin) containing 40 ml sterilized maximal recovery diluent (MRD; 1.0 g Bacto™ Peptone [Difco] and 8.5 g sodium chloride [Fisher Scientific, Houston, TX] in 1 l distilled water) and homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min for microbiological analysis.

### Microbiological analysis

For microbiological analysis, 1 ml of the homogenized sample was serially diluted in 9 ml of sterile 0.1% buffered peptone water (BPW; Difco, pH 7.2) and appropriate dilutions were plated by use of a Spiral Plater™ (Spiral System®, Spiral Systems, Inc., Cincinnati, OH) onto TSA (Difco) for enumeration of *E. coli* O157:H7 and *Salmonella* populations. Colonies formed on plates were automatically counted (CASBA™ 4, Spiral Biotech, Inc., Norwood, MA) after incubation at 35°C for 48 h. The detection limit of the microbiological analysis was 1.26 log CFU/$cm^2$ for cells attached to the beef tissue. The pH value of each homogenized sample was measured after microbiological analysis, using a digital pH meter (*Ultra*Basic, UB-10; Denver Instrument) with a glass

**TABLE 2. Efficacy of decontamination treatments classed according to reduction categories (of 0.5 log CFU/cm²) against *Escherichia coli* O157:H7 and *Salmonella* (TSA) on beef carcass tissue after 30 s exposure**

| Reduction categories (log CFU/cm²) | *Escherichia coli* O157:H7 Treatments | *Salmonella* Treatments |
|---|---|---|
| 0.0–0.5 | Acidified oxidized water (23°C; AOW) | LA / BOW |
| | 0.1% Ammonium hydroxide (23°C; AH) | W |
| | Water (23°C; W) | AOW |
| | Basic oxidized water (23°C; BOW) | AOW / LA |
| | AOW / BOW | BOW |
| | AH / W | AH |
| | BOW / AH | AH / BOW |
| | AOW / AH | AOW / AH |
| | AOW / W | W / AH |
| | BOW / W | W / AOW |
| | W / BOW | AH / AOW |
| | AH / BOW | AOW / W |
| | AOW / W | AH / W |
| | W / AH | BOW / AH |
| | W / AOW | AOW / BOW |
| | BOW / AOW | W / BOW |
| | | BOW / AOW |
| | | BOW / W |
| 0.6–1.0 | 2.5% Lactic acid (55°C; LA) | LA |
| | LA / AH | AH / LA |
| | LA / AOW | LA / AH |
| | LA / W | LA / W |
| | AH / LA | LA / AOW |
| | LA / BOW | W / LA |
| | AH / AOW | |
| 1.1–1.5 | 4% Sodium metasilicate (82°C; SM) / AOW | BOW / LA |
| | W / LA | AOW / SM |
| | BOW / LA | BOW / SM |
| | AOW / LA | SM |
| | SM / LA | SM / BOW |
| | AH / SM | SM / W |
| | SM / BOW | SM / W |
| | SM / W | SM / AH |
| | SM / AH | AH / SM |
| | | SM / AOW |
| | | SM / LA |
| 1.6–2.0 | SM | W / SM |
| | W / SM | LA / SM |
| | AOW / SM | |
| | LA / SM | |
| 2.1–2.5 | BOW / SM | |

**TABLE 3. Least squares means (± standard deviations) indicating survival and reduction of *Escherichia coli* O157:H7 on beef carcass tissue after exposure to sodium metasilicate (SM) at different concentration-temperature combination**

| Temperature (°C) | Concentration (%) | Survival (log CFU/cm²) |
|---|---|---|
| Nontreated control | | 6.0a (0.4) |
| 4 | 4 | 3.9b (0.2) |
| 23 | 4 | 4.1b (0.3) |
| 55 | 1 | 4.2b (0.2) |
| | 4 | 3.9b (0.4) |
| 82 | 1 | 3.6b (0.4) |
| | 4 | 3.9b (0.3) |

abc – Means within the same column with different letters are different ($P < 0.05$)

pH electrode (pH/ATC Electrode #300729.1; Denver Instrument).

### Statistical analysis

Populations of bacteria were expressed as mean log CFU/cm² with associated standard deviations. Values for the mean log and standard deviation of each set of bacterial counts were calculated on the assumption of a log-normal distribution of microorganisms. In phase I, two replicate experiments were conducted with 10 samples per treatment for each organism. Preliminary analysis of fixed effects using the GLM procedure of SAS® v.8.2 *(32)* indicated that log CFU/cm² populations were dependent on pathogen and/or treatment. In phase II, two replicate experiments were conducted with 10 samples per treatment for each pathogen. Preliminary analysis of fixed effects, using the GLM procedure of SAS® v.8.2 *(32)*, indicated that log CFU/cm² populations were dependent on pathogen and treatment. The viable population data were separated by pathogen and evaluated by use of nonparametric one-way ANOVA procedures of SAS® v.8.2 *(32)* to test treatment differences between least squares means. All differences were reported at a significance level of alpha = 0.05.

## RESULTS AND DISCUSSION

### Phase I

Reductions in *E. coli* O157:H7 and *S.* Typhimurium populations on beef tissue exposed to decontamination treatments were similar (Table 2). Overall, individual applications of W, AOW, BOW, or AH for 30 s at ambient temperature (23°C), along with sequential application of each combination of two solutions, resulted in ≤ 0.6 log CFU/cm² pathogen reductions. Generally, treatments that combined W, AOW, BOW or AH with LA or SM resulted in greater ($P < 0.05$) pathogen reductions on beef tissue than single applications or combinations of W, AOW, BOW or AH treatments. Sequential treatments combining LA or SM with other chemicals had no additional effects beyond those of the individual treatments, but treatments that used SM as the second intervention tended to result in higher reductions, indicating that a residual bactericidal effect may have ensued (Table 2). In contrast, it appeared that use of SM alone resulted in higher pathogen reductions than those combination treatments in which SM was applied first (especially SM/LA, SM/AOW and SM/AH). This suggested a potential neutralization or "quenching" effect on SM (more specifically, the basic pH) when it was followed by a subsequent intervention, in particular, one with a lower pH. It should be noted that effectiveness of the LA, and especially the SM, treatment may have been a function of temperature of application (55 and 82°C, respectively).

Several previous studies *(8, 11, 12, 20, 21, 26, 27, 31, 33, 35, 37, 38)* have documented the effectiveness of electrolyzed oxidizing water against *E. coli* O157:H7 and *Salmonella* in or on various liquid and solid surfaces. However, in this study, AOW and BOW treatments were not effective at reducing pathogen populations on beef tissue. Application time may influence efficacy of electrolyzed oxidizing water treatments in reducing bacterial populations; Venkitanarayanan et al. *(37, 38)* used long application times (> 5 minutes) compared to the 30 s used—to simulate realistic plant application times—in the present study. Additionally, organic materials associated with beef tissue may inactivate chlorine or other oxidizing compounds during decontamination, thus reducing efficacy of such treatments on beef carcasses. In the beef industry, the length of time needed to apply a treatment is important and a 5-min application time is too long at current line speeds; additionally, the presence of fecal contamination on beef carcasses may increase the organic load and result in greater inactivation of an electrolyzed oxidizing water treatment, thereby rendering it ineffective as a decontaminant.

Treatments involving high pH (e.g., ammonium hydroxide, trisodium phosphate, or sodium metasilicate) are thought to be more effective against Gram negative than Gram positive cells, as these tratments readily solubilize the outer membrane of Gram negative cell walls, resulting in damage to the wall and consequential disruptions in the cytoplasmic membrane *(23)*. In this study, treatment of beef tissue with AH did not result in reduced ($P \geq 0.05$) pathogen populations. Ammonia has been used to treat beef trimmings (for use in production of lean fine-textured beef) to reduce microbial populations *(24)*. For ammonia treatments to have an immediate bactericidal (6 log reduction) effect on pathogens in the latter application, meat pH must be > 9.0 *(24)*. In our study, the ammonium hydroxide solution had a pH of 10.89, which may be expected to cause destruction or at least injury of cells; however, considering that the beef tissue used contained a high proportion of adipose tissue (approximately pH 5.7), AH was able to raise the pH of the tissue only to 7.6, which was clearly not high enough to affect pathogen loads.

Sodium metasilicate (pH 12.3) had a greater effect than did other treatments on pathogen populations in this study (Table 2). Different application temperatures (4, 23, 55 or 82°C) of 4% SM did not cause any difference ($P \geq 0.05$) in pathogen population reduction (Table 3). Thus, it appeared that application temperature did not influence antimicrobial properties of SM; however, it is possible that antimicrobial activity was limited at the application concentration of 4% and was not enhanced by temperature. When SM was evaluated with different concentration and temperature combinations (1% at 55°C, 1% at 82°C, 4% at 55°C and 4% at 82°C), SM concentration influenced plate

**TABLE 4.** Least squares means (± standard deviations) and pooled (mean values for samples from both pathogens) pH of samples indicating survival and reduction of *Escherichia coli* O157:H7 and *Salmonella* on beef tissue not dipped (control) or after dipping (for 30 s in 1.5 L) in various treatment systems*

| | Treatment Systems* Indicating Solution and Stage of Application | | | | | |
|---|---|---|---|---|---|---|
| Organism | Application #1 | Application #2 | Application #3 | Application #4 | Survival (log CFU/cm$^2$) | Pooled Sample pH |
| *E. coli* O157:H7 | Undipped | Undipped | Undipped | Undipped | 5.7a (0.2) | 5.54 (0.09) |
| | Undipped | 82°C W | Undipped | Undipped | 4.0b (0.4) | 5.62 (0.12) |
| | 82°C W | 82°C W | 55°C W | 55°C W | 3.9b (0.3) | 6.02 (0.23) |
| | 82°C SM | 82°C W | 55°C LA | 55°C LA | 3.8b (0.7) | 5.69 (0.12) |
| | Undipped | 82°C W | 55°C LA | 55°C LA | 3.7b (0.3) | 4.46 (0.15) |
| | 82°C SM | Undipped | 55°C LA | 55°C LA | 3.6b (0.3) | 3.80 (0.20) |
| | 82°C SM | 82°C W | Undipped | 55°C LA | 2.8c (0.3) | 4.08 (0.10) |
| | 82°C SM | 82°C W | 55°C LA | Undipped | 2.7c (0.2) | 4.21 (0.43) |
| | 82°C W | 82°C W | 55°C LA | 55°C LA | 2.7c (0.4) | 3.90 (0.20) |
| | 82°C SM | 82°C W | 55°C W | 55°C W | 2.6c (0.4) | 3.70 (0.40) |
| | Undipped | Undipped | 55°C LA | Undipped | 2.6c (0.2) | 4.00 (0.10) |
| | Undipped | Undipped | Undipped | 55°C LA | 2.5c (0.4) | 4.10 (0.09) |
| | Undipped | Undipped | 55°C LA | 55°C LA | 2.5c (0.5) | 4.24 (0.07) |
| | Undipped | 82°C W | Undipped | 55°C LA | 2.4c (0.6) | 3.90 (0.10) |
| *Salmonella* | Undipped | Undipped | Undipped | Undipped | 5.6a (0.3) | 5.54 (0.09) |
| | Undipped | 82°C W | Undipped | Undipped | 3.7b (0.5) | 5.62 (0.12) |
| | 82°C W | 82°C W | 55°C W | 55°C W | 3.7b (0.3) | 6.02 (0.23) |
| | 82°C SM | 82°C W | 55°C LA | 55°C LA | 3.6b (0.3) | 5.69 (0.12) |
| | Undipped | 82°C W | 55°C LA | 55°C LA | 3.5b (0.4) | 4.46 (0.15) |
| | 82°C SM | Undipped | 55°C LA | 55°C LA | 3.3b (0.4) | 3.80 (0.20) |
| | 82°C SM | 82°C W | Undipped | 55°C LA | 3.2bc (0.2) | 4.08 (0.10) |
| | 82°C SM | 82°C W | 55°C LA | Undipped | 2.9c (0.2) | 4.21 (0.43) |
| | 82°C W | 82°C W | 55°C LA | 55°C LA | 2.9c (0.5) | 3.90 (0.20) |
| | 82°C SM | 82°C W | 55°C W | 55°C W | 2.8cd (0.5) | 3.70 (0.40) |
| | Undipped | Undipped | 55°C LA | Undipped | 2.8cd (0.4) | 4.00 (0.10) |
| | Undipped | Undipped | Undipped | 55°C LA | 2.8cd (0.4) | 4.10 (0.09) |
| | Undipped | Undipped | 55°C LA | 55°C LA | 2.5d (0.4) | 4.24 (0.07) |
| | Undipped | 82°C W | Undipped | 55°C LA | 2.3d (0.4) | 3.90 (0.10) |

*Treatment systems indicating sequence of four possible dipping applications that simulate decontamination of beef at a pre-evisceration stage (Application #1), followed 20 min later by a post-evisceration stage (Application #2), followed 10 min later by a pre-chill organic acid rinse stage (Application #3), and lastly 48 h later* by a post-chill organic acid rinse stage (Application #4)

abcd – Means within the same column for each pathogen with different letters are different ($P < 0.05$)

LA: 5% lactic acid

SM: 1% sodium metasilicate

W: water

counts less at the higher application temperature (Table 3). Thus, efficacy of SM applied at 1% may be more dependent upon application temperature than efficacy of SM applied at 4%. During the experiment, it was noted that treatment of beef tissue with SM resulted in adverse visual appearance, including grayish color and wrinkling of the adipose tissue.

### Phase II

Adverse visual effects of sodium metasilicate on beef tissue (noted in Phase I) were reversed when simulated spray-chilling was included in the processs. *Escherichia coli* O157:H7 and *Salmonella* had similar survival trends in response to application of interventions in a multiple-hurdles systems. In general, it appeared that the multiple-hurdles systems were separated into two groups based on efficacy in decontaminating beef tissue. The first group included treatment systems using one or more thermal (82°C) application with no LA, or a single 5% LA application, which resulted in pathogen reductions of 1.7 to 2.1 log CFU/cm² (Table 4). The second, and more effective, group included treatment systems combining two 5% LA applications, or a thermal (82°C) and 5% LA application; these resulted in pathogen reductions of 2.3 to 3.3 log CFU/cm² (Table 4). Use of a single hot (82°C) water treatment did not result in different levels of pathogen reduction ($P \geq 0.05$) than use of two hot treatments and two warm (55°C) water treatments (Table 4). This implies that the amount of pathogen reduction due to application of hot (82°C) solutions is limited and cannot be further enhanced by additional thermal (hot [82°C] or warm [55°C]) treatments; it is possible that a resistant pathogenic sub-population exists which, after selection by certain interventions, may be tolerant to subsequent interventions.

Pathogen load reductions caused by treatment systems comprised of one or more thermal treatments with no 5% LA application were similar ($P \geq 0.05$) to the reduction resulting from a single application of 5% LA. Thus, hot (82°C) water and 5% LA may have similar ($P \geq 0.05$) decontaminating effects on beef tissue, but it appeared that reductions due to treatment with 5% LA were further enhanced by subsequent 5% LA applications. This finding may have resulted from the time lapse between the pre-chill application of 5% LA and the post-chill application of the acid, as the 48-h spray-chilling process may have produced a stressful environment for the mesophilic pathogen and therefore may have sensitized cells to the subsequent acid treatment. Under the conditions of this study, hot (82°C) water treatment followed by the use of 5% LA, or the exclusive use of two 5% LA treatments, appeared to enhance pathogen reduction and should be considered when selecting interventions for incorporation in a multiple-hurdles system.

The use of multiple-hurdles systems increases the chances of beef carcasses meeting established regulatory requirements for *E. coli* O157:H7 and *Salmonella*. The intervention systems evaluated in the present study could easily be incorporated into a multiple-hurdles system in most US beef packing plants; furthermore, the alternative approaches shown in this study to result in similar levels of pathogen reduction may provide options from which industry may select systems that best fit individual plant scenarios.

Individual application to beef tissue of W, AOW, BOW or AH, or sequential combinations of treatments with these compounds, did not dramatically reduce pathogen contamination from inoculated levels, but combined applications of 2.5% LA at 55°C, and especially 4% SM at 82°C, resulted in reasonable pathogen reductions due to the additive effects of the chemical and temperature of application. Under the conditions of this study, it appeared that the temperature of application (4, 23, 55, or 82°C) did not affect the antimicrobial activity of 4% SM, but 1% SM appeared to be more effective when applied at 82°C than when applied at 55°C. Although electrolyzed oxidative water may be an effective pathogen decontaminant when applied to food contact surfaces, produce, or poultry (applied as a submersion bath), organic matter associated with beef tissue, combined with the difficulty of applying the compound for extended periods of time, may result in sufficient inactivation of chlorine (or other oxidizing agents) to render it ineffective when applied to beef carcasses. Results indicated that there was a limit to the pathogen reduction resulting from a single hot (82°C) water treatment, so that additional hot or warm (55°C) water treatments did not result in additional reductions. This suggested the existence of a resistant pathogenic sub-population that may be tolerant to certain interventions and, as such, might be selected to dominate product surfaces and resist subsequent similar interventions. Conversely, it was apparent that reductions due to treatment with 5% LA may be further enhanced by a subsequent 5% LA treatment, possibly due to the effects of cold stress placed on the pathogen between acid applications.

Under the conditions of this study, pathogen reductions were greatest with use of multiple-hurdle systems that combined two 5% LA applications or a hot (82°C) water/SM application plus a 5% LA application, followed by those systems with one or more hot (82°C) water applications or a single 5% LA application. Use of one hot (82°C) water application followed by one 5% LA application was sufficient to achieve the maximum reductions encountered with use of any of the compounds tested in this study. Currently, the most commonly applied decontamination interventions in the industry include an application of hot water followed by lactic acid spraying, and results of this study indicate that additional applications of hot water or lactic acid do not enhance the effectiveness of such programs. For beef processing plants with limited opportunity to incorporate several decontamination interventions, there are options for applying one or two interventions that may result in comparable pathogen reductions.

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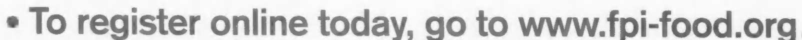

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# Guidelines for *Listeria* Testing of Environmental, Raw Product and Finished Product Samples in Smoked Seafood Processing Facilities

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## SUMMARY

The Smoked Seafood Working Group (SSWG), a collaboration of two US industry trade organizations, smoked seafood processors and academia, developed guidelines for controlling *Listeria monocytogenes* in smoked seafood operations. To minimize the potential for *L. monocytogenes* contamination of finished products, it is necessary to have sanitation procedures that prevent contamination of product contact surfaces and eliminate niches where *L. monocytogenes* can become established, grow, and persist. Environmental testing can be used to help identify problem areas or locate contamination sources in the plant, and to confirm that problem-solving procedures have been effective. Raw seafood and finished product testing can be used to evaluate raw product suppliers and verify the effectiveness of control procedures. Regular testing can also help to track performance over time and identify new sources or reservoirs of contamination in the processing plant environment. This paper describes considerations for developing effective environmental and product testing programs for *L. monocytogenes* and provides four examples to illustrate how testing programs could be structured for various types of smoked seafood processors.

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## INTRODUCTION

*L. monocytogenes* contamination has been found in both hot- and cold-smoked seafood *(2, 6, 8)*. Efforts to control *Listeria monocytogenes* in the food processing plant environment can reduce both the frequency and level of contamination in smoked fish products, but it is not possible, given current technology, to completely eliminate the organism from the processing plant environment or totally eliminate the potential for contamination of finished products *(4, 20)*. Although the process of producing cold smoked products does not include a heating step that will eliminate *L. monocytogenes* that may be present on the raw material, *L. monocytogenes* contamination during processing appears to be a major source of finished product contamination *(5, 14)*. The potential for contamination of cold-smoked product after the smoking process must thus be evaluated and minimized. Although hot smoked seafood products do reach a high enough temperature for sufficient time (145°F (62.8°C) for 30 minutes) to kill *L. monocytogenes*

*(10)*, these products can also be contaminated at processing steps that occur after the product is smoked *(16)*. To minimize the potential for *L. monocytogenes* contamination of finished products, it is necessary to have sanitation controls that prevent contamination of product contact surfaces and eliminate niches where *L. monocytogenes* can establish itself, grow, and persist *(5, 22)*. Environmental testing can be used to help identify problem areas or locate contamination sources in the plant, and to confirm that problem solving procedures have been effective *(20, 21)*. An ongoing testing or monitoring program can be used initially to help determine what control measures are most effective and where changes or modifications in plant procedures are needed. When these measures have been implemented, regular testing can then help to track performance over time and identify new sources or reservoirs of contamination in the processing plant environment *(3, 19, 20)*.

Since 2001, representatives of the National Fisheries Institute and the National Food Processors Association, individuals from at least 10 smoked seafood firms, and food safety or seafood specialists from Cornell University, Virginia Tech and the Sea Grant programs in New York and Delaware have been working together as the Smoked Seafood Working Group (SSWG) to develop guidelines for the control of *Listeria monocytogenes* in smoked seafood manufacturing plants. This initiative was also conducted as part of a Cornell University project to develop control strategies for *Listeria monocytogenes* in food processing environments, funded under the National Food Safety Initiative in 2000 by the Cooperative State Research, Education and Extension Service of USDA, Project Number 00-51110-9768.

The following information is designed to offer smoked fish processing plants a variety of options for developing an effective environmental and product monitoring program. Examples are provided to illustrate different types of environmental testing programs for smoked fish processing operations. An effective monitoring program should include decisions as to:

- what areas of the plant are to be tested,
- the frequency of testing,
- what testing procedures will be used,
- how test results will be evaluated, and
- what actions will be taken when test results are positive.

A brief description of these elements, illustrated by four different examples of how these components could be integrated into a complete plan, follows.

## ESTABLISHING A SAMPLING/MONITORING PROGRAM

### Deciding on the test organism

One of the first steps in establishing a sampling/monitoring program is to determine whether to test for *Listeria* species (*Listeria* spp., or generic *Listeria*) or for *L. monocytogenes*. Within the genus *Listeria*, only *L. monocytogenes* is considered a foodborne pathogen *(13)*. The term "*Listeria* spp." includes *L. monocytogenes* along with non-pathogenic species. Generally, non-pathogenic species of *Listeria*, in particular *L. innocua*, are found more frequently than *L. monocytogenes* in many processing plants, *(7, 15, 16, 19)* but this can be plant specific. In some circumstances and in some types of samples only a small fraction (< 5 to 10%) of samples positive for *Listeria* spp. are actually also positive for *L. monocytogenes* *(14, 15, 19)*. On the other hand, in some situations the majority (> 70–80%) of the samples positive for *Listeria* species may also be positive for *L. monocytogenes* *(14, 15, 19)*.

Although newer, rapid systems are becoming available, differentiation of *L. monocytogenes* from other *Listeria* species and specific detection of *L. monocytogenes* through the use of traditional cultural methods is generally time consuming, often requiring at least 7 days. In contrast, testing for *Listeria* spp., which would include *L. monocytogenes*, is less expensive and generally requires only 2 to 3 days. Most environmental testing programs in the US food industry use tests for *Listeria* spp. as an indicator for the potential presence of *L. monocytogenes*. However, some companies elect to test for *L. monocytogenes*, as this is the organism of concern. A *Listeria* spp.-positive sample should be interpreted as an indicator of *potential*, not presumptive, *L. monocytogenes* contamination. Depending on the location of the positive, it may be appropriate to determine whether a sample that is positive for *Listeria* spp. contains *L. monocytogenes* or to treat it as if it were *L. monocytogenes*. For finished product testing, it is generally more appropriate to test specifically for *L. monocytogenes* rather than only for *Listeria* spp., unless product in which *Listeria* spp. is found is treated as if *L. monocytogenes* had been found.

### Deciding what to monitor or test

A monitoring/testing program for smoked seafood may involve selecting and testing several different kinds of samples, including:

- Raw products,
- Non-food contact surfaces in the processing plant environment,
- Food contact surfaces, and
- Finished ready-to-eat products.

Some monitoring programs may not test all types of samples. A processor must always remember that the goal of testing is to find the organism if it is present, not to obtain "negative" test results.

### Raw seafood testing

Research has shown that *L. monocytogenes* can be isolated from many of the types of raw fish commonly used for smoking *(1, 4, 9, 14, 17, 18, 19)*. These raw products can be one source of *L. monocytogenes* contamination that is constantly being introduced into a plant. Many smoked seafood facilities receive raw products that have undergone some processing (heading and gutting, filleting). Contamination levels can be higher if the raw product is not handled properly during harvesting and primary processing. Testing for *Listeria* spp. or *L. monocytogenes* in raw seafood can help processors understand contamination sources associated with different species or types of raw products and monitor the performance of suppliers. Raw seafood testing is predominantly used in the production of cold smoked fish, as there is no processing step lethal to *L. monocytogenes*. Raw seafood found to be positive for *L. monocytogenes* should not be used to produce cold-smoked products unless the raw products can be treated to reduce the risk (e.g., chemical washing steps, freezing product, addition of growth inhibitors or combination treatments *(12)*).

### Non-food contact surface testing

Research has shown that *L. monocytogenes* can frequently be isolated from various areas in the smoked fish processing plant environment and can persist in niches in certain areas of the plant *(1, 9, 16, 18, 19, 22)*. These areas can include

floors, floor mats, walls, drains, tubs or totes, conveyances used to move product from one area of the plant to another, racks, cooler coils and condensate collectors, seams and crevices in processing machinery, and sponges, mops and other cleaning utensils. Each plant should determine appropriate environmental sites to sample, as well as appropriate sampling frequencies based on the potential for finished product contamination and based on knowledge of the specific operation and controls that are in place, along with any microbiological data available. Sampling locations can include the areas noted above, equipment support structures, structures over areas where product will be exposed, and, in particular, the wheels and vertical supports on racks. Sufficient samples should be taken to be representative of the plant environment. Testing non-food contact environmental surfaces can help processors understand contamination patterns, identify *L. monocytogenes* niches, and evaluate the effectiveness of sanitation procedures.

When conducting environmental testing of non-food contact surfaces, weekly sampling is recommended initially for most wet areas, where *L. monocytogenes* can grow; in dry-cleaned areas, sampling may be less frequent. The number of sampling locations and the frequency of sampling may be adjusted based on sampling results obtained over time. For example, repeated negative findings may suggest reducing the frequency of sampling in a particular area or elimination of a sampling site. When potential *L. monocytogenes* contamination problems are identified, the number of samples and sampling frequency may need to be increased to pinpoint contamination sources and then to demonstrate that the control measures used to eliminate *L. monocytogenes* were effective.

Non-food contact surface samples may be taken at different times during production: pre-operational (pre-op), during operation and at the end of the production shift, prior to cleanup. Companies need to consider what information can be obtained from each type of test when setting up the sampling program. Pre-op sampling reflects the efficacy of cleaning and sanitation, but it usually provides little information on sites that potentially harbor *L. monocytogenes*. Generally, sampling several hours into production allows time for *L. monocytogenes* to work its way out of any harborage sites in which it may be present and contaminate the environment, the processing line, and, potentially, product *(20)*. Thus, sampling during production or at the end of the shift prior to cleaning and sanitation can provide the best indication of the presence of *L. monocytogenes* in the processing environment and help processors identify new or persistent niches in equipment or the plant environment. Sampling drains is another means of determining the presence of *L. monocytogenes* in the processing environment, since drains can serve both as harborage sites and as a collection point for microorganisms in the plant when they get flushed to the drain during cleanup.

Data from non-food contact surface monitoring should be tracked over time to identify the need to take action and to identify trends that may not be obvious from a single day's monitoring *(3, 20)*. Detection of *Listeria* spp. in an environmental monitoring sample does not necessarily indicate a microbiological control problem; however, it does indicate that additional investigation should be undertaken *(21)*. Plants should determine the action to be taken in the event that *Listeria* spp. is detected at frequencies exceeding the upper control limit, target, or "trigger" that the plant has set (although attention should be given to cleaning and sanitizing an area when any positive is found). Because the reasons for a positive finding are likely to be plant-specific, actions taken in response to positives will vary. Consider the following points in determining remedial actions for non-food contact surface positives *(21)*:

- When results indicate a trend toward an increased incidence of *Listeria* spp. in the environment, or repetitive positives in a particular area, plants should investigate to determine the reason(s) for the increase and should take action to reduce the level again.
- Additional samples should be taken from the environmental area where the positive was detected. These samples may indicate that additional actions are needed in this area.
- If, after a remedial action has been applied, additional samples are positive, the environment should be intensively cleaned and re-tested.
- Plants should consider the need to sample food contact surfaces in the areas where environmental positives are detected.

Floor drains, floors, and floor mats represent almost constant problem areas *(19, 20, 21)*. A separate sampling program with specific goals for each of these areas may be appropriate. Actions taken in response to a positive in these areas, especially drains, may also be less stringent than for positives in other areas in the environment; for example, while positives in these areas may result in additional sanitation, these areas may not need to be re-sampled daily until a specified number of negative samples is achieved.

### Food contact surface testing

It is recommended that food contact surfaces be sampled routinely for *Listeria* spp. to verifiy that environmental and sanitation controls are preventing *L. monocytogenes* contamination of surfaces. Although some facilities choose to sample food contact surfaces only when monitoring of non-food contact surfaces suggests that there may be a problem, this approach is not recommended, since food contact surface contamination is not necessarily preceded by non-food contact surface contamination. In addition to routine testing, many processors conduct food contact surface sampling to verify the effectiveness of sanitation procedures used to solve specific problems or to eliminate persistent contamination sources identified by routine sampling of non-food contact surfaces.

Plants should determine the locations to sample, the time of day for sampling, and the frequency of sampling based upon knowledge of the specific operation and the controls in place, as well as any available microbiological data. When testing equipment, it is best to run the units for a period of time prior to swabbing/sponging, as the movement of parts and equipment vibrations may dislodge microorganisms from harborage sites. A pre-determined plan of action should be developed to address the finding of food contact surface positives. It is particularly important that plants investigate the reason(s) for all positives on food contact surfaces. Investigational sampling must be capable of identifying equipment that contains niches where *L. monocytogenes* has become established. Examples of steps that may need to be taken as a result of positives on food contact surfaces include modifying cleaning and sanitizing procedures, re-designing equipment, and re-training employees to improve adherence to Good Manufacturing Practices (GMPs) and other policies, practices and programs. Finding *Listeria* spp. on food contact surfaces may indicate the need for product testing for *L. monocytogenes*. Factors to be consid-

ered when making this decision would include whether there are other positive tests that suggest this is not a sporadic positive, the likelihood that the *Listeria* spp. would be *L. monocytogenes* (based on knowledge about the prevalence of *L. monocytogenes* and *Listeria* spp. in the specific facility), the likelihood of transfer from the food contact surface to product, and whether product storage, handling and use could increase the risk of illness if low levels of *L. monocytogenes* were present.

### Finished product testing

Manufacturers periodically test finished products to verify that sanitation and other *L. monocytogenes* control measures (both prerequisite programs and HACCP controls, if implemented) are effective. Some manufacturers may use finished product testing as part of their product release program ("hold and test," wherein product is held until test results are available). Many manufacturers conduct product testing at the request of their customers. Firms that have a solid environmental monitoring program (food contact surfaces and non-food contact surfaces) with appropriate remediation strategies may be able to convince customers that reducing the frequency of finished product tests would not compromise the safety of the product.

Because current US regulatory policies require that any lot of product in commerce that tests positive for *L. monocytogenes* be recalled from the market, it is imperative that each firm adequately define what constitutes a production lot when finished product testing is conducted. Further, the product lot sampled should be held until laboratory test results are available.

When product is sampled, representative samples should be collected from the lot. Sampling plans may be based on information from the International Commission on Microbiological Specifications for Foods (ICMSF) *(11)*. ICMSF categorizes microbial hazards according to risk – moderate, serious and severe – and it ranks *L. monocytogenes* as either a serious hazard in foods for the general population or a severe hazard in foods for restricted populations (high risk groups, e.g., those in hospitals and nursing homes). ICMSF describes 15 different "cases" of sampling plans, with sampling plan stringency based on degree of risk and the effect on risk of the conditions of use of the product. With respect to *L. monocytogenes* in refrigerated smoked seafood for the general population, case 12 could be applied (serious hazard that could increase since refrigerated storage would allow growth of *L. monocytogenes*); the sample size (n) would be 20, and c (number of units that could be positive) would be 0 *(11)*. If product is to be held frozen, case 11 (n = 10, c = 0) could be applied (the risk does not increase since the product is held frozen.). If more stringent sampling is desired (e.g., product for nursing homes), sample size could be increased to 30, or even 60, samples. To reduce testing costs it may be possible, based on data for meat (R. Huffman, American Meat Institute Foundation, personal communication), to composite up to five samples (up to 125 g) for testing as a single unit without sacrificing sensitivity. It is highly recommended that, to minimize the chance of contaminating the samples, intact samples be sent to the laboratory and that, if any compositing is done, the laboratory do it. Samples from different lots should not be composited, since this could delay identification of which lots are contaminated when a positive occurs.

## TESTING PROCEDURES

### Sampling guidelines for *Listeria* testing

For environmental sampling, sponge samples are generally preferred to swab samples, as sponges can cover larger areas. However, swabs are useful to sample cracks and crevices that can serve as harborage sites for *L. monocytogenes*. When taking swab or sponge samples, a scientifically acceptable method should be used. Consistent sampling techniques should be used to ensure that results can be compared over time. It may be necessary for smoked seafood processors to get additional guidance or training on proper sampling techniques from a testing laboratory or from other food safety professionals. Product contact surface and non-product contact surface samples should be taken from an area as large as practical. Unless a processor is attempting to enumerate *L. monocytogenes* in a specific location (an expensive procedure not generally needed), a consistent-sized area need not be sampled.

### Determining who will conduct the tests

Companies need to carefully assess whether the samples they collect will be tested at their own in-house facility or sent out to a contract laboratory. In most instances the latter will be preferable, as this will eliminate the risk of the laboratory serving as a source of *L. monocytogenes* contamination for the plant. Special precautions must be taken if a laboratory that is located in a plant conducts pathogen testing. The laboratory may need to be completely separated from the plant, and control protocols will need to be implemented to ensure that people, sampling equipment, etc. do not carry pathogens from the laboratory to the plant. Actual costs for *Listeria* spp. and *L. monocytogenes* tests will depend on variables such as the amount and frequency of testing, test methods used, the sample collection supplies provided, and shipping costs. Before implementing a testing program, it is prudent for any company to discuss its testing needs with several laboratories to evaluate and determine which laboratory has the best price, services, and logistical arrangements to meet the company's needs. However, primary consideration should be the laboratory's capability to conduct accurate testing for *Listeria* using good laboratory practices and to handle the company's volume of tests in a timely manner. Consideration may be given to the use of accredited laboratories (e.g., to ISO 17025), although this is not essential.

### Actions taken based on sampling results

Firms should clearly recognize that the purpose of sampling and testing for *Listeria* spp. is to gather information that can be used to identify and eliminate potential sources of *L. monocytogenes* contamination. The goal of this testing is to find the organism if it is present so that the potential for contamination of the finished product can be minimized. Each firm should expect to find positives and determine, prior to starting such a testing program, the type of response or action that will be taken when test results are positive. The type of response will be different depending on whether tests are positive for *Listeria* spp. or *L. monocytogenes* and depending on the potential implications for finished product contamination.

For example, a firm that routinely monitors for the presence of *Listeria* spp. on non-food contact surfaces should decide on an appropriate "trigger" for further actions based on the number of positive test results and their location. Positives from non-food contact surfaces may trigger additional environmental testing, testing of food contact surfaces, and, in

some cases, testing of product. Positive tests for *Listeria* spp. do not necessarily indicate that finished products may be contaminated, but it may indicate that specific sanitation control measures to eliminate *Listeria* are not effective or are not being conducted properly or that personnel are not observing appropriate practices to minimize *L. monocytogenes* contamination. As noted previously, further investigation and sampling should be conducted to identify the contamination source and eliminate it. If environmental testing is conducted for *L. monocytogenes* instead of *Listeria* spp., processors will need to evaluate the source of any positive sample and determine the likelihood that product contact surfaces or finished products may have been contaminated. In addition to actions to eliminate *Listeria* at the site, more intensive sampling of the area may need to be conducted, as well as testing of product contact surfaces and possibly finished product(s). The finding of *Listeria* on food contact surfaces, particularly when there are multiple positives on a line, or after actions have been taken as the result of a positive sample, should be more likely to trigger product testing than the finding of a positive on a non-food-contact surface.

### Problem solving

When an effective control program for *L. monocytogenes* is in place, finding multiple positives in the environment or product may indicate that the primary source of *L. monocytogenes* is a harborage site where the organism has become established and is multiplying. This can lead to line-specific contamination *(21)*, in which the contamination will often flow downstream along a processing or packaging line. Mapping of the contamination sites on a layout of the area can assist in locating the source of contamination or, at least, suggest additional sites to sample *(20)*. It is critical that the harborage site be found and eliminated. This usually means taking many samples of food contact surfaces along the line and in the adjacent environment. Line samples should be taken throughout the day (e.g., every 2 hours). To pinpoint the location of the harborage site, samples should be analyzed individually, not as composites. Suspected pieces of equipment should be torn down, and samples from suspicious sites or materials should be collected. Equipment should be cleaned and sanitized as it is being reassembled; the equipment should then be re-sampled. This is the preferred approach to finding *Listeria* on equipment surfaces and is usually adequate to eliminate the contamination *(20)*. However, if this process is unsuccessful, it may be necessary to remove sensitive electronics, oil and grease and to heat equipment surfaces to 160°F (71.1°C) for 20–30 minutes *(5, 20)* Lower temperatures for longer times may also be effective. Small parts can be placed in an oven or a hot water bath; larger equipment can be shrouded with a heat-resistant tarp and steam introduced under the tarp. It is also possible that employee practices may be a factor involved in contamination incidences. Refresher training in the controls necessary to prevent *L. monocytogenes* contamination may thus be indicated if repeat positive samples are found.

## EXAMPLES OF *LISTERIA* MONITORING PROGRAMS IN SMOKED FISH PLANTS

The following examples describe four different hypothetical monitoring and testing programs for smoked fish operations to illustrate the guidelines provided above.

Example 1 (Company A) illustrates a program in a high volume plant that produces only cold-smoked salmon.

Example 2 (Company B) is a program in a medium-sized plant that produces 12 different types of hot- and cold-smoked fish.

Example 3 (Company C) describes a program in a small plant that produces 5 different types of hot smoked fish.

Example 4 (Company D) is a program in a medium-sized plant that uses a "zone" concept for its testing program.

It is important to keep in mind that these examples are provided for information purposes only. As noted previously, there is no one sampling or testing program that is appropriate for all smoked fish operations or even specific types or sizes of operations. The examples do not cover all possible scenarios that may arise during such testing programs. It is unlikely that any one of the examples will exactly match the unique conditions or procedures used in any particular plant. Rather, they are intended to help firms evaluate testing options and develop their own monitoring and testing programs as one component of a complete *Listeria* control plan. Cost estimates (which may be highly variable, depending on the number and types of tests conducted, logistics and testing method used) are included to help processors understand the costs that may be associated with various testing strategies.

## EXAMPLE I

Company A produces a variety of cold-smoked salmon products for sale to retail stores, restaurants, catering companies, and institutional food service customers. Over 1 million pounds (approximately 450,000 kg) of headed and gutted (H&G) frozen salmon are purchased from 8 different suppliers in North America, South America and Europe each year. Frozen, brined salmon fillets are purchased from 2 large international suppliers. The plant operates all year and has 50 employees, all of whom work on a single shift (7 a.m. to 4 p.m.), except for 3 individuals who monitor the smokers in the evening and at night. The plant has a loading dock where raw products are delivered and stored in designated freezers. Raw product is thawed and prepared for brining in a raw material handling room. Products are brined in tubs in a cooler designated for brining. Brined products are placed on racks in the raw material room and moved into the smoking chambers. After the smoking cycle is completed, the smoked fish is moved to a designated finished product cooler. Smoked product is sliced and packaged in a finished product handling room. Company A has 3 slicing machines. Finished product is portioned and weighed by hand and then vacuum packed. Product is then stored at 36°F (2.2°C) for 2–3 days and shipped or is frozen until shipment. Company A has a sampling and testing program that includes routine testing of environmental (non-food contact) sites and product contact surfaces, periodic testing of finished product, and routine testing of raw seafood (Fig. 1).

### Routine environmental testing

Each week Company A collects 12 samples from 6 different types of non-food contact sites in the exposed finished product handling area and tests them for *Listeria* spp. All swab or sponge samples are collected before processing. Two environmental samples are collected from each of the following five sampling sites: floors near the slicing machines; the wheels of carts used to transport in-process products and packaged products; coolers where smoked product is stored before it is packaged; the edges of and underneath tables where finished products are portioned and weighed; and underneath product conveyor belts. In addition, 2 samples are taken from floor drains, but results are treated differently (focused cleaning and testing only when there are positives). Test results are evaluated by tracking the total number of positives at each site over time. Whenever a

positive is detected (including in samples from drains), special attention is focused on cleaning and sanitizing that site. If 2 or more samples, including those from floor drains, are positive, or if the same site comes up positive two or more times in a month, extra attention is given to cleaning and sanitizing those sites. Except when the positives are from a floor drain, swab samples are then taken daily until the samples are negative for three consecutive days, and the routine weekly monitoring schedule is resumed. If there are any positive test results in 3 consecutive days, trouble-shooting procedures are implemented, which include shutting down lines in the affected area; using different sanitizers; more aggressive cleaning and application of sanitizer; use of heat sanitizing if necessary and feasible; or using other methods until there are 3 consecutive negative test results.

### Product contact surface testing

Samples for *Listeria* testing are collected each week from 6 different product contact surfaces in the area of the plant where exposed finished products are handled and processed. A total of 12 different test sites on slicers, conveyor belts, scales, skinning machines and trim knives have been identified. Swab or sponge samples are taken at six of these sites each week so that all sites are sampled twice per month. Two of the monthly samplings are done before processing begins and two at mid-shift break, i.e., twice a month all the sample sites are tested before processing begins, and twice a month the sites are sampled at the mid-shift break. For machinery or equipment with moving parts, when pre-op samples are taken, the equipment is run for 15–30 minutes without product prior to sampling in order to dislodge any contamination from hidden, inaccessible areas. A pre-op positive from a piece of equipment suggests poor cleaning and sanitation or possibly persistent contamination (a harborage site). Re-sampling at selected sites using historical data to identify potential hot spots may help identify the contaminated area. When a product contact surface sample is positive, extra attention is given to the area, breaking the equipment down as necessary and cleaning and sanitizing this site. Pre-op samples from this site are then tested daily for 3 consecutive days. If results of at least 2 days of tests are negative, then routine sampling of the area is continued. If positives are found on 2 or more days during this 3-day testing period, the line is shut down; equipment is disassembled and thoroughly cleaned and sanitized with a different sanitizer than the one routinely used. Swab samples are taken again before the line is put back into production and for 3 consecutive days after production has resumed. If tests are negative on two or more days, the routine sampling schedule is resumed. If positives still occur on two or more of these testing days, samples of finished product produced since the line was restarted are taken (20 packages, tested as 4 composites of 5 samples) and the corresponding lot of product is held until test results are obtained. Product that is negative is released. Product that tests positive must be destroyed or cooked, since reprocessing as cold smoked fish is not an option.

### Finished product testing

Company A tests a random sample from a single lot of finished product once each quarter for *L. monocytogenes*. This company has determined that a lot is identified as a single type of product from one processing line produced during a specified period of time (usually a single day of production). Four composite samples, each consisting of 5 finished packaged products from a single lot, are collected. The lot from which the samples are taken is isolated until test results are obtained. The composite samples are tested for *L. monocytogenes*. If test results are negative, routine monitoring continues. If one or more of the samples test positive, the lot of product that the sample was taken from must be reprocessed into a hot-smoked product or fully cooked, or destroyed. Monitoring of product contact surfaces for *Listeria* spp. is then conducted daily for one week using the weekly testing protocol. If a positive test result is found, intensive sanitation procedures are conducted at the site. When test results are negative for three consecutive days, routine sampling is resumed.

### Routine raw product testing and screening for new suppliers

The raw product testing and new supplier sampling program of Company A is as follows:

### Screening new suppliers

Company A has a policy requiring that samples of product be tested for *Listeria* contamination before the company establishes a business relationship with and accepts large shipments of frozen H&G salmon or brined fillets from any new supplier. This initial screening process requires that 6 samples from at least three different lots of product be tested for *Listeria* species. If 5 or more of the samples from each lot of product are negative, the new supplier will be incorporated into Company A's routine raw material testing program. If more than 3 samples from any one of the three lots or more than 6 samples overall are positive, then Company A will not accept product from this supplier until they are able to demonstrate that effective *Listeria* control measures have been implemented and the screening process is repeated to confirm supplier controls are effective. If 2 to 3 tests from any of the initial lots of product are positive, additional samples are taken from two new lots of product from that supplier. If at least 5 or more of the samples from each of these additional lots are negative, the supplier can be incorporated into the routine raw material testing program. If 2 or more samples per lot from these additional tests are positive, Company A will not accept product from the supplier until it can demonstrate that effective *Listeria* control measures have been implemented and the screening process has been repeated and passed. Lots that test positive for *Listeria* spp. are returned to the supplier.

### Routine raw product testing

Samples are taken randomly from 3 different suppliers on a quarterly basis. Six samples are taken from a single lot from each supplier, for a total of 18 routine raw material samples per quarter. Lots that test positive for *Listeria* spp. are returned to the supplier or sold to local restaurants to be used for cooked product. If 5 or more of the samples from a single supplier are negative, the supplier is returned to the routine testing schedule. If 2 to 3 samples from a supplier are positive, two new lots of product from this supplier will be tested. If 5 or more of the samples in each of the additional lots are negative, the supplier is returned to the routine testing schedule. If 2 or more of these additional tests are positive, Company A will notify the supplier of the problem and work with them to ensure that effective *Listeria* control measures are being used. When assurance is received that problem-solving measures have been implemented, Company A will then re-test the supplier. If more than 3 of the initial samples from a single supplier are positive, then Company A will notify the supplier of the problem and work with it to ensure that effective *Listeria* control measures are being used. When assurances have been received that problem-solving measures have been implemented, Company A will then re-test the supplier.

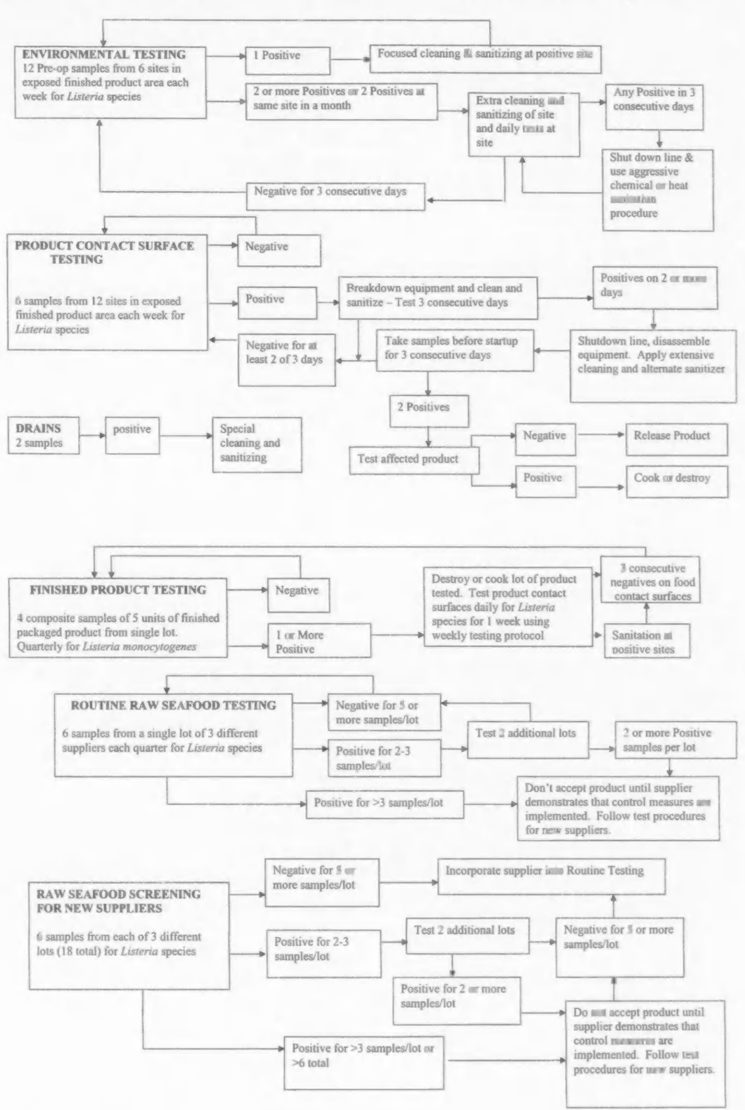



**Figure 1.** Flow Diagram for *Listeria* Testing – Company A (Example 1)

## Testing program costs

Based on the sampling program outlined, Company A estimates that 1,248 environmental samples, 72 routine raw material samples, and 72 screening samples from four new suppliers will be tested per year for *Listeria* species. This represents approximately 1,400 routine *Listeria* species tests annually. At a cost of $25 per test, the annual cost would be approximately $35,000. In addition, Company A estimates that up to 75 additional tests will be needed to solve problems when occasional test results are positive. The cost of these additional tests at $25 per test would be $1,875. Finally, 16 finished product composite samples will be tested for *L. monocytogenes*. At a cost of $30 per test, the annual cost would be $480. Additionally the company budgets for 8 product composites to be tested in the event of product contact surfaces testing positive ($240). Based on these estimates, Company A determined that it must budget an additional $40,000 annually in operating expenses specifically for the *Listeria* testing program outlined in this example.

## EXAMPLE 2

Company B annually produces approximately 400,000 pounds (~181,400 kg) of smoked fish products that are sold to retail stores, delicatessens and restaurants. Approximately 40% of the finished product is vacuum packed cold-smoked salmon, 15% is vacuum-packed sablefish and the remainder is air packed hot-smoked products such as trout, whitefish, mackerel, salmon, bluefish and eels. Salmon is received in the H&G frozen form from suppliers in North and South America. Sablefish is obtained frozen from suppliers in the Pacific Northwest. Frozen trout fillets are received from domestic suppliers, and all other products are received as fresh whole fish from suppliers in the US and Canada. The plant operates all year and has 26 employees. There is one shift from 8:00 a.m. to 5:00 p.m., with 2 employees managing the smoking operation and 3 employees assigned to sanitation duties conducted in the evening and at night.

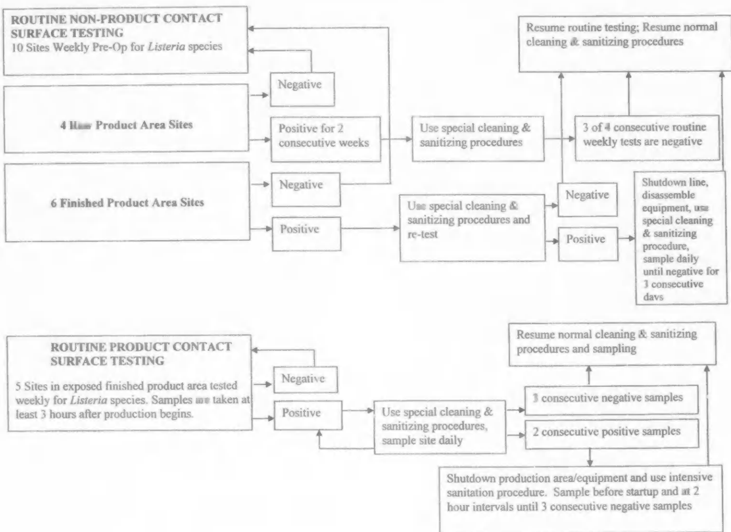
Raw fresh fish are received and stored in a designated cooler before processing. Frozen salmon and trout are delivered daily from a nearby storage warehouse to meet daily production needs. All raw products are handled and processed in a raw production room. One side of the room is designated for thawing frozen products and filleting and trimming products to be cold smoked, and the other side of the room is used for cleaning and preparing whole fish for brining and hot smoking. All products are wet brined and stored in a designated brining cooler. After smoking, all finished products are stored in a designated finished product cooler. Finished products are trimmed, sliced (if necessary), and packed in a separate packing room. Two slicers are used to prepare cold-smoked salmon. All individual packages are portioned and weighed by hand by plant workers and either vacuum or air packed. Finished products are stored in a refrigerated cooler set at 32°F for orders that will be shipped within one week. Some product will be frozen for longer-term storage.

Company B tests non-product contact surfaces and product contact surfaces for *Listeria* spp. (Fig. 2). The company assumes that, given the mixture of raw products used in the plant, all species of seafood may contain *Listeria*, and it has implemented an aggressive routine sanitation program in both the raw and finished product handling areas to control the organism. Products to be cold smoked are treated with an alkaline treatment (lime solution, pH 12) to reduce *Listeria* contamination levels *(12)*.

### Non-Product contact surface testing

Company B monitors 10 different environmental non-food contact sites in its plant on a weekly basis. Six sites are tested

**Figure 2.** Flow Diagram for *Listeria* Testing – Company B (Example 2)




in the finished product area and 4 sites are tested in the raw material handling area. Pre-op sponge samples are taken in the finished product area as follows: 1 sample on a slicing machine from an area that does not directly touch product; 2 samples from the edges and underneath work stations where product is packed and weighed; 2 employee contact surfaces such as the door handle to finished product coolers, sites on the slicer or skinner or a knife handle; and 1 sample from wheels or surfaces of carts used to move finished product. Sites in the raw product room include 1 sample from each of the following areas: the edges and underneath tables used to prepare raw products; the floor of the brining cooler; the raw product cooler; and the frame of a smoker rack. All samples are taken before production begins.

Test results are monitored over time. While raw-material areas are expected to have a higher frequency of positive samples, they represent a lower risk for contributing to finished product contamination. Finished product areas are expected to have a lower frequency of positive results, but these areas pose a greater risk for finished product contamination. Tests in the raw material area are used to monitor patterns of contamination. Sites that have a positive result for 2 consecutive weeks will receive a more stringent cleaning and sanitizing procedure, along with sanitizer rotation until at least 3 of 4 consecutive tests are negative, at which time the normal cleaning and sanitizing procedures will be resumed. When a positive is detected in the finished product area, the sanitation crew is immediately notified and that site will receive special attention in the cleaning and sanitation protocol until the results are available from the re-test of this site, which is done within 24 hours of finding a positive. If the re-test result is negative, normal procedures are resumed. If the re-test result is positive, a supervisor will shut down the line, if necessary, and ensure that additional procedures for sanitation and equipment disassembly are implemented. Daily sampling of this site will occur until 3 consecutive negative samples demonstrate that the contamination source has been eliminated, and then the normal cleaning and sampling routine will be resumed.

### Product contact surface testing

Five product contact surfaces are tested weekly in the finished product handling area only. A swab or sponge sample is taken from the following areas: the blade of a slicing machine, a scale used to weigh product before packaging, a conveyor belt (skinning machine belt or packaging belt), a trimming knife, and one of the totes or racks used to move cold smoked products. All samples are taken at least 3 hours after processing has started. If a sample is positive, intensive cleaning and sanitizing procedures are focused on that area and samples are taken daily from the positive site. This process continues if there are any positives. When 3 consecutive days of negative samples are obtained, normal cleaning and sanitizing and sampling procedures are resumed. If samples are positive on 2 consecutive days, the area is shut down and extensive sanitation procedures are implemented. Swabs are taken before start-up and at two-hour intervals until negative samples for 3 consecutive days demonstrate that the contamination source has been eliminated, after which routine testing is resumed.

### Finished product testing and raw material testing

Company B does not perform any routine testing of finished products or raw products.

### Testing program costs

Based on the sampling program outlined above, Company B estimates that 780 environmental samples will be tested per year for *Listeria* species. At a cost of $25 per test, the annual cost would be approximately $19,500. In addition, Company B estimates that up to 50 additional tests will be needed to solve problems when occasional test results are positive. The cost of these additional tests at $25 per test would be $1,250. Based on these estimates, Company B has determined that it must budget an additional $21,000 annually in operating expenses specifically for the *Listeria* testing program outlined in this example.

## EXAMPLE 3

Company C is a small "boutique" processor that produces 10,000 to 20,000 pounds (approximately 4,500 to 9,000 kg) annually of hot smoked fish products for sale to area retail stores, restaurants and catering operations. The primary products are hot smoked salmon, trout, eel, bluefish and mackerel; fish are purchased fresh from local fishermen or wholesalers. The plant is a single large room with 4 employees. Production occurs daily from May to October, and a single batch of product is smoked each day. One to three batches are smoked per week during the remaining months of the year. Raw products are prepared in a designated area for brining. Because space and equipment constraints do not allow complete separation, all products (raw fish, products being brined, and finished products) are stored in the same cooler (which presents a higher risk of recontamination of finished product). All finished products are placed in open plastic containers after smoking.

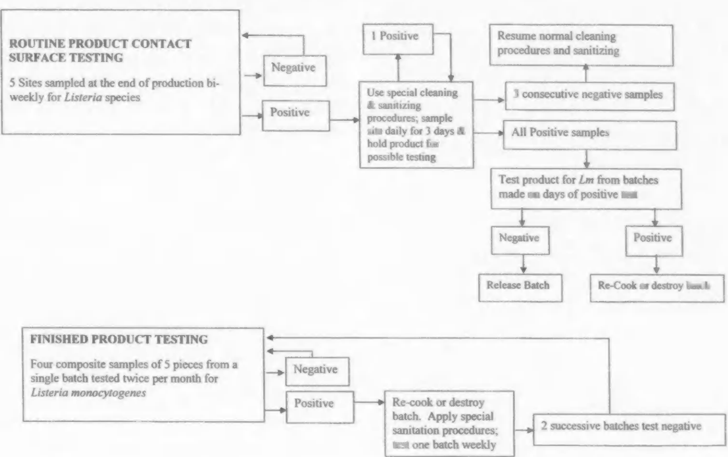



**Figure 3.** Flow Diagram for *Listeria* Testing – Company C (Example 3)

After an initial 8-hour cooldown period, lids are placed on finished product containers during storage to minimize the potential for cross contamination. Customer orders are assembled just prior to delivery, and all finished products are air packed.

Company C has a monitoring program that involves testing product contact surfaces, with periodic finished product testing (Fig. 3). All of the products produced by this firm undergo a step that requires the internal product temperature to reach a minimum of 145°F (62.8°C) for 30 minutes, which is lethal to *L. monocytogenes*. For this reason the primary concern for this firm is post-processing contamination of finished products from the plant environment, and the firm does not test raw products. Testing product contact surfaces is used to demonstrate that the firm's *L. monocytogenes* control measures are effective. Periodic finished product testing is used for further confirmation of the effectiveness of these control measures.

### Product contact surface testing

Company C swabs 5 different product contact surfaces on a bi-weekly basis and has them tested for *Listeria* spp. Swab or sponge samples are taken at the end of production, prior to cleaning and sanitizing, at the following sampling sites (one sample per site): the table used to pack orders, two different cutting boards used to trim or cut product into portion sizes, one of the containers used to store smoked products, and the scale used to weigh customer orders. If a positive test result is obtained, the affected site or equipment is thoroughly cleaned and sanitized using intensive procedures. Daily swabs are taken for 3 days. This process continues if there are any positives. When 3 consecutive days of tests are negative, routine sampling and cleaning and sanitizing procedures are resumed. Finished product from the batch produced when the sample was taken is held and tested for *L. monocytogenes* if a product contact surface is positive; consequently, whenever product contact surfaces are tested product is placed on hold. If tests are negative the product is released. If tests are positive the lot is destroyed or re-processed with a full cook reaching a minimum internal temperature of 145°F (62.8°C) for 30 minutes.

### Finished product testing

Four composite samples consisting of five different 25-g pieces from a single batch of finished product is tested twice each month for *L. monocytogenes*. A lot is comprised of a single batch of product smoked in the processor's single smokehouse. The lot is held until test results are obtained. Additional lots produced at the same time may also be tested. If any product test is positive, the product is destroyed or re-cooked if possible through the full cycle to ensure that it reaches a minimum internal temperature of 145°F (62.8°C) for 30 minutes, and special sanitation procedures are used until 2 successive batches test negative.

### Testing program costs

Based on the sampling program outlined above, Company C estimates that 130 environmental samples will be tested per year for *Listeria* species. At a cost of $25 per test, the annual cost would be approximately $3,250. In addition, Company C estimates that up to 20 additional tests will be needed to solve problems when occasional test results are positive. The cost of these additional tests at $25 per test would be $500. Costs for finished product testing when food contact surfaces are positive are estimated to be $600. Company C also will test 96 (8 composites per month × 12 months) routine finished product samples per year for *L. monocytogenes*. The cost of these finished product tests at $30 per test would be $2,880. Company C has also budgeted for additional testing if product were to test positive. They estimate that this might happen twice a year, requiring 4 additional tests of product composites for a cost of $120. Based on these estimates, Company C has determined that it must budget an additional $7,350 annually in operating expenses specifically for the *Listeria* testing program outlined in this example.

## EXAMPLE 4

Company D produces cold-smoked salmon and a variety of different hot smoked ready-to-eat products for sale to retail stores, restaurants and commissary operations. The primary raw material used in the plant is frozen H&G salmon and brined salmon fillets from suppliers in North and South America. Trout is purchased from aquaculture suppliers in the US and Canada; raw products for other specialty items are both wild caught and farm raised. The plant operates year round and has 50 employees, all of whom work on a single shift, except for the cleaning crew and the smokehouse operators. Whole salmon and fillets are stored in a frozen storage warehouse and delivered to the plant to meet production needs. Other raw products are stored either in the in-plant freezer or a raw material cooler. Frozen products are thawed and prepared for brining in a raw material handling area. From there, product moves into an in-process area where brine is prepared and fish are rinsed after brining and loaded onto racks for smoking. After smoking, the finished product is moved to a designated cooler for holding. Smoked product is then moved into a finished product handling and packing room

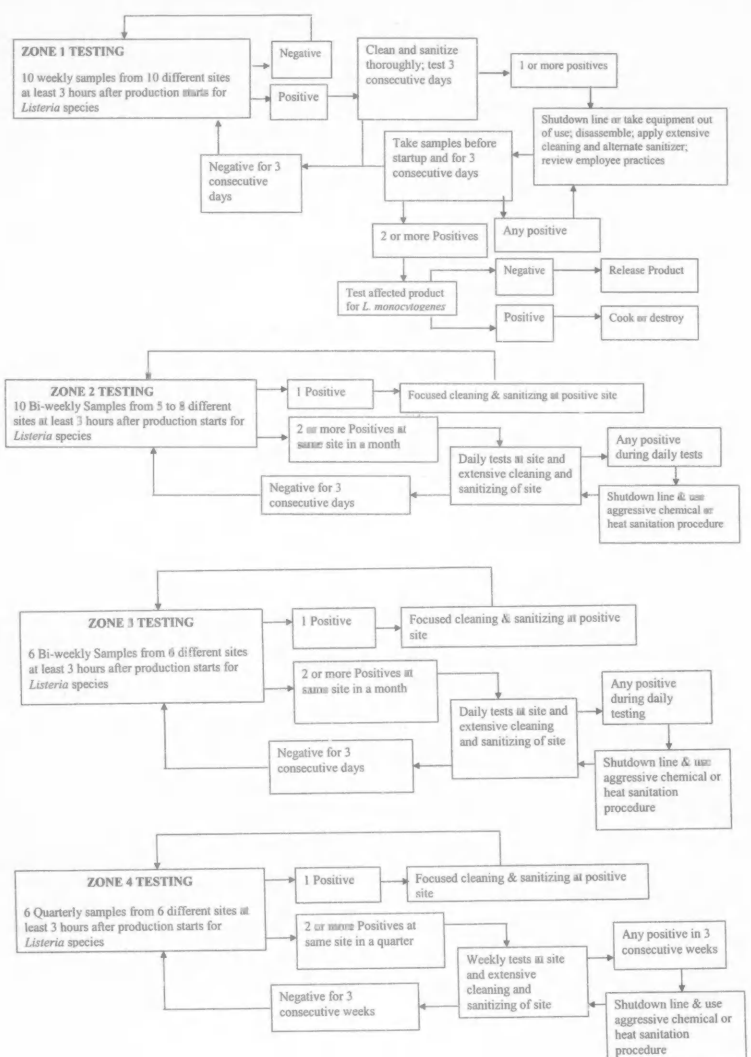



**Figure 4.** Flow Diagram for *Listeria* Testing – Company D (Example 4)

where the product is trimmed, sliced, portioned and packed. Finished vacuum- and air-packed product is either stored at 36°F (2.2°C) or frozen until orders are packed and product is shipped to customers.

Company D has implemented an environmental *Listeria* testing program that divides plant operations into four different zones that were identified by evaluating the relative potential risk that they represent in terms of possible direct finished product contamination. Zone 1 includes all direct product contact surfaces in the finished product handling area that could harbor *Listeria* and directly contaminate finished product, including equipment such as slicers, skinners, trimming knives, scales, work tables, conveyor belts, carts, racks, totes used to transport finished product, and employee hands or gloves. Zone 2 includes non-food-contact surfaces in the finished product handling area in close proximity to product contact surfaces that could indirectly contaminate food contact surfaces or finished products, such as the exterior of equipment, floors, stress mats, cart wheels, metal framework, coolers where finished product is stored, drains, employee aprons, and shoes. Zone 3 includes product contact surfaces in the in-process areas of the plant that could harbor *Listeria*, including fillet tables and knives, smokehouses, brine tubs, brining coolers, smoker racks, and employee aprons, as well as drains in the in-process area. Zone 4 includes those areas that are remote from the finished product handling areas, such as raw material storage coolers, thawing tubs, storage areas for ingredients and packaging materials, and staging areas. Company D's environmental *Listeria* testing program identifies how and when testing will occur and appropriate responses to test results for each plant zone (Fig. 4).

## Zone 1

Company D collects a single swab or sponge sample from each of 10 different sites in Zone 1 weekly and tests them for *Listeria* spp. Equipment samples from slicer blades, skinning machines, etc. are taken after at least three hours of production and up until the end of the day's production to "shake-out" any potential contamination that may not have been eliminated from the previous day's cleaning and sanitizing activities (due to a harborage site) as well as to pick up contamination that occurs during production. Sites included in each weekly sample collection include at least 2 samples from slicer blades, 1 sample from the skinning machine, 2 samples from work tables and/or conveyor belts, 1 sample from a scale, 1 sample from a randomly selected employee's hands, 1 sample from a trimming knife, and 2 samples from carts, totes, or racks used to transport exposed finished products. If a sample is positive (other than an employee's hands or a trimming knife), special attention is devoted to cleaning and sanitizing procedures and the site is re-tested for 3 consecutive days. If the site is negative for 3 consecutive days, routine testing at that site is resumed. If results of any tests are positive tests, the equipment or line will be shut down and intensive cleaning and sanitizing procedures will be applied, including disassembly of the slicer or skinning machine, if positive, and heat or chemical sterilization if possible. An additional sample is then taken before startup and again for three consecutive days, holding product produced on the line those days, until 3 consecutive negative samples are obtained. If any positive is found, sanitation and test procedures continue, with more aggressive cleaning and sanitation and more extensive sampling in the area to determine the root cause of the positive. If 2 or more additional positive samples are found during the 3 days of testing, the lot of product produced on that line or piece of equipment is tested for *L. monocytogenes*. If test results are negative, product can be released and intensive cleaning and sanitizing procedures

and daily testing are reapplied until consecutive negative results for three days are found. If the product test for *L. monocytogenes* is positive, the isolated lot is destroyed or cooked or hot smoked to a minimum internal temperature of 145°F (62.8°C) for at least 30 minutes. If a trimming knife is positive, employee practices are reviewed and revised as needed and employee refresher training is conducted; the type of sanitizer used for trim knives may be changed. If an employee's hand or gloves tests positive, a supervisor will review company hand washing and personal hygiene policies at the work site and re-test the same employee the following week.

### Zone 2

Company D collects 10 samples every two weeks from 5 to 8 different non-food contact surfaces in the finished product handling area. Swab or sponge samples are collected during production and tested for *Listeria* species. Sample sites include 2 samples from non-food contact sites on equipment used for finished product such as slicers, packaging equipment etc.; 1 sample from metal framework of work tables or packaging equipment; 1 sample from stress mats or the floor near slicers; 1 sample from an employee apron or shoes; 1 sample from the wheels of carts used to transport exposed finished product; 1 sample from the cooler used to store exposed finished product; and 1 drain sample. If a site tests positive, focused cleaning and sanitizing procedures are used at this site until the results of the next scheduled test are obtained. If this subsequent test result is negative, routine procedures are resumed. If 2 positive samples at the same site are obtained in the same month, intensive cleaning and sanitizing procedures are implemented at this site and, except when the positive samples are from a drain, daily tests are conducted. If test results are negative for 3 consecutive days, routine sanitation and testing procedures are resumed. If any test is positive during this daily testing, the line is shut down and heat or intensive chemical sanitation procedures is applied until daily tests are negative for 3 consecutive days.

### Zone 3

Company D collects 6 samples every two weeks from 6 different sites in this zone. Swab or sponge samples are collected after at least three hours of production and tested for *Listeria* species. Sample sites include 1 sample from a fillet table; 1 sample from a brine tub; 1 sample from a drain; 1 sample from the brining cooler; 1–2 samples from smoker racks or the smokehouse; and 1 sample from an employee apron or gloves. The same protocol for responding to positive samples described for Zone 2 is used for Zone 3.

### Zone 4

Company D collects 6 samples quarterly from 6 different sites in this zone. Swab or sponge samples are collected at the same time samples are being taken from other zones and tested for *Listeria* species. Sample sites include 1 sample from raw material storage cooler; 1 sample from empty raw material thawing tubs; 1 sample from drains in the thawing area; 1 sample from empty tubs or totes used to move thawed product into the in-process area; 1 sample from wheels of carts used to move product into the in-process area; and 1 sample from a bathroom door. A protocol similar to that described for Zones 2 and 3 is used to respond to positive samples from Zone 4. However, in Zone 4, samples are taken quarterly rather than every two weeks, and the re-sampling frequency for responding to a positive test result is weekly rather than daily.

### Raw and finished product testing

Raw products are treated with an alkaline treatment (lime solution, pH 12) to reduce *Listeria* contamination levels *(12)*, and no raw product or supplier testing is conducted. Company D does not conduct any routine finished product testing; such testing may be conducted in conjunction with Zone 1 positives, as noted above.

### Testing program costs

Based on the sampling program outlined above, Company D estimates that 520 samples will be tested per year for *Listeria* species in Zone 1; 260 samples in Zone 2; 156 samples in Zone 3 and 24 samples in Zone 4. The total number of samples tested for *Listeria* species per year is 960. At a cost of $25 per test, the annual cost would be $24,000. In addition, Company D estimates that up to 60 additional tests will be needed to solve problems when occasional test results are positive. The cost of these additional tests at $25 per test would be $1,500. The company does not anticipate the need to test product; however, it includes $1000 in the budget as a contingency. Based on these estimates, Company D has determined that it must budget an additional $26,500 annually in operating expenses specifically for the *Listeria* testing program outlined in this example.

## ACKNOWLEDGMENTS

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# Symposium Series on Food Microbiology

*sponsored by the*

## International Life Sciences Institute ILSI North America Technical Committee on Food Microbiology in Conjunction with the International Association for Food Protection 90th Annual Meeting

## August 10–13, 2003

**Hilton New Orleans Riverside**
**New Orleans, Louisiana, USA**

### PREFACE

The *Symposium Series on Food Microbiology* consisted of four international symposia sponsored by the North American Branch of the International Life Sciences Institute (ILSI N.A.) Technical Committee on Food Microbiology at the International Association for Food Protection (IAFP) 90th Annual Meeting, held August 10–13, 2003, in New Orleans, Louisiana, USA. Sessions covered the use of food safety objectives and other risk-based approaches to reduce foodborne listeriosis, new horizons in diagnostic food microbiology, shelf-life dating of ready-to-eat refrigerated foods, and the evolution of foodborne pathogens.

The North America branch of the International Life Sciences Institute (ILSI North America or ILSI N.A.) is a public, non-profit scientific foundation. ILSI N.A. advances the understanding and application of scientific issues related to the nutritional quality and safety of the food supply as well as health issues related to consumer self-care products. The organization carries out its mission by sponsoring relevant research programs, professional education programs and workshops, seminars, and publications, as well as providing a neutral forum for government, academic, and industry scientists to discuss and resolve scientific issues of common concern for the well-being of the general public. ILSI N.A. also strives to foster the career development of outstanding new scientists. ILSI N.A.'s programs are supported primarily by its industry membership.

The ILSI N.A. Technical Committee on Food Microbiology was formed in 1987 to address issues related to microbial food safety hazards. The committee has funded over two million dollars worth of research on several important foodborne pathogens and has sponsored numerous scientific meetings in the area of microbial food safety. Since 1993, the committee has collaborated with IAFP by sponsoring an annual international symposium series on food microbiology. ILSI N.A. and the Technical Committee on Food Microbiology hope that making the abstracts and extended abstracts of the presentations in these symposia available to the public will provide important information to a worldwide audience and will help stimulate initiatives to improve the safety of our global food supply.

# ABSTRACTS AND EXTENDED ABSTRACTS

## USE OF FOOD SAFETY OBJECTIVES AND OTHER RISK-BASED APPROACHES TO REDUCE FOODBORNE LISTERIOSIS

**Introductory Remarks**

ISABEL WALLS, ILSI Risk Science Institute, Washington, D.C., USA

**Achieving Continuous Improvement in Reductions in Foodborne Listeriosis: A Risk-based Approach—Overview of Expert Panel Report**

MICHAEL P. DOYLE, Center for Food Safety, University of Georgia, Griffin, Georgia, USA

**Hazard Characterization Issues: Virulence, Pathogenicity, and Modeling Dose-Response**

CATHERINE W. DONNELLY,* Richard Raybourne, Mary Alice Smith, Martin Wiedmann, Department of Nutrition and Food Science, University of Vermont, Burlington, Vermont, USA

**Factors Affecting Exposure of Individuals to *Listeria monocytogenes***

KATHERINE M.J. SWANSON, General Mills, Inc., Minneapolis, Minnesota, USA

**Use of Food Safety Objectives as a Tool for Reducing Listeriosis**

ROBERT L. BUCHANAN, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, USA

**Prevention and Control Strategies for Reducing Foodborne Listeriosis**

DON L. ZINK, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, USA

**Education Strategies for Reducing Foodborne Listeriosis**

LYDIA C. MEDEIROS, Department of Human Nutrition, Ohio State University, Columbus, Ohio, USA

## NEW HORIZONS IN DIAGNOSTIC FOOD MICROBIOLOGY

*The assistance of Les Smoot in developing this program is gratefully acknowledged.*

**Development, Validation, and Impact of Rapid Methods on Testing for Pathogens in Foods**

PETER FENG, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, USA

**Real-Time PCR**

PINA M. FRATAMICO, Agricultural Research Service, U.S. Department of Agriculture, Wyndmoor, Pennsylvania, USA

**Biosensor Detection of *Salmonella* in Sprouts**

MARIANNE F. KRAMER* and Daniel V. Lim, Department of Biology, University of South Florida, Tampa, Florida, USA

**Molecular Identification of *Salmonella* Serotypes**

PATRICIA I. FIELDS,* Collette Fitzgerald, and John R. McQuiston, Foodborne and Diarrheal Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

**Biochip/Microarray Applications for the Food Industry**

CLAUDE MABILAT, bioMérieux SA, Venissieux, France

**International Standardization and Harmonization of Detection Methods**

MICHAEL H. BRODSKY, Brodsky Consultants, Thornhill, Ontario, Canada

## SCIENCE-BASED SHELF LIFE DATING OF READY-TO-EAT REFRIGERATED FOODS

*The assistance of Paul Hall in developing this program is gratefully acknowledged.*

**History of Use and Consumer Perception of Code Dates**

JILL HOLLINGSWORTH, Food Marketing Institute, Washington, D.C., USA

**Microbiological Concerns Related to Refrigerated Ready-to-Eat Foods**

MICHAEL P. DOYLE, Center for Food Safety, University of Georgia, Griffin, Georgia, USA

**Principles for Determining If a Product Requires Shelf Life Dating**

RICHARD C. WHITING, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, USA

**Protocols to Establish and Validate Safety-based Shelf Life Dating**

MARK W. CARTER, Kraft Foods NA, Glenview, Illinois, USA

**Alternatives to Safety-based Shelf Life Dating**

TED LABUZA,* Dan Belina, and Dr. Francisco Diez-Gonzalez, Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota, USA

**European Perspectives on Shelf Life Dating**

ROY P. BETTS, Campden & Chorleywood Food Research Association, Chipping Campden, United Kingdom

**The speaker's name is capitalized, and only the speaker's affiliation is listed.*

## THE EVOLUTION OF FOODBORNE PATHOGENS

**Mutators and Bacterial Promiscuity: Some Overlooked Facts in Pathogen Evolution**

THOMAS A. CEBULA, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, USA

**Evolution of *Escherichia coli* O157:H7 and Other *E. coli***

THOMAS S. WHITTAM, National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan, USA

**Evolution of *Salmonella* Virulence and Host Adaptation**

ANDREAS J. BÄUMLER, Department of Medical Microbiology and Immunology, Texas A&M University, College Station, Texas, USA

**Multilocus Sequence Typing for Evolutionary Analysis and Outbreak Tracking**

KATE E. DINGLE* and Martin C.J. Maiden, Nuffield Department of Clinical Sciences, University of Oxford, Oxford, United Kingdom

**Molecular Evolution of *Listeria monocytogenes***

MARTIN WIEDMANN*, Katy Windham, and Kendra Nightingale, Department of Food Science, Cornell University, Ithaca, New York, USA

## USE OF FOOD SAFETY OBJECTIVES AND OTHER RISK-BASED APPROACHES TO REDUCE FOODBORNE LISTERIOSIS

### INTRODUCTORY REMARKS

ISABEL WALLS, ILSI Risk Science Institute, One Thomas Circle, NW, Ninth Floor, Washington, D.C. 20005, USA

In 2002, the ILSI Risk Science Institute initiated a project to consider ways to minimize the likelihood of an outbreak of listeriosis and to reduce the number of sporadic cases. A steering committee was convened to help frame the specific objectives and scope of the project, to identify the questions that should be answered by an expert panel, and to provide input on the selection of the panel. The steering committee met in April 2002. After a series of presentations by committee members and a brainstorming discussion, the committee agreed that an expert panel should be convened to address the question "How can we achieve continuous improvement in reductions in listeriosis?"

A risk-based approach should be used that would include discussions to identify public health goals, the use of food safety objectives to achieve these goals, optimization of resources, and ways to measure success, i.e., how to ascertain that sustained reductions in the level of listeriosis are occurring.

The ILSI Risk Science Institute convened an expert panel that has met three times over the past year, both in plenary session and in breakout groups, to review the state of the science and address the question posed by the steering committee. The following five breakout groups were established: (1) Setting Public Health Goals for *Listeria monocytogenes,* (2) Exposure Assessment Issues, (3) Hazard Characterization Issues, (4) Prevention/Control Strategies, and (5) Education Strategies. A draft report has been prepared and will be presented at this meeting. Expert panel members will meet one more time to finalize the report, which should be completed by December 2003. Plans are going forward to publish the report as a special supplement to the *Journal of Food Protection.*

#### *Listeria Monocytogenes in Foods* — Steering Committee Members

- Dr. David W.K. Acheson, Center for Food Safety and Applied Nutrition, Food and Drug Administration
- Dr. Robert L. Buchanan, Center for Food Safety and Applied Nutrition, Food and Drug Administration
- Dr. Michael P. Doyle, Center for Food Safety, University of Georgia
- Dr. Paul A. Hall, Kraft Foods NA
- Dr. Paul S. Mead, Division of Vector-borne Infectious Diseases, Centers for Disease Control and Prevention
- Dr. Marguerite A. Neill, Brown University and Memorial Hospital of Rhode Island
- Ms. Catherine Nnoka, ILSI North America
- Dr. R. Bruce Tompkin, ConAgra Refrigerated Prepared Foods (retired)
- Dr. Isabel Walls, ILSI Risk Science Institute
- Dr. Don L. Zink, Center for Food Safety and Applied Nutrition, Food and Drug Administration

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- Dr. Christopher Griffith, University of Wales Institute, Cardiff
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## ACHIEVING CONTINUOUS IMPROVEMENT IN REDUCTIONS IN FOODBORNE LISTERIOSIS: A RISK-BASED APPROACH—OVERVIEW OF EXPERT PANEL REPORT

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Continued efforts are needed to ensure steady reductions in the incidence of listeriosis because of its high mortality rate. The focus of this report is the use of a risk-based approach that will have the greatest impact on reducing foodborne listeriosis. Three landmarks were identified to use in developing a road map to provide continuous reductions in foodborne listeriosis:

(1) identifying a baseline for the number of cases of listeriosis to use for comparison purposes (e.g., with FoodNet data)

(2) defining populations at risk of listeriosis:
- (a) exquisitely sensitive
- (b) intermediately sensitive
- (c) normal healthy individuals
- (d) unique high-risk subpopulations that will require different control strategies

(3) defining "high risk" foods, those that:
- (a) have the potential for contamination with *Listeria monocytogenes*
- (b) support the growth of *L. monocytogenes* to high numbers
- (c) are ready to eat
- (d) require refrigeration
- (e) are stored for an extended period of time

Dose-response data will be useful for estimating the impact of reducing the numbers of *L. monocytogenes* in foods. Control strategies (routes on the road map) likely to have a major impact on reducing foodborne listeriosis include (1) reducing the number of servings of high-risk foods and (2) educating at-risk populations.

The most effective strategies to control/eliminate listeriae in high-risk foods include (1) reformulating foods to include antimicrobials to prevent/retard the growth of listeriae to high numbers, (2) using postpackaging treatments to destroy listeriae on products, and (3) establishing acceptable storage times for foods that support the growth of listeriae to high numbers. Educating at-risk populations will require targeted messages specific to each population.

In risk management terms, this road map is a food safety objective for *L. monocytogenes*.

## HAZARD CHARACTERIZATION ISSUES: VIRULENCE, PATHOGENICITY, AND MODELING DOSE-RESPONSE

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Despite extensive research on *Listeria monocytogenes*, determination of the infectious dose of this organism remains elusive. Host susceptibility clearly influences infectious dose, along with virulence differences between strains, stress response, and the influence of food matrices. Invasive listeriosis occurs primarily in individuals with some form of immune system compromise. High-risk individuals include pregnant women, the elderly, organ transplant patients, patients with cancer, diabetics, those with HIV/AIDS, and people undergoing treatments with steroids/cytotoxic drugs. Should establishment of a food safety objective for *L. monocytogenes* be primarily focused on susceptible populations? For highly susceptible individuals, any level established under a food safety objective framework would not be protective, so strict avoidance of high-risk foods may be necessary for these people. Improved understanding of the range of virulence and of the factors that make a population susceptible may lead to further reductions in human listeriosis. Increased active surveillance of high-risk populations may lead to the identification of foods not previously linked to sporadic cases of listeriosis.

## FACTORS AFFECTING EXPOSURE OF INDIVIDUALS TO *LISTERIA MONOCYTOGENES*

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*Listeria monocytogenes* risk assessments suggest that the probability of contracting listeriosis increases as an individual is exposed to higher numbers of organisms through consumption. Understanding the factors that influence exposure is therefore important in identifying risk-based strategies to reduce illness. Among the principal considerations that impact exposure are the prevalence and level of contamination, the amount and frequency of consumption, and the potential for growth of *L. monocytogenes* under refrigeration. The number of organisms is influenced by four factors: (1) the number of organisms present on the incoming ingredients, (2) the potential for the addition of new organisms through cross-contamination, (3) the potential for an increase in numbers owing to growth or concentration, and (4) the potential for a reduction in numbers through cooking, acidification, and other treatments. These four factors must be considered in manufacturing, at retail, in food service settings, and in the home for maximum public health benefits, because all sites provide the potential for contamination, growth, and inactivation.

Opportunities to reduce exposure exist throughout the farm-to-table continuum. Manufactured products must be produced in a manner that minimizes the potential for contamination, retail and food service products must be handled so as to minimize recontamination that could result in subsequent growth during storage, and consumers must avoid recontamination from raw product sources and minimize the potential for growth during refrigerated storage. Mathematical modeling can be a useful tool for assessing the potential for growth during storage and thus for determining appropriate product storage time and temperature conditions for at-risk

consumers. The amount and frequency of consumption can be influenced by regional consumption patterns, specific food preferences for subpopulations, and restriction diets for exquisitely sensitive individuals such as transplant patients. Targeted educational strategies for susceptible populations to use to minimize the potential for *L. monocytogenes* growth (e.g., refrigerated storage temperatures, freezing, or reduced time of refrigerated storage) can reduce exposure and thereby improve public health.

## USE OF FOOD SAFETY OBJECTIVES AS A TOOL FOR REDUCING LISTERIOSIS

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The emergence of analytic tools such as quantitative microbial risk assessment is making it increasingly feasible to evaluate the level of public health protection achieved by food safety systems. This capability has, in turn, stimulated interest in metrics for articulating the level of stringency expected of food safety systems. One such metric, discussed widely on an international basis, is the food safety objective (FSO). The Codex Alimentarius Committee on Food Hygiene has proposed a definition of the FSO as "the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides the appropriate level of health protection." The point of consumption was selected because it can be directly related, via an appropriate dose-response relationship, to predicted public health outcomes. An FSO can be viewed as the integrated stringency expected for the entire farm-to-fork food safety system.

There have been extensive discussions of the desirability of establishing an FSO for *Listeria monocytogenes* in ready-to-eat foods. The recent availability of the risk assessments from the Food and Drug Administration's Food Safety Inspection Service and from the Food and Agriculture Organization of the United Nations and the World Health Organization has allowed evaluation of the impact of different proposed FSOs. It is apparent from these analyses that in addition to the direct impact of various proposed FSO values, the overall public health impact must also take into account the percentage of the food servings consumed that do not achieve the FSO. Potentially, a less stringent FSO could enhance food safety if it leads to a greater degree of compliance. Although FSOs are a useful concept, their actual implementation will likely have to be achieved through development of performance criteria and microbiological criteria based on the FSO through a risk assessment process.

## PREVENTION AND CONTROL STRATEGIES FOR REDUCING FOODBORNE LISTERIOSIS

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The greatest impact on improving public health relative to listeriosis will be achieved by reducing the exposure of high-risk individuals to foods that contain high cell numbers of *Listeria monocytogenes*. An ILSI Risk Science Institute expert panel identified three areas as critical to achieving this goal: (1) preventing the growth of *L. monocytogenes* in contaminated foods, (2) preventing the contamination of foods in which the organism can grow, and (3) targeting education messages to high-risk consumers.

Given its ubiquity in the environment, in raw materials, and in human and animal sources, recontamination of food with *L. monocytogenes* is always a possibility despite the best efforts to keep it out. Food processors should use various control strategies (good manufacturing practices, sanitation standard operating procedures, etc.) to minimize environmental *L. monocytogenes* contamination and to prevent cross-contamination, or they can pasteurize products to eliminate the pathogen. This latter control strategy is less feasible for food service, retail, and home environments. Separation of cooked or ready-to-eat products from raw foods, along with effective sanitation, can minimize the potential for contamination in these settings.

Preventing *L. monocytogenes* growth in ready-to-eat foods is the most significant consideration in reducing foodborne listeriosis. Prevention strategies can and should be implemented in manufacturing sites, in retail and food service settings, and in the home to minimize the likelihood that the organism will grow to high numbers. Prevention of growth to high numbers can be achieved through time/temperature controls, including freezing, and through formulating foods so that they do not support growth.

Combinations of interventions to prevent both contamination and growth will be much more effective than any single intervention in mitigating the potential contamination of ready-to-eat products with *L. monocytogenes* and in reducing the subsequent risk of illness and death.

For control measures to be effective, they must be implemented correctly and at all points where control strategies are needed, i.e., at postharvest, in processing plants, throughout distribution, at the retail level, and in the home. An intensive environmental sampling program is necessary to minimize the potential for environmental contamination with *L. monocytogenes* in foods during processing. Not only must an appropriate testing program be in place, but equally important, an effective action plan must be implemented to take corrective action when results indicate that environmental controls are not working.

## EDUCATION STRATEGIES FOR REDUCING FOODBORNE LISTERIOSIS

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Of all the educational messages given to consumers to control foodborne illnesses, those related to control of *Listeria monocytogenes* are the most complex. About 20% of the American population is at risk for listeriosis because of pregnancy *(1)*, aging *(2)*, or illnesses and medications affecting the immune system *(3)*. The diversity of these groups, as well as food-related risk and the extent to which efforts are made to control risk, gives emphasis to the complexity of the message. Even though voluntary and regulatory steps are being taken in the food production and retail sectors to reduce risk, the food handler and the end-user control ultimate risk.

Human behavior is a factor in the continued incidence of listeriosis. In recent studies it was found that individuals in high-risk groups were generally unaware of food safety guidance regarding listeriosis and were vulnerable to misinformation about food handling practices *(4–6)*. Pregnant women did not consider themselves to be "ill" and did not perceive the risk to themselves or the baby. They did indicate that they would make changes in their practices if they knew that the health of their child could be endangered. Seriously ill patients viewed food safety as secondary and their underlying condition as their

primary concern; however, they were motivated overall by the desire to improve or maintain their health. Health behavior and promotion models advocate incorporation of motivating information into education that appeals to the desire of an individual to maintain or return to good health *(7)*. A medical condition or life-stage may impose many restrictions on daily life, but if given a satisfactory explanation of the consequences of consuming high-risk foods, the message is more likely to be accepted and practiced by a consumer.

High risk end-users, however, are not the only group who need motivation to follow safe food handling. All food handlers, including those in food manufacturing, retail, and food service, need education and training to ensure product safety. There is no assurance that voluntary and regulatory control of the product and food environment will protect end-users if individuals handling food are not appropriately informed of potential risks, trained in effective control measures, and properly motivated to be responsible for the safety of the food that others will consume.

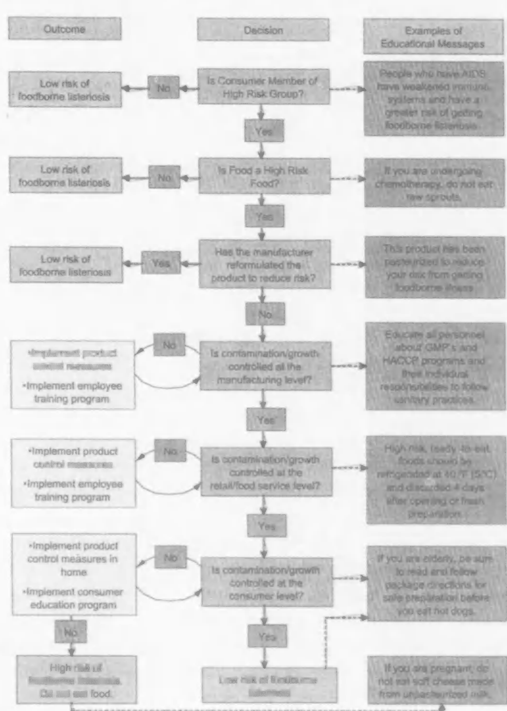
Effective educational strategies should focus on high-risk, ready-to-eat foods known to be sources of *Listeria monocytogenes*, cleaning and sanitizing, storage and shelf life, and practical information to aid the end-user in selection, purchase, and preparation of foods prepared for or eaten away from home. Effective messages communicate risk associated with consumption of a high-risk food, and should be customized to the learning needs and situation of the individuals who will be expected to implement the practice. Credible messages are most effective when backed with the strongest research-based information available so that food handlers and end-users will respect the information and be motivated by that credibility to follow what they are being asked to incorporate into their daily work and home life.

A key feature of an effective educational strategy is its flexibility. Static messages become obsolete as new research information becomes available, as regulatory requirements change, or as new product design and formulations become available. Food handling guidance sometimes changes long before messages ever reach the end-user; new and innovative information may never reach them if the venue for information delivery is not effective. A method that facilitates effective communication, is customized for situation and audience, maximizes the credibility of the message, and is inherently flexible is one that we expect to be a successful educational strategy. As an outcome of the ILSI Expert panel to consider how to ensure further reductions in foodborne listeriosis, we are advocating a method for others to use when developing educational strategies. The method uses a decision tree approach (Fig. 1).

The first step when planning food safety educational messages aimed at controlling listeriosis is to consider the end-user of the food product. If the end-user is not a member of a high-risk group, the probability of that individual contracting listeriosis is relatively low. There is no need for a precautionary educational message. If the end-user is a member of a high-risk group, education is needed. An example of a general message is, "People who have AIDS have weakened immune systems and have a greater risk of getting foodborne listeriosis" *(8)*. However, although factual, this does not provide advice that will result in continuous improvement in the incidence of listeriosis. The decision-making process must continue.

The next step is to consider whether a food that will be consumed by the high-risk individual is also a high-risk food, or one that has a greater probability than other foods to be

**Figure 1.** A decision tree for planning food safety education messages for control of *Listeria monocytogenes*




contaminated with an infective dose of *Listeria monocytogenes*. If the food is not considered by food scientists or regulatory agencies to be a high-risk food, then there is no need for a precautionary educational message, and the food could be consumed. If the food meets the definition of a high-risk food for *Listeria monocytogenes* contamination, education is needed. Combining the decision that the end-user is a member of a high-risk group and that the food in question is a high-risk food, an example of an educational message could be, "If you are undergoing chemotherapy, do not eat raw sprouts" *(9)*. This could be an effective education message that should be delivered if a food is inherently unsafe for a high-risk consumer to eat, although this is rarely the case because product control and risk reduction measures could be used to reduce the risk of a food product. Thus, the decision process continues.

Has the manufacturer reformulated the product to reduce risk? If yes, then there is no need to advocate to high-risk end-users to avoid that product; however, the educator will have difficultly making this decision if there is no way to know that a previously high-risk food has been reformulated. Clear labeling and product information communicating the fact of reformulation is an effective education strategy. An example of a message on a product label could be, "This product has been pasteurized to reduce your risk from getting foodborne illness." If a similar message or information is not available to the educator or the end-user, the decision process must proceed to the next step.

Now the decision requires information on product control measures and the training that food handlers receive before it can be decided whether a food is safe for a high-risk individual to consume. The first critical control points for the reduction of *Listeria monocytogenes* contamination or control occur at the

manufacturing level. If the quality control program of the manufacturer has evidence that the foods produced are not safe, then additional product controls and/or education and training of employees will be needed. The verification of control measures may indicate that a satisfactory safety level has not yet been achieved, even after educational or product control steps have been implemented. Until verified as safe, the decision tree cycles back to education and product control. Guidance for food manufacturers could be, "Educate all personnel about GMP's and HACCP programs and their individual responsibilities to follow sanitary practices." Has this been done? Once this question can be answered in the affirmative, the decision process continues.

The next step is for retail and food service establishments, where successfully processed foods can become contaminated as the result of further handling. The decision process is the same at this level as for the food manufacturers. Is an acceptable level of safety achieved through product controls and educational efforts? If yes, then proceed with the decision tree. Advice to employees handling food at the retail or food service levels could be, "High risk, ready-to-eat foods should be refrigerated at 40°F (4.4°C) and discarded 4 days after opening or fresh preparation."

Consumers have ultimate control over the safety of the food in their home. Safe food brought into the home and mishandled is no longer safe. The decision tree continues with the same question that was asked of food manufacturers and retail or food service establishments. If the answer is no, the consumer will need to control contamination and growth of *Listeria monocytogenes* in the home, but they will need education to learn how to do this effectively. If this has been assured and all questions above this one in the decision tree have been satisfactorily answered, the food is low risk for causing foodborne listeriosis and could be eaten. A sample message is, "If you are elderly, be sure to read and follow package directions for safe preparation before you eat hot dogs."

If the answer to the question is no and the high-risk end-user does not take measures to control contamination and growth of *Listeria monocytogenes* in the home, then there is no guarantee that the food is safe, even if the previous answers on the decision tree are affirmative. The food should not be eaten. A sample message could be, "If you are pregnant, do not eat soft cheese made from unpasteurized milk."

The decision to advise a high-risk end-user to consume a high-risk food is complex. How does an educator or high-risk consumer know what the food scientists know, especially how and when risk of consuming a food product has been reduced through product or environmental control measures or reformulation? This will require a commitment on the food industry's part to communicate that information to the public and to those who advise the public, such as health care providers. The two questions that consumers so often ask those of us who are educators, specifically "How do I know a food is safe to eat?" and "How long is it safe to keep a food before I need to throw it away?" can be answered more effectively if the information is readily available in convenient places, such as the product label.

High-risk consumers want to do the correct things that will enhance their health. If they know and understand the risk and if they have the knowledge, ability and information to distinguish high-risk foods, they will alter their behavior. The burden is on us as food safety professionals to give them the chance to help themselves.

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## *NEW HORIZONS IN DIAGNOSTIC FOOD MICROBIOLOGY*

### DEVELOPMENT, VALIDATION AND IMPACT OF RAPID METHODS ON TESTING FOR PATHOGENS IN FOODS


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Microbiological analysis for pathogens and their toxins in foods remains a challenging task. Foods come in all physical forms and are composed of an infinite array of ingredients, many of which can hamper or interfere with assays or with proper mixing, resulting in heterogeneous samples and irreproducible analytical results. These problems are compounded by the non-uniform distribution of bacterial populations in foods, the presence of low levels of pathogens among a large population of indigenous microflora and the possibility that the organisms are stress-injured by the food processing procedures, therefore making them difficult to detect *(1)*. To overcome these problems, conventional microbiological assays have included several sequential culture enrichment steps, which, although effective in enhancing the detection efficiency for pathogens, are labor-intensive and time-consuming. Complexity of food matrices therefore continues to be one of the greatest complications in food testing.

The advent of biotechnology introduced many new technologies that are being used in so called "Rapid Methods" for detecting foodborne pathogens and their toxins *(2)*. The pace with which these assays were developed surprised many,

as did the popularity of rapid methods, which currently experiences worldwide interest. However, it is not easy to define exactly what a rapid method is because the term "rapid" is subject to interpretation. As a result, "rapid method" encompasses a large group of tests ranging from assays that give very rapid results to those that simply shorten the assay time of conventional procedures *(3)*.

The technologies and the assay formats used in rapid methods are equally diverse. There are specialized chromogenic and fluorogenic *(4)* substrates that provides presumptive identification of organisms; miniaturized biochemical assays that simplifies identification of pure culture isolates; and antibody *(5, 6)* as well as nucleic acid-based tests *(7, 8)* that are highly specific for their targets. All these technologies are used in various assay formats, ranging from specialized culture media to simple home pregnancy-like devices, and some even use fairly sophisticated instrumentation and are automated. In a broader sense, all these are rapid methods as they do expedite the testing procedure, but, from a more stringent perspective, none of these are truly "rapid methods." The process of culture isolation required for rapid identification tests continues to be conventional and slow, and most assays designed to detect specific pathogens in foods still require cultural enrichment in order to circumvent the problems associated with complex food matrices. In other words, the assays may be performed in only minutes or hours, but the total analysis time is much longer because of the need for culture isolation or growth enrichment.

The number of rapid methods available for detecting bacterial pathogens and toxins in foods is impressive. Over 50 assays are commercially available for testing for *Salmonella* or for *E. coli* O157; and assays exist for virtually every major foodborne pathogen and toxin *(3)*. Rapid methods are usually more sensitive and specific than conventional microbiological methods used for the detection of pathogens and toxins in foods. The detection sensitivities for bacterial cells range from $10^4$ to $10^7$ cells, with most assays detecting around $10^5$. For toxins, most assays can detect mg of protein but some can attain pg levels of detection. However, despite the abundance of tests that afford better sensitivity, there are advantages and limitations to using rapid methods in food testing. For example, the continued reliance on culture enrichment is a time-limiting step for rapid methods, but enrichment provides essential benefits, such as diluting out effects of inhibitors, differentiating viable from non-viable cells and allowing the repair of stress-injured cells. Most rapid methods are single target assays and therefore ideally suited for screening large numbers of food samples for the absence of a particular pathogen, but they are less well suited for investigations in which the causative agent of an illness or outbreak is unknown. As a screening tool, negative results by rapid methods are accepted but positive results are regarded only as presumptive and need to be confirmed. Although confirmation, often done by conventional microbiological procedures, is time consuming and will extend analysis by several days, this may not be an imposing limitation, inasmuch as negative results are most often encountered in food testing. Conversely, however, the acceptance of negative results may be precarious, as samples giving false-negative results are not recognized and the product, if consumed, may result in human infections. It is therefore critical that the rapid method is fully evaluated for that specific commodity, as the detection efficiencies of these methods, many of which use different technologies and formats, can be food dependent. In the United States, methods that have been subjected to the collaborative study program of the Association of Official Analytical Chemists International are regarded as official or standard methods of analysis. This validation process entails a multi-lab, comparative evaluation of the new method versus standard method using multiple replicates of various food types, seeded with different levels of the target pathogen *(9)*. Also, once official status is granted, the methods must be performed exactly as specified in the protocol.

As detection methods improve, the level of detection sensitivity also increases, which can create some interesting problems. For instance, the current specification for many pathogens and toxins in ready-to-eat foods is "zero" or "absence". Since this criterion is determined by testing, the establishment of "absence" depends on the sensitivity of the assay. The problem with increased assay sensitivity is that it may give rise to situations in which foods previously analyzed by one method and found to have no pathogens may no longer meet the same specifications if a more sensitive method is used. This can create interesting challenges to the quality control programs of the food industry and to the regulatory positions of the state and federal health agencies, and may become a recurrent situation each time a more sensitive method is introduced.

As technology continues to advance at a rapid pace, next generation assays are already being developed that can detect specific targets with extreme sensitivity and in a short time. For example, biosensors use a biological component such as an antibody for specificity, coupled with a sensing component that converts biological signals into measurable, digital electronic readings *(10, 11)*. Some biosensors for detecting foodborne pathogens are already commercially available, and these assays can detect low levels of cells quickly and potentially may enable in-line monitoring for pathogens and toxins during food processing *(12)*.

Similarly, the rate-limiting gene amplification process in polymerase chain reaction (PCR) assays has been significantly reduced by the introduction of real-time PCR assays that not only allow rapid amplification of target genes but also provide real-time results. A number of real-time PCR assays using various detection technologies, such as Sybrgreen, FRET hybridization probes, TaqMan, molecular beacon, etc. have already been developed and are commercially available for testing for some of the major pathogens in foods.

The development of microarray technology has also augmented our capability of rapid characterization of foodborne pathogens. Phenotypic microarrays can simultaneously analyze a bacterial isolate for 2000 phenotypes, ranging from metabolic and growth requirements to antibiotic resistance *(13)*. Similarly, DNA microarrays, also known as gene chips, can perform several hundreds of thousands of tests in one reaction, so that potentially, it can not only detect but also genetically characterize multiple pathogens simultaneously *(14, 15)*. Currently, microarrays are used mostly in laboratory research and few diagnostic assays have yet been developed for testing for pathogens in foods.

It is clear that developments in rapid methods continue to advance at a great pace and to have a tremendous impact on diagnostic methods used in food testing. The various technologies used in these methods are giving us faster and more sensitive assays; however, shortening the testing time merely overcomes one of the hurdles in food testing, for, regardless of how sophisticated the technology or the assay format, the problems associated with the complexity of food matrices, including sampling and sample preparation, continue to present formidable challenges to developing methods to test for pathogens and toxins in foods.

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## REAL-TIME PCR


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Detection and identification of microorganisms in foods, animal feces, and environmental samples is a difficult task at best, and complications in sampling, low concentrations of target molecules, and interference from the sample matrix add to the complexity of detection methods. A straightforward approach for conceptualizing detection technologies and their feasibility is to categorize them into three groups: (1) traditional cultural methods, which involve enrichment for the target organism in liquid medium, plating onto selective agar/s, and confirmation of the isolate by use of a series of biochemical and other tests; (2) immunological-based assays, which rely on the binding of an antibody to an antigen of the bacterium; and (3) genetic-based methods, which rely on binding of segments of nucleic acids to bacterial DNA or RNA targets. Genetic methods include the polymerase chain reaction (PCR) and DNA hybridization assays. The PCR is considered a "rapid method", referring to a method that expedites the detection process.

### PCR

The PCR is a powerful technique that has transformed basic biology and has been incorporated into methods for the diagnosis of microbial infections and genetic diseases, as well as for detection of pathogens in food and environmental samples. Assays based on the PCR are now accepted methods for rapidly confirming the presence or absence of specific pathogens in foods. Conventional PCR methods for pathogen detection generally involve four steps: (1) nucleic acid extraction; (2) DNA amplification; (3) product detection by agarose gel electrophoresis; and (4) amplicon confirmation. Generally, the amplified DNA fragments are visualized by agarose gel electrophoresis and staining with ethidium bromide, which also allows determination of the PCR product size, or by hybridization of the amplicon with a labeled nucleic acid probe. Additionally, combining the PCR with a hybridization step enhances assay sensitivity and specificity. One disadvantage of traditional PCR is that post-PCR processing steps may result in transfer of small amounts of DNA from one run to the next, leading to false positive results.

### Real-Time PCR

An important advance in recent years is the development of homogenous assays for real-time fluorescence detection of PCR products in a closed-tube format. Real-time PCR has many applications, including gene expression analysis *(1)*, single nucleotide polymorphism (SNP) typing *(2)*, and pathogen detection *(3–6)*. Although real-time PCR is not currently used widely for routine pathogen testing by the food industry, an increasing demand for high-throughput screening in the clinical and pharmaceutical industries has produced several technological developments in methods for detecting and analyzing biological molecules, many of which could be applied to problems in the food industry. Compared to conventional PCR, real-time PCR methods offer the following advantages: (1) use of closed systems; therefore, lower potential for cross contamination and false positive results; (2) shorter analytical turnaround time; (3) higher sensitivity and precision; (4) no need for post-PCR processing steps, such as agarose gel electrophoresis to visualize and size the amplicon or Southern blotting to verify the amplified product; (5) greater assay capabilities (qualitative, quantitative, mutation, and multiplex assays performed using the same instrument); and (6) greater quantitation range for real-time PCR compared to traditional PCR (5–6 logs versus 2–3 logs, respectively).

Real-time PCR systems rely upon detection and quantitation of signals generated from a fluorescent reporter. The signal produced by the reporter increases in proportion to the amount of PCR product produced. The product yield (fluorescence) is plotted against cycle number, yielding a curve that represents the accumulation of PCR product over the duration of the PCR reaction. The log-linear phase of the reaction is used to determine the cycle threshold ($C_t$) for each sample. The $C_t$ is defined as the first cycle in which there is a significant increase in fluorescence above a specified threshold. For quantifying the PCR product, a standard curve is generated using $C_t$ values for

a series of reactions containing a known quantity of target DNA. Quantification is performed by comparing the $C_t$ values of unknown samples against the standard curve or against the $C_t$ values of an internal standard.

Different fluorescence systems can be employed for detection of production of the PCR product, and among the various chemistries available, SYBR Green I is the most economical and convenient to use. SYBR Green I is a thermostable intercalating dye that binds double-stranded DNA, resulting in an increase in fluorescence as the amount of PCR product increases. In assays using SYBR Green I, products are detected by programming the real-time PCR instrument to perform a melt curve at the end of the reaction. A drop in fluorescence is observed at the point in which the PCR product melts because of dissociation of the dye from the double-stranded DNA. Because the specific PCR product has a unique $T_m$, melt curves can distinguish between specific and non-specific products, including primer dimers. Dual-labeled (TaqMan) probes or molecular beacons are oligonucleotides that contain fluorescent and quenching dyes at the 5' and 3' ends, respectively. TaqMan probes bind to an internal region of the PCR product. During replication of the template, the exonuclease activity of the polymerase results in cleavage of the probe separating the reporter and quenching dyes, resulting in a measurable increase in fluorescence intensity. Real-time PCR assays based on the use of TaqMan probes for detection of food-borne pathogens have been described *(4, 6)*. Molecular beacons are oligonucleotides with a hairpin structure consisting of a sequence-specific portion (loop) and complementary arm sequences located on each side of the probe sequence. The complementary arm sequences that form the stem of the haripin are end-labeled with the fluorophore and the quencher dyes. During the reaction, the probe sequence in the loop hybridizes to a complementary sequence within the PCR product. The conformational change that occurs distances the quencher from the reporter dye, yielding fluorescence *(5, 7, 8)*. The fluorescence resonance energy transfer (FRET) principle makes use of two oligonucleotide probes, one labeled with a donor fluorochrome at the 3' end and the other labeled with an acceptor dye at the 5' end. The probes hybridize to the target sequences so that they are distanced by one or a few bases and are oriented head-to-tail. When in that position, the energy emitted from the donor excites the acceptor dye, which then emits fluorescent light at a longer wavelength. The amount of target DNA produced is proportional to the ratio of the fluorescence of the donor and the acceptor. Fluorescence is measured after the annealing step of the PCR when both probes are hybridized to the target DNA *(9)*. Other types of fluorescence systems developed for real-time PCR assays include Scorpion probes, LUX (Light Upon Extension) primers that are designed to be self quenched until they are incorporated into the PCR product resulting in an increase in fluorescence due to a change in the secondary structure, Amplifluors, MGB Eclipse probes *(10)*, and others.

Several instruments currently available for performing real-time PCR include the LightCycler (Roche Diagnostics Corp.), the RAPID (Idaho Technologies), the iCycler iQ (Bio-Rad), the MX4000 (Stratagene), the Rotor Gene (Corbett Research), the ABI Prism 7000 and 7900HT (Applied Biosystems), and the Smart Cycler (Cepheid, Inc.). The RAPID and Smart Cycler instruments, originally designed in conjunction with the military to detect biological warfare agents in the field, are available as portable instruments. The design of the Smart Cycler is unique compared to other real-time PCR platforms because each processing block contains 16 independently controlled, programmable I-CORE (**I**ntelligent **C**ooling/heating **O**ptical **R**eaction) modules. Sixteen different PCR protocols can be run simultaneously, which facilitates optimization of PCR assays. Up to 6 Smart Cycler processing blocks can be linked together, allowing simultaneous analysis of 96 discrete samples. The LightCycler and the instruments from Applied Biosystems can be coupled with automated nucleic acid extraction instruments called the MagNa Pure LC and ABI Prism 6700 or 6100, respectively.

Real-time reverse transcriptase PCR (RT-PCR) targeting viral RNA or bacterial mRNA instead of DNA can also be performed. Reverse transcriptase is used to amplify RNA into cDNA. This is followed by real-time PCR, which copies the cDNA while incorporating fluorescent dyes or probes into the product. Fabre et al. (2003) used a one-step real-time RT-PCR assay employing TaqMan probes for detection and quantitation of the Barley yellow dwarf virus. The assay was 10 to 1000 times more sensitive than standard RT-PCR and ELISA assays. In multiplex real-time PCR assays, multiple sequences are amplified simultaneously in a single reaction, by use of probes labeled with different colored fluorophores that have unique emission spectra. A multiplex PCR assay employing TaqMan probes was developed to detect *Ralstonia solanacearum (11)*. One probe, labeled with the FAM dye, was used to detect all biovars of the organism, while the other probe, labeled with the VIC dye, detected only biovar 2A. A third primer set and probe set targeting the potato cytochrome oxidase gene was used as an internal control for the real-time PCR assay. Bellin et al. *(13)* developed a multiplex PCR targeting the genes encoding Shiga toxin 1 and Shiga toxin 2 in Shiga toxin-producing *E. coli* using FRET hybridization probes and a LightCycler instrument.

## Detection of *E. coli* O157:H7 by Multiplex Real-Time PCR

A multiplex real-time PCR assay employing TaqMan probes was developed to detect *E. coli* O157:H7 in foods. Four target sequences of the *E. coli* O157:H7 $fliC_{h7}$, $rfbE_{O157}$, $stx_1$, and $stx_2$ genes were amplified simultaneously; the sizes of the PCR products were 171, 114, 199, and 157 bp, respectively. The probe for the $fliC_{h7}$ PCR product was labeled with 6-carboxy-4,7,2',7'-tetrachlorofluorescein (TET) and the Black Hole Quencher 1 dye, the probe for $rfbE_{O157:H7}$ with 6-carboxyfluorescein (FAM) and Black Hole Quencher 1, and the probes for $stx_1$ and $stx_2$ were both labeled with Texas Red and Black Hole Quencher 2. Ground beef samples (25 g) were inoculated with ca.1 to 5 CFU of a cocktail of three strains of *E. coli* O157:H7, stored at 4°C for 72 h or at -20°C for 2 weeks, then subjected to enrichment in 225 ml of Rapid-Chek *E. coli* O157:H7 enrichment medium, BCM O157:H7(+) broth, and modified *E. coli* broth containing novobiocin for 8 and 20 h at 42°C at 150 rpm. DNA extraction using the PrepMan Ultra (Applied Biosystems) reagent was performed, using 1 ml of the enrichment. *E. coli* O157:H7 was detected in enrichments incubated for 8 h by the real-time multiplex PCR assay using the Smart Cycler. Thus, the assay can be employed for rapid detection of *E. coli* O157:H7 in ground beef, and potentially other types of samples as well (unpublished results).

## CONCLUSIONS

Although conventional PCR or real-time PCR systems can theoretically detect 1 copy of the target sequence, in practice this is generally not possible when dealing with food, fecal, or environmental samples. Enrichment of the sample for at least 6 to 8 hours in a suitable enrichment medium must be performed

prior to nucleic acid extraction and DNA amplification. PCR-based assays are generally more sensitive than culture-based methods, and numerous reports have appeared in recent years on the use of the PCR for pathogen detection. In addition, many conventional and real-time PCR-based kits for pathogen testing are becoming commercially available. Compared to conventional PCR, real-time PCR can reduce overall assay time by eliminating post-PCR processing steps such as agarose gel electrophoresis and Southern blotting and also permits reliable quantification of template DNA. However, for the use of real-time PCR assays for routine screening of samples for the presence of pathogenic organisms to become a reality, additional research in the development of rapid, simple, and inexpensive assay systems for high-throughput automated sample processing and detection of pathogens in foods and other types of samples is critical.

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## BIOSENSOR DETECTION OF *SALMONELLA* IN SPROUTS


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### Fiber-optic biosensors

Evanescent wave fiber-optic biosensors use an emerging innovative technology to rapidly detect specific microorganisms and their toxins. These biosensors can identify target analytes in minutes directly from complex matrix samples, significantly improving the detection sensitivity, selectivity, and speed. The biosensors use a 635-nm laser diode to provide the excitation light that is launched into the proximal end of a waveguide, an injection molded optical polystyrene fiber. Fluorescent molecules within approximately 100-1000 nm of the waveguide surface are excited by an evanescent field, and a portion of their emission energy recouples into the fiber. A photodiode allows for quantitation of the collected emission light at wavelengths above 650 nm. The fiber-optic biosensor assay is based on a sandwich immunoassay that utilizes antibodies or other molecules to capture and detect the target pathogen. The captured target analyte is tagged with cyanine 5-labeled (Cy5) antibody. Emission from the Cy5 is recorded in picoAmps (pA). The data are expressed as increases in fluorescence that are proportional in rate and magnitude to the target pathogen concentration. The complex nature of many sample matrices as well as the presence of particulate matter in samples often severely reduces the sensitivity and specificity of conventional bacterial detection systems. Relatively dirty sample homogenates can be rapidly tested directly by use of the evanescent wave fiber-optic biosensor. In addition, live microbial targets can be recovered from fiber optic waveguides to determine microorganism viability, confirm identification, and preserve as evidence *(1)*. The evanescent wave biosensor (Analyte 2000) manufactured by Research International (Monroe, Washington, USA) has been used to detect toxins such as staphylococcal enterotoxin B *(2)* and pathogens such as *Escherichia coli* O157:H7 *(3–5)*.

The usefulness of the fiber-optic evanescent wave biosensor detection system has increased dramatically with the manufacture of an automated portable device for biowarfare/bioterrorism agent detection in the battlefield environment (RAPTOR, Research International). This instrument simplifies the assay even further by automatically performing a user-defined, multi-step assay protocol to interrogate four distinct assays for a single sample or for four different samples. To perform an assay, the user snaps in a disposable pre-prepared coupon, adds a liquid sample, and pushes the "run assay" button. The assay is completed in five to fifteen minutes. The positive, negative or suspect positive results for each of the four waveguides are displayed in the LCD panel of the machine. The data can also be downloaded to a personal computer for more detailed analysis if desired. The coupon can be reused up to 40 different assays as long as the pathogen(s) under interrogation is not detected.

### Sprouts and spent irrigation water

Raw seed sprouts are perceived as a healthy and beneficial food. However, recent outbreaks of *Salmonella*, *Bacillus cereus*, and *Escherichia coli* O157:H7 infections in the United States and abroad have been linked to consumption of raw sprouts *(6–9)*. The Food and Drug Administration (FDA) has issued health warnings for consumption of sprouts, stating that persons in high-risk categories (i.e., children, the elderly, and the immunocompromised) and persons who wish to reduce their

risk of foodborne illness should not eat raw or lightly cooked sprouts *(10, 11)*. The evidence points to sprout seeds as the source of the contamination *(12)*. Soaking sprout seeds in a 20,000 mg/liter (ppm) calcium hypochlorite solution for 15 minutes is the recommended chemical seed treatment currently approved *(1)*, but no FDA approved treatment eliminates all bacteria from sprout seeds *(6, 13)*. When even small numbers of pathogenic bacteria are present on sprout seeds, the bacteria multiply exponentially to infectious doses because of the microbiologically favorable warm, moist, nutrient-rich conditions found during the sprouting process *(7)*.

The bacterial counts of the spent irrigation water used in the sprouting process have been shown to be within approximately one log of the bacterial counts found in the sprouts *(14, 15)*. The spent irrigation water used to irrigate sprouts can be a carrier of many microorganisms, including pathogenic strains of *Escherichia coli* and *Salmonella enterica*. Sprout producers have been advised by the FDA to include microbiological testing of spent irrigation water at least 48 hours after seeds have germinated as part of an overall strategy to enhance the safety of sprouts *(1)*. Microbial analysis for the detection of specific microorganisms is labor intensive, typically requires highly trained individuals, and takes many days to complete *(16)*. Therefore, there is a need for a rapid and automated assay targeted to detect potential pathogens in sprout spent irrigation water.

### Testing Spent Irrigation Water Using the Biosensor

A rapid (20 minute) RAPTOR assay targeted to detect *Salmonella* in sprout spent irrigation water has been developed. The spent irrigation water collected from sprouts grown from alfalfa seeds contaminated with various concentrations of *S.* Typhimurium was assayed by use of the RAPTOR. The sandwich immunoassay consisted of a polyclonal antibody, affinity purified for common structural antigen-1 of *Salmonella* (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) used for *Salmonella* capture and a monoclonal antibody to *S.* Typhimurium (BiosPacific, Emeryville, CA) used for detection. This antibody combination could directly detect a minimum of $10^5$ CFU/ml when *S.* Typhimurium was inoculated directly into alfalfa sprout spent irrigation water. *S.* Typhimurium was then inoculated into seeds, and the seeds were germinated. *S.* Typhimurium could be positively identified in spent irrigation water that had been collected 67 hours after seed germination when the *S.* Typhimurium concentration in the seeds was only 50 CFU *S.* Typhimurium/g of seed. The specificity of the biosensor assay described here lies in the specificity of the BiosPacific antibody. The assay did not detect the background bacterial flora indigenous to sprout spent irrigation water. There was no positive detection when the biosensor assay was used to test uninoculated seeds even though the spent irrigation water recovered during the sprouting of uninoculated seeds contained high levels ($10^5$–$10^6$ CFU/ml) of background bacteria. In addition, it was possible to recover *S.* Typhimurium colonies from waveguides after completion of the biosensor assay when the spent irrigation water that was tested had been collected from sprouts that were grown from seeds contaminated with at least 5 CFU *S.* Typhimurium/g of seeds.

The alfalfa sprouting process takes five to seven days, so that even after three days, the seeds have not finished sprouting. Collection and biosensor testing of sprout spent irrigation water from three-day-old sprouts would therefore be a very workable and commercially feasible alternative solution for the sprouting industry. Ideally, an in-line biosensor assay should be capable of detecting *Salmonella* in spent irrigation water produced from seeds contaminated with levels even lower than 50 CFU *Salmonella*/g of seeds. Studies to improve the sensitivity of the biosensor assay are on-going in our research laboratories. The assay does, however, show proof-of-concept of how a biosensor assay that detects *Salmonella* could be utilized. The automated detection system could potentially be set up to run automatically in-line in the spent irrigation water piping system in order to have the capability to continuously detect the presence of pathogens in the spent irrigation water.

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## MOLECULAR IDENTIFICATION OF *SALMONELLA* SEROTYPES

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Serotyping has been the cornerstone of characterization of *Salmonella* isolates for epidemiologic surveillance and outbreak investigations for almost 80 years *(1)*. Serotype is determined in an immunologic assay that detects variable epitopes on two surface structures, LPS (O antigen) and flagella (H antigen) *(2)*. While serotyping has been of great value in understanding the epidemiology of *Salmonella* and is technically quite simple to perform, it has several drawbacks. Typically, it takes a minimum of three days to determine all antigens. Obtaining the variety, quality, and quantity of the antisera required to perform serotyping has been problematic. Outside the public health field, demand for these reagents is low; thus, commercial suppliers have reduced or stopped regular production of essential typing reagents. Hundreds of different antisera are required for routine serotyping, each of which must be produced and continuously quality controlled independently.

To circumvent the technical problems associated with production and maintenance of high quality serotyping reagents, we initiated the development of a DNA-based system for the determination of serotype that could be used to complement or replace traditional methods. Molecular identification of the genes responsible for expression of serotype antigens, rather than serologic identification of the antigen itself, has several advantages. The probes are DNA molecules rather than absorbed sera, making their production and quality control easier and more reproducible than is the case for antisera. The reagents required and techniques employed to perform DNA-based assays are fairly universal and becoming common in the clinical lab; thus, "molecular serotyping" could be performed in more laboratories than traditional serotyping. Molecular methods have the potential to be faster and more specific than traditional methods, to be automated, and to identify rough and problematic isolates.

The genetic basis for serotype is well understood. Many of the genes involved in O antigen biosynthesis are organized in a large regulon, the *rfb* region *(3)*. Although *rfb* regions can be quite diverse, they are flanked by the same two genes in *Salmonella (4)*. A single set of primers corresponding to the sequences flanking the *rfb* region can be used to amplify the *rfb* region from different O groups by use of the polymerase chain reaction (PCR). Salmonellae are unique among enteric organisms in that many serotypes possess two different flagellar antigens. They are encoded by two genes, *fliC*, which is common to all enteric organisms and *fljB*, which is unique to *Salmonella (5)*. These two genes are located in different regions of the genome; they are flanked by different sequences, so they can be amplified by PCR and sequenced independently. A substantial amount of DNA sequence data for genes responsible for serotype is already available in public databases. Both *rfb* regions and flagellin alleles exhibit substantial diversity, supporting the idea that molecular serotyping is technically feasible.

To identify sequences specific for serotype antigens in *Salmonella*, we sequenced the *rfb* region and alleles of *fliC* and *fljB* and from a panel of *Salmonella* serotypes, with the goal of sequencing genes representative of the 119 flagellar antigens and the 46 O antigen groups. Our initial focus has been on the "top 100" serotypes, which represent 98% of the clinical isolates in the United States. DNA was extracted from bacterial cells grown overnight on trypticase soy agar. Primers corresponding to sequences flanking *fliC*, *fljB*, and the *rfb* region were used to amplify the three loci by PCR. A combination of subcloning and primer walking was used to sequence *rfb* regions. A panel of eight sequencing primers was used to sequence most *fliC* and *fljB* alleles. Additional primers were used as required for specific alleles. DNA sequences were assembled and analyzed with the use of Lasergene 99 (DNASTAR, Inc, Madison, WI) software and the Wisconsin Package version 10.1 (Genetics Computer Group, Madison, WI). The National Center for Biotechnology Information (NCBI) BLAST server (http://www.ncbi.nlm.nih.gov/BLAST/) was used to search GenBank for sequence homologies.

The complete nucleotide sequence was determined for the *rfb* region from ten O serogroups: serogroups F, G, H (2 sequences), I, J, K, O, P, S, and Y. They ranged from 6 to 14 kb, with an average length of 11 kb. An additional 8 *rfb* regions sequence determinations are in various stages of completion; enough DNA sequence data has been determined for 6 of these serogroups to identify serogroup-specific targets. These sequences, plus the ten *rfb* region sequences that were determined by other groups *(4, 6–8)* represent all the O serogroups found in the top 100 serotypes, and 28 of the 46 identified O serogroups.

Comparisons of the *rfb* sequences determined here with those in GenBank identified many sequences potentially unique to a serogroup. *wzx* (flippase, exports the O subunit to the cell surface) and *wzy* (the O antigen polymerase) were the most diverse genes and were typically targeted in prototype group-specific PCRs. Serogroup-specific PCR tests were developed and/or validated for 19 serogroups: 16 serogroup-specific PCRs were developed based on the sequences determined here; four serogroup-specific PCRs were based on published sequences; and three serogroup-specific PCRs that had been reported in the literature (Luk et al.,1993) were validated. The specificity of the PCR primers were validated on a panel of 398 strains that represented the top 100 serotypes plus all other serogroup/subspecies combinations.

The complete nucleotide sequence was determined for approximately 400 flagellin alleles (1.5 kb each), representing at least two alleles each for most antigenic types and all flagellar antigens represented in the top 100 serotypes. Analyses of the DNA sequences determined here and the flagellin alleles available in the GenBank database identified sequences potentially unique to a flagellar antigen type. The specificity of the flagellar antigen target sequences were tested in a PCR DNA-enzyme immunoassay (EIA) format. A multiplex PCR specific for *fliC* and *fljB* was performed, followed by hybridization of the PCR fragments with antigen-specific probes in the EIA. Sixteen probes that identify H antigens from the top 100 serotypes as well as 3 additional probes have been validated against a representative panel of isolates that included all H antigen types. In addition, five probes, each detecting a group of related antigens (the L complex, the 1 complex, the EN complex, the G complex and the $Z_4$ complex) have been designed and validated.

We have begun the development of a serotype determination method that is based on DNA markers. One of our goals for a DNA-based system is that it correlates with the current serotyping scheme as closely as possible, which will allow the results from

the two serotyping systems to be compared. Compatibility between molecular and traditional serotyping methods will ensure continued worldwide surveillance and outbreak identification based on serotype and will preserve the value of historical serotype datasets.

As appropriate DNA targets are identified for individual serotype antigens, reference laboratories should be able to determine serotype by using the PCR (for O antigens) and PCR-probe (for H antigens) formats described here. However, in order to transfer the technology to clinical and public health laboratories, all the DNA targets must be brought together is a unified, simple format. Microarray technology is the most promising of those currently available, since it allows the probing of hundreds to thousands of oligonucleotides in a single, automatable step *(9)*. Alternatively, technology to detect up to a hundred different fluorescent probes in a single reaction is also being developed *(10)*.

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## BIOCHIP/MICROARRAY APPLICATIONS FOR THE FOOD INDUSTRY


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DNA chips have been described for almost 10 years now. This abstract aims to present the principle and current applications of DNA chips, especially in the field of food applications.


### Implications of sequence databases evolution for molecular diagnostics

For many years, molecular biology techniques have been used for research purposes. In addition to being an essential knowledge-seeking tool, they have definite diagnostic value. In fact, this value is constituted by the nucleotide sequence itself. It is the specific combination of the four nucleotides which provides a unique genetic fingerprint for differentiating bacterial species, strains, and virulence factors, for assessing antibiotic or anti-viral resistances, etc.

Therefore, knowing the exact nucleotide sequences is the essential prerequisite for developing a molecular diagnostic test. Regarding taxon-specific probes, not only is it necessary to know the sequence representing the targeted element, e.g., the 16S ribosomal RNA sequence of *Listeria monocytogenes*, but also those of the neighboring taxonomic species, e.g., 16S sequences from other *Listeria* species, in order to address the specificity of the probe in complex matrices such as food or food enrichments.

The academic need to know the "tree of life" (see the related website) in terms of nucleotide sequences has led over the past twenty years to exponential growth in public and private sequence databases (EMBL, Genbank, TIGR and other banks compiled by therapeutic or diagnostic companies). Information processing tools now known as "bioinformatics" have followed this trend (tools such as ENTREZ are accessible to anyone on the Internet).

Having this knowledge, access to target sequences in clinical, food or environmental samples is obtained by use of various techniques, depending on the level of information required. Qualitative detection of a specific target will, for example, require a hybridization probe (a restriction fragment from the invasine *inv*A gene of *Salmonella* for its detection and/or confirmation), an oligonucleotide, a SSCP-gel ("Single Strand Conformation Polymorphism") or enzymatic sequencing on gel according to the Sanger method to determine sequence variations within a polymorphic marker.

Apart from this qualitative aspect (presence/absence of the sequence and therefore of the organism containing it), the quantitative aspect is important in some contexts, e.g., determination of the *Legionella pneumophila* load in water as a decision threshold. Hence the variety of molecular diagnostic techniques is endless *(1)*, which is a sign that they are still perfectible and have not yet reached their maximum potential *(2)* and explains why such methods are still not used on a wide scale routinely. However, the constant investments made by public and industrial research centers are justified by the enormous potential involved:

1. sensitivity due to target amplification, still limited by sampling/extraction technologies,
2. exquisite specificity due to the sequence databases knowledge
3. detection of non- or fastidious- culturable organisms (viruses, parasites)
4. multi-detection possible from the same sample (bacteria, virus, parasites, yeasts, animals, plants)
5. versatility/high resolution (detection in the same test of an important single point mutation of organism 1 and of serotypes sequence polymorphisms of organism 2)

These last 2 points are the privileged areas of DNA chips.

### What are "DNA chips"?

This generic term refers to an evolution in the reverse dot-blot hybridization technique that aims to analyze natural or amplified nucleic acids *(3)*. The words DNA "micro-arrays" or "biochips" are also used, although a biochip can also integrate other functions of molecular diagnostics, such as the extraction and the amplification. DNA chips are flat surfaces, generally very small (less than 1 cm²), that contain tens to millions of oligonucleotide DNA probes. Therefore, in practice their manufacturing is a fantastic technological evolution combining the expertise of different fields. Biophysics and biochemistry are

used to synthetize nucleic acids and control their adsorption on solid surfaces, to fragment and label amplicons, in micro-manufacturing techniques to deliver small surface disposables with a high probe density, in micro-optics to catch very low signals, in bioinformatics technology to design these micro-arrays and to interpret the hybridization data, etc. The basic notion underlying DNA chips is "a lot of materialized genetic information."

### Why DNA chips? Applications in the food industry

The most important application of DNA chips today lies in the acquisition of knowledge in basic biological science through the "expression monitoring" technique. It consists in the comparison of the relative quantities of messenger RNA expressed by several genes of interest (up to several thousands!) at the same time in the same experiment. Comparison of the data between a reference sample and a test sample allows one to infer the effect of a given variable. It is mainly used to evaluate the therapeutic efficacy of various anti-tumor or anti-infectious substances *(4)*. In the field of food safety/risk assessment, a few applications have been described. Van Hal et al. *(5)* checked unintended side effects by comparing individual nutrient effects on the genetic make up of GMO or natural tomatoes. Another team analyzed the effect of food components by studying functional and toxicological effects, using human intestine cells cultures (CaCo-2). In the field of food quality, people try to better manage microbial growth in food by developing gene expression databases for microbial response to pH, temperature, and water content.

With regard to diagnostics, DNA chips can be used to characterize more exhaustively a single isolate vis-à-vis several criteria: species identification, antibiotic susceptibility status, and even strain epidemiology. We applied this approach to fastidious organisms such as mycobacteria *(6)*. Strains of a single species can be differentiated by use of MLST, or Multi Locus Sequence Typing *(7)* whereby polymorphisms of several loci of one bacterial genome are aggregated to cluster a given individual. Our team applied this principle by use of the DNA chip format for the differentiation of *Staphylococcus aureus* strains *(8)*. Using the published genome of *Salmonella* Typhimurium materialized on one chip as a reference, a Stanford University team is currently differentiating the *Salmonella* genotypes *(9)*. Differentiation on a chip of O157:H7 genotypes from other pathogenic and non-pathogenic *E. coli* was achieved by Call et al. *(10)* and applied to poultry carcass rinse by Chandler et al. *(11)*.

The versatile power of this technique can also be used to screen for the presence of very different organisms directly in a sample in a single test, provided all the previous steps are available. For example, we showed the feasibility of the sensitive detection of 13 bacteria, virus and parasites in a single test in one day to assess the microbiological quality of tap water (unpublished results). Virus detection required numerous probes in order to detect comprehensively all variants of one group, whereas enterotoxigenic *Enterobacteriaceae* demanded the robust detection of one specific point mutation. Such a multi-detection approach to the detection of pathogens or spoilage organisms is being evaluated by several teams on samples amenable to direct nucleic acid extraction, such as liquids (milk, water rinses of food materials), for simultaneous detection of pathogens such as *Salmonella, Listeria, Campylobacter*, and Noroviruses.

Complex microbial communities can now be observed with a more comprehensive eye than is possible with the biased culture technique. Randazzo et al. *(12)* compared the bacterial ribosomal 16S gene content of raw milk at various fermentation stages of a traditional Italian cheese in order to assess a traceable and reproducible quality index. Although it utilized gels (DGGE), this application could well benefit from the DNA chip format more suitable for diagnostics. A Lawrence Livermore team has built a bacterial air database using an Affymetrix chip, identifying 450 taxons, and has sampled 8 sites (city and country) using a 2.5-day protocol that uses eubacterial 16S PCR. This will allow the establishment of the baseline of a future monitoring system (ASM 2003 annual meeting poster Q-221, Andersen et al.).

Because nucleic acids are universal to living organisms, the genetic characterization of a food sample might also include quality information relating to its plant and animal ingredients. Today, GMO tracking requires the screening of more and more authorized and non-authorized ingredients. Tomorrow, allergens (of plant or animal origin) might be tested for because of future regulations.

Finally, with DNA chip technology, limitations are no longer put on the knowledge of the genetic content of a sample. The remaining challenges now lie in the previous steps: statistically representative sampling, efficient (sensitive) nucleic acid extraction directly from the sample, and multiplex amplification, as well as in their integration into user-oriented solutions *(13)*.

## CONCLUSION

DNA chip technology is now a reality in research. It has been shown to be robust enough to be amenable to molecular diagnostics. Because of its specific quality of high genetic information content, it allows a more precise and comprehensive risk assessment: multi-detection of several pathogens of different nature can now be detected in one test.

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## INTERNATIONAL STANDARDIZATION AND HARMONIZATION OF DETECTION METHODS

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Users of laboratory data must believe that results are reliable and reproducible and they must be confident that the laboratory is technically competent to produce that data. Even in the best of times microbiological results may not be reliable. Microorganisms are not uniformly distributed in supporting matrices and therefore their detection is very much dependent on the source, number and volume of samples collected for analysis. Transportation method, time and temperature can also influence recovery, as many organisms are sensitive to environmental conditions and stress. Cultural conditions for the expression of virulence characteristics may differ from those that simply support growth. In addition, plasmid-mediated virulence factors may be lost because of inappropriate incubation conditions or excessive subculturing. Even within the same genus and species, microorganisms display an incredible diversity of phenotypes, serotypes and genotypes that frequently require special analytical techniques to elucidate. To further compound the problem, microbial taxonomists regularly rename organisms as new genetic information is uncovered, and those who are not aware of these changes become confused with the new names.

Regardless of these other considerations, one of the most significant factors that contributes to the lack of confidence in data reliability is the choice of the methods used by different laboratories. Traditional cultural methods often lack the sensitivity of more rapid techniques such as immunoassays, polymerase chain reaction (PCR) and bio- and chemiluminescence, but are often the methods of choice because of historical use. Laboratories have many options to choose from when selecting methods, but often the choice is determined by the client and/or by a mistaken assumption that the only valid methods are those that are used or recognized by regulatory agencies. In spite of the plethora of method options and the number of different situations to which they may be applicable, such as regulatory enforcement, screening, outbreak investigation, etc., the concept of "fit for purpose" has been difficult for both laboratories and their clients to accept.

There are many elements to ensure a laboratory's technical competence, giving credence to its analytical results, including the use of appropriate methods, validation of laboratory capability and ensuring analyst competency. The use of appropriately validated methods is one of the essential components required by ISO Standard 17025 for laboratory accreditation *(3)*. Currently, very little guidance is given to laboratories for choosing methods that are "fit for purpose". Although there are some common elements to choose from when validating methods, such as precision, matrix effects, sensitivity and specificity, these may vary depending on whether the method is qualitative or quantitative. Similarly, laboratories do not know how the validation of methods from one organization compare with the validation criteria used by another. In addition, methods developed in one laboratory, e.g., to address emergency situations, are frequently not communicated to other laboratories dealing with the same or a similar situation. In order for microbiology laboratories to more effectively co-operate on food safety and security issues, a mechanism needs to be developed that encourages the use and sharing of appropriately validated analytical methods. Using harmonized method validation criteria would provide consistency in the evaluation of new/modified methods worldwide.

In October 2001, AOAC International was awarded a 3-year cooperative agreement with the United States Department of Agriculture, Food Safety and Inspection System to develop such a mechanism *(1)*. Why was AOAC chosen for this task? AOAC started in 1884 as the Association of Official Agricultural Chemists under the auspices of USDA to develop standardized methods for fertilizers. Dr. Harvey W. Wiley, the Director of the Bureau of Chemistry, was also Director of AOAC. The methods were not formally published until 1920, with the first edition of AOAC Official Methods of Analysis or OMA. With the issuance of the Pure Food and Drug Act in 1906, AOAC was charged with developing standardized analytical methods for food additives, pesticides, chemical contaminants, natural toxicants and microbiological contaminants. In 1927, the Food and Drug Administration assumed responsibility for AOAC and legislated the use of AOAC-OMA for all regulatory actions. In 1965, the name of the organization was changed to Association of Official Analytical Chemists to better reflect its major activities. AOAC became independent from FDA and began to operate as a not-for profit association. In 1991, the association adopted just the acronym, AOAC International, as its official name to illustrate the broader scope of its membership and method validation programs. That year also saw the implementation of the AOAC Research Institute (RI) to address the issues around the emergence and validation of rapid, proprietary methods. The majority of these methods were and continue to be microbiological. Since the birth of the Performance Testing Program, the RI has validated more than 52 rapid, proprietary microbiological assays, including 10 for *Listeria*, 15 for *Salmonella* and 5 for *Escherichia coli*. In 1998, AOAC was accredited for their proficiency-testing program by A2LA in accordance with ISO 43-1. With the recognition that acronyms don't always reflect the focus of an organization, AOAC adopted the tag line "Association of Analytical Communities" in 2001. This history and experience has put AOAC in a unique position to become the "gatekeeper" for analytical methods. With the help of federal funding and the support of the international scientific community, AOAC initiated the e-CAM project, an internet-based, interactive methods classification system that will serve as a repository for methods classified by validation criteria to be used by the global analytical community.

Under e-CAM, there will be five separate categories of methods. One category is designated REG for regulatory methods. These are methods currently used or specified in regulations by national and international regulatory agencies for enforcement purposes.

The other 4 categories are differentiated by the degree of validation.

### Harmonized collaboratively validated (HCV) methods:

Methods validated through a full collaborative study that meet the standards set forth in the international AOAC/IUPAC/ISO harmonized protocol or ISO 5725 for chemistry methods and ISO 16140, or AOAC Methods Committee Guidelines for

Validation of Qualitative and Quantitative Microbiological Methods for full collaborative study *(2)*.

**Multiple-laboratory validated (MLV) methods:**

Methods validated through multiple laboratory studies not meeting the standards set forth in the international AOAC/IUPAC/ISO harmonized protocol or ISO 5725 for chemistry methods or ISO 16140, or AOAC Methods Committee Guidelines for Validation of Qualitative and Quantitative Microbiological Methods, but following a written, accessible validation protocol.

**Single-laboratory validated (SLV) methods:**

Methods validated through single laboratory studies meeting the standards set forth in the international AOAC/IUPAC/ISO harmonized protocol for chemistry methods or ISO 16140 or AOAC Methods Committee Guidelines for Validation of Qualitative and Quantitative Microbiological Methods. Microbiology methods must include a methods comparison study conducted in a single laboratory.

**Developmental non validated (DNV ) methods:**

Methods that appear useful or applicable to meet an analytical need but have not been validated to a level that meets Single-laboratory Validation criteria. Includes the most up-to-date methods, which may not yet be optimized or fully characterized with respect to performance characteristics.

**e-CAM's content and features** *(1)*

The entire database will be designed to be user-friendly. The Home Page will provide background information on the method validation program and outline the purpose of e-CAM. The database contains instructions for submitting a method, together with a template for doing so directly on-line. It will also contain links to discussion groups, including user feedback on the performance of the method and Buyers' Guide (instrumentation and services vendors).

In addition to using validated methods, laboratories need to adopt harmonized procedures to verify their capabilities and to ensure the competency of their analysts. Internal quality control programs, which are designed to ensure that all critical aspects of the laboratory are under control (Laboratory HACCP), are essential prerequisites. Interlaboratory Proficiency Testing programs are useful for estimating the relative accuracy and precision of results between laboratories and to verify a laboratory's capabilities for designated analytes in selected matrices. A well-designed Intralaboratory Proficiency Testing program, however, will not only provide an ongoing assessment of analytical performance capability by individual analysts within the laboratory, but will also generate data that can be used for the estimation of uncertainty of measurement.

## CONCLUSION

International standardization and harmonization of detection methods initially requires a globally recognized classification system to assist laboratories in choosing methods that are fit for purpose. Participation in proficiency testing programs, as part of laboratory accreditation to the specifications in ISO Standard 17025, is an important component to validate laboratory capability and adherence to a Quality System. However, developing and maintaining analyst competency for each method, on an ongoing basis, is perhaps the most critical challenge faced by most laboratories, regardless of accreditation status.

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# *SCIENCE-BASED SHELF LIFE DATING OF READY-TO-EAT REFRIGERATED FOODS*

## HISTORY OF USE AND CONSUMER PERCEPTION OF CODE DATES

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Open dating of perishable foods became popular in the 1960s when dates were first put on milk. Dates are now found on almost all perishable products sold at retail. The terminology varies, including "sell by," "use by," and "best if used by." The basis for dating has been the optimal quality of the product. Dates are put on products by the manufacturer or the retailer. Several efforts have been made to standardize the use of code dates, including those of the Conference of Weights and Measures and the Food and Drug Administration. However, there is no uniform national approach. Consumers strongly favor dates on products, although there is inconsistent understanding among the public of what the dates mean. Consumers often equate the product date with safety. Dating food for safety presents new challenges that must be based on science. Consumer education is also needed to promote understanding of the use of product dates and the relationship between time/temperature storage and food safety.

## MICROBIOLOGICAL CONCERNS RELATED TO REFRIGERATED READY-TO-EAT FOOD

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Among the principal considerations processors should address before investing in new product lines of ready-to-eat refrigerated foods is the microbiological safety of such products. Several bacterial pathogens, such as *Listeria monocytogenes* and nonproteolytic *Clostridium botulinum*, can grow at refrigeration temperature. In addition, temperature control in refrigeration units of retail outlets and homes is often unacceptable for foods that rely solely on refrigeration temperature to control foodborne pathogens. Under these conditions, additional pathogens such as *Salmonella* spp. and *Escherichia coli* O157:H7 would also be of concern because of their ability to grow at 7–8°C. The types of foods of greatest concern for contamination by psychrotrophic pathogens are those that support pathogen growth to large numbers or toxin production during the refrigerated shelf life of such products. This is of utmost concern when hazardous levels of pathogens or toxins occur and the product remains edible. Strategies to extend the shelf life of foods by eliminating or suppressing spoilage microorganisms could result in conditions that favor pathogen growth or toxin production if contamination by harmful psychrotrophs occurs. Examples may include foods that are packaged under modified atmosphere or that are precooked or partially processed and lack suitable antimicrobial properties to suppress pathogen growth. Shelf life limitations can be an important safety net for processors of ready-to-eat foods, especially those at high risk of pathogen contamination and growth.

## PRINCIPLES FOR DETERMINING IF A PRODUCT REQUIRES SHELF LIFE DATING

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The recently completed Food and Drug Administration/ Food Safety Inspection Service *Listeria monocytogenes* risk assessment for ready-to-eat foods reinforces the belief that most cases of listeriosis result from the consumption of high numbers of the pathogen by susceptible persons. The risk of illness per food serving is sensitive to the initial contamination levels and any growth that occurs during storage. Although an effective control is the prevention of growth, *L. monocytogenes* will grow in many ready-to-eat foods, particularly if growth to high numbers is possible without the food appearing spoiled. Therefore, warning consumers to avoid excessive storage times is a potential control in growth-permitting foods. Determining likely growth depends on pathogen strain, inoculum method, cell history, competitive flora, time to spoilage, and anticipated time/temperature profiles during storage. A decision on how much growth would be tolerated depends on the desired degree of protection. A validated process risk assessment using scientific data, probable parameter values (such as those used for temperatures), variation/uncertainty in the parameters, and a desired level of protection can determine an appropriate shelf life date. Dating should be considered an additional protective step for food safety, because dating cannot overcome the consequences of other factors (temperature) being excessive.

## PROTOCOLS TO ESTABLISH AND VALIDATE SAFETY-BASED SHELF LIFE DATING

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Traditionally, shelf life dating has been based on quality parameters of the product. Protocols have been established to determine the shelf life of minimally processed refrigerated foods based on microbiological challenge studies with pathogens such as *Clostridium botulinum*. Typically, product formulations are challenged with multiple-strain spore cocktails and incubated under several conditions that mimic normal storage and storage under temperature abuse. The product formulations are tested until toxin is detected in the samples. Shelf life is then determined by adding a margin of safety (measured in days or weeks) based on the time to first appearance of toxin. Protocols such as those based on the "verifiable secondary barrier" approach may be used to validate which formulation parameter(s) actually are controlling product safety. Microbiological challenge protocols and predictive microbiological models based on vegetative pathogens such as *Listeria monocytogenes* can also be used to establish the shelf life of refrigerated, ready-to-eat products such as processed meats. In this presentation, we examine the usefulness of these protocols in delivering safe and wholesome refrigerated ready-to-eat products to consumers.

## ALTERNATIVES TO SAFETY-BASED SHELF LIFE DATING

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### INTRODUCTION

An open date on a food package implies something about the shelf-life or safety of the food to a consumer (Szybist, 1999). To food producers it represents the time at which the loss of desired quality occurs, based on the percentage of consumers they are willing to displease for a given distribution. To the food retailer, says something about how fast to move the product to get it into the consumer's home before it spoils. The date also implies that the product should be stored properly, e.g., that the temperature in the refrigerated cabinet is maintained at 4°C or less. If not maintained properly, the food may spoil before the date, leading to a disgruntled consumer or to a food poisoning incident. If no date is present, consumers may sort for those packages that are dated.

The presence or absence of a date has legal implications, with respect to being either misleading or misbranded. Specifically, as stated in Section 201(n) of the Food, Drug and Cosmetic Act, the definition of misbranding says:

If an article is alleged to be misbranded because the labeling or advertising is misleading, then in determining whether the labeling or advertising is misleading, there shall be taken into account (among other things) not only representations made or suggested by statement, word, design, device, or any combination thereof, but also the extent to which the labeling or advertising fails to reveal facts material in the light of such representations or material with respect to consequences which may result from the use of the article to which the labeling or advertising relates under the conditions of use prescribed in the labeling. This implies that if a date is on the product, and especially if it is in conjunction with open dating of the food, processors would need to have the relevant data to back up the statement. Setting a date thus becomes a problem if the food product is temperature sensitive, as are all refrigerated foods. Does one set the time for the optimal temperature, for an effective temperature or for an abuse temperature? A food that is not held at proper conditions to meet the promise of that date, i.e., that is abused by improper transportation and storage temperatures, would become adulterated. Since it is well documented that temperature abuse occurs in distribution, this becomes a critical issue in open dating *(1, 2)*. One solution to this problem is to have a device on the package that integrates the time temperature exposure (TTI). This tag will show a color change at end of shelf life or when the pathogen reaches a critical value, e.g., the time to be able to detect (TTD). A second solution is a device that shows a color change when the pathogen reaches a critical value, based on sensing the pathogen itself or one of its metabolites. To employ these solutions, certain critical factors which are discussed next, must be known.

### Microbial Shelf life

Shelf-life prediction is an essential feature in marketing refrigerated and Ready to Eat (RTE) products *(3, 4)*. In shelf life testing of these products, there are at least three quality parameters that need to be evaluated at various temperatures during the test period: microbial safety, microbial spoilage and overall organoleptic changes *(5)*. When microbial hazards are minimal, tests for spoilage and organoleptic change take precedence in shelf life determination. Such testing may be accomplished by monitoring microbial growth; changes in color, odor, and texture; and overall acceptability of the product.

Where a product may pose a health risk before sensory expiration, the indicator's shelf life prediction must be conservative enough to ensure that the indicator will predict the end point before a risk develops. For example, Kraft General Foods, Inc., used a safety margin of between 1/3 to 1/4 of the product's organoleptic shelf life as their printed shelf life date in light of the fact that the needed data was missing *(6)*.

To model pathogen growth for the design of a tag, the following is needed *(6)*:

a. time to detection (TTD) as a function temperature (or other factors)
b. lag time and log phase growth as a function of the same parameters
c. an estimate of the initial microbial load
d. the final microbial load that the safety shelf life is based on

Note that if the TTD of a pathogen is known, modeling shelf life beyond that time is only an exercise, since the food would be considered adulterated at that point, i.e., the law technically doesn't care that the infectious dose is higher than one organism per serving.

There are many models used to predict the growth curve and its temperature dependence *(7, 8)*, have shown that the Arrhenius model (log k vs 1/T K) and the square root model work very well and have predicted spoilage to within ±2 hours for milk stored in a ±4 C sine wave temperature regime. All the other models (Gompertz, Logistic etc.) have not been tested well for variable temperature history because of their complexity. Also, all models have ignored the influence of prior temperature history on the growth rate at future temperatures *(10)*. Our recent work shows that, at least for *Listeria monocytogenes* strain 7776 isolated from the Bil Mar outbreak, the growth rates for the temperature regimes at subsequent times were for the most part within the 95% CL of the rates if always at that temperature, at least in the Log phase (Table 1). It is not known if this would be the case in the below detection limit phase or lag phase. In the cases where the growth was less at the new temperature, using the original rate for that temperature would overpredict growth, thus being on the conservative side.

The second parameter that is largely unknown is the rate of growth or the time to detect below the detection limit (e.g., 1 CFU/25 g). Recently we accomplished this for the same *Listeria* strain as the one identified in Table 1. Product was inoculated at below detection levels and then representative samples (at seven temperatures) were taken over time until detection. Figure 1 shows the Arrhenius plot for this (log 1/TTD vs 1/T in °K). Figure 2 shows the shelf-life plot (log TTD vs T°C). Both gave good straight lines, as also found by Fu et al., *(11)* for pseudomonas growth in milk. This relationship is required in TTI tag design.

### Chemical Time-Temperature Integrators (TTI)

Time-temperature integrators (TTI) are small physical devices that are placed on the food package to measure the temperature history of a product, indicate a definitive change at the end of shelf-life through "integration" of the time-temperature exposure, and give a warning e.g., "Use food by July 30, 2004 unless dot turns red" *(4, 12–14)* TTIs are reliable indicators of end of shelf-life for food products if they have similar temperature sensitivities ($E_a$) with regard to the food deterioration mechanism *(15)*. The devices can used on individual consumer packages, so that they establish a control system, important because not all products will receive uniform handling, distribution and time-temperature effects *(15)*. As a result, TTIs can increase the effectiveness of quality control in distribution, stock rotation practices of perishable foods in grocery stores, and efficiency in measuring freshness by the consumer *(16–18)*. Taoukis and Labuza *(19–20)* showed that for the most part, the commercially available TTIs are both reliable and applicable for use in combination with open dating of refrigerated foods. Malcata *(21)*, in addition showed that although the tags respond more quickly than the actual food to temperature abuse because the tags are on the surface of the package, the response is on the conservative side of safety, i.e., the tag shows an endpoint before the food is spoiled. The Campden Food and Drink Association in the United Kingdom has developed technical standards for the evaluation of TTIs *(22)*. The three major manufacturers of chemical TTIs are 3M *(23)*, Lifelines *(24–25)*, and VITSAB *(26)* as shown in Fig. 3.

TTIs can play a critical role in food safety *(27–28)*. There are potential dangers with controlled atmosphere packaged (CAP/MAP) refrigerated RTE meals and temperature abuse. Improper conditions can lead to the growth of harmful pathogens or botulinum toxin, especially under anaerobic conditions *(29–30)*. FSIS has recommended monitoring the temperature of meat in the processing room during the entire grinding process of meats, as established in the "Guidance for Beef Grinders to Better Protect Public Health" (Guidance for Minimizing Impact Associated with a Food Safety Hazard in Raw Ground Meat and Other FSIS Regulated Products). The document specifically mentions the use of TTIs on packages as an indicator of adequate temperatures of the meat during storage, distribution, and display of the products in grocery and other retail establishments.

Chemical TTIs have the following problems when used for predicting safety *(7, 9, 11)*:

a. *Activation energy ($E_a$)*: The activation energy ($E_a$) for the chemical reaction of the tag must be the same as that of the pathogen. This is a problem since $E_a$ can vary with stage of growth, i.e., TTD, lag and log phases. Our work with *Listeria* shows 23, 20.4 and 18.7, respectively, for these three stages, based on seven storage temperatures. These were not different statistically but could pose a problem. However, if only the TTD is used, they would be functional.
b. *Run out time:* The run out time varies with each growth phase, creating the same problem as in a. This cannot be accounted for in a chemical tag.
c. *End point:* The end point must be all or none; otherwise, sorting will increase.

It should be pointed out that these tags have been successfully used for food and drug quality. For example, they are used on MREs by the military, on polio vaccine vials by WHO, and by the Monoprix chain in France for all their deli items.

### RFID tags

Since 1998 there has been a revolution in new materials and processes that have driven down costs dramatically for memory chips, batteries and circuitry. This has led to the Radio Frequency

**TABLE 1.** Influence of temperate shifts on growth of *Listeria monocytogenes* (Strain 7776) as compared to the constant temperature growth rate

| T °C | k observed $hr^{-1}$ | k constant $hr^{-1}$ | significance |
|---|---|---|---|
| 4 | 0.0374±0.0013 | 0.0354±0.001 | NSD |
| 36 | 0.756±0.084 | 1.184±0.043 | NSD |
| 4 | 0.041±0.286 | 0.0354±0.001 | NSD |
| 36 | 0.952±0.286 | 1.184±0.043 | S |

**Figure 1.** Arrhenius plot for time to detect *Listeria monocytogenes* (strain 7776) when inoculated at < 1 CFU/25 g at different temperatures

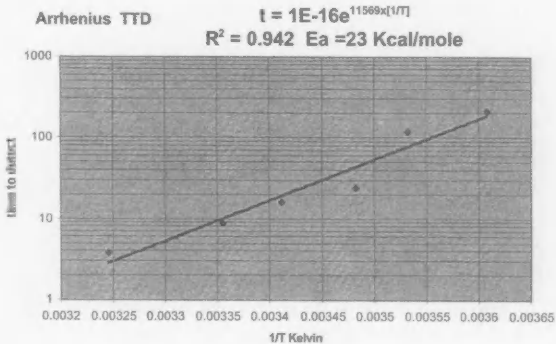
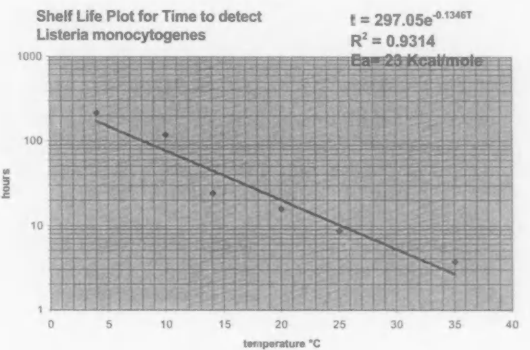
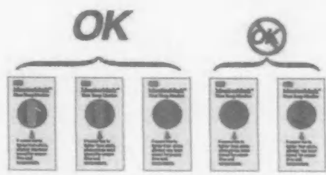



**Figure 2.** Shelf-life plot of time to detect *Listeria monocytogenes* (strain 7776) when inoculated at < 1 CFU/25 g at different temperatures

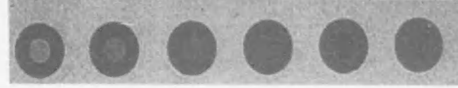



Identification (RFID) or so-called Smart Label revolution *(31)*, with predictions that such tags will replace the UPC code within three years. There are several companies are developing two-way RFID temperature-recording sensor tags that can be download at various receiver ports along the way for abuse detection. Several other companies are taking temperature monitoring one step further, i.e., integration as is done in the chemical tag, using the microbial kinetic parameters in an on-board chip. The advantage is that such tags can integrate over the whole three stages of growth and can have an exact good/no good indicator (light) to eliminate sorting. In addition, these tags will store the whole temperature-time sequence of exposure, thus allowing the processor to determine where abuse occurred. By downloading the RFID t-T data at various points in the chain, product close to being unsafe can be removed before the last stage, thus ensuring a safe food supply. Giannakourow et al. *(32)* has shown that using a "least shelf life left" delivery system based on TTIs can save on the cost of distribution and reduce the probability of a Listeria outbreak. The current price for the t-T recording tags is around $2, making them viable only for cases or pallets, but the price should be under 5¢ by 2005, driving this technology.

**Figure 3.** Three commercial types of TTIs




a. a. 3M Monitor Mark showing change in color as the product ages



b. Lifeline TTI showing change in color as the product ages

c. VITSAB TTI showing change in color as the product ages

## Metabolite sensors

With revolutions in nano-technology, electronics, microbial genetics and chemistry, we also now have the ability to build sensors that can directly detect a specific pathogen strain using an antibody-antigen (AB-AG) sensor that detects a specific microbe surface protein. These have led to new pathogen detection techniques that have markedly reduced the time of analysis. Researchers at several universities and organizations have given presentations and press releases on their advances in building an in-package sensor for pathogens. This seems to be the ultimate way to build in an end-of-shelf-life on-package tag, as it detects the real thing. Other sensors in development have relied on detection of microbial metabolic products, using a certain level as an endpoint. However, there are some critical questions that must be addressed.

a. *Topology:* to be sensed by AB-AG, the organism has to be directly under the sensor or the sensor has to touch the whole food surface. This assumes that contamination will be isotropic-homogeneous, an assumption for which there is no proof; rather, a non-homogeneous distribution is likely, and in foods such as hamburger, contamination can be inside at the center. No data exist for the rate of displacement for a microbe in or on solid foods. Assuming it can move at a speed of 1 length per sec (1 μm/sec) it will travel 2.8 cm per hour. If the probe were a surface of 1 cm × 1 cm exactly 1 cm away as a band on the radius, and the microbial cell took a straight-line run toward it, the chances of hitting are less than 1 in 200 million. If a Monte Carlo approach were assumed, the chances could be more than 1 in 10 billion.

b. *Metabolite sensor:* These sensors assume that any chosen detectable metabolite is produced only by that particular pathogen and that there is a direct correlation with growth level. This is an area of needed research, but its high degree of uncertainty precludes this approach for a successful shelf life indicator. Some have assumed they can use volatile metabolites, but reaction, internal volume of headspace, scalping and diffusion out of the package will all occur, making any prediction of shelf life fraught with error. A more general approach would be to

measure surface pH or conductance *(33)* but this would not be pathogen specific.

c. *Cost:* Given a and b, the cost of these sensors is unknown and probably high because of power requirements.

d. *Chemical safety:* Electronic and chemical TTIs can be on the outside of a package, while metabolite sensors need to be in direct contact with the surface of the food. This creates problems of migration from the tag of compounds not allowed to contact food.

## CONCLUSIONS

We are close to the time when the use of on-package sensors for shelf life and safety will become a possibility. As seen in this review, electronic time-temperature integrators make the most sense, along with chemical TTIs if time to detect is selected as an endpoint. The chief drawback to their use will be in ensuring against sorting, getting the price low and overcoming the liability factor for the manufacturer by indemnifying them for temperature abuse in distribution.

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# EUROPEAN PERSPECTIVES ON SHELF LIFE DATING

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## Legislation in Europe

The European Union (EU) currently consists of fifteen member states. There are at present thirteen new applicants who will be admitted to the Union within the next few years. Each member within the European Union state will have its own national legislation; however, the European Union will also draw up and implement legislation of a variety of types. This European legislation will need to be implemented in all member states within the Union. Such harmonization of legislation is designed to help free trade within the Union. European legislation is applied through EU Regulations, EU Directives, EU Decisions, and Recommendations and Opinions. Regulations are applied directly throughout the EU without the need for any national measures to implement them in member states. Directives bind Member States to the objectives to be achieved, while leaving the national authorities free to choose the form and means to be used. Decisions are binding on all those to whom they are addressed. Recommendations are not binding.

## Shelf Life and European Legislation

Within the EU, shelf-life dating is covered under EU Directive 2000/13/EEC, which covers the labelling, presentation and advertising of foods offered for sale to the ultimate consumer. Article 3 of that Directive states that pre-packaged foods shall bear a durability mark (i.e., a "best before" date); it also states that highly perishable foods must have a 'use by' date. The Directive states that the date, and thus the shelf life, is the responsibility of the producer.

A second EU Directive that has an impact on shelf life, Directive 92/59/EEC, covers general product safety. This states that it is the responsibility of the manufacturer to put safe products on the market, and it further defines "safe products" as anything that, under normal or reasonably foreseeable conditions of use, including duration, presents minimum risks and a high level of protection.

As described previously, EU Directives are enforced through national legislation. In the UK, for example, the requirements of Directive 2000/13/EEC are enforced through the UK Food Labelling Regulations (1996). These regulations state that durability indications must take one of two forms:

(1) "Best Before" plus a date to which food can be expected to retain its specific properties if properly stored.

(2) "Use By" plus a date to which a microbiologically perishable food, if properly stored, is recommended for use.

## What legislation means to the producer

The effect of this legislation is to direct manufacturers to consider inherent changes over the course of storage, in the food that they produce, and to determine when such change makes the food unacceptable to consumers.

The reason for unacceptability could be due to one or more factors:

— organoleptic change (e.g., rancidity)

— microbiological change (which could lead to an organoleptically unacceptable product, or in a worst case scenario, an unsafe product)

Manufacturers have to consider such changes over the life of their products and must, therefore, consider the effects of:

— processing and packing
— distribution
— retail sale
— consumer practice

All will have some effect on product life, and the only way to assess such effects is to undertake shelf-life testing.

Shelf-life testing tends to be done routinely in Europe to answer the question, "How long does the food remain within defined quality specifications throughout normal production and storage?" Such testing will usually utilize 'normally' produced foods carrying their natural microbiological flora. Pathogens will not usually be considered, as these will be assumed to be eliminated through use of risk management tools such as HACCP.

In some cases it may be necessary to assess the response of a particular microorganism or group during shelf-life. As 'natural' contamination with specific organisms cannot be guaranteed, the product will have to be inoculated to undertake such a 'challenge test.' Challenge testing is often used to assess the potential for growth of specific pathogens, if they were able to contaminate a food, or to look at the potential for growth of specific spoilage organisms that may be a major concern for certain product types. Whether undertaking shelf-life testing or challenge testing, there are a number of significant experimental questions that must be addressed. Incorrect assessment will result in the practical experimentation giving a false indication of shelf-life and this could result in economic losses due to too short a life being given, or severe commercial problems due to spoilage, if the life given is too long.

## Determination of shelf life

Both shelf life and challenge testing have key experimental parameters that must be defined before testing begins. These relate to the storage times and temperatures that should be used during trials. Considering production, distribution, retail sale and consumer storage, it is clear that a food will not be stored at a constant temperature throughout its life. The variations in temperature must be estimated and included in shelf life or challenge tests to get a more realistic determination; indeed, it is inherent within Directive 92/59/EEC that 'reasonably foreseeable conditions of use' must be considered.

When considering storage temperature, estimates must be made of product storage temperatures in a number of locations and the length of time the product will remain at those temperatures must be approximated.

— storage at the producer's goods-out area
— within distribution (temperature controlled vehicles, etc.)
— within retail display cabinets
— during purchase and transport to the home
— during storage by the final consumer

This is complicated by the variety of national legislation in Europe covering 'chilled' storage temperatures, which range from ≤ 4°C in France to ≤ 8°C in the UK and Finland.

In order, therefore, to gain a realistic estimation of shelf life through use of experimentation, a product must be held at a series of defined temperatures and times to 'mimic' real storage regimes that food will see during distribution.

In Europe an issue has been noted when different manufacturers/retailers use different time/temperature regimes during shelf-life testing. Some initial experimental trials done at Campden & Chorleywood Food Research Association (CCFRA) have indicated that, if the same type of food is tested using a variety of real time/temperature regimes used by European producers, the determined shelf life could vary by up to four days. Therefore, depending on which testing method is used, a

product may be given too short a life (resulting in good product quality but potentially an economic loss to the producer) or too long a life (resulting in a potentially poor quality product and possible organoleptic spoilage within a marked life).

### The way forward

It would seem clear that the way to progress is to ensure that producers understand the requirements of setting experimental regimes to assess product shelf life, in a scientifically sound and transparent way, and to attempt to standardize such procedures. At present CCFRA is working with European food producers and retailers to define a standardized testing protocol for the determination of product life. This has been published *(1)* and will help to ensure that product life is assessed in a standardized manner, using scientifically sound principles. In addition, the increased development and use of validated predictive models for microbiological safety and spoilage will aid the decision-making process.

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## *THE EVOLUTION OF FOODBORNE PATHOGENS*

## MUTATORS AND BACTERIAL PROMISCUITY: SOME OVERLOOKED FACTS IN PATHOGEN EVOLUTION


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The survival of a microbe in a demanding environment ultimately rests on the degree of diversity within the microbial population at large. Genetic change (mutation) and exchange (recombination) generate the genetic diversity on which selection works to cull and establish successful microbes in distinct niches. An adverse environment should favor a microbe endowed with a high mutation rate, since it would increase the stochastic probability of spawning the rare mutant needed to survive, whether to escape immune surveillance, elude therapeutic intervention, or evade the manifold barriers meant to keep microbial populations in check. Yet, this seems counterintuitive, for a high mutation rate should lead to deleterious or lethal consequences. In this context, the importance of particular mutators in microbial evolution is explored, specifically those defective in methyl-directed mismatch repair (MMR). MMR mutators are unique in that not only are they hypermutable (i.e., they exhibit high mutation rates), but they are promiscuous as well (i.e., they show an increase in recombination). Such properties could speed the evolutionary process. MMR reservoirs then may be the "mixing pools" where the horizontal transfer of genes within and among species takes place.

## EVOLUTION OF *ESCHERICHIA COLI* O157:H7 AND OTHER *E. COLI*


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*Escherichia coli* O157:H7 is a source of food and waterborne illness that was first linked to human disease in 1982. This organism has caused serious outbreaks of diarrhea, bloody diarrhea, and hemolytic uremic syndrome, and is now recognized as a public heath problem worldwide. The full extent to which this pathogen differs from normally harmless *E. coli* has recently been revealed through genome sequencing. These studies have shown that pathogenic strains often contain hundreds of different genes not found in commensal *E. coli*.

How do new pathogens such as *E. coli* O157:H7 accumulate such genetic differences? To address this question, we have developed a two-pronged approach. First, we examine variation in DNA sequences for conserved genes that form the backbone of the bacterial genomes. This variation is used to construct an evolutionary framework for modeling the history of strain divergence. Second, we investigate the distribution of genes that contribute to virulence and map this distribution onto the evolutionary framework. Many of the virulence genes are associated with mobile genetic elements, including plasmids, phages, and pathogenicity islands. This two-pronged approach allows us to test hypotheses about how and when virulence elements have been acquired in the emergence of new pathogens.

For *E. coli* O157:H7 we have developed a model in which this pathogen has evolved through a series of stages by the acquisition of mobile virulence elements, including a pathogenicity island that mediates bacterial attachment to the intestinal epithelia, a virulence plasmid that may contribute to host range, and several distinct bacteriophages that encode Shiga toxin genes. Continued flux of mobile elements and loss of genetic material result in rapid diversification of O157:H7 genomes. Similar events have occurred in other branches of the *E. coli* tree that have resulted in the formation of new pathogenic varieties. The extent to which these new pathogens contribute to foodborne infectious disease is not well understood and warrants more investigation.

## EVOLUTION OF *SALMONELLA* VIRULENCE AND HOST ADAPTATION


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*Salmonella enterica* subspecies I serotypes are frequently isolated from food animals and account for more than 99% of human cases of disease. In contrast, serotypes of *S. enterica* subspecies II-VII and *Salmonella bongori* are commonly isolated from cold-blooded vertebrates but are only rarely isolated from patients. One difference between *S. enterica* subspecies I serotypes and reptile-associated serotypes of *S. enterica* subspecies II-VII and *S. bongori* is the presence of the *ShdA* gene in the former group. The *ShdA* protein promotes colonization of the murine cecal mucosa at areas of epithelial erosion where the extracellular matrix is exposed to the intestinal lumen, thereby resulting in prolonged intestinal carriage.

Characterization of *ShdA* binding to fibronectin (Fn) proteolytic fragments identified the Hep-2 domain as the primary binding site for this adhesin. Binding of *ShdA* to the Hep-2 domain of Fn and to a second extracellular matrix protein, collagen I, was found to be heparin sensitive. The Hep-2 domain contains a high-affinity heparin-binding site, the cationic cradle, that is conserved among Fn sequences from frogs to humans. Amino acid substitutions of basic residues that form the cationic cradle of the Fn Hep-2 domain that inhibit heparin binding also abrogated binding of *ShdA*. Molecular mimicry of a host polysaccharide ligand by *ShdA* may be a mechanism to increase intestinal persistence by accessing binding sites in the extracellular matrix, thereby enabling *S. enterica* subspecies I serotypes to expand their host range to include warm-blooded animals.

# MULTILOCUS SEQUENCE TYPING FOR EVOLUTIONARY ANALYSIS AND OUTBREAK TRACKING

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## INTRODUCTION

Bacterial foodborne disease remains a major, and increasing, challenge to the food industry and to public health systems worldwide. In the United Kingdom, for example, reported cases of *Campylobacter jejuni* gastroenteritis have risen steadily during the past 20 years, with 54,000 cases, corresponding to 103.7 cases per 100,000 population, reported in 2000. Even this large figure is likely to be an underestimate as great as eight-fold *(1)*. Despite this high incidence, the relative contribution of the various possible sources of human infection have yet to be elucidated, although it is thought that poorly-cooked food, especially poultry products, are a major source of infection. One of the most important requirements for epidemiological tracking is accurate isolate characterization, which has been lacking for *C. jejuni* for a number of reasons *(2)*.

### Population-based approaches to bacterial typing

Bacterial isolate characterization was greatly influenced by the early success of the Kaufmann-White serotyping scheme for *Salmonella enterica (3)* and serological characterization became the predominant paradigm for bacterial characterization. However, much of the success of this scheme was due to the fact that the serological differences indexed by the scheme correlated with genetic lineages of *Salmonella enterica (4)*, which themselves correlated with biological properties of interest to the clinical microbiologist. As more data on the population biology of bacterial pathogens has become available *(5)* it has become increasingly clear that bacteria can exhibit a range of population structures that do not necessarily correlate with serological variation.

### Multilocus sequence typing (MLST)

MLST was developed as a generic typing scheme that was applicable to a wide variety of bacterial pathogens regardless of their population structure *(6)*. The approach indexes variants at multiple, usually seven, housekeeping loci (i.e., genes encoding metabolic functions) to provide information representative of the genome as a whole. This variation is measured by direct nucleotide sequence determination, which provides a number of advantages, including: 100% typability of isolates; high discrimination; easy quality assurance and comparability of data; scalability from a few to hundreds or even thousands of isolates. Each unique allele at each locus is assigned a number, and the seven numbers are combined to produce an allelic profile which is assigned a sequence type (ST) designation *(7)*. These data are easily compared over the internet *via* a central database (http://pubmlst.org/campylobacter) and can be analyzed with a variety of techniques. MLST data can be complemented with data from other loci, e.g., the *flaA* gene short variable region (*flaA*SVR) *(8)*.

### Clonal complexes

MLST is highly discriminatory; at the time of writing, the PubMLST database for *Campylobacter* contained 2415 isolates and a total of 1085 unique sequence types on 12-9-04. However, this diversity is extensively structured. Some STs are much more common and are isolated in geographically and temporarily diverse samples. When population data are analyzed by a variety of techniques, it appears that most of the low frequency genotypes have arisen from these frequent persistent STs by changes in up to three of the seven loci. Clonal complexes comprise these 'cental genotypes', from which they derive their name, and their variants. When analyzed by clonal complex, MLST datasets become much simpler. In one study of 814 *C. jejuni* isolates, 379 unique STs were found; however, 92% of the isolates were resolved into only 17 clonal complexes. Six of these clonal complexes accounted for 63% of the human isolates examined *(9)*.

### Epidemiology and clonal complexes

As just noted, certain clonal complexes appear to be associated with human disease and there is preliminary evidence that these vary between continents. The neuropathies Guillain-Barré syndrome and Miller Fisher syndrome are not strongly associated with particular clonal complexes, although one clonal complex is over-represented in isolate collections sourced from such patients *(10)*. There is some evidence for association of particular clonal complexes with particular food animals and retail food, providing information relevant to understanding the routes of human infection. In general, serotype is poorly correlated with clonal complex, providing an explanation for the difficulties of epidemiological analysis on the basis of serotype data alone *(9)*. Clonal complex is also useful in the investigation of outbreak clusters, although, as a small number of clonal complexes cause the majority of human infections, analysis by ST or by the addition of the *flaA* SVR sequence may be necessary to unambiguously resolve an outbreak.

### Population and evolutionary analysis

As MLST data are nucleotide sequence-based they can be analyzed by evolutionary and phylogenetic techniques *(10, 11)*. These analyses have confirmed that the different allelic combinations present are generated by recombination and indicate that *C. jejuni* shares some alleles with related species such as *Campylobacter coli*. Much of the within-allele variation is also a consequence of recombination. Comparisons of MLST and pulse field gel electrophoresis data have provided evidence for chromosome rearrangements within bacteria belonging to the same ST, with implications for using PFGE fingerprints for epidemiological purposes.

## CONCLUSIONS

The application of nucleotide sequence-based isolate characterisation techniques, especially MLST, provide unified approaches to bacterial typing that are cost-effective, rapid and highly discriminatory. The data produced are comparable *via* the Internet and can be used in a variety of applications, ranging from small-scale outbreak investigation and the examination of epidemiological trends to studies of the evolutionary and population biology of enteric pathogens.

## ACKNOWLEDGMENT

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## MOLECULAR EVOLUTION OF *LISTERIA MONOCYTOGENES*

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### INTRODUCTION

Classically, *L. monocytogenes* has been differentiated into 13 serotypes, with more than 90% of human listeriosis cases caused by serotype 1/2a, 1/2b, and 4b strains. While the majority of human clinical infections occur as sporadic cases human listeriosis can also occur in large epidemics. Most sporadic human listeriosis cases and large human foodborne listeriosis epidemics have reportedly been caused by *L. monocytogenes* serotype 4b *(1)*. Serotypes 1/2a and 1/2b are also responsible for significant numbers of sporadic cases of human illness, and a serotype 1/2a strain was responsible for a recent multistate human listeriosis outbreak in the US *(2)*. Serotyping data collected by the CDC in 1986 showed that serotypes 1/2a (30%), 1/2b (32%), and 4b (34%) represented the majority of isolates from 144 human sporadic cases *(3)*. Of 1,363 human isolates collected in the UK, 15% were 1/2a, 10% were 1/2b, and 64% were 4b *(4)*. The remaining ten currently recognized *L. monocytogenes* serotypes have only rarely been linked to human disease. This apparent association between a few specific *L. monocytogenes* strains and most cases of human listeriosis raises the intriguing challenge of identifying unique characteristics enabling these strains to be more effective than others in causing human disease. Two hypotheses could explain the apparent predominance of serotype 4b strains in human epidemic listeriosis and of 4b, 1/2a, and 1/2b strains in sporadic human cases: (1) humans are more commonly exposed to these subtypes than to other *L. monocytogenes* serotypes, i.e., these strains are found in foods more frequently than other serotypes; and/or (2) these subtypes have a unique pathogenic potential for humans.

Molecular subtyping methods have consistently grouped *L. monocytogenes* into two major lineages. Multilocus enzyme electrophoresis (MEE), PFGE, ribotyping and amplified restriction fragment length polymorphism analysis (AFLP) all show that *L. monocytogenes* can be separated into two major genetic groups *(1)*. Allelic analyses of several virulence genes as well as ribotyping revealed a third phylogenetic lineage within *L. monocytogenes*. Specifically, a combination of virulence gene alleles and ribotype patterns allowed separation of *L. monocytogenes* strains into three distinct lineages, designated I, II, and III *(1, 5)*.

Our laboratory has focused on a comprehensive approach using sequencing of multiple virulence, stress response, and housekeeping genes, genomic DNA microarray analysis, and tissue culture characterization of selected *L. monocytogenes* isolates from humans and animals with clinical listeriosis, foods, and environmental samples to probe the evolution and population genetics of *L. monocytogenes* and to define the virulence characteristics of different *L. monocytogenes* lineages and strains. The long-term goal of our research efforts is to better understand the ecology, evolution, and virulence characteristics of distinct *L. monocytogenes* clonal groups in order to better understand the transmission and sources of human foodborne listeriosis cases. This paper summarizes some recently published and unpublished data from our group on the ecology and evolution of *L. monocytogenes*.

### *Listeria monocytogenes* lineages

Evolutionary analysis of both multilocus sequencing (Cai et al., unpublished) and microarray data *(6)* confirmed the existence of two distinct *L. monocytogenes* lineages, previously termed lineages I and II *(5)*. These two lineages correlate with serotype groupings; lineage I comprised serotypes 1/2b, 3b, 3c and 4b; lineage II comprised serotypes 1/2a, 1/2c *(7)*. Evolutionary analysis of the virulence genes *actA* and *inlA* and the *hly-mpl* and *plcA-hly* intergenic regions, in addition to *recA*, *sigB*, and *prs*, revealed a pre-dominantly clonal population structure for *L. monocytogenes* that is characterized by two distinct lineages (I and II) with limited recombination between these two lineages (Cai et al., unpublished). Based on preliminary microarray analyses we also identified a considerable number of contigs present in lineage II strains, but absent from lineage I strains *(6)*. Further analysis suggested a model in which the ancestor of the two lineages had the 1/2 somatic serotype, and the regions absent in the lineage I genome arose by loss of ancestral sequences *(6)*. *L. monocytogenes* isolates previously designated as lineage III, which includes the rare *L. monocytogenes* serotypes 4a and 4c, appear to have a mosaic genome structure based on DNA sequence analyses. Phylogenies based on non-virulence genes further indicate that lineage III strains may be classified into two distinct groups, including at least one group that appears to be closely related to *L. innocua*.

Characterization of a total of 42 human clinical isolates and 502 isolates from ready-to-eat foods collected in Maryland and California during 2000 and 2001 *(8)*, as well as of 450 additional human clinical isolates collected throughout the U.S. between 1997 and 2002, was performed to probe associations between specific subtypes and human listeriosis. Genotypic analyses of isolates by automated *Eco*RI ribotyping and PCR-RFLP analysis

of the *hly* gene *(5)* allowed assignment of isolates into one of 63 different *Eco*RI ribotypes and into one of three previously described genetic lineages. Statistical analyses showed that, while exclusive associations were rare, the majority of subtypes were significantly associated with isolation from either foods or humans. Using a large isolate set, this study not only confirmed our previous data *(9)* that lineage I strains are significantly associated with human listeriosis cases compared to both animal listeriosis cases and isolation from foods, but also provided specific data on the comparative prevalence of different ribotypes among human clinical and food isolates. Most strikingly, one specific *Eco*RI ribotype (DUP-1062A) was found to represent 30.1% of food isolates, but only 1.8% of human isolates, a highly significant difference in prevalence ($P < 0.0001$). Further studies to define whether isolates with this ribotype show attenuated virulence and to probe the genetic basis of their virulence attenuation are currently in progress in our laboratory.

### Evolutionary Analyses

Evolutionary analyses of the stress response gene *sigB* and the virulence genes *actA* and *inlA* indicated that, while evolution of *sigB* followed a molecular clock model, evolution of *actA* did not. *actA* appeared to be under positive selection ($P < 0.005$) as determined by using a likelihood ratio test. By use of an empirical Bayes approach, 8 ActA amino acid sites with posterior probabilities > 95% of being positively selected were identified. Posterior probability plots revealed both highly conserved regions and regions with a significant frequency of positively selected aa sites in *actA* (Cai et al., unpublished data). These data indicate that active evolution of *actA* may play a role in the development of strain-specific virulence characteristics.

## CONCLUSIONS

In conclusion, available data indicate that *L. monocytogenes* is characterized by a predominant clonal population structure (particularly for lineages I and II), although strains previously characterized as lineage III show more diversity and indications for recombination. Both evolution by gene loss and active evolution of specific virulence genes (e.g., *actA*) appear to contribute to evolution of *L. monocytogenes*, including evolution of virulence related characteristics. Our data also support the hypothesis that the previously observed high prevalence of specific *L. monocytogenes* subtypes (e.g., serotype 4b strains) among human listeriosis cases and outbreaks appears to at least partially represent unique virulence characteristics of these subtypes rather then their abundance in contaminated food products.


## ACKNOWLEGMENTS

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# Award Nominations

The International Association for Food Protection welcomes your nominations for our Association Awards. Nominate your colleagues for one of the Awards listed below. You do not have to be an IAFP Member to nominate a deserving professional. To request nomination criteria, contact:

International Association for Food Protection
6200 Aurora Ave., Suite 200W
Des Moines, Iowa 50322-2864
Phone: 800.369.6337; 515.276.3344
Fax: 515.276.8655
Web site: www.foodprotection.org
E-mail: info@foodprotection.org

You may make multiple nominations. All nominations must be received at the IAFP office by March 14, 2005.

- Persons nominated for individual awards must be current IAFP Members. Black Pearl Award nominees must be companies employing current IAFP Members. NFPA Food Safety Award nominees do not have to be IAFP Members.
- Previous award winners are not eligible for the same award.
- Executive Board Members and Awards Committee Members are not eligible for nomination.
- Presentation of awards will be during the Awards Banquet at IAFP 2005 – the Association's 92nd Annual Meeting in Baltimore, Maryland on August 17, 2005.

**Nominations deadline is March 14, 2005**

# Nominations will be accepted for the following Awards:

**Black Pearl Award** — Award Showcasing the Black Pearl

Presented in recognition of a company for its outstanding commitment to, and achievement in, corporate excellence in food safety and quality.

*Sponsored by Wilbur Feagan and F&H Food Equipment Company*

**Fellows Award** — Distinguished Plaque

Presented to IAFP Members who have contributed to the Association and its Affiliates with distinction over an extended period of time.

**Honorary Life Membership Award** — Plaque and Lifetime Membership in IAFP

Presented to IAFP Members for their dedication to the high ideals and objectives of the International Association for Food Protection and for dedicated service to the Association.

**Harry Haverland Citation Award** — Plaque and $1,000 Honorarium

Presented to an active IAFP Member for many years of dedication and devotion to the Association and its ideals and objectives.

*Sponsored by Zep Manufacturing Company*

**Harold Barnum Industry Award** — Plaque and $1,000 Honorarium

Presented to an active IAFP Member for dedicated and exceptional service to IAFP, the public, and the food industry.

*Sponsored by Nasco International, Inc.*

**Educator Award** — Plaque and $1,000 Honorarium

Presented to an active IAFP Member for dedicated and exceptional contributions to the profession of the Educator.

*Sponsored by Nelson-Jameson, Inc.*

**Sanitarian Award** — Plaque and $1,000 Honorarium

Presented to an active IAFP Member for dedicated and exceptional service to the profession of Sanitarian, serving the public and the food industry.

*Sponsored by Ecolab, Inc., Food and Beverage Division*

**Maurice Weber Laboratorian Award** — Plaque and $1,500 Honorarium

Presented to an IAFP Member for dedicated and exceptional contributions in the laboratory. The Award recognizes a commitment to the development and/or application of innovative and practical analytical approaches in support of food safety.

*Sponsored by Weber Scientific*

**International Leadership Award** — Plaque, $1,000 Honorarium and Reimbursement to attend IAFP 2005

Presented to an IAFP Member for their dedication to the high ideals and objectives of the International Association for Food Protection and for promotion of the mission of the Association in countries outside of the United States and Canada.

*Sponsored by Unilever – Safety and Environmental Assurance Centre*

**Food Safety Innovation Award** — Plaque and $2,500 Honorarium

Presented to an individual or organization for creating a new idea, practice, or product that has had a positive impact on food safety, thus, improving public health and the quality of life.

*Sponsored by 3M Microbiology*

**NFPA Food Safety Award** — Plaque and $3,000 Honorarium

This Award honors an individual or a group or organization for preeminence in and outstanding contributions to the field of food safety. The award will be presented in 2005 to an individual in recognition of a long history of outstanding contributions to food safety research and education.

*Sponsored by National Food Processors Association*

# Highlights of the Executive Board Meeting November 15, 2004

Following is an unofficial summary of actions from the Executive Board Meeting held via teleconference on November 15, 2004:

**Approved the following:**

- Minutes of August 6-12, 2004 Executive Board Meeting
- Minutes of September 20, 2004 Executive Board Meeting teleconference
- Providing support for the FSnet Newsletter

**Discussed the following:**

- E-mail votes taken since the last meeting
- Committee Member appointments for 2005-2006
- Nominating Committee report
- IAFP 2005 planning (tours, Monday Night Social, Tuesday Event, expo service, etc.)
- IAFP 2005 Ivan Parkin Lecturer
- IAFP 2005 recording sessions
- Possible new Affiliate organizations
- Affiliate organizations not in compliance with IAFP Bylaws
- *JFP* Online review of manuscripts
- Audit report for year ending August 31, 2004
- IAFP 2008 location
- Awards criteria and jury instruction rework
- Awards honorarium
- Trade arrangements with other organizations
- 3-A Sanitary Standards, Inc. update
- Food Research Coalition
- Rejected symposium
- Publication of sensitive materials policy compliance

**Reports received:**

- *Food Protection Trends*
- *Journal of Food Protection*
- IAFP Web Site
- Membership update
- Advertising update
- Financial statements for period ending September 30, 2004
- Board Members attending Affiliate meetings
- Affiliate Newsletter
- Future Annual Meeting schedule
- Exhibiting (IAFP On the Road)
- Future Board meeting dates

Next Executive Board meeting: January 23, 2005

# NEW MEMBERS

## CANADA

**Nasrin Honardar**
Maxxam Analytics Inc.
Mississauga, Ontario

## MEXICO

**Carmen Hernandez-Brenes**
ITESM
Monterrey, Nuevo Leon

## UNITED KINGDOM

**Kaarin E. Goodburn**
Kettering, Northants

## UNITED STATES

### FLORIDA

**Jan R. Bennett**
University of Florida
Naples

**Tracy W. Fisher**
Orlando Culinary Academy
Orlando

### GEORGIA

**Hoikyung Kim**
University of Georgia
Griffin

### MARYLAND

**Shira Kramer**
Sterilex Corporation
Owings Mills

### MINNESOTA

**William R. Krueger**
Minnesota Dept. of Agriculture
St. Paul

### MISSOURI

**Deborah J. Longoria**
Hiland Dairy
Clever

### OREGON

**Rhoda Sithole**
Turtle Island Foods Inc.
Hood River

### TENNESSEE

**Michele L. McLeskey**
Sara Lee Foods
Newbern

### WASHINGTON

**Michele R. Maddox**
Starbucks Coffee Company
Renton

### WISCONSIN

**Michael O'Brien**
Foremost Farms
Sparta

# GOLD SUSTAINING MEMBER

(The following company recently became a Gold Sustaining Member)

**Ecolab Inc.,** St. Paul, MN, USA

# SILVER SUSTAINING MEMBER

(The following company recently became a Silver Sustaining Member)

**BD Diagnostics, Inc.,** Sparks, MD, USA

# NEW SUSTAINING MEMBERS

**Mark Moorman**
Kellogg Company
Battle Creek, MI, USA

**David M. Shepherd**
Maxxam Analytics Inc.
Mississauga, Ontario, Canada

**Jarret D. Stopforth**
Institute for Environmental Health
Lake Forest Park, WA, USA

## Douglas Campbell, CFSP Appointed Vice President of Environmental Health Testing

Environmental Health Testing, LLC announced the appointment of Douglas Campbell to the position of vice president of business development. Campbell, who will assume the role immediately, will be responsible for new business development for Environmental Health Testing and its subsidiaries including National Registry of Food Safety Professionals.

In addition to his work in the food safety certification business, Doug worked for the United States Department of Agriculture, Food Safety Inspection Service for almost 15 years in areas including microbiology, food technology and meat and poultry inspection. He was recognized twice for his work there with the USDA's Superior Service Award, their second-highest award.

Doug has authored and co-authored numerous technical articles on food safety and microbiology that have appeared in international, juried journals. He has also been an active member of the Conference of Food Protection (CFP) including service on Council 2 and the Standards Committee.

## Nilfisk-Advance America Promotes Jessica Abel to Marketing Communications Manager

Nilfisk-Advance America has promoted Jessica Abel to marketing communications manager. In her new position, Abel is responsible for the strategic planning, management, and execution of all of the company's marketing activities, including advertising, direct mail, public relations, and employee relations.

During her four years with the company, Abel has served as marketing services coordinator, public relations coordinator, and advertising assistant. Previously, Abel served as an account manager for Fortress Systems International, Charlotte, NC for one year.

Abel is a graduate of Millersville University, Millersville, PA, and holds a Bachelor of Science degree in communications, with a minor in business administration, and a focus on marketing.

## Food Technology Appoints New Executive Editor and Editor-in-Chief

Veteran editor Neil Mermelstein has been named executive editor of *Food Technology* magazine. Joining IFT and assuming the duties of editor-in-chief is respected publication's professional Bob Swientek.

As editor-in-chief, Swientek will focus his energies on *Food Technology's* readability, overseeing and working in collaboration with staff, reporters and contributing editors on all aspects affecting content and design of the venerable publication.

Swientek brings to IFT 20 years of experience in print publications, having served as chief editor at *Food Processing* and *Prepared Foods* magazines, and most recently at *Brand Packaging*. Swientek will be *Food Technology's* ninth chief editor in its 58-year history.

Mermelstein's responsibilities as executive editor will include editing and transition work. He will also undertake special projects and writing assignments.

Mermelstein joined *Food Technology* in 1971 as associate editor and has been editor since March 2001.

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**www.foodprotection.org**

## 2005 Crumbine Award Criteria Released

The Foodservice & Packaging Institute, Inc. (FPI) has released the criteria for the 2005 Samuel J. Crumbine Award for Excellence in Food Protection at the local level, which annually recognizes excellence in food protection services by local environmental health jurisdictions in the United States and Canada.

Entries for the Crumbine Award competition are limited to United States and Canadian local environmental health jurisdictions (county, district, city, town, or township) that provide food protection services to their communities under authority of a statute or ordinance. Past winners may apply five years after receiving the award.

The winner of the Award is selected by an independent panel of food protection practitioners who are qualified by education and experience to discern excellence in a program of food and beverage sanitation. They represent various interests, including leading public health and environmental health associations, past Crumbine Award winners, consumer advocates and the food industry. The jury makes its award selection each spring in a judging process administered by FPI. The application deadline for the award is March 15, 2005.

The Crumbine Award is supported by the Conference for Food Protection in cooperation with the American Academy of Sanitarians, American Public Health Association, Association of Food & Drug Officials, Foodservice & Packaging Institute, Inc., International Association for Food Protection, International Food Safety Council, National Association of County and City Health Officials, National Environmental Health Association, NSF International, and Underwriters Laboratories, Inc.

For more information about the Crumbine Award, including the 2005 award criteria, go to FPI's Web site at www.fpi.org (in the "Award Programs" section); or contact Lynn Rosseth at FPI at 703.538.2800, or by E-mail at lrosseth@fpi.org.

## President Bush Nominates Nebraska Governor, Mike Johanns as US Secretary of Agriculture

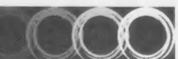
President Bush nominated (Nov. 2004) Nebraska Governor Mike Johanns, a Republican attorney who grew up on an Iowa dairy farm, as US Secretary of Agriculture to oversee the nation's farm and food programs.

Agriculture Secretary Ann Veneman said at the time, "I congratulate Governor Johanns on being named by the President. He is a good friend whom I've worked with closely over the years to advance agriculture policy and trade opportunities for America's farmers and ranchers. Governor Johanns brings tremendous strength to this position and will serve the President well during the next four years. I have no doubt that he will continue the strong leadership tradition at USDA and build on many accomplishments this Administration has achieved during its first term."

## Safefood Study Finds Room for Improvement in Food Production Safety Systems

Safefood, the Food Safety Promotion Board of Ireland has found that a shortage of technical expertise is preventing food manufacturing plants on the island from achieving the highest standards in food safety policy. The research into HACCP (Hazard Analysis Critical Control Point) systems published by Safefood looked at safety systems of food production companies in the Republic of Ireland and in Northern Ireland. The findings were launched in Dublin by Safefood at a HACCP seminar, which also included workshops aimed at improving HACCP awareness and best practice. The research found that 95% of companies do have a formal food safety policy, which was generally well communicated to staff. It also found that staff monitoring the critical control points of the food safety system performed well, with 95% understanding their role and their importance in the HACCP system. The study found that there was no difference between companies in the Republic of Ireland and Northern Ireland in terms of HACCP implementation and maintenance. Nor was there any real difference between small production plants (less than 250 people) and large corporations in terms of food safety policy.

Despite these positive baseline findings, the study found shortcomings in the areas of training and verification schedules, which would help to ensure that HACCP is



properly implemented and maintained. A lack of formal training was believed to be responsible for many of the deficiencies in HACCP systems. The study found that 16% of team leaders and 24% of team members within food production plants had no formal training in HACCP food safety systems.

The majority of companies did not perform well in the area of verification. This means that a constant audit of the HACCP plan is conducted, including sampling and checking data trends to ensure that it remains appropriate. Forty-eight percent did not have an appropriate verification procedure in place, while 23% of the verification auditors were not trained. Safefood stressed that these results were not a cause for alarm, but did indicate areas which could be improved upon. Safefood has produced a number of workshops designed to address the deficiencies highlighted in this report, the first took place in Dublin October 13th.

Dr. Thomas Quigley, director of science & technical, Safefood, said, "The primary objective of the study was to assess the effectiveness with which HACCP has been implemented and maintained in food manufacturing plants on the island of Ireland. This study is unique in that it involved an in-depth, two-day evaluation of HACCP on site in food production companies. This approach has been extremely successful in providing a wealth of knowledge on the ways in which HACCP systems are being implemented at present. This is the first study of this kind in Europe and it is expected that there will be European-wide interest in the study with other countries following suit in benchmarking HACCP implementation."

The study concluded that a shortage of technical expertise was a key factor contributing to many of the deficiencies observed. There are also limitations in the currently available HACCP training. The results are by no means any cause for alarm, but they do show areas which could be improved upon. The results of the study have been passed to the enforcement agencies in the South and North and should be very useful in helping to further improve food safety practices on the island. Safefood is immediately addressing the issues raised by the report and will facilitate the resolution of the deficiencies identified. Safefood is holding a one-day practical workshop, the first in a series for the industry, enforcement agents, HACCP trainers and auditors on issues of training, verification and maintenance. The workshop is a learning based approach that will provide practical solutions to the inadequacies of the current systems.

## New WHO 5 Keys Strategy for Safer Food

Each year, unsafe food makes at least two billion people ill worldwide, or about one third of the global population. Yet five simple prevention techniques could significantly reduce this burden of disease. On the occasion of the Second Global Forum of Food Safety Regulators, WHO launched its 5 Keys Strategy – a series of 5 simple actions which people can undertake at home or at work while preparing and consuming food. These are: keep hands and cooking surfaces clean; separate raw and cooked food; cook food thoroughly; keep food stored at safe temperatures; and use safe water and raw ingredients. WHO has produced a basic training manual to ensure that member states can use and disseminate effectively the information contained in the 5 Keys Strategy. It is meant for food safety professionals, teachers and interested organizations to use in train-ing selected target groups (including foodhandlers and schoolchildren, for example). Field testing of Bring Food Safety Home – How to Use the WHO 5 Keys to Safer Food is now starting around the world. Countries where field testing will occur include Argentina, Bolivia, Guyana, Haiti, Honduras and Nicaragua in the Americas; and Bangladesh, Bhutan, India, Indonesia, Maldives, Nepal and Timor-Leste in Southeast Asia. Even though the actions are applicable everywhere, WHO recognizes that the way food is prepared and the type of food which is eaten varies enormously across and within countries. The 5 Keys Strategy, consequently, does not set out prescriptions, and the implementing manual is a reflection of globally validated best practice, emphasizing 5 main messages which member states are encouraged to apply to local conditions. WHO regional offices are working to produce more specific versions of the 5 Keys Strategy and the manual. The five main messages are being translated into over 25 languages. While the global manual looks at the core messages, for example the WHO Regional Office for Southeast Asia, based in New Delhi (India), has produced a version which emphasizes the best way to adapt these messages to the local situation, where many people cannot afford the detergents and soaps generally recommended in preventing the spread of foodborne diseases.

## Officials Inaugurate Food Safety Institute of the Americas

United States Department of Agriculture Deputy Secretary Jim Moseley and Under Secrtary for Food Safety Dr. Elsa Murano, along with elected officials and dignitaries from throughout the Western Hemisphere, inaugurated the Food Safety Institute of the Americas (FSIA) to develop and promote effective food safety education and training programs throughout the Americas. "This institute is the first of its kind and seeks to provide a cooperative, educational-oriented relationship with the nations in the Western Hemisphere," Moseley said. "FSIA will address food safety and public health concerns by establishing and enhancing important networks among regulatory officials, researchers, public health officials, consumers, meat, poultry and egg processors and producers, as well as animal producers." Murano said the establishment of the institute supports priorities established by Agriculture Secretary Ann M. Veneman to spur the exchange of information and technology among countries around the world, which was the centerpiece of ministerial level meetings on science and technology held in Sacramento, CA, in June 2003 and follow-up meetings in May 2004 in Costa Rica and Burkino Faso in June 2004.

The grand opening activities began with a ceremonial signing of cooperative agreements between USDA, the University of Florida and Miami-Dade College, designed to reflect relationships between the cooperators to carry out educational or special studies programs to improve the safety and security of the food supply in the Americas. The University of Florida and Miami-Dade College are the first institutions to partner with FSIA in this endeavor.

USDA has worked to improve food safety programs in the Western Hemisphere by working with governments to raise the level of food safety activities and become active participants in international food standard setting bodies like the Codex Alimentarius Commission. In June, Murano signed a Memorandum of Understanding with the Pan American Health Organization to improve the safety of meat and poultry products that are traded among the nations of the Western Hemisphere.

The establishment of FSIA also is an objective outlined in the recently released "Fulfilling the Vision: Initiatives in Protecting Public Health," Murano said. This document reviews recent successes and builds on the course the Bush Administration set last year to improve the prediction and response to food safety challenges and further reduce the rate of foodborne illness.

## Food Safety: USDA and FDA Need to Better Ensure Prompt and Complete Recalls of Potentially Unsafe Food

Weaknesses in USDA's and FDA's food recall programs heighten the risk that unsafe food will remain in the food supply and ultimately be consumed. Specifically, USDA and FDA do not know how promptly and completely the recalling companies and their distributors and other customers are carrying out recalls, and neither agency is using its data systems to effectively track and manage its recall programs. For these and other reasons, most recalled food is not recovered and therefore may be consumed. GAO's analysis of recalls in 2003 showed that about 38 percent and 36 percent of recalled food was ultimately recovered in recalls overseen by USDA and FDA, respectively. These agencies also told GAO of instances in which companies were slow to reveal where they had distributed the food or provided inaccurate customer lists. That distribution information is critical because USDA's and FDA's primary role in recalls is to monitor the effectiveness of a company's recall actions. To do so, the agencies contact a sample of the distribution chain from these lists to verify that customers in the food distribution chain received notice of the recall, and that they located the food and removed it from the marketplace. However, the methodology that the agencies use for selecting the customers to check can result in entire segments of complex distribution chains being overlooked. Moreover, GAO found that the agencies did not complete verification checks for some recalls before the shelf life of the food expired. In addition, consumer groups and others question the usefulness of USDA's and FDA's efforts to communicate with the public, suggesting alternatives such as posting notices in grocery stores and direct notification of consumers. Agencies responsible for the safety of products, such as toys, heart pacemakers, and automobiles, have specific recall authority not available to USDA and FDA for food. This includes the authority to (1) require a company to notify the agency when it has distributed a potentially unsafe product, (2) order a recall, (3) establish recall requirements, and (4) impose monetary

penalties if a company violates recall requirements. For example, by law, companies must promptly notify the Consumer Product Safety Commission after learning that a product may pose an unreasonable risk of serious injury or death, or face penalties of up to $1.65 million. Likewise, FDA has recall authority for unsafe biological products, medical devices, radiation emitting electronic products, and infant formula. Moreover, in contrast to its inability to penalize a company that is slow to conduct a food recall, FDA can impose penalties of up to $100,000 per day for a company that fails to recall a defective biological product, such as a vaccine.

Two large food recalls completed in 2003 were associated with 8 deaths and nearly 100 serious illnesses in at least 16 states. Manufacturers voluntarily recall potentially unsafe food by notifying their customers to return or destroy it. The US Department of Agriculture (USDA), for meat, poultry, and egg products, and the Food and Drug Administration (FDA), for other food, have programs to monitor voluntary food recalls, verify that companies contact their customers, and maintain recall data. GAO (1) examined the recall programs and procedures USDA and FDA use to protect consumers from unsafe foods and (2) compared their food recall authority with the authority of agencies to recall other consumer products.

GAO proposes that Congress consider legislation requiring a company to notify USDA or FDA if it discovers it has distributed unsafe food and giving agencies authority to order food recalls, and recommends that the agencies take actions to ensure prompt, complete recalls and better recall monitoring. USDA said the report was generally accurate and its May 2004 directive will address weaknesses GAO found. FDA did not believe its system lengthened recalls or its processes reduced recovery. FDA disagreed with some recommendations. GAO continues to believe its recommended actions are needed to protect consumers. The full report can be found at: http://www.gao.gov/cgi-bin/getrpt?GAO-05-51.

## Kansas State Researchers Seek to Improve Food Safety Practices of Restaurant Employees

The numbers are downright sickening. An estimated 76 million foodborne illness cases occur in the United States every year. That's one in four Americans who will contract a foodborne illness annually after eating foods contaminated with such pathogens as *E. coli* O157:H7, *Salmonella*, hepatitis A, and *Listeria*. Here's some more food for thought: Approximately 325,000 people are hospitalized with illnesses and 5,000 die. The estimated costs in medical expenses and lost wages or productivity are between $6.5 and $34.9 billion. While most foodborne illness cases (or food poisoning as it is sometimes called) go unreported to health departments, nearly 13.8 million cases a year are caused by known agents — 30 percent by bacteria, 67 percent by viruses, and three percent by parasites. Toss in the fact that approximately 75 percent of all food consumed away from home is prepared in a restaurant, deli, cafeteria or institutional food service operation and that more than 11.7 million individuals are employed in the food service industry, the potential for foodborne illness outbreaks is significant.

Despite those numbers, few Americans understand the impact a foodborne illness could have on themselves or their families, especially children. But a three-year, $482,763 grant received by researchers at Kansas State University (K-State) from the United States Department of Agriculture, seeks to improve food safety practices of restaurant employees by using the theory of planned behavior. The grant was one of 26 totaling more than $12 million awarded to 19 colleges and universities throughout the US and its territories through the National Integrated Food Safety Initiative, announced by the USDA. The goal of these grants is to improve the efficiency and effectiveness of food safety programs.

According to Carol Shanklin, associate dean of the K-State Graduate School and a professor of hotel, restaurant, institution management and dietetics and Kevin Roberts, an instructor in hotel, restaurant, institution management and dietetics, the overall intent of the grant is to look at restaurant employee knowledge, attitude and practices related to food safety.

Both Roberts and Shanklin said the three most common risk factors implicated in foodborne diseases are directly related to the food handling practices of foodservice employees. These risk factors — time/temperature abuse, cross contamination and personal hygiene, hand washing in particular — are preventable if proper food safety practices are followed. Shanklin, Roberts, and the grant's other researchers, Betsy Barrett, an associate professor of hotel, restaurant, institution

management and dietetics, and Laura Brannon, an associate professor of psychology, will develop a training program designed to overcome employee barriers to food safety implementation in restaurants and increase the frequency of food safety practices utilized in restaurants. Researchers will evaluate employees' knowledge and observe their practices before and after attending a training seminar; and consult with them on why they are not using correct food handling practices.

Based upon the results of that study, a training program will be developed to specifically address those barriers and those critical behaviors that need to be implemented by the employees in order to prevent foodborne illness outbreaks among consumers. "Our ultimate goal is to develop tools that restaurant managers can use in training and supervising employees that would reinforce appropriate food handling practices to decrease consumers' risk of foodborne illness when they dine away from home. Foodborne illness outbreaks can have a negative effect on a restaurant because word of mouth is the most positive or negative advertisement that a business can have," Shanklin said.

The grant was the only one funded that was specifically targeted towards commercial restaurants. All others focused on food processing operations. "I think this speaks well for K-State's reputation for quality food safety research at USDA by the reviewers. I think even though 20 restaurants will be involved in the study, a sampling of restaurants within three states — Kansas, Iowa and Missouri — the results will benefit the whole industry and ultimately consumers," Shanklin said.

## OzFoodNet – Foodborne Illnesses in Australia 2003

Foodborne disease is a significant health issue both in Australia and overseas. OzFoodNet (established in 2000) estimates that each year approximately 5.4 million people become ill as a result of contaminated food. In addition, foodborne illness results in an estimated 17,770 hospitalizations and 125 deaths each year. The majority of people do not seek medical attention and do not appear in official notification statistics.

OzFoodNet has recently published Foodborne disease investigation across Australia: Annual report of the OzFoodNet network, 2003 in Communicable Diseases Intelligence 28 2004 359. The report is also available at: http://www.health.gov.au/internet/wcms/Publishing.nsf/Content/cda-pubs-cdipu bs.htm.

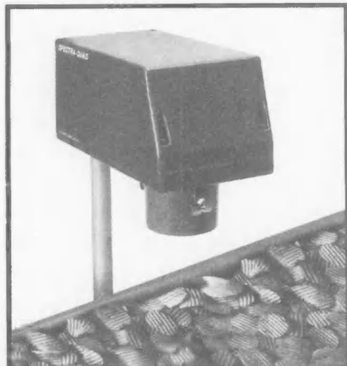
The Annual Report states that in Australia, doctors and laboratories are required to notify cases of certain diseases to state and territory health departments. In 2003, there were 23,250 reported cases of potentially foodborne diseases reported. The majority of these were bacteria that cause gastroenteritis. The two most commonly reported causes of gastroenteritis were *Campylobacter* and *Salmonella* bacteria that were responsible for 67 percent and 30 percent of these reports respectively.

Listeriosis is another important foodborne disease caused by infection with *Listeria monocytogenes* that can result in meningitis, septicaemia or abortion. While *Listeria* infections are uncommon, they pose particular risks to pregnant women, aged people, and those with weakened immune systems. In 2003, there were 72 listeriosis cases notified, with twelve infections in pregnant women or their fetuses. This was slightly higher than the historical reports average of 60 cases per year.

The rates of notified foodborne infections in Australia are similar to those reported in New Zealand, but considerably higher than rates reported for the United States. Reasons for this could include differences in laboratory testing between both countries or higher level of exposure to these infections in Australia.

In 2003, contaminated food in Australia was responsible for 99 outbreaks of gastroenteritis affecting 1,686 people, including 105 who were hospitalized and 6 who died. The types of contaminated food causing these outbreaks included fish, prawns, salad, chicken, tofu, fried rice, pizza, raw eggs and tahini. Significantly, frozen oysters from Japan were responsible for three outbreaks of Norovirus infection. The most common place where outbreaks occurred was restaurants (34 percent), in private homes (20 percent) and events catered by professional companies (14 percent). These settings, along with aged care facilities, are high risk for serious outbreaks of foodborne disease.

In view of the overall burden of illness and the serious risks concerning some foodborne infections, OzFoodNet's work will continue to be important for monitoring, investigating and responding to foodborne illness.



*Thermo Electron Corporation*

## Thermo Electron Corporation's Spectra-Quad Analyzer Provides Real-time Information on Moisture and Other Critical Product Parameters

Thermo Electron Corporation's Spectra-Quad On-Line Moisture and Constituent Analyzer incorporates a patented, industry-proven optical system and other quality innovations, making the Spectra-Quad an industry standard for on-line process analysis.

Using advanced filter technology and specialized measurement algorithms, the Spectra-Quad offers the highest performance on a broad range of applications. The system will measure product constituents such as moisture, coating weight, film thickness as well as many others.

Continuous product monitoring of the various key process variables allows immediate production line adjustments to be made. This avoids extended periods of off-specification production that can occur with manual sampling and analysis. Production line start-ups can be controlled to reach optimum conditions in the shortest possible time.

**Thermo Electron Corporation**
763.783.2630
Minneapolis, MN
www.thermo.com

## New Purity* FG Synthetic Grease and Purity* FG Synthetic EP Gear Fluid from Petro-Canada Provide Extreme Temperature Protection for Food Protection Processing Equipment

Petro-Canada has launched two new synthetic lubricants to the food processing industry that provide added protection in extreme temperature applications while maintaining the highest food-industry safety standards.

Purity* FG Synthetic Grease provides continuous protection under normal operating conditions from –45°C (–49°F) to 200°C (392°F), and up to 250°C (482°F) for transient periods. Purity* FG Synthetic EP Gear Fluid provides continuous protection under normal operating conditions from –35°C (–31°F) to 121°C (250°F).

"With processing equipment being pushed to their limits, it's essential to use lubricants that can meet or surpass operating conditions," said James McLean, category manager, Specialty Fluids, Petro-Canada. "We have formulated our products to do just that."

Purity* FG Synthetic Grease and Purity* FG Synthetic EP Gear Fluid have also demonstrated better wear protection and load-carrying capability in ASTM tests versus leading competitive synthetic products. In addition, Purity* FG Synthetic EP Gear Fluid outperformed the best competitive product by almost 50 percent in preventing sludge and varnish build up.

Petro-Canada's attention to food safety ensures that Purity* FG Synthetic Grease and Purity* FG Synthetic EP Gear Fluid fit perfectly in HACCP and GMP plans. They are H1 registered by NSF, free of genetically modified substances (GMS), and maintain food allergy safety. They are also certified Kosher and Pareve by Star K.

Purity* FG is a full line of food grade products including hydraulic fluids, compressor fluids, EP gear fluids, grease, light and heavy chain fluids, MF aerosol spray, white mineral oils, heat transfer fluid and trolley fluid.

**Petro Canada**
888.284.4572
Mississauga, Ontario, Canada
www.petro-canada.com

## Micropump's New I-Drive® — Greater Performance in a Smaller Package

Micropump® announces the release of its new I-Drive® electromagnetically driven pump, offering a significantly smaller package size without sacrificing power or capacity.

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*The publishers do not warrant, either expressly or by implication, the factual accuracy of the products or descriptions herein, nor do they so warrant any views or opinions offered by the manufacturer of said articles and products.*

This innovative design utilizes several unique features, including over-molded NdFeB magnets, next-generation surface mount technology, and an enhanced heat-sink, all of which lead to a smaller, more reliable, and better performing drive and pump/drive assembly.

The new I-Drive® combines a pumphead, a brushless DC motor, and an electronic controller into a single, compact unit that provides outstanding functionality and delivers smooth, pulseless flow. In this unique patented design, the 40W or 70W motor drives the pump electromagnetically, eliminating all physical contact with the motor. This means that there are absolutely no moving parts in the drive unit, leading to outstanding service life. The thermal and overload protected electronic controller provides variable speed drive options of 0-5 VDC, 4-20 mA remote control signal, or local manual control. Mounting options include a motor flange attachment or a removable bracket.

Available in standard or custom OEM configurations, the I-Drive® has a flow rate up to 3.2 l/min (0.85 gpm) and can handle a maximum differential pressure of 7 bar (100 psi). The simplicity of the built-in speed control and the tachometer output signal allows easy integration into PLC- or PC-controlled machines or end-user installations. The I-Drive carries CE, LVD, EMC, and UL safety approvals; the enclosure is IP55 rated.

**Micropump Inc.**
360.253.2008
Vancouver, WA
www.micropump.com

*Synbiosis*

## Synbiosis' New Automated Colony Counter

Synbiosis, a manufacturer of automated microbiological systems, is pleased to announce its ProtoCOL SR automated colony counter is being used at the College of Agriculture, Food and Rural Enterprise, Loughry Campus, Northern Ireland, United Kingdom, to enumerate different colored colonies of bacteria and guarantee the highest food safety standards.

Microbiologists at Loughry Campus are using the versatile ProtoCOL SR to automatically count a wide range of bacteria and yeast cells on spiral, pour and spread plates. One of the main uses of the ProtoCOL SR is to differentiate and count the numbers of red colonies from a background of blue colonies plated on selective chromogenic plates.

Since these bacteria are isolated from meat and shellfish destined for supermarkets, being able to use the ProtoCOL SR to quickly count colonies means we can ensure food going on sale is of the highest quality.

**Synbiosis**
301.662.1538
Frederick, MD
www.synbiosis.com

## Sigma-Aldrich Introduces Pre-poured Agar Plates for the Rapid Growth of Bacteria for Isolation and Selection

Sigma-Aldrich Corporation proudly introduces its new line of pre-poured plate media for use in molecular biology. The new line of pre-poured agar plates will allow researchers the benefit of sterile packaging, lot-to-lot consistency and time-saving convenience. In addition to LB and LB Ampicillin Agar, Sigma-Aldrich will offer custom plates covering a wide range of media formulations and antibiotics.

Pre-poured agar plates eliminate the need for mixing various components, autoclaving, pouring and cooling the plates. This provides the ultimate in convenience and efficiency for the researcher. Each lot of plates is tested with several *E. coli* strains to insure the highest quality and performance.

**Sigma-Aldrich Corporation**
314.771.5765
St. Louis, MO
www.sigma-aldrich.com

## Multisorb Technologies Introduces the FreshPax™

Dairy processors and cheese manufacturers can now protect dairy foods including all natural cheeses from spoilage with FreshPax™ oxygen sorbents from Multisorb Technologies, Inc., the world leader in active packaging solutions. FreshPax oxygen sorbents naturally maintain the shelf life and quality of packaged cheese and cheese products without

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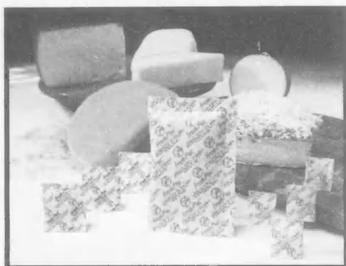

*Multisorb Technologies*

additives or preservatives. FreshPax retains food taste and color by effectively removing oxygen from the interior packaging environment. FreshPax protects packaged dairy foods such as cheeses, fermented dairy products such as yogurt and sour cream, and other dairy foods from spoilage, mold growth and other aerobic degradation.

FreshPax oxygen sorbent sachets are available in a wide variety of sizes and fills to ensure adequate oxygen absorber capacity and extend the shelf life of all natural dairy products. The sorbent sachets are inserted into almost any package to rapidly reduce oxygen levels and maintain the oxygen content within packaging to below 0.01 percent. FreshPax's oxygen scavenger technology prevents the growth of aerobic pathogens and organisms and controls the oxidative chemical and biochemical spoilage reactions that occur in products within a sealed package. FreshPax reduces or eliminates the need for food additives and preservatives, and is ideal for use with all natural dairy products.

The oxygen sorbent sachet can be used by itself or in conjunction with vacuum/gas flushed packaging to further reduce ambient oxygen present at the time of packaging to absorb virtually all-residual oxygen within packaging. The removal of oxygen from packaging retains the natural color and preserves nutritive value of food products. FreshPax works to continually absorb oxygen that permeates packaging throughout the shelf life of a product, minimizing the need for BHA, BHT, sulfur dioxide, sorbates, benzoates, and other food additives. FreshPax is made entirely of food grade ingredients.

**Multisorb Technologies**
888.SORBENT
Buffalo, NY
www.multisorb.com

## Surface Environmental Monitoring from International pbi S.p.A.

Contaminated surfaces by pathogenic microorganisms may produce infections, diseases, intoxications, food and dairy deteriorations. It is therefore necessary a periodic monitoring of the surfaces that can be contaminated by *Salmonella, E. coli, Listeria* and other germs.

The new "agar-blister" contact plates of International pbi are specially developed for this purpose, thanks to its single wrapped package that allows each single plate to be used one at a time, it can be adopted either by a small or big laboratory or quality control department.

The shelf life of 7 months at room temperature guarantees an easy and safe storage.

The "agar-blister" contact plates are also available in irradiated multi-packaging for clean room use.

More than ten different media are available for different microorganisms identification.

**International pbi S.p.A.**
39.02.48.779.1
Milano, Italy
www.rapidmicrobiology.com

## Hardy Diagostics Offer Contact Plates for Environmental Monitoring

The contact plate is a petri dish with a diameter of 60 mm, slightly overfilled with a nutrient agar. The petri plate has a grid molded into the bottom to aid in the counting of microorganisms. The Tryptic Soy Agar with Lecithin and Tween contact plate is useful in monitoring total microbial contamination and to assist in determining surface sanitation. Tryptic Soy Agar provides amino acids and other nitrogenous compounds making it a nutritious medium for many microorganisms.

Germicidal or disinfectant residue (quaternary ammonia compounds, hexachlorophene, and ethanol) is neutralized by the addition of Lecithin and Tween. TSA with Lecithin and Tween is available as a 15 x 60 mm contact plate.

For cleanroom applications, TSA with Lecithin and Tween contact plates are available double bagged and gamma irradiated.

**Hardy Diagnostics**
800. 266.2222 ext. 7696
Santa Maria, CA
www.hardydiagnostics.com

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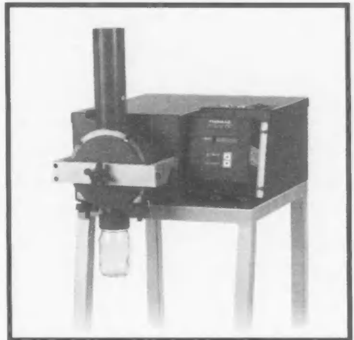

*Thomas Scientific*

### Thomas Scientific Introduces the Digital Ed-5 Wiley Mill

The Digital ED-5 Wiley Mill from Thomas Scientific is designed for reducing a wide range of difficult animal and plant materials as well as plastics. The ED-5 is the only grinder available with a digital speed controller that allows continuous variation of the cutter head between 650 and 1140 rpm. It uses four stationary steel blades mounted on its rotating cutting head that work against four adjustable blades mounted on the circumference of its 5 inch cutting chamber to efficiently chop the sample. The ED-5 is equipped with a powerful but quiet 1 HP motor that handles 5 to 250 g samples. For critical operations, the Digital ED-5 is available with stainless steel contact parts. The mil is solidly built using all steel construction and comes mounted on a heavy-duty steel frame with independent interlocking casters.

**Thomas Scientific**
800.345.2100
Swedesboro, NJ
www.thomassci.com

### Extra Corrosion Protection with Water-displacing Food Grade Lubricant from Cortec Corporation

Cortec® CorrLube® is a light lubricating and deep penetrating oil specially formulated to the needs of food and beverage processing applications. The product provides superior corrosion protection and retards bacterial growth. Having a USDA H-1 rating, CorrLube® can be used for a variety of machinery and equipment in processing food, meat, poultry and beverages.

CorrLube® features deep penetrating capabilities to attack rust and loosen frozen parts. Its powerful penetrating agents quickly free rusted and corroded fasteners to facilitate easy operation of air-actuated pins and guides. Once parts are free of rust, the product provides superior corrosion protection for ferrous and nonferrous metals to inhibit new attack of rust and corrosion, CorrLube® even provides protection to stainless steel and aluminum moving parts. It serves as an excellent anti-corrosion film on equipment during cleaning and idle periods.

CorrLube® can be used for both dry and wet equipment. When applied to wet equipment and components, it displaces the water on the surfaces and replaces it with lubricant and protective film. It lets metal equipment and components shed water and run off the metal surface. Its anticorrosion film helps prevent any residual attack to equipment from water, misting, and other corrosive agents in oils and greases.

In addition, CorrLube® is formulated with an H-1 approved bacteriostatic preservative. The preservative retards the growth of bacteria which are often present in conventional lubricants. It helps reduce breeding sites for microbes and minimize the spread of bacteria.

**Cortec® Corporation**
800.426.7832
White Bear Lake, MN
www.CortecVCI.com

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**IMPORTANT! Please read this information before completing your registration form.**

## MEETING INFORMATION

Register to attend the world's leading food safety conference.

Full Registration includes:

- Technical Sessions
- Symposia
- Poster Presentations
- Ivan Parkin Lecture
- John H. Silliker Lecture
- Awards Banquet
- Exhibit Hall Admittance
- Cheese and Wine Reception
- Exhibit Hall Reception
- Program and Abstract Book

## 4 EASY WAYS TO REGISTER

Complete the Attendee Registration Form and submit it to the International Association for Food Protection by:

**Online: www.foodprotection.org**

Fax: 515.276.8655

Mail: 6200 Aurora Avenue, Suite 200W
Des Moines, IA 50322-2864, USA

Phone: 800.369.6337; 515.276.3344

The early registration deadline is July 13, 2005. After this date, late registration fees are in effect.

## REFUND/CANCELLATION POLICY

Registration fees, less a $50 administration fee and any applicable bank charges, will be refunded for written cancellations received by July 29, 2005. No refunds will be made after July 29, 2005; however, the registration may be transferred to a colleague with written notification. Refunds will be processed after August 22, 2005. Event and tour tickets purchased are nonrefundable.

## STUDENT FUND RAISER

Help support the students with their annual fund raiser. See page 78 to order T-shirts or polo shirts.

## EXHIBIT HOURS

| | |
|---|---|
| **Sunday, August 14, 2005** | 8:00 p.m. - 10:00 p.m. |
| **Monday, August 15, 2005** | 9:30 a.m. - 1:30 p.m. |
| | 3:00 p.m. - 6:30 p.m. |
| **Tuesday, August 16, 2005** | 9:30 a.m. - 1:30 p.m. |

## DAYTIME TOURS – Lunch included

| | |
|---|---|
| **Saturday, August 13, 2005** | 9:00 a.m. - 5:00 p.m. |
| Welcome to Washington | |
| **Sunday, August 14, 2005** | 10:00 a.m. - 2:00 p.m. |
| Baltimore City Tour by Land and by Sea | |
| **Monday, August 15, 2005** | 9:00 a.m. - 2:00 p.m. |
| Annapolis Past and Present | |
| **Tuesday, August 16, 2005** | 9:00 a.m. - 1:00 p.m. |
| A Taste of Baltimore from the Inside | |
| **Wednesday, August 17, 2005** | 10:00 a.m. - 1:00 p.m. |
| Chesapeake Bay Cooking Class | |

## EVENING EVENTS

| | |
|---|---|
| **Saturday, August 13, 2005** | |
| Baseball Game | 3:30 p.m. - 7:30 p.m. |
| **Sunday, August 14, 2005** | |
| Opening Session | 7:00 p.m. - 8:00 p.m. |
| Cheese and Wine Reception<br>*Sponsored by Kraft Foods North America* | 8:00 p.m. - 10:00 p.m. |
| **Monday, August 15, 2005** | |
| Exhibit Hall Reception | 5:00 p.m. - 6:30 p.m. |
| Monday Night Social - Harbor Cruise | 6:30 p.m. - 10:00 p.m. |
| **Tuesday, August 16, 2005** | |
| Little Italy Walking Tour and Dinner | 6:00 p.m. - 10:00 p.m. |
| **Wednesday, August 17, 2005** | |
| Awards Banquet Reception | 6:00 p.m. - 7:00 p.m. |
| Awards Banquet | 7:00 p.m. - 9:30 p.m. |

## GOLF TOURNAMENT

| | |
|---|---|
| **Saturday, August 13, 2005** | |
| Golf Tournament at Waverly Woods Golf Club | 8:30 a.m. - 4:30 p.m. |

## HOTEL INFORMATION

For reservations, contact the hotel directly and identify yourself as an IAFP 2005 attendee to receive a special rate of $149 per night, single/double or make your reservations online. This special rate is available only until July 13, 2005 or until sold out.

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# Attendee Registration Form

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❐ IAFP occasionally provides Attendees' addresses (excluding phone and E-mail) to vendors and exhibitors supplying products and services for the food safety industry. If you prefer NOT to be included in these lists, please check the box.

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| Registration | $ 385 ($435 late) | $ 583 ($633 late) | ______ |
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| One Day Registration: ❐ Mon. ❐ Tues. ❐ Wed. | $ 210 ($235 late) | $ 320 ($345 late) | ______ |
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| Children 15 & Over* (Names): ______ | $ 25 ($ 25 late) | $ 25 ($ 25 late) | ______ |
| Children 14 & Under* (Names): ______ | FREE | FREE | ______ |
| *Awards Banquet not included | | | |

| EVENING EVENTS: | | # OF TICKETS | |
|---|---|---|---|
| Golf Tournament (Saturday, 8/13) | $ 125 ($135 late) | ______ | ______ |
| Baseball Game (Saturday, 8/13 - 3:45 p.m.-7:3 0 p.m.) | $ 26 ($ 36 late) | ______ | ______ |
| Student Luncheon (Sunday, 8/14) | $ 5 ($ 15 late) | ______ | ______ |
| Monday Night Social - Harbor Cruise (Monday, 8/15) | $ 45 ($ 55 late) | ______ | ______ |
| Children 14 and under | $ 40 ($ 50 late) | ______ | ______ |
| Tuesday Evening - Little Italy Walking Tour and Dinner (Tuesday, 8/16) | $ 92 ($102 late) | ______ | ______ |
| Additional Awards Banquet Ticket (Wednesday, 8/17) | $ 50 ($ 60 late) | ______ | ______ |

| DAYTIME TOURS: (Lunch included in daytime tours) | | | |
|---|---|---|---|
| Welcome to Washington (Saturday, 8/13) | $ 89 ($ 99 late) | ______ | ______ |
| Baltimore City Tour by Land and by Sea (Sunday, 8/14) | $ 74 ($ 84 late) | ______ | ______ |
| Annapolis Past and Present (Monday, 8/15 | $ 125 ($135 late) | ______ | ______ |
| A Taste of Baltimore from the Inside (Tuesday, 8/16) | $ 80 ($ 90 late) | ______ | ______ |
| Chesapeake Bay Cooking Class (Wednesday, 8/17) | $ 99 ($109 late) | ______ | ______ |

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Purchase an IAFP 2005 long-sleeve T-shirt or Polo Shirt from the Student PDG to help raise money in support of our Students. Pre-ordered T-shirts are $18.00 and Polo shirts are $25.00. Shirts will be available for pick-up from the SPDG booth throughout IAFP 2005. All order forms are due by July 13th. If you have any questions, contact Renee Raiden at rraiden@vt.edu.

## IAFP SPDG Shirt Order Form

If you choose to pay by credit card, make sure you include the amount to be charged. If you are paying by check make checks payable to IAFP and enclose the check with your order form. Please mail order forms for receipt by July 13, 2005 for pre-orders.

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# COMING EVENTS

## FEBRUARY

- **7–8, HACCP IV: Validation & Verification of Your HACCP Plan,** Guelph Food Technology Centre, Guelph, Ontario, Canada. For more information, contact Marlene Inglis at 519.821.1246; E-mail: minglis@gftc.ca.
- **8–10, Principles of Food Microbiology,** Seattle, WA. For more information, call 708.957.8449; Web site: www.silliker.com.
- **8–11, Food Pasteurization with Electronic Irradiation,** College Station, TX. For more information, contact Tom A. Vestal at 979.458.3406; E-mail: t-vestal@tamu.edu.
- **10–12, Expo Carnes 2005,** Cintermex, Monterrey, N.L., Mexico. For more information, outside Mexico call +52.81.83.69.66.60.64 y 65; E-mail: lizapex@cintermex.com.mx.
- **15–17, NFPA's 2005 Food Claims and Litigation Conference,** Ojai, CA. For more information, call 202.639.5950; Web site: www.nfpa-food.org/documents/FoodLitRegForm05.pdf.
- **16–17, American Association of Cereal Chemists Nutritional Importance of Carbohydrate Quality in Cereal Foods Symposium,** Novotel Tour-Noire, Brussels, Belgium. For more information, contact Jody Grider at 651.454.7250; E-mail: jgrider@scisoc.org.
- **16–17, Arizona Environmental Health Association Southwest Food Safety Conference and Exhibition,** Riverside Resort Hotel & Casino, Laughlin, NV. For more information, contact Chris Reimus at 480.820.7655 ext. 202; E-mail: creimus@mail.maricopa.gov.
- **17, Georgia Association for Food Protection Annual Spring Meeting,** University of Georgia, Food Science Bldg., Athens, GA. For more information, contact Mark Norton at 404.656.3621; E-mail: mnorton@agr.state.ga.us.
- **22–24, Kentucky Association of Milk, Food & Environmental Sanitarians Annual Spring Meeting,** Executive Inn West, Louisville, KY. For more information, contact Laura Strevels at 859.363.2022; E-mail: laura.strevels@ky.gov.
- **28–March 1, Effective Food Security,** Guelph Food Technology Centre, Guelph, Ontario, Canada. For more information, contact Marlene Inglis at 519.821.1246; E-mail: minglis@gftc.ca.

## MARCH

- **10–13, IAFIS 2005 Annual Conference,** San Francisco Fairmont, San Francisco, CA. For more information, call 703.761.2600 or go to www.iafis.org.
- **14–15, Microbiology IV: Sampling & Interpreting Results,** Guelph Food Technology Centre, Guelph, Ontario, Canada. For more infomation, contact Marlene Inglis at 519.821.1246; E-mail: minglis@gftc.ca.
- **16–18, Food Safety Summit,** Washington, D.C. Convention Center, Washington, D.C. For more information, call 800.746.9646 or go to www.foodsafetysummit.com.
- **31, Foodborne Illness & Food-Related Injury: Investigation & Resolution for Food Service & Retail,** Guelph Food Technology Centre, Guelph, Ontario, Canada. For more infomation, contact Marlene Inglis at 519.821.1246; E-mail: minglis@gftc.ca.
- **31, UKAFP Call for Abstracts,** Cardiff, Wales. For more information, contact Gordon Hayburn at 44.(0) 2920.416456; E-mail: ghayburn@uwic.ac.uk.

## APRIL

- **6–8, Missouri Milk, Food and Environmental Health Association Educational Conference,** Ramada Inn, Columbia, MO. For more information, contact Marsha Perkins at 573.874.7346; E-mail: mlp@gocolumbiamo.com.

## MAY

- **12–17, The 30th National Conference on Interstate Milk Shipments,** Hyatt on Capitol Square, Columbus, OH. For more information, contact Leon Townsend at 502.695.0253; E-mail: ltownsend@ncims.org.
- **17–18, Pennsylvania Association of Milk, Food and Environmental Sanitarians Annual Spring Meeting,** Penn State University, State College, PA. For more information, contact Gene Frey at 717.397.0719; E-mail: erfrey@landolakes.com.
- **23–26, 3-A SSI Annual Meeting,** Four Points by Sheraton Milwaukee, Milwaukee, WI. For more information, contact Timothy Rugh at 703.790.0295; E-mail: trugh@3-a.org.
- **23–26, AOAC Midwest Section Meeting and Expo,** Kansas City, MO. For more information, contact Ron Jenkins at 816.891.0442; Web site: www.midwestaoac.org.
- **24, Associated Illinois Milk, Food and Environmental Sanitarians Annual Spring Meeting,** Bloomington, IL. For more information, contact Don Wilding at 217.785.2439; E-mail: dwilding@idph.state.il.us.
- **24–26, Penn State Food Microbiology Short Course Detection and Control of Foodborne Pathogens,** Penn State University, Berks-Lehigh Valley College, Reading, PA. For more information, contact Dr. Hassan Gourama at 610.396.6121; E-mail: hxg7@psu.edu; http://foodsafety.cas.psu.edu.

## JUNE

- **12–15, 4th IDF International Mastitis Conference,** Maastricht, The Netherlands. For more information, go to www.fil-idf.org/mastitis2005.
- **13–14, Brazil Association for Food Protection Annual Meeting,** Conselho Regional de Quimica do Estado de Sao Paulo, R. Oscar Freire 2309, Sao Paulo, SP, Brazil. For more information, contact Maria Teresa Destro at 55.113.091.2199; E-mail: mtdestro@usp.br.
- **16–24, XXV Quarter Century Gala International Workshop/Symposium on Rapid Methods and Automation in Microbiology,** Kansas State University, Manhattan, KS. For more information, contact Daniel Y. C. Fung at 785.532.5654; E-mail: dfung@oznet.ksu.edu.

IAFP UPCOMING MEETINGS

**AUGUST 14-17, 2005**
Baltimore, Maryland

**AUGUST 13-16, 2006**
Calgary, Alberta, Canada

**JULY 8-11, 2007**
Lake Buena Vista, Florida

## Assistant Professor
## Food Safety/Poultry Products

**The Poultry Science Department at Auburn University** has established a "Peak of Excellence" program in Poultry Products Safety and Quality and is seeking candidates for an Assistant Professor, 9 month tenure-track faculty position in the area of food microbiology and/or safety. Position is available August 16, 2005. **Women and Ethnic Minorities are encouraged to apply.**

Responsibilities include; developing an instructional, research and outreach program in further processing, value-added poultry products with an emphasis in product microbiology; teaching and developing undergraduate and graduate courses in Poultry Products Safety.

Minimum qualifications include a Ph.D. in Poultry Science, Animal Science, Food Science, Food Microbiology or a closely related area with documented experience in food microbiology and/or food safety. Knowledge and ability to develop effective research, teaching and outreach programs in food safety with an emphasis in poultry processing and poultry products should be demonstrated; evidence of individual and collaborative research; excellent interpersonal skills and communication skills; ability to effectively interact with diverse audiences; and ability to communicate effectively, both orally and in writing. The candidate selected for this position must be able to meet eligibility requirements for work in the United States at the time appointment is scheduled to begin and must be able to communicate in English.

Salary commensurate with education and years of experience. Candidates should submit a letter of application, current resume, all transcripts, and names, phone numbers, addresses, and e-mail addresses of five references to:

Dr. Shelly McKee, Search Committee Chair
Department of Poultry Science
236 Upchurch Hall
Auburn University, AL 36849
PH: (334)844-2765
FAX: (334)844-2641
E-mail: mckeesr@acesag.auburn.edu

Review will begin December 1, 2005 and continue until a suitable candidate is selected. The position start date is August 16, 2005.

Information on the AU Poultry Product Safety and Quality Program can be found at www.ag.auburn.edu/dept/ph/peak

**Auburn University is an Affirmative Action/Equal Opportunity Employer.**

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- Perform food safety and quality audits at retail shops throughout North America. All audits include training activities for franchisees, Market Teams and Corporate personnel.
- Manage various improvement tests and projects including such things as: new cleaning chemicals, sanitation testing equipment, electronic data collection, sanitary smallwares, training tools, 3rd party audit programs, outside lab testing, rapid method testing, etc.
- Provide technical expertise and review for all new shop designs, training materials and manuals, new products, equipment, shop processes and operating systems.
- Represent Dunkin' Brands on various regulatory and industry associations such as the Conference for Food Protection. Provide technical guidance on regulatory and retail compliance.
- Develop and communicate audit plans for all Markets, maintain schedules and electronic maps, communicate results
- Develop, manage and communicate Corrective Action Plans with all Markets, Training, Construction services and Restaurant Operating Systems to drive improvement

Job Requirements:

- Bachelor of Science or Master of Science in Food Science, Biology or related scientific field.
- 5 or more years experience in retail regulatory, food safety and quality assurance.
- Experience in project management, auditing, and product risk evaluation.
- Thorough knowledge of Hazard Analysis of Critical Control Point (HACCP) programs, Good Manufacturing Practices (GMP's), ISO Standards, Food and Drug Administration (FDA) Model Food Code, Code of Federal Regulations (CFR) and general microbiology.
- Experience in working on standing or ad hoc regulatory advisory committees related to the Conference for Food Protection and Association of Food and Drug Officials
- Participation and leadership in industry associations such as the National Restaurant Association QA Study Group and International Association for Food Protection
- Ability to navigate and understand a complex regulatory landscape
- Thorough knowledge of the FDA Model Food Code, FDA Plan Review Guide, NSF sanitary standards, and retail HACCP
- Ability to travel extensively (40%-50%), handle heavy workload and meet high expectations
- Proficient computer skills to use the Quality Management Information System, Reflexis audit database, Lotus Notes PIM, Microsoft Office, MapPoint mapping with database integration
- Strong interpersonal skills in mediation and negotiation to build and maintain relationships with franchisees, internal employees and external partners
- Written and verbal communication and presentation skills

**To be considered for this position, please apply using our online application at www.careers-adqsr.com or send your resume to mquine@adrus.com**

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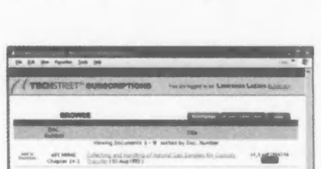

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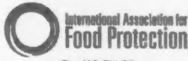


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**ERRATA**

In the article "Effect of Thermoultrasonication on *Salmonella enterica* Serovar Enteritidis in Distilled Water and Intact Shell Eggs," by Cabeza et al., *Journal of Food Protection* 67(9):1886–1891, the last equation in Table 2 (p. 1889) should have read log $D_M$ = 7.4527−0.1388*T*, not log $D_M$ = 7.4527−0.1355*T* as presented.

An acknowledgment for the article "Spectroscopic Quantification of Bacteria Using Artificial Neural Networks" by Gupta et al., *Journal of Food Protection* 67(11):2550–2554 is as follows:

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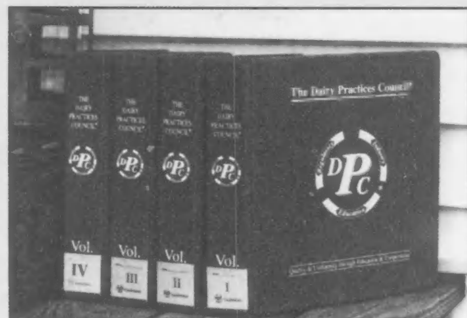

# IAFP

## Offers

## "Guidelines for the Dairy Industry"

## from

# The Dairy Practices Council®

***This newly expanded Four-volume set consists of 70 guidelines.***

1 Planning Dairy Freestall Barns
2 Effective Installation, Cleaning, and Sanitizing of Milking Systems
3 Selected Personnel in Milk Sanitation
4 Installation, Cleaning, & Sanitizing of Large Parlor Milking Systems
5 Directory of Dairy Farm Building & Milking System Resource People
6 Natural Ventilation for Dairy Tie Stall Barns
7 Sampling Fluid Milk
8 Good Manufacturing Practices for Dairy Processing Plants
9 Fundamentals of Cleaning & Sanitizing Farm Milk Handling Equipment
10 Maintaining & Testing Fluid Milk Shelf-Life
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12 Tunnel Ventilation for Dairy Tie Stall Barns
13 Environmental Air Control and Quality for Dairy Food Plants
14 Clean Room Technology
15 Milking Center Wastewater
16 Handling Dairy Products from Processing to Consumption
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22 Control of Antibacterial Drugs & Growth Inhibitors in Milk and Milk Products
23 Preventing Rancid Flavors in Milk
24 Troubleshooting High Bacteria Counts of Raw Milk
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IAFP has agreed with The Dairy Practices Council to distribute their guidelines. DPC is a non-profit organization of education, industry and regulatory personnel concerned with milk quality and sanitation throughout the United States. In addition, its membership roster lists individuals and organizations throughout the world.

For the past 34 years, DPC's primary mission has been the development and distribution of educational guidelines directed to proper and improved sanitation practices in the production, processing, and distribution of high quality milk and milk products.

The DPC Guidelines are written by professionals who comprise six permanent task forces. Prior to distribution, every guideline is submitted for approval to the state regulatory agencies in each member state. Should any official have an exception to a section of a proposed guideline, that exception is noted in the final document.

The guidelines are renown for their common sense and useful approach to proper and improved sanitation practices. We think they will be a valuable addition to your professional reference library.

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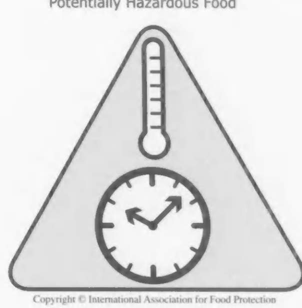
Potentially Hazardous Food



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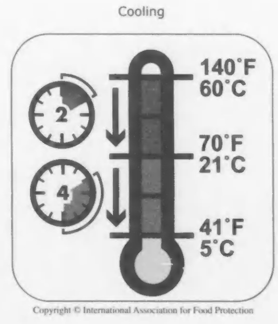


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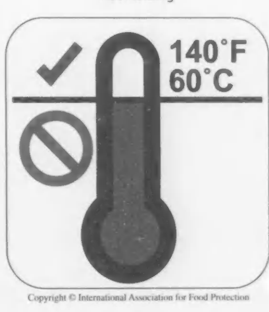


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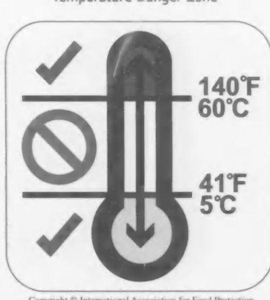


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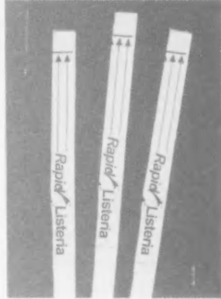


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