New Technologies in Food Testing

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June 27, 2005
Why do we need new technology?

Regulatory food testing:
- Requires confirmed results
- Requires viable organisms (for example, PFGE)
  - Nonviable bacteria/viruses not hazardous, except toxins
  - Sometimes need bacterial counts (MPN)
- Requires sensitive methods
  - Traditional methods require enrichment
  - Stressed organisms, overgrowth, low numbers
- Requires rapid methods
  - Traditional methods have built-in delays due to enrichment, growth requirements, ID methods
- Requires testing of high numbers of samples

Bioterrorism *demands* rapid, sensitive, specific testing
Overview

- Immunomagnetic Separation (IMS)
- Magnetic immunoassay/concentration
- Chromogenic agar
- PCR
- Real-time PCR/molecular beacons
- Electrochemiluminescence
- Real-time PCR
Immunomagnetic Separation (IMS) Overview
The IMS Principle

- Uniform, superparamagnetic, monodisperse, polymer beads coated with a ligand directed against the specified target, e.g. antibody against surface antigen.

- Dynabeads will bind to the target, making it “magnetic”, forming a target-bead complex.

- Target is then easily isolated from the sample using a magnet particle concentrator (MPC).
Concept of IMS

- PCR
- ELISA
- Plating
- Other Methods
Flexibility of IMS

Any sample matrix provided sample pre-enrichment is done
(Food, Water, Fecal material, Blood, Soil)

Any detection system
(Culture media, PCR, ELISA, ATP, Luminometry, Microscopy)
Advantages of Bacterial IMS

- Increased recovery rate
- Isolation and concentration
- Flexible
- Versatile
- Inexpensive
- Simple
- Reliable
- Minimum space requirements
IMS Bacterial Microbiology

Products

- **Dynabeads anti-Salmonella**
  - Approvals: AOAC, NMKL

- **Dynabeads anti-E.coli O157**

- **Dynabeads EPEC/VTEC (O145, O111, O103, O26)**
  - Products for rapid selective concentration of important emerging human pathogens. Difficult to detect strains as they lack phenotypic characteristics, unlike O157

- **Dynabeads anti-Listeria**
Selective Enrichment/Concentration of Bacterial Pathogens

Pre-enrichment of Sample
(6-24 h, 30-42°C)

Selective Enrichment
(18-48 h)

Detection
5 min - 24 h

IMS
30 min, 18-28°C
Bacterial Manual IMS Procedure

- Sample pre-treatment (enrichment)
  - Add 1mL aliquot of pre-enriched sample to 20uL Dynabeads in a microcentrifuge tube.
  - Incubate for 10 minutes at room temperature using a Dynal Sample Mixer.
- Perform immunomagnetic separation using the MPC-S.
  - Aspirate supernatant and wash with PBS Tween buffer.
  - Re-suspend in 100uL of wash buffer and then complete end detection.

Pre-enrichment → Immunocapture → Separation → Wash and Concentration → Detection

Plating
ELISA
PCR
Other methods
Automated IMS (AIMS) Dynal BeadRetriever

- All steps are automated. Simply load and go.
- Pre-programmed to work with all the Bacterial Microbiology Dynabeads kits currently available.
- Processes 15 samples within 25 minutes.
- Completely closed system without aerosol formation.
- Utilizes inverse IMS with increased bead recovery & reduced contamination of culture plates.
- Continuous operation at room temperature.
- Small footprint for unit and consumables.
Advantages of BeadRetriever Unit

- **Minimizes:**
  - Biological hazards
  - Hands-on time
  - Technician error
  - Physical resources

- **Prevents:**
  - Cross-contamination
  - Background flora

- **Increases:**
  - Safety
  - Throughput
  - Ease of use
  - High fat, high particulate samples

- **Improves:**
  - Bead recovery
  - Consistency
Magnetic Immunoassay/Concentration Overview
Pathatrix

For the rapid detection of a range pathogenic and/or spoilage bacterium in food samples.

Antibody coated paramagnetic particles selectively binds and purifies the target organism from a comprehensive range of complex food matrices.

Only microbial detection system that can analyse the entire 225ml + 25g sample simultaneously by re-circulating the sample through a "capture phase" where the antibody coated magnetic beads are immobilized.

By providing heat to the system the organisms can be cultured and captured simultaneously, thus increasing the method sensitivity.

Use with a variety of detection methods (direct plating onto selective media, COLOURTRIX; FLURATRIX (fluorescence microscopy); serology; PCR; ELISA; and/or DNA probe).
Figure 1: Antibody coated beads capturing on surface of capture phase
The beads are added immediately prior to re-circulating the sample
**Figure 2: Capture of Target in Food**

The sample is re-circulated repeatedly across the capture phase with the whole 250ml sample passing over the phase approximately twice every minute.
Figure 3: Captured target bacteria after wash

When the re-circulation is complete the captured bacteria (bound to the antibody coated magnetic beads) can be washed extensively.
**Figure 4: Captured target bacteria are eluted**

The magnet is removed and the bead / bacteria complexes are washed off the capture phase, collected and concentrated in a wash tube (supplied as part of the standard consumable).
Figure 5: Detection Solutions for purified target organisms
Chromogenic Agar
Overview

E. Coli O157:H7
Chromogenic substrates

- Plating Medium
  - Selective
  - Differential
  - Specific
  - Sensitive
  - Screening for preliminary confirmation
    - Saves times
    - Saves costs
  - Detection/differentiation by utilizing specific enzyme activities
**Figure 1.** *Bacillus anthracis* ANR-1 and *Bacillus thuringiensis* ATCC 33679 co-spiked into PBS and then plated onto ACA.
Presumptive Positive Colonies of *Bacillus anthracis*

Cream to pale teal-blue-Colored Colonies of *Bacillus anthracis* after 20-24 hours at 35-37°C

Teal-Blue Colonies of *Bacillus anthracis* after 36-48 hours at 35-37°C

Dark Teal-Blue Colonies of *Bacillus cereus/Bacillus thuringiensis* after 20-24 hours at 35-37°C

After 20-24 hours at 35-37 C
Presumptively positive colonies of *Escherichia coli* O157:H7 appear as blue-black domed colonies 1.5 to 2.5 mm in diameter with a black precipitate after 20-24 hours incubation at 35°C.
Enterobacter sakazakii

Presumptively positive colonies of Enterobacter sakazakii appear as blue-black raised to domed colonies 1.0 to 2.0 mm in diameter with or without colorless halos after 24 hours at 35°C

Enterobacter sakazakii
(Pure Culture)

Enterobacter sakazakii
+Salmonella (Yellow)
+Escherichia Coli (Green)

Melibiose

Sucrose

Enterobacter sakazakii Screening Medium
After 6 hours at 35°C - Positive Growth on Sugars
Chromogenic medium

- Enterobacter sakazakii
- Bacillus anthracis (R & F Laboratories)
- Listeria monocytogenes
- Bacillus cereus/Bacillus thuringiensis
- Escherichia coli O157:H7
- Salmonella
- Yersinia pestis (out soon-R & F Laboratories)
PCR Overview
Qualicon Products
BAX® System

Automated detection of bacteria in food
Worldwide adoptions and certifications

- **AOAC International Official Method**
  Salmonella #2003.09; L. Monocytogenes #2003.12

- **AOAC-RI Performance Tested Method**
  Salmonella #100201; Listeria monocytogenes #070202; E. Coli O157:H7 #010401; Genus Listeria #030502

- **USDA-FSIS Adoption**
  Salmonella #MLG 4C.00; Listeria monocytogenes #MLG 8A.00

- **Health Canada Certification**
  Salmonella #MFLP-29; Listeria monocytogenes #MFLP-28 E. coli O157:H7 #MFLP-30; Enterobacter sakazakii #MFLP-27
Genetics based detection

Food or Environmental Sample → Enrichment → Is it there?

Yes

High Sensitivity
Find the target organism at very low levels

High Specificity
Find only the target organism, even among high levels of competitors

No
Available BAX® System Assays

- Salmonella
- Genus Listeria
- Listeria monocytogenes
- E. coli O157:H7
- Enterobacter sakazakii
<table>
<thead>
<tr>
<th>BAX® System</th>
<th>Other Methods</th>
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<tbody>
<tr>
<td><em>Salmonella</em> + Confirmation</td>
<td>Next day</td>
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<tr>
<td><em>E. coli O157:H7</em> 8-hr enrichment media</td>
<td>Next day</td>
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<td><em>L. monocytogenes</em> Confirmation</td>
<td>48 hours</td>
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<td><em>Listeria</em> Genus Confirmation 24-hr enrichment media</td>
<td>Next day</td>
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<tr>
<td><em>E. sakazakii</em> Confirmation</td>
<td>Next day</td>
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PCR made easy with the BAX® system

- Tableted reagents packaged in PCR tubes
  - provide for process control
  - eliminate multiple transfers
  - reduce operator error
Simplicity and Ease of Use

- Minimal sample handling
- NO DNA ISOLATION!
- Pipetting important user skill
- Assays allow multichannel pipetting to minimize hands-on time
- Tools developed to simplify manipulations
- Uniform protocols for sample types
- Definitive, not presumptive results
- High throughput – 96 tests/batch (pooling)
- Screen for multiple organisms simultaneously
Automated cycler/detector

- Universal cycling protocol allows amplification of multiple targets in one batch
- Sophisticated algorithms analyze melting curves of fluorescent detection
- Windows-based interface, with Wizards that prompt you through the protocol.
- Color-coded graphic display of yes-or-no results in less than four hours

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No  →  Yes
Future targets for the BAX® system

- Yeast and Mold (just out last week)
  - Shortened enrichment + PCR assay for quicker time to result

- Campylobacter
  - PCR assay to detect *C. jejuni* and *C. coli* in poultry
  - Estimated delivery - mid 2005

- Vibrio
  - PCR assay to detect regulated strains in seafood
  - Estimated delivery - late 2005
Electrochemiluminescence Overview
Electrochemiluminescent Label

BV-TAG™ label

UNIQUE LABEL CHEMISTRY

• Stable non-isotopic label (ruthenium)
• Unique light emitting characteristics
• Variety of linking chemistries
• Size: ~1000 daltons
• Solubility: Aqueous and Organic solvents
Electrochemiluminescence Process

- Ruthenium and TPA are oxidized at the surface of the electrode when voltage is applied. TPA loses a proton which reduces the ruthenium to an excited state, causing light to be emitted. Ruthenium is not consumed, so it can be oxidized and excited again as long as TPA is present.

- Multiple excitation/emission cycles amplifies the light emitted and increase sensitivity.

- Emitted light is measured to determine concentration of analytes in sample.
BioVeris Technology™ – Read Cycle

Simple automated read cycle
- Precise
- Sensitive
- No operator interpretation
M1M Portability

- Molded transport case
- Analyzer can be used in the transport case
- The instrument is ready to run after <15 min.
- Case Dimensions: 29” X 15” x 28”
- Total power consumption < 75 W
- Case contains sufficient bulk reagents to run over 300 tests
BioVeris Available Assays for Food

- Staphylococcal enterotoxin A
- Staphylococcal enterotoxin B
- Botulinum neurotoxins A, B, E, & F
- *E. coli* O157
- Anthrax
- Anthrax Lethal Factor Enzymatic Assay
- Botulinum neurotoxin A & B Enzymatic Assays
- Ricin
- Salmonella
- Listeria
- Campylobacter
Real-time PCR/Molecular Beacons Overview
WARNEX RAPID PATHOGEN DETECTION
SENTINEL - ABOUT THE SOFTWARE

DETECTION PROCESS

SIMULTANEOUS DNA AMPLIFICATION + RESULT ANALYSIS

NO HANDS-ON TIME
### THE DETECTION PROCESS:

**Step 1 + 2 + 3**

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
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<tbody>
<tr>
<td>Enrichment</td>
<td>DNA extraction</td>
<td>DNA amplification &amp; detection &amp; analysis</td>
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<tr>
<td><strong>18-24 hrs</strong></td>
<td><strong>20 min</strong></td>
<td><strong>2 hrs</strong></td>
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RESULTS WITHIN 3 HOURS POST ENRICHMENT

ON-LINE SUPPORT

FROM SAMPLE

CERTIFICATE OF ANALYSIS

ENRICHMENT

REAL-TIME PCR DETECTION

DNA EXTRACTION

BACTERIAL GROWTH
READY TO USE PLATES & FLEXIWELLS
CONTAINING ALL REQUIRED REAGENTS

Controls
Salmonella spp.
E. coli O157:H7
E. coli O157
L. monocytogenes
Listeria spp.
Campylobacter spp. In validation

U.S. AOAC RI performance test
<table>
<thead>
<tr>
<th><strong>Warnex SUMMARY</strong></th>
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<tbody>
<tr>
<td><strong>SPEED</strong></td>
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<tr>
<td><strong>VERSATILITY</strong></td>
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<tr>
<td><strong>SIMPLICITY</strong></td>
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<tr>
<td><strong>ACCURACY</strong></td>
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<tr>
<td><strong>Real-Time PCR</strong></td>
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<tr>
<td><strong>Integrated platform</strong> (microplates and flexiwell)</td>
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<tr>
<td><strong>Ready to use microplates</strong></td>
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<tr>
<td><strong>Automatic analysis of results</strong></td>
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<td><strong>Automatic printout of CoA</strong></td>
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<tr>
<td><strong>Double specificity concept from Primers and Molecular Beacons decrease false + results</strong></td>
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<tr>
<td>Primers select pathogens</td>
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<tr>
<td>Molecular beacons confirm its presence</td>
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Real-time Pathogen Detection Kits Overview
New Applied Biosystems
TaqMan® Pathogen Detection Kits
Salmonella enterica, E. coli O157:H7, Listeria monocytogenes, Campylobacter jejuni
available Fall 2005

- **ease-of-use:**
  - single, closed tube analysis
  - one single program for all bacteria
  - integrated and automated amplification, detection, data collection and analysis
  - no post-PCR processing required

- **increased specificity**

- **faster results:**
  - save days until TTR

- **better results:**
  - Internal Positive Control to help prevent false negatives
Principle:
- combines the amplification power of PCR (sensitivity) with a hybridization reaction (specificity) in one tube

Experience:
- proven 3rd generation technology from the inventors of real-time PCR

Advantages:
- minimizes hands-on needs compared to conventional PCR
- no carry-over contamination: single, closed tube analysis
- increased specificity: addition of a fluorogenic TaqMan® probe between PCR primers confirms the presence of the correct PCR product only
For more information…

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FL DACS Foodborne Pathogen Analysis Conference
July 17-20, 2005 St. Pete Beach, FL
www.flworkshop.com