



Matrix MicroScience, Inc.

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BIBLIOGRAPHY

Escherichia coli O157:H7

MULT-1 PARTON, A. & BETTS, R. (2002) **A Practical Solution to the Problems Associated with Rapid Pathogen Detection.** Matrix MicroScience, Newmarket, U.K. and Campden & Chorleywood Food Research Assoc., U.K. (IAFP Annual Meeting, San Diego, CA June 30 – July 3, 2002)

Summarized the data that led to the granting of AOAC-RI approvals for Salmonella, Listeria, E.coli O157:H7 and the “Dual” Salmonella/Listeria tests, with a sensitivity of 1 -10 cfu / 25 gram sample.

MULT-2 MAKS-WARREN, N., PARISI, B., SLADE, P., & FU, T-J. (2005) **Evaluation of an Automated Immuno-magnetic Separation System for Selective Isolation of Escherichia coli O157, Salmonella, and Listeria monocytogenes from Inoculated Spent Sprout Irrigation Water.** Food and Drug Administration & National Center for Food Safety & Technology, Summit-Argo, IL USA. (IAFP Annual Meeting, Baltimore, MD, August 14-17, 2005)

Irrigation waters were pre-concentrated prior to the Pathatrix IMS step. In doing so, levels of Salmonella and Listeria as low as 1 cfu per 2.5 L could be found in sprout water.

MULT-3 PARTON, A., MURRAY, J., PRENTICE, N., SCOTT, M., & COOMBS, P. (2005) **Evaluation of a Novel Sample Pooling Strategy for High Volume Screening and Detection of Escherichia coli O157, Salmonella spp. and Listeria spp. in Food.** Matrix MicroScience: Newmarket, UK. (IAFP Annual Meeting, Baltimore, MD, August 14-17, 2005)

Presented data to support the pooling strategy approach for the detection of E.coli O157:H7, Salmonella spp. and Listeria spp. from a variety of food types. This could provide the food industry with a more rapid and cost effective means for screening for these pathogens.

MULT-5 **Pathatrix Recirculating Immuno-magnetic Separation – A Unique and Versatile System for the Rapid Detection of Foodborne Pathogens in Leafy Produce, Herbs and Spices (2008)**

J. MURRAY, N. PRENTICE, P. BENTON, K. BRZEGOWA, B. HOUSTON, M. VAN WART, M.F. SCOTT, ADRIAN PARTON, Matrix MicroScience Ltd., Lynx Business Park, Fordham Road, Newmarket, Cambs, CB8 7NY, UK (IAFP Annual Meeting, Columbus, OH, August 3 - 6, 2008)

The simultaneous isolation and detection of E. coli O157:H7 and Salmonella from pooled (125 g) fresh produce samples was comfortably achieved within a working day (< 8 h) using RIMS linked to RT-PCR. The data also shows that RIMS linked to RT-PCR can be successfully used to isolate and detect Listeria spp. and Salmonella in post growth pooled spice samples when target pathogens are initially present at low levels (1-10 CFU in 1875 g)

- EC-1** WU, V.C.H., GILL, V., OBERST, R., PHEBUS, R., & FUNG, D.Y.C. (2004) **Rapid Protocol (5.25 H) for the Detection of *Escherichia coli* O157:H7 in Raw Ground Beef by an Immuno-capture System (Pathatrix) in combination with Colortrix and CT-SMAC.** *Journal of Rapid Methods and Automation in Microbiology* **12**, 57-67

*Validated the protocol first developed by Matrix Microscience for the rapid detection of low numbers of *E. coli* O157:H7 from raw ground beef in 5.25 hours.*

- EC-2** ARTHUR, T.M., BOSILEVAC, J.M., NOU, X., & KOOHMARAIE, M. (2005) **Evaluation of Culture- and PCR-Based Detection Methods for *Escherichia coli* O157:H7 in Inoculated Ground Beef.** *Journal of Food Protection* **68**, 1566-1574.

Demonstrated that Pathatrix (either in combination with PCR or with agar plates) detected a greater number of positive samples than other methods; and when linked with PCR, gave the fastest results. The Pathatrix protocol was also able to test 375 gram composite samples and detect levels of 10 cfu/ 375 gram.

- EC-3** ELLINGSON, J.L.E. (2005) **Marshfield Clinic *E. coli* O157:H7 Test method.** *Inside Laboratory Management* Nov/Dec 2005, 18-20.

*Summarized the results of the study that led to AOAC-RI approval for the combination of Pathatrix and real-time PCR for the detection of *E. coli* O157:H7 from ground beef in as little as 8 hours.*

- EC-4** DURSO, L.M. & KEEN, J.E. (2005) **A Comparison of Immunomagnetic Separation (IMS) Techniques for the Detection of Shiga-Toxigenic *Escherichia coli* O157 (STEC O157) from Feces of Naturally-Infected Cattle.** USDA – Meat Animal Research Center, Clay Center, NE (ASM Annual Meeting, Atlanta, GA June 6 – 10, 2005)

Demonstrated that Pathatrix was more sensitive than other IMS methods, although they had reservations about sample throughput. [Since addressed by the pooling strategy – Ed.]

- EC-5** DAMMANN, H., KOZICZKOWSKI, J., CLARK, D., RADCLIFF, R.P., & ELLINGSON, J.L. (2005) **PCR Detection of *Escherichia coli* O157:H7 in Ground Beef: Individual and Pooled Samples.** Marshfield Clinic Laboratories Food Safety Services: Marshfield, WI, USA. (IAFP Annual Meeting, Baltimore, MD, August 14-17, 2005)

*Summarized their work to validate the use of Pathatrix to detect *E. coli* O157:H7 in both single and pooled samples. By coupling with PCR, it was possible to detect 8 cfu in both individual and pooled samples within 8 hours. Due to this level of accuracy and sensitivity, pooling across lots may be a way for companies to reduce the cost of testing for this pathogen.*

- EC-6** PRENTICE, N., MURRAY, J., SCOTT, M.F., COOMBS J.P., & PARTON, A. (2006) **Rapid Isolation and Detection of *Escherichia coli* O157:H7 in Fresh Produce.** *Journal of Rapid Methods and Automation in Microbiology* **14**, 299 – 308.

*Described a method for the rapid isolation and detection low levels of *E. coli* O157:H7 (0.04 – 0.4 cfu / g) from fresh salad, bean sprouts and spinach in less than 7 hours using a combination of Pathatrix and real-time PCR.*

- EC-7** LAU, H.K., LIN, A., & HIMATHONGKHAM, S. (2006) **Detection of *Escherichia coli* O157:H7 in Produce Rinse by Pathatrix™ Immunomagnetic Extraction and Conventional Methods.** *FDA/ORA/DFS Laboratory Information Bulletin* #4371

Compared a modified BAM method with Pathatrix for the detection of E. coli O157:H7 from three different types of lettuce at different levels (1 cfu / 25 g and 10 cfu / 25 g) and incubation periods (6 h & 24 h). Reported that the advantage of Pathatrix was the short incubation period (6 h) coupled with a high isolation rate.

- EC- 8** HIMATHONGKAM, S., YEE, J., LAU, H., LIN, A. & LAU, D.K. (2006) **Rapid and Effective Method to Improve Detection and Isolation of *E.coli* O157:H7 from Fresh Produce.** CA Dept of Health Services and FDA, Alameda, CA. (IAFP Annual Meeting, Calgary, Alberta August 13-16, 2006)

Demonstrated successful recovery of E.coli O157:H7 at levels of 15 cfu / 150 gram romaine lettuce or spinach with 5 hours incubation followed by Pathatrix and real-time PCR.

- EC-9** FEDIO, W. M., JINNEMAN, K. C., CARRILLO, C., YOSHITOMI, K. J., ZAPATA, R., WENDAKOON, C. N., BROWNING, P. & WEAGANT, S. D. (2007) **Detection of *E. coli* O157:H7 in Ground Beef by Pathatrix™ Immunomagnetic-capture, Real Time PCR and Cultural Methods.** New Mexico State University, Las Cruces, NM; Food and Drug Administration, Bothell, WA. (ASM Annual Meeting, Toronto, Ont May 20 - 26, 2007.)

Evaluated different pre-enrichment broths and incubation times with and without Pathatrix IMS in ground beef artificially inoculated with 0.1 cfu/g and 1.0 cfu/g. 24 hours enrichment followed by IMS provided the greatest number of positive detections.

- EC-10** GILL, A. (2007) **Evaluation of Cationic Magnetic Separation Beads for the Capture of *Escherichia coli* O157:H7.** Health Canada, Ottawa, Ont. (IAFP Annual Meeting, Orlando, FL July 8 - 11, 2007.)

Compared the ability of cationically- charged particles to capture E.coli O157:H7 from ground beef. Showed that the beads had a low affinity for the pathogen but that background flora did not interfere with capture.

- EC-11** WEAGANT, S.D., YOSHITOMI, K.J., CARILLO, C., ZAPATA, R., WENDAKOON, C., BROWNING, P., JINNEMAN, K.C. & FEDIO, W.M. (2007) **Detection of *Escherichia coli* O157:H7 in Artificially Contaminated Spinach by Pathatrix Immunomagnetic-capture, Real-time PCR and Cultural Methods.** New Mexico State University, Las Cruces, NM, (IAFP Annual Meeting, Orlando, FL July 8 - 11, 2007.)

Were able to detect E.coli O157:H7 at 0.1 cfu/g in artificially contaminated spinach using modified Buffered Peptone Water combined with IMS, real-time PCR and selective agars. The FDA BAM method provided equivalent sensitivity but required additional time to complete.

- EC-12** DURSO, L.M. & KEEN, J.E. (in press) **Shiga-toxigenic *Escherichia coli* O157 and non-Shiga-toxigenic *E.coli* O157 respond differently to culture and isolation from naturally contaminated bovine faeces.** *Journal of Applied Microbiology* available on line: doi:10.1111/j.1365-2672.2007.03473.x

Reported that the combination of 6-hr enrichment in Gram-negative broth containing vancomycin, cefixime and cefsulodin, large volume IMS (more sensitive than a conventional low volume IMS) and selective plating on TCA (Tellurite-Chromogenic agar) maximized the recovery of STEC O157 from naturally-contaminated cattle fecal specimens.

- EC-13** M.T. JAY, M. COOLEY, D. CARYCHAO, G.W. WISCOMB, R.A. SWEITZER, L. CRAWFORD-MIKSZA, J.A. FARRAR, D.K. LAU, J. O'CONNELL, A. MILLINGTON, R.V. ASMUNDSON, E.R. ATWILL, R.E. MANDRELL (2008) ***Escherichia coli* O157:H7 in Feral Swine near Spinach Fields and Cattle, Central California Coast.** California Dept. of Public Health, Richmond, CA; Univ. of California, Davis, CA; US Dept. of Agriculture, Albany, CA; US Dept. of Agriculture, Sacramento, CA; Univ. of North Dakota, Grand Forks, ND; California Dept. of Public Health, Sacramento, CA & US Food

and Drug Administration, Alameda, CA, USA (*Emerging Infectious Diseases*, 2007, 13, N° 12, p1908-1911)

The involvement of feral swine in the Escherichia coli O157:H7 contamination of agricultural fields and surface waterways was investigated after a nationwide outbreak was traced to bagged spinach from California. Isolates from feral swine, cattle, surface water, sediment, and soil at one ranch were matched to the outbreak strain.

- EC-14** S. HIMATHONGKHAM, M.L. DODD, J.K. YEE, D.K. LAU, R.G. BRYANT, A.S. BADOIU, H.K. LAU, L.S. GUTHERTZ, L. CRAWFORD-MIKSZA, M.A. SOLIMAN, (2007) **Recirculating Immunomagnetic Separation and Optimal Enrichment Conditions for Enhanced Detection and Recovery of Low Levels of *Escherichia coli* O157:H7 from Fresh Leafy Produce and Surface Water.** Food and Drug Laboratory Branch, California Dept. of Public Health, Richmond, CA & U.S. Food and Drug Administration, San Francisco District Laboratory, Alameda, CA, USA (*Journal of Food Protection*, 2007, 70, N° 12, p2717-2724)

This study demonstrated the development of a rapid, simple method for enhanced detection and isolation of low levels of Escherichia coli O157:H7 from leafy produce and surface water using Pathatrix recirculating immunomagnetic separation (RIMS) coupled with real-time PCR and a standard culture method.

- EC-15** **Detection and Recovery of *Escherichia coli* O157:H7 in Artificially Contaminated Alfalfa Sprouts by PATHATRIX Immunomagnetic Separation, Real-Time PCR and Cultural Methods** (2008) S.D. WEAGANT, K.J. YOSHITOMI, K.C. JINNEMAN, R. ZAPATA, C. WENDAKOON, P. BROWNING, W.M. FEDIO, New Mexico State University, P.O. Box 30003, Las Cruces, NM 88003, USA (IAFP Annual Meeting, Columbus, OH, August 3 - 6, 2008)

*O157:H7 capture by Pathatrix was assessed in conjunction with various media. After 24 h, mBPWp greatly improved detection and recovery of *E. coli* O157 over EEB. Pathatrix treated enrichments increased recovery of *E. coli* O157:H7 over non-IMS treatments.*

- EC-16** **Addressing Potential Contaminants in Soil for the Study of Pathogenic *Escherichia coli* O157 and O8 Strains** (2008) A. LAYCOCK, M. SHARMA, K. KNIEL, University of Delaware, 044 Townsend Hall, 531 South College Ave., Newark, DE 19717, USA (IAFP Annual Meeting, Columbus, OH, August 3 - 6, 2008)

*APEC O157 (1.3×10^6 CFU/g) and O8 (3.0×10^6 CFU/g) could be recovered from soil inoculated with 10^8 CFU/g, using *E. coli* O157 Pathatrix beads and plated onto TSA-NA without the need for further selective media.*

- EC-17** **Inclusivity of Three Immunomagnetic Beads for Forty Strains of *Escherichia coli* O157** K. J. YOSHITOMI, S. D. WEAGANT, C. N. WENDAKOON, C. CARRILLO, K.C. JINNEMAN, R. ZAPATA, P. BROWNING, W.M. FEDIO, New Mexico State University, P.O. Box 30003, Las Cruces, NM 88003, USA (IAFP Annual Meeting, Columbus, OH, August 3 - 6, 2008)

*Pathatrix successfully recovered all 40 strains of *Escherichia coli* O157 and also captured a greater percentage of target organism compared to the other IMS beads tested.*

- EC-18** **Same Day Detection of low level *E. coli* O157:H7 in Raw Ground Beef using post pre-enrichment sample pooling, re-circulating IMS and Real Time PCR.** (2008) J. MURRAY, N. PRENTICE, P. BENTON, K. BRZEGOWA, B. HOUSTON, M. VAN WART, M.F. SCOTT, ADRIAN PARTON, Matrix MicroScience Ltd., Lynx Business Park, Fordham Road, Newmarket, Cambs CB8 7NY, UK (Presentation; Food Micro, Aberdeen, Scotland, September 1-4, 2008)

The detection of low level E. coli O157:H7 contamination in post growth pooled (1875 g) raw ground beef samples was comfortably achieved within one working day (<7 h) using the Pathatrix pooling strategy linked to real-time PCR.

Enterobacter sakazakii

MULT-4 PARTON, A., MURRAY, J.S., PRENTICE, N. & HALL, P.A. (2007) **Development of a Rapid Method for the Isolation and Detection of *Enterobacter sakazakii* and *Salmonella* spp. in Xanthan Gum.** Matrix Microscience, Newmarket, U.K. (IAFP Annual Meeting, Orlando, FL July 8 - 11, 2007.)

*Data showed that Pathatrix-RIMS coupled to selective agars could detect *Salmonella* spp. and *E. sakazakii* at low levels (1 – 10 cfu/25 g) and correlated 100% with standard methods. The test allowed for the rapid analysis of larger sample sizes.*

ESAK-1 MULLANE, N.R., MURRAY, J., DRUDY, D., PRENTICE, N., WHYTE, P., WALL, P.G., PARTON, A., & FANNING, S. (2006) **Detection of *Enterobacter sakazakii* in Dried Infant Milk Formula by Cationic-Magnetic-Bead Capture.** *Applied and Environmental Microbiology*, **72**, 6325-6330.

*Using non-selective cationically-charged particles, demonstrated that the Pathatrix protocol reliably detected 1 to 5 cfu of *E. sakazakii* in 500 gram of infant milk formula in less than 24 hours.*

ESAK-2 MURRAY, J., PRENTICE, N., PARTON, A. & HALL, P. (2006) **The Detection of *Enterobacter sakazakii* and Other *Enterobacteriaceae* from Milk Powders Using Paramagnetic Cationic Particles.** Matrix MicroScience: Newmarket, UK, and Golden, CO & Univ College, Dublin, Ireland. (IAFP Annual Meeting, Calgary, Alberta August 13-16, 2006)

*Showed that using non-specific cationically-charged particles, the Pathatrix method was at least as sensitive as the FDA BAM method at low levels of 1 – 10 cfu / 100 gram (1 – 10 cfu / 500 gram with pooling.) Test times were 24 hours compared with 96 hours or more for the BAM method. As well as *E. sakazakii*, the method also captured *Salmonella* spp. and other *Enterobacteriaceae* simultaneously.*

ESAK-3 TALL, B.D., KOTHARY, M.H., RESTAINO, L., CARTER, L., DEER, D., EWING-PEEPLES, L., FRAZAR, C. & McCARDELL, B.A. (2007) **Isolation and Detection of *Enterobacter sakazakii* from Cereals and Cereal Ingredients Using a Novel Enrichment Broth, Cationic Paramagnetic Capture, a Chromogenic Agar and Real Time PCR.** Food and Drug Administration, Laurel, MD & R&F Laboratories, Downers Grove, IL. (ASM Annual Meeting, Toronto, Ont May 20 - 26, 2007.)

*Demonstrated that the recovery of *E. sakazakii* from cereal could be improved by the use of a higher dilution of pre-enrichment broth (1:20 instead of 1:10) and by the use of filter stomacher bags. Using Pathatrix cationic beads, were able to capture all 15 *E. sakazakii* biotypes. And the sensitivity of the method could be improved from 0.1 cfu / gm to 0.4 cfu / gm by extending the pre-enrichment period from 6 hours to 24 hours.*

Listeria spp.

MULT-1 PARTON, A. & BETTS, R. (2002) **A Practical Solution to the Problems Associated with Rapid Pathogen Detection.** Matrix MicroScience, Newmarket, U.K. and Campden & Chorleywood Food Research Assoc., U.K. (IAFP Annual Meeting, San Diego, CA June 30 – July 3, 2002)

*Summarized the data that led to the granting of AOAC-RI approvals for *Salmonella*, *Listeria*, *E. coli* O157:H7 and the “Dual” *Salmonella/Listeria* tests, with a sensitivity of 1 -10 cfu / 25 gram sample.*

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*Irrigation waters were pre-concentrated prior to the Pathatrix IMS step. In doing so, levels of *Salmonella* and *Listeria* as low as 1 cfu per 2.5 L could be found in sprout water.*

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MULT-5 Pathatrix Recirculating Immuno-magnetic Separation – A Unique and Versatile System for the **Rapid Detection of Foodborne Pathogens in Leafy Produce, Herbs and Spices** (2008)

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*The simultaneous isolation and detection of *E. coli* O157:H7 and *Salmonella* from pooled (125 g) fresh produce samples was comfortably achieved within a working day (< 8 h) using RIMS linked to RT-PCR. The data also shows that RIMS linked to RT-PCR can be successfully used to isolate and detect *Listeria* spp. and *Salmonella* in post growth pooled spice samples when target pathogens are initially present at low levels (1–10 CFU in 1875 g).*

LIST-1 MURRAY, J., PRENTICE, N., GORDON, C.M. & PARTON, A. (2004) **Detection of *Listeria* spp. in Environmental Samples by a Combination of Wet Composites and a Novel Immuno-Capture Method.** Matrix Microscience, Newmarket, U.K. & Golden, CO. (IAFP Annual Meeting, Phoenix, AZ August 8 – 11, 2004)

*Demonstrated a novel approach to testing environmental samples for *Listeria* spp. and introducing the wet compositing/pooling strategy for increased sample throughput.*

LIST-2 WU, V.C.H., KARY, D., POPE, E., KIM, B. & ANDERSON, G. (2005) **Development of a Rapid Protocol for the Detection of *Listeria monocytogenes* by Optimal Enrichment Procedures with Immuno-Capture Systems.** University of Maine, Orono. (AOAC International Annual Meeting, Orlando, FL September 11 -15, 2005)

*Showed that Universal Pre-Enrichment Broth supplemented with Oxyrase™ prior to Pathatrix effectively captured low numbers or injured organisms of *Listeria monocytogenes*.*

LIST-3 MURRAY, J.S., PRENTICE, N., SCOTT, M.F., HALL, P.A. & PARTON, A. (2007) **Development of a Rapid Assay for the Detection of *Listeria* spp. in Environmental Swabs Using Re-circulating Immuno-magnetic Separation Linked to Real-Time PCR (RT-PCR).** Matrix Microscience, Newmarket, U.K. (IAFP Annual Meeting, Orlando, FL July 8 - 11, 2007.)

*Reported a 100% correlation between Pathatrix-RIMS and either RT-PCR or selective agars and the BAM method testing environmental swabs for *Listeria* spp. at levels of 1 – 10 cfu / swab. The rapidity and sensitivity of the method offers significant benefits to food producers.*

- LIST-4** CARTER, M.W, HALL, P.A. KUPSKI, B. & THOMPSON, L. (2007) **Evaluation of Pathatrix (Immunomagnetic Concentration) and BAX (PCR) Using Two Nonselective Enrichment Broth for the 24-hour Recovery of Stressed *Listeria* Species from Artificially Contaminated Sponges.** Silliker, Inc., South Holland, IL. (IAFP Annual Meeting, Orlando, FL July 8 - 11, 2007.)

*Evaluated two non-selective pre-enrichment broths (BPW and UPB) for the recovery of *Listeria* spp. from artificially contaminated sponges. It was concluded that both broths showed promise when combined with immunoconcentration and PCR.*

Mycobacterium avium* subsp. *paratuberculosis

- MAP-1** STEPHAN, R., SCHUMACHER, S., TASARA, T. & GRANT, I.R. (2007) **Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Swiss Raw Milk Cheese Collected at the Retail Level.** *Journal of Dairy Science* **90**, 3590 – 3595.

Used a combination of Pathatrix and PCR to isolate MAP from Swiss cheese. Although no viable cells could be cultured, 4.2% of the raw milk cheese samples tested positive with the F57-based real-time PCR system, providing evidence for the presence of MAP in the raw material.

***Salmonella* spp.**

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Data showed that Pathatrix-RIMS coupled to selective agars could detect Salmonella spp. and E. sakazakii at low levels (1 – 10 cfu/25 g) and correlated 100% with standard methods. The test allowed for the rapid analysis of larger sample sizes.

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SAL-1 YUK, H., WARREN, B., & SCHNEIDER, K.R. (2006) Preliminary Evaluation of Flow-Through Immunocapture followed by Real-Time PCR for the Detection of Salmonella Serovars on Tomato Surfaces within 8 Hours. Journal of Food Protection 69, 2253-2257.

Illustrated that the combination of Pathatrix and PCR was superior to the BAM culture method for the detection of Salmonella serovars on tomato surfaces and was completed within 8 hours.

SAL-2 WARREN, B.R., YUK, H-G. & SCHNEIDER, K.R. (2006) Sensitive and Specific Detection of Salmonella from Ground Beef and Potato Salad Samples within Eight Hours. University of Florida. (IAFP Annual Meeting, Calgary, Alberta August 13-16, 2006)

Reported that Pathatrix and real-time PCR could yield Salmonella results within 8 hours in ground beef and potato salad.

SAL-3 WARREN, B.R., YUK, H-G. & SCHNEIDER, K.R. (2007) Detection of Salmonella by Flow-Through Immunocapture Real-Time PCR in Selected Foods within 8 Hours Journal of Food Protection 70, 1002 – 1006.

Demonstrated the ability to improve both conventional and real-time PCR methods for the detection of Salmonella from tomato surfaces, potato salad and ground beef. Presumptive colonies on XLD agar were isolated 48 hours faster than by the BAM method and Pathatrix-PCR could be completed within 8 hours.

SAL-4 MURRAY, J.S., PRENTICE, N., SCOTT, M.F., HALL, P.A. & PARTON, A. (2007) Rapid Isolation and Detection of Salmonella spp. from Chocolate Crumb, Cocoa Liquor and Cocoa Butter Using Re-circulating Immunomagnetic Separation linked to Real-time PCR. Matrix Microscience, Newmarket, U.K. (IAFP Annual Meeting, Orlando, FL July 8 - 11, 2007.)

Data indicated that there was 100% correlation between the reference method and either RIMS linked to RT-PCR or selective agar plates at low levels (1 – 10 cfu / sample) for all sample types. The approach offers significant benefits to chocolate producers because pooling allows for larger sample sizes.

SAL-5 High Throughput Salmonella Testing Using a 10 Sample (Post Pre-Enrichment) Pooling Strategy Linked to Re-Circulating IMS and Real Time PCR (2008) J. MURRAY, N. PRENTICE, P. BENTON, K. BRZEGOWA, B. HOUSTON, M. VAN WART, M.F. SCOTT, ADRIAN PARTON, Matrix MicroScience Ltd., Lynx Business Park, Fordham Road, Newmarket, Cambs CB8 7NY, UK (IAFP Annual Meeting, Columbus, OH, August 3 - 6, 2008)

The data showed that Pathatrix can successfully isolate Salmonella serovars from 10-pooled samples in a variety of different food matrices. Detection of the low level inoculums was achieved using either Real Time PCR or selective agar plates.

SAL-6 Detection of low level Salmonella contamination in cocoa products, chocolate and peanut butter using re-circulating Immunomagnetic Separation and PCR (2008) N. PRENTICE, J. MURRAY, P. BENTON, K. BRZEGOWA, B. HOUSTON, M. VAN WART, M.F. SCOTT, ADRIAN PARTON, Matrix MicroScience Ltd., Lynx Business Park, Fordham Road, Newmarket, Cambs CB8 7NY, UK (Food Micro, Aberdeen, Scotland, September 1-4, 2008)

This study demonstrated that Pathatrix® RIMS can be effectively coupled to RT-PCR or agar plate for the rapid, cost effective isolation and detection of Salmonella from a range chocolate, cocoa products and peanut butters when low level Salmonella contamination is initially present i.e. <10 cfu/sample.

Shigella spp.

SHG-1 WARREN, B.R., YUK, H-G. & SCHNEIDER, K.R. (2006) Detection of Shigella sonnei in Selected Foods by Flow-Through Immunocapture Followed by Real-Time Polymerase Chain Reaction or Isolation on MacConkey Agar *Journal of Rapid Methods and Automation in Microbiology* **14**, 309 – 324.

Compared PCR with plating after Pathatrix using commercially-available polyclonal and monoclonal antibodies. Pathatrix-PCR achieved equal detection of S. sonnei from potato salad, and superior detection from ground beef and tomato surfaces. But in all cases, Pathatrix-PCR gave results faster than the BAM method.

SHG-2 MADSON, S.M., LASTER, E.W., THOMAS, M.Z., WATTS, K.A. & SOFOS, J.N. (2006) A Preliminary Limited Study on Detection of Shigella sonnei from Cantaloupe Rinse by Culturing with or without Pre-enrichment, Cell Capture, and Aerobic or Anaerobic Enrichment. Food and Drug Administration, Denver, CO & Colorado State Univ. (12th Annual FDA Science Forum, April 18 – 20, 2006)

Evaluated the use of non-specific cationically-charged particles which captured shigellas, but too many competing flora as well. Suggested that more specific beads and anaerobic enrichment would be more effective.

Viruses

VIR-1 PLANTE, M., BIDAVID, S., FARBER, J.M. & KARTHIKEYAN, K. (2005) Rapid Extraction and Detection of Hepatitis A Virus from Food Samples. Health Canada, Ottawa, Canada. (IAFP Annual Meeting, Baltimore, MD August 14-17, 2005)

In combination with RT-PCR, showed that Pathatrix (using cationically-charged particles) could detect low levels of Hepatitis A (10 pfu / 25 g samples) in fruits, vegetables and ready-to-eat foods. Results were obtained in 5 hours. The use of this approach allowed for rapid, simple and sensitive isolation of HAV from foods.

- VIR-2** PLANTE, M., KARTHIKEYAN, K. BIDAWID, S., MATTISON, K. & FARBER, J.M. & (2005) **Development of Methods for Norovirus Detection from Various Outbreak Foods** Health Canada, Ottawa, Canada. (IAFP Annual Meeting, Baltimore, MD August 14-17, 2005)

Showed that Pathatrix was successful in detecting noroviruses from foods implicated in an outbreak, and recommended that in future studies, Pathatrix should continue to be used to concentrate noroviruses from foods.

- VIR-3** PAPAFRAGKOU, E., VINGE, J. & JAYKUS, L-A. (2005) **Use of the Feline Calicivirus Model in Evaluating Methods to Extract and Detect Noroviruses in Foods.** North Carolina State University, Raleigh, NC. (IAFP Annual Meeting, Baltimore, MD, August 14-17, 2005)

Included data that also showed that Pathatrix plus cationically-charged particles could detect very low levels of Hepatitis A. Detection levels of 10^0 were found, which is especially noteworthy for this important viral pathogen.

- VIR-4** HIRNEISEN, K.A., HOOVER, D.G. & KNIEL, K.E. (2007) **Isolation and Infection of Potential Foodborne Viral Pathogens.** Univ. of Delaware, Newark, DE. WA. (IAFP Annual Meeting, Orlando, FL July 8 - 11, 2007.)

Reported that cationic beads captured a variety of viruses from ready-to-eat foods and that they could subsequently be removed from the beads for use in cell culture. This allowed for the levels of active virus to be determined.

- VIR-5** PAPAFRAGKOU, E., ELHANAFI, D. & JAYKUS, L-A. (2007) **Persistence of Hepatitis A Virus on Foods and Food Preparation Surfaces.** North Carolina State Univ., Raleigh, NC. (IAFP Annual Meeting, Orlando, FL July 8 - 11, 2007.)

Used Pathatrix in combination with cationic beads to demonstrate that HAV is quite environmentally stable, a characteristic that likely contributes to its transmissibility by foodborne routes.

- VIR-6** A.Y. ASAHINA, R. FUJIOKA, A. HENRY, P.C. LOH (2007) **Using Indigenous Mollusks and Pathatrix to Detect Pathogens in Water.** Dept. of Microbiology, University of Hawaii-Manoa, USA (34th Annual International Global Health Conference, Washington, D.C., USA, May 29 - June 1, 2007)

Pathatrix can be used to concentrate viruses from mollusks to determine the hygienic water quality in any area where indigenous mollusk populations occur. Initial results show that Pathatrix is capable of capturing viable virus at an initial inoculum concentration of 2.6×10^6 pfu per 10 grams of isognomon flesh.

- VIR-7** E. PAPAFRAGKOU, M. PLANTE, K. MATTISON, S. BIDAWID, K. KARTHIKEYAN, J.M. FARBER, L.A. JAYKUS (2008) **Rapid and Sensitive Detection of Hepatitis A Virus (HAV) in Representative Food Matrices.** Dept. of Food Science, North Carolina State University, Raleigh, NC, USA & Health Canada, Food Directorate, Bureau of Microbial Hazards, Ottawa, Ont., Canada (*Journal of Virological Methods*, 2008, 147, p177-187)

Pathatrix was used to isolate HAV from 25 g samples of artificially contaminated lettuce, strawberries, green onions, deli-turkey, oysters, and frosted cake. Using Pathatrix linked to RT-PCR, HAV was consistently detected at input levels corresponding to 10^2 PFU/25 g of food sample and at levels as low as 10^{-1} PFU/25 g for some matrices. The assay was also used to confirm viral contamination of produce items associated with a recent HAV outbreak. This represents the first application of Pathatrix to isolate HAV from foods.

VIR-8 The Use of Feline Calicivirus as an Internal Control for the Detection of Hepatitis A Virus with the Pathatrix System (2008) VANESSA MORTON, Michelle Driscoll, Kirsten Mattison, Sabah Bidawid and Jeffrey M. Farber, Health Canada, 251 Sir Frederick Banting Driveway, Ottawa, ON K1A 0L2, Canada (IAFP Annual Meeting, Columbus, OH, August 3 - 6, 2008)

A standardized HAV extraction protocol was developed utilizing Pathatrix.. Detection of FCV from each sample demonstrated that the virus extraction and detection procedure was successful and that no PCR inhibitors were present.

Biothreat Agents - Yersinia pestis, etc

BA-1 HANES, D.E., SAYLOR, M.L., KOTHARY, M.H., LORENZO, M.E. & TALL, B.D. (2005) **Isolation of Yersinia pestis from Food Using Immuno-magnetic Capture.** Food and Drug Administration, Laurel, MD. (ASM Annual Meeting, Atlanta, GA June 6 – 10, 2005)

Together with antibodies raised by the authors, showed that Pathatrix could successfully isolate Y. pestis from food matrices and provided a viable alternative to previous methods which were time-consuming and laborious. An important advance in the development of better methods to detect biothreat agents in food.

BA-2 HANES, D.E., KOTHARY, M.H., TALL, B.D., EWING-PEEPLES, L., CARTER, L., WEAGANT, S.D. & TOROSIAN, S. (2007) **Isolation of Yersinia pestis from Foods Using a Novel Enrichment Broth and Immunocapture.** Food and Drug Administration, Laurel, MD, Bothell, WA & Winchester, MA. (ASM Biodefense Meeting, Washington, DC Feb 27 – Mar 2, 2007.)

Demonstrated that buffered YpE broth coupled with Pathatrix can be used to isolate low levels of Yersinia pestis in foods.

BA-3 TALL, B.D., HANES, D.E., KOTHARY, M.H., EWING-PEEPLES, L., BURR, D.H., CARTER, L. & WEAGANT, S.D. (2007) **Evaluation of the FDA-FERN Screening Method for the Isolation and Identification of Yersinia pestis from Foods.** Food and Drug Administration, Laurel, MD, Madison, WI & Seattle, WA. (ASM Biodefense Meeting, Washington, DC Feb 27 – Mar 2, 2007.)

Reported the results of a collaborative study involving 18 laboratories testing five different food matrices with three levels of Yersinia pestis. Concluded that immunocapture [Pathatrix] can be used to identify Y pestis from a variety of food matrices by culture and PCR..

BA-4 D.E. HANES, L. EWING-PEEPLES, M.H. KOTHARY, B.D. TALL (2008) **Isolation of Francisella tularensis from Foods Using the Pathatrix Immunomagnetic Capture System.** Food and Drug Administration (FDA), Laurel, MD, USA (6th ASM Biodefense & Emerging Diseases Research Meeting, Baltimore, MD, USA, February 24 – 27, 2008)

The study examined the use of Pathatrix for the isolation of Francisella tularensis (Ft) from liquid infant formula (IF), a tomato-based vegetable juice (VJ), milk and baby food (BF). The results show that Pathatrix successfully captures F. tularensis, but the food matrix influences the recovery of viable organisms.

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ADDENDUM

The following papers do not include the use of the Pathatrix immuno-capture system but they do report concepts that are included in the Pathatrix approach and protocols.

GEN-1 NARANG, N. & CRAY, W.C. (2006) **Evaluation of Hand Mixing of Ground Beef and Poultry Samples as an Alternative to Stomaching for the Detection of *Salmonella*.** *Food Protection Trends* **26**, 14 -19.

Demonstrated that all of the Salmonella isolates tested could be detected by both treatment methods (stomaching and hand mixing) in the three meat matrices tested. (ground beef, ground chicken, ground turkey).

GEN-2 OGDEN, I.D., MacRAE, M., HEPBURN, N.F. & STRACHAN, N.J.C. (2000) **Improved isolation of *Escherichia coli* O157 using large enrichment volumes for immunomagnetic separation** *Letters in Applied Microbiology* **31**, 338-341.

*Showed that increasing the volume tested from 1 mL to 10 mL and to 50 mL improved recoveries of *E. coli* O157, but they stated that the 50 mL could not be recommended until a suitable magnetic separation device had been developed. [Pathatrix is capable of handling 50 mL or 250 mL.]*

GEN-3 GUERINI, M.N., ARTHUR, T.M., SHACKLEFORD, S.D. & KOOHMARAIE, M. (2006) **Evaluation of *Escherichia coli* O157:H7 Growth Media for Use in Test-and-Hold Procedures for Ground Beef Processing** *Journal of Food Protection* **69**, 1007-1011.

In this study, a 1:3 ratio of pre-enrichment broth to ground beef worked as well as a 1:10 ratio. Thus, only 1000 mL (instead of 3.375 L was required to pre-enrich a 375 gram sample.

GEN-4 WEAGANT, D.S. & BOUND, A.J. (2001) **Evaluation of techniques for enrichment and isolation of *Escherichia coli* O157:H7 from artificially contaminated sprouts** *International Journal of Food Microbiology* **71**, 87-92.

*Demonstrated that a pre-enrichment incubation temperature of 42°C was better than 37°C for the recovery of *E.coli* O157:H7 from bean sprouts.*

GEN-5 STEVENS, K.A. & JAYKUS, L-A. (2004) Bacterial Separation and Concentration from Complex Sample Matrices: A Review *Critical Reviews in Microbiology* **30**, 7-24.

Suggested that an ideal sample preparation method would be able to both concentrate pathogens and remove matrix-associated inhibitors. Further, that it would be universal (applicable to multiple food types and microorganisms), simple, rapid and inexpensive. All with a view to decreasing or eliminating the need for cultural enrichment.

GEN-6 D'AOUST, J-Y., PAGOTTO, F., AKHTAR, M., BUSSEY, J., COOPER, C., McDONALD, C., MEYMANDY, M. & TYLER, K. (2007) Evaluation of the BAX Gel and Fluorimetric Systems for the Detection of Foodborne Salmonella *Journal of Food Protection* **70**, 835-840.

Suggested that the potential benefits of immunomagnetic separation of Salmonella in preenrichment cultures, and the use of larger portions of test materials in PCR analyses should be investigated

GEN-7 Effect of Zero-Valent Iron on Removal of Escherichia coli O157:H7 from Agricultural Waters (2008) ALEXANDRA M. DEREVIANKO, J. HANDLIN, A. YOSKOWITZ, Y. JIN, P. CHIU, M. SHARMA, K. E. KNIEL, University of Delaware, 044 Townsend Hall, 531 South College Ave., Newark, DE 19711, USA (IAFP Annual Meeting, Columbus, OH, August 3 - 6, 2008)

This study was initiated to determine the effectiveness of zero-valent iron in the removal and inactivation of E. coli O157:H7 in a simulated irrigation system. Pathatrix was used to determine the level of viable O157 remaining after filtration.

GEN-8 Salmonella Testing of Pooled Pre-Enrichment Broth Cultures for Screening Multiple Food Samples (1972) W. R. PRICE, R. A. OLSEN, J. E. HUNTER, Winton Hill Technical Centre, The Procter & Gamble Company, Cincinnati, Ohio (*Applied Microbiology*, **23**, 679-682)

The procedure offers a simple, yet effective, way to increase sample capacity in the Salmonella testing of foods, particularly where a large proportion of samples ordinarily is negative. This article provides the first published description of wet compositing (Pooling).

GEN-9 Trends and Opportunities in Food Pathogen Detection (2008) S.R. NUGEN, A.J. BAEUMNER, Department of Biological & Environmental Engineering, Cornell University, 318 Riley-Robb Hall, Ithaca, NY 14853, USA (*Anal. Bioanal. Chem.*, **391**, 451-454)

Automated systems using immunomagnetic beads such as Pathatrix (Matrix MicroScience) are effective for isolating microorganisms from food systems such as fresh salad, ground beef potato salad and mashed potatoes. This system offers the advantage of a large 250-mL sample size.