

Microbiological Rapid Methods for Seafood Processing Facilities

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Microorganisms of Concern

- Spoilage
 - Molds
 - Bacteria
- Foodborne pathogens
 - *Salmonella* species
 - *Listeria monocytogenes*
 - *Campylobacter jejuni*
 - *Staphylococcus aureus*
 - *Shigella* species
 - *Clostridium botulinum*
 - *Vibrio vulnificus*, *V. parahaemolyticus* and *V. cholera*

Microorganisms of Concern

- *Salmonella* species
 - Symptoms of the disease diarrhea, vomiting, spiking fever, abdominal pain, and headache
 - Natural reservoirs for this pathogen are animals and birds
 - Mainly introduced by fecal contamination

Microorganisms of Concern

- *Listeria monocytogenes*
 - Flu-like symptoms in healthy individuals
 - Stillbirths or miscarriages in pregnant women
 - Meningitis in elderly and immunocompromised individuals
 - Widely distributed on plant vegetation and in soil
 - Introduced through environmental contamination

Microorganisms of Concern

- *Campylobacter jejuni*
 - Symptoms of the disease fever, abdominal cramps and diarrhea (with or without blood)
 - *Campylobacter* infection may mimic acute appendicitis
 - Associated with warm blooded animals
 - Introduced through fecal contamination
 - *Campylobacter spp.* were found in 42% of 380 shellfish (Wilson and Moore, Epidemiol. Infect. 1996)

Microorganisms of Concern

- *Staphylococcus aureus*
 - Symptoms include vomiting, cramps, chills, diarrhea, sweating and fever
 - Contamination of foods due to humans, animals, environmental sources

Microorganisms of Concern

- *Shigella species*

- Symptoms of the disease are abdominal pain, diarrhea, and bloody diarrhea
- Rare cases children may have seizures
- Fecal contaminated water and unsanitary handling by food handlers are the most common causes of contamination

Microorganisms of Concern

- *Clostridium botulinum*
 - Symptoms of the disease are vomiting, fatigue, dizziness, paralysis of muscles, double vision, finally respiratory failure
 - Incidence of the disease is low, but the mortality rate is high
 - Type E mainly associated with seafood
 - Contamination from soil and water

Microorganisms of Concern

- *Vibrio vulnificus*
 - Causes wound infections, gastroenteritis, or primary septicemia
 - Primary septicemia has 55% mortality
 - Found in estuarine environments and associated with oysters, clams, crabs and finfish

Microorganisms of Concern

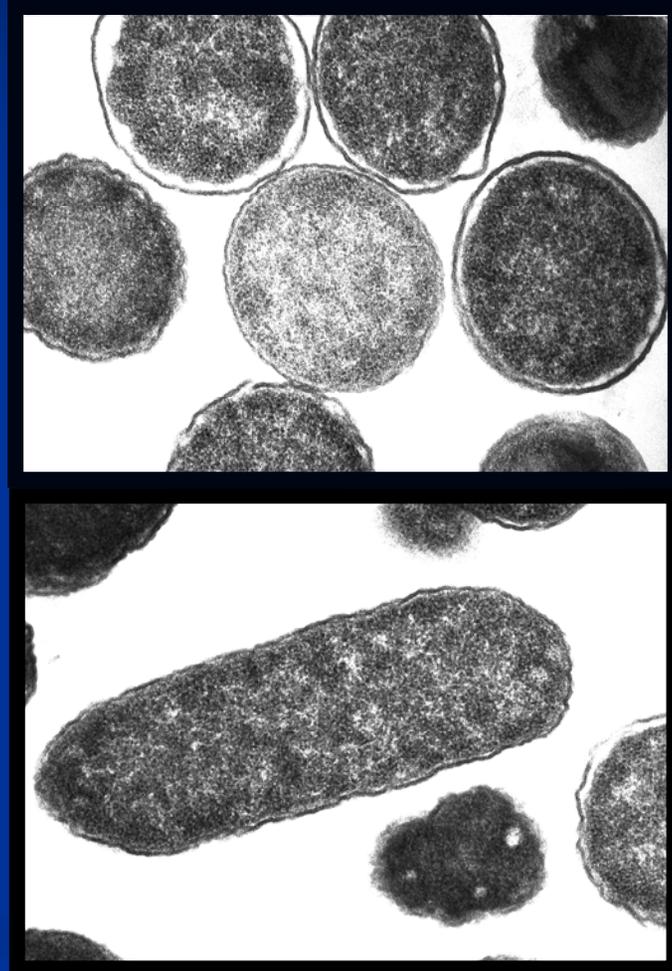
- *Vibrio parahaemolyticus*
 - Frequently isolated from the estuarine and marine environment of the United States
 - Causes diarrhea, abdominal cramps, nausea, vomiting, headache, fever, and chills
 - Major outbreaks occur during warmer months

Microorganisms of Concern

- *Vibrio cholerae*
 - Associated with raw shellfish or from shellfish either improperly cooked or re-contaminated after proper cooking
 - Generally a disease spread by poor sanitation, resulting in contaminated water supplies

Rapid Methods used for HACCP

- Support sanitation
- Measure process control
- Identification of Foodborne Pathogens



Rapid Methods for Sanitation Testing

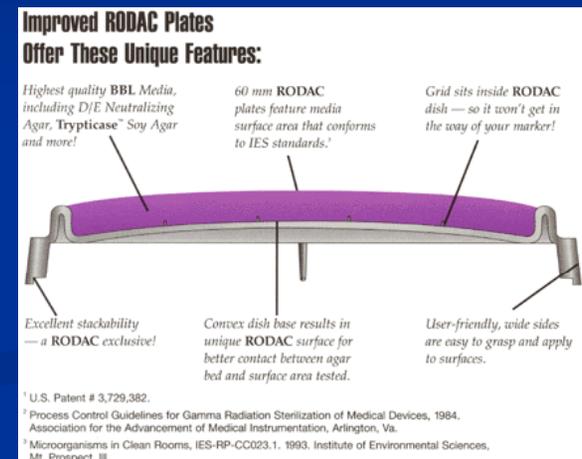
- Environmental microbiological monitoring:
 - Efficiency of sanitation
 - Frequency required for sanitation
 - Environmental sources of microorganisms
 - Frequency required for special maintenance procedures (air filters)
 - Sanitary design of equipment

Rapid Methods for Sanitation Testing

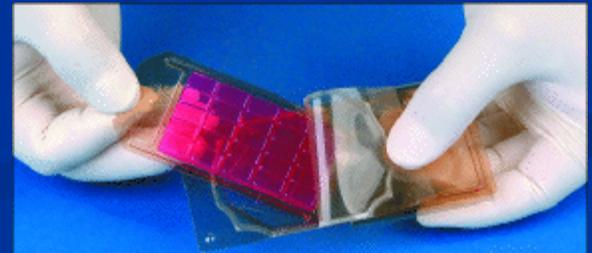
■ Contact Plate

- Pressing a solidified nutrient agar against environmental surface for 5 seconds
- Plates incubated and direct microbial count determined
- Rodac® contact plate
- Hycon® contact slide

Rodac® contact plate



Hycon® contact slide



Rapid Methods for Sanitation Testing

- **Contact Plate (microbial analysis):**
 - **3M Petrifilm™ Plates**
 - **Open film**
 - **Place 1 ml, sterile DI H₂O on surface**
 - **Close film and allow agar medium to rehydrate**
 - **Open film, exposing rehydrated medium**
 - **Apply to equipment surface**
 - **Close film and incubate**

Rapid Methods for Sanitation Testing

- **Sponge (microbial analysis):**
 - Rehydrate sponge in Neutralization broth
 - Collect sample and place in sterile bag
 - Add enrichment broth
 - Incubate bag
 - Plate the growth on selective media



Rapid Methods for Sanitation Testing

- Swabbing (microbial analysis)
 - Wet the swab with broth in tube
 - Rub swab over the desired surface
 - Return swab to tube
 - Release bacteria from the swab
 - Pour 1 ml onto plate
 - Incubate plates
 - Calculate bacteria

Rapid Methods for Sanitation Testing

- **Protein or Carbohydrate Analysis**
 - Sample a surface and expose to chemicals
 - Color change indicates presence of protein and/or carbohydrates
 - Not appropriate for surfaces soiled by fat
 - VERIclean® (Charm Sciences Inc.)
 - FLASH® (BIOcontrol)

FLASH® (BIOCONTROL)



Rapid Methods for Sanitation Testing

- **ATP Bioluminescence Technology**
 - Indicate if sanitation of processing equipment is acceptable
 - Determine if incoming products conform to acceptable standards
 - Several companies manufacture portable hand-held instruments

Rapid Methods for Sanitation Testing

- **ATP Bioluminescence Technology**
 - The surface of the processing equipment is swabbed.
 - The swab placed into a reagent that extracts the ATP from microorganisms.
 - ATP reacted with Luciferin-luciferase.
 - Measure of light emitted that is proportional to the amount of ATP and therefore biomass.

Rapid Methods for Sanitation Testing

- **Luminator-T™ Luminator-T™, Firefly™ and LuMinator-K™ (Charm Sciences)**
 - Download to CharmLink™ or any standard spreadsheet program
 - Allows trending and analysis of individual measurement parameters
 - Identify potential cause/effect relationships

Firefly™



Luminator-T™



Rapid Methods for Measuring Process Control

- Monitor quality of incoming product
 - Meets standards
- Verify processing
 - Controls spoilage and pathogenic bacteria
- Monitor finished product quality
 - Shelf-life determination

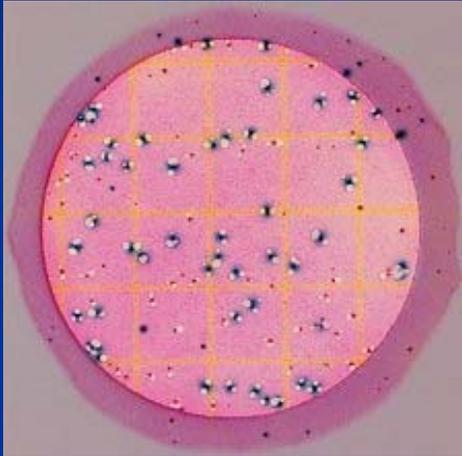
Rapid Methods for Measuring Process Control

- 3M™ Petrifilm™, *E. coli* / Coliform Count Plate:
 - Contains modified Violet Red Bile (VRB) nutrients
 - *E. coli* and total coliform results in one test
 - Red colonies with gas are generally coliforms; blue colonies with gas are typically *E. coli*
 - Incubation time 24 – 48 hours
 - AOAC® Official MethodSM

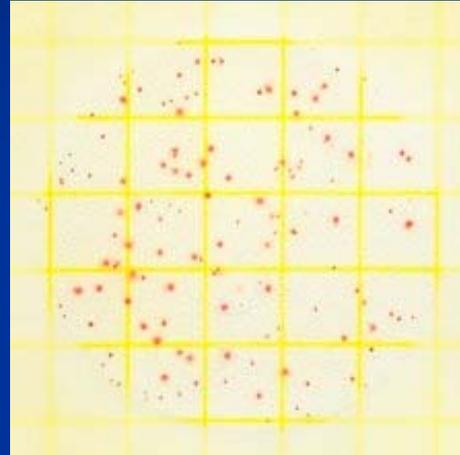
3M Petrifilm

AOAC® Official MethodSM

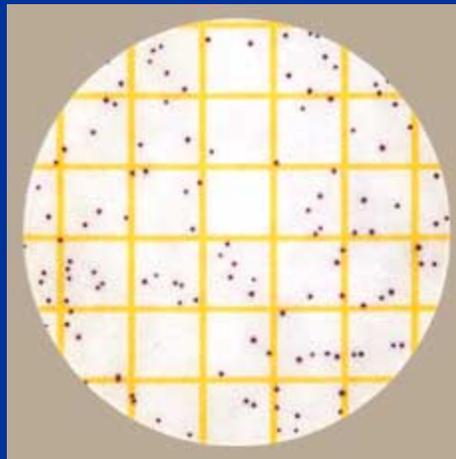
E. coli / coliform



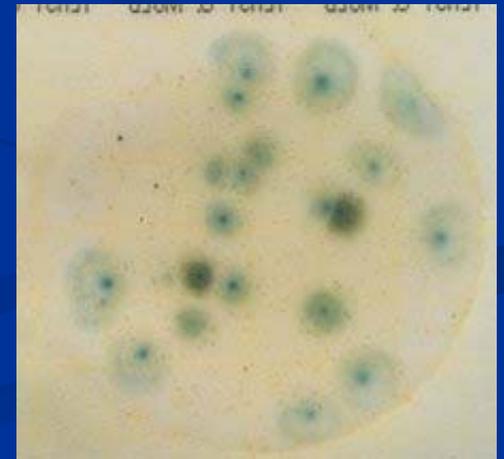
Aerobic



S. aureus



Yeast and Mold



Rapid Methods for Measuring process control

- SimPlate (Biocontrol)
 - Food sample added to CEC media (coliform & *E. coli*)
 - Media added to plates
 - Excess media poured out of the plates
 - Plates incubated for 24 hours
 - Results read:
 - Coliforms = orange to red
 - *E. coli* = wells that fluoresce when exposed to UV light

SimPlate (Biocontrol)

AOAC® Official MethodSM



Coliform results

E. coli results

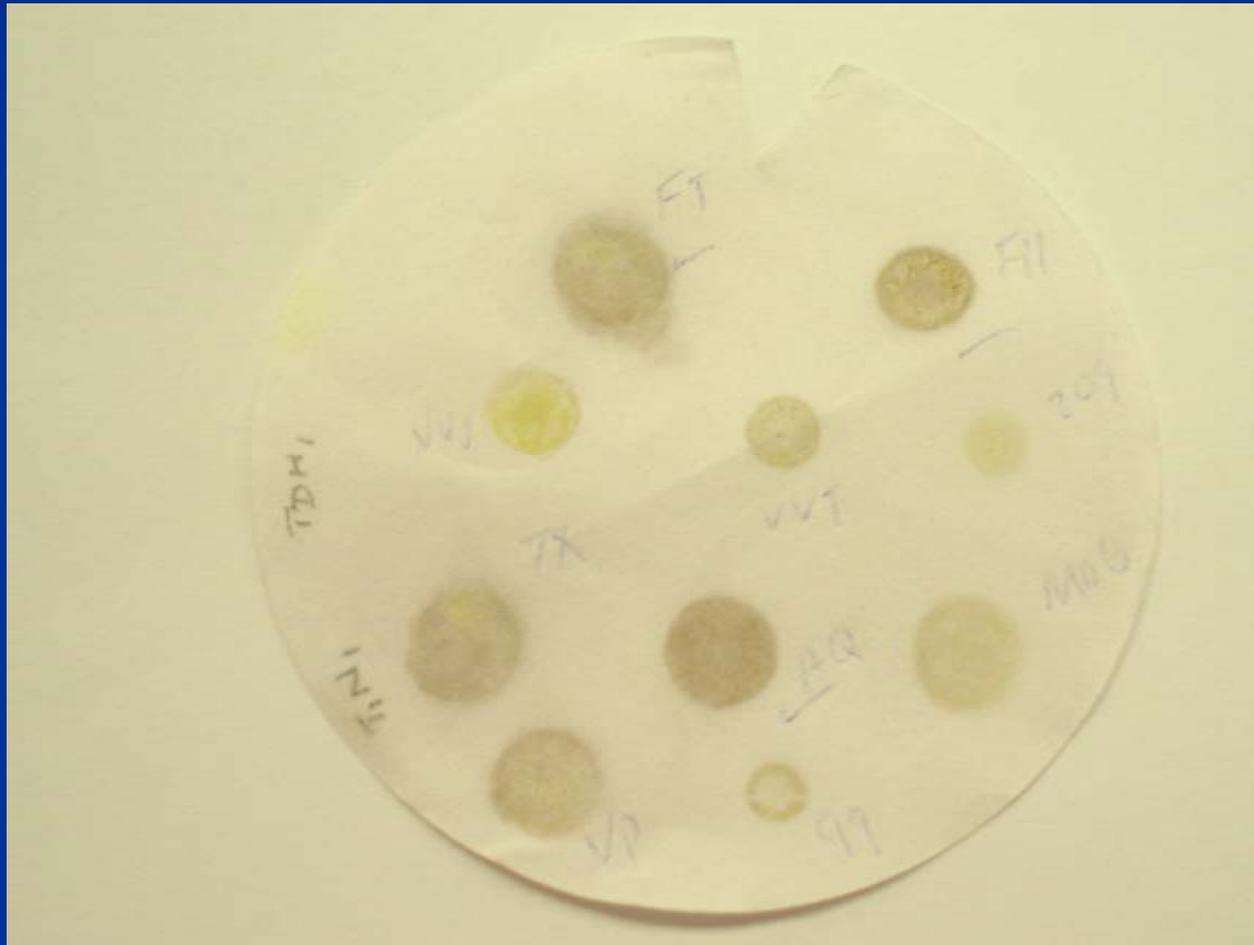
Rapid Methods for Identification of Foodborne Pathogens

- DNA based methods
 - PCR (Polymerase Chain Reaction)
 - DNA probe
- Antibody-based methods
 - Several methods available
- Dehydrated media minikit
 - Pure bacterial cultures can be identified to the species level within 24 hours.

DNA Based Methods

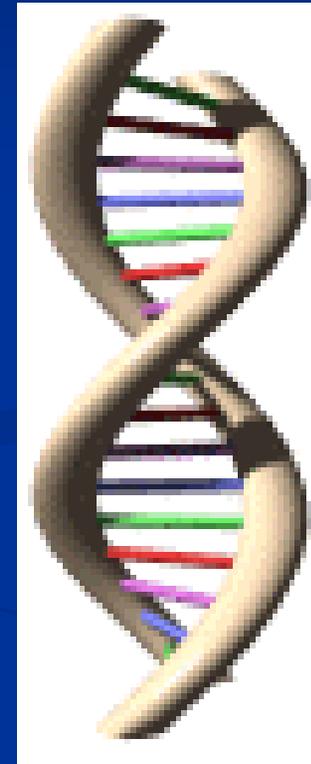
- DNA Probe Hybridization
 - Direct plating using DNA probes for identification
 - Can determine the amount of bacteria in a food sample
 - Results within 8 hours
 - Methods can be developed for most bacteria
 - *Vibrio vulnificus* and *Vibrio parahaemolyticus* method available on Bacteriological Analytical Manual website (BAM)

DNA Probe membrane detecting
Vibrio parahaemolyticus
thermostable direct hemolysin gene (TDH)



DNA Based Methods

- **Polymerase Chain Reaction (PCR)**
 - Extremely rapid
 - Amplify specific sequences of DNA
 - Need an enrichment step (PCR can detect dead microorganisms)



PCR Based Methods

- BAX® (Du Pont Qualicon) AOAC® Official MethodSM
- 96 different samples analyzed every 4 hours
- Results expressed as positive or negative on monitor

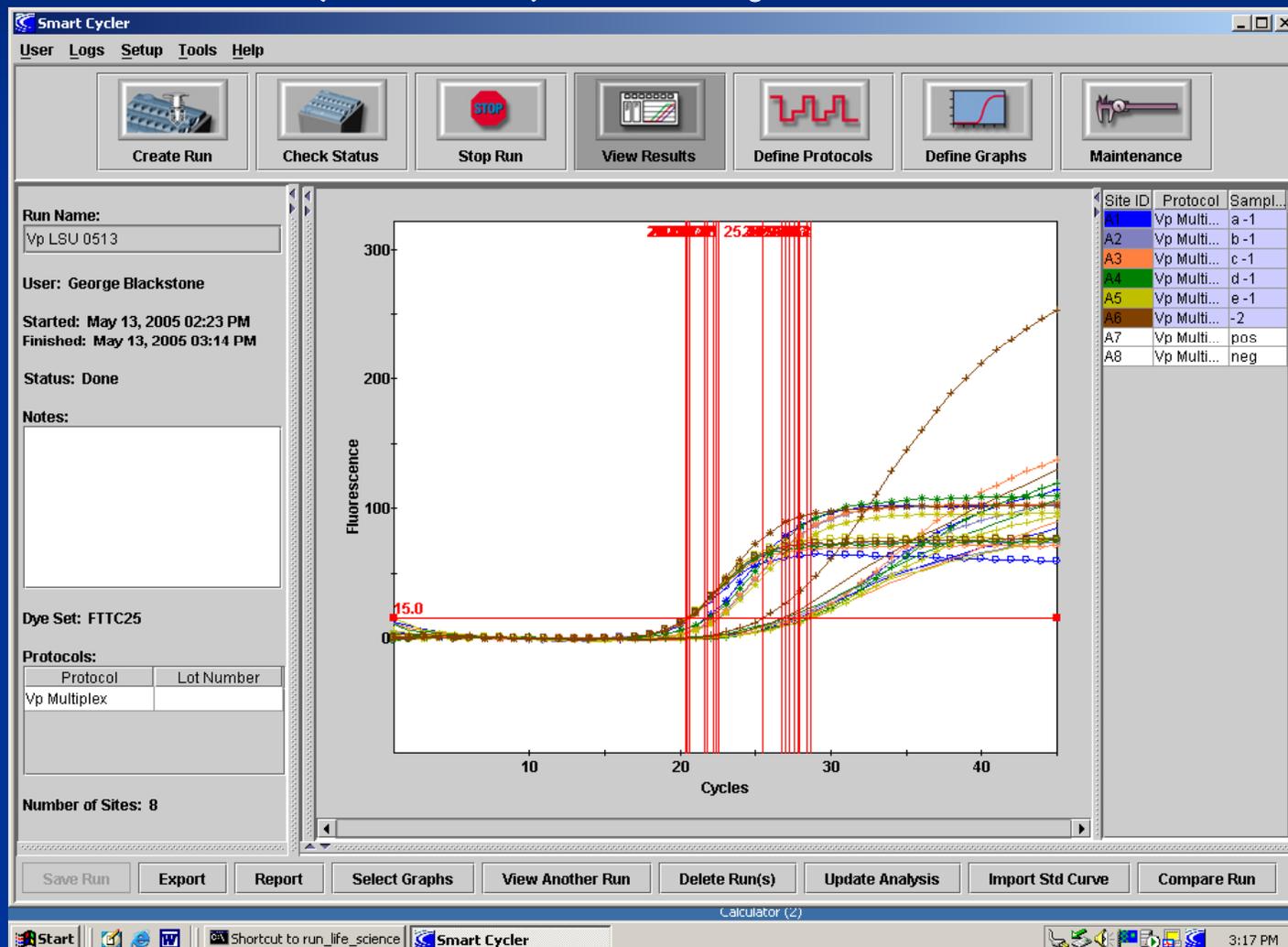


PCR Based Methods

■ Smart Cycler

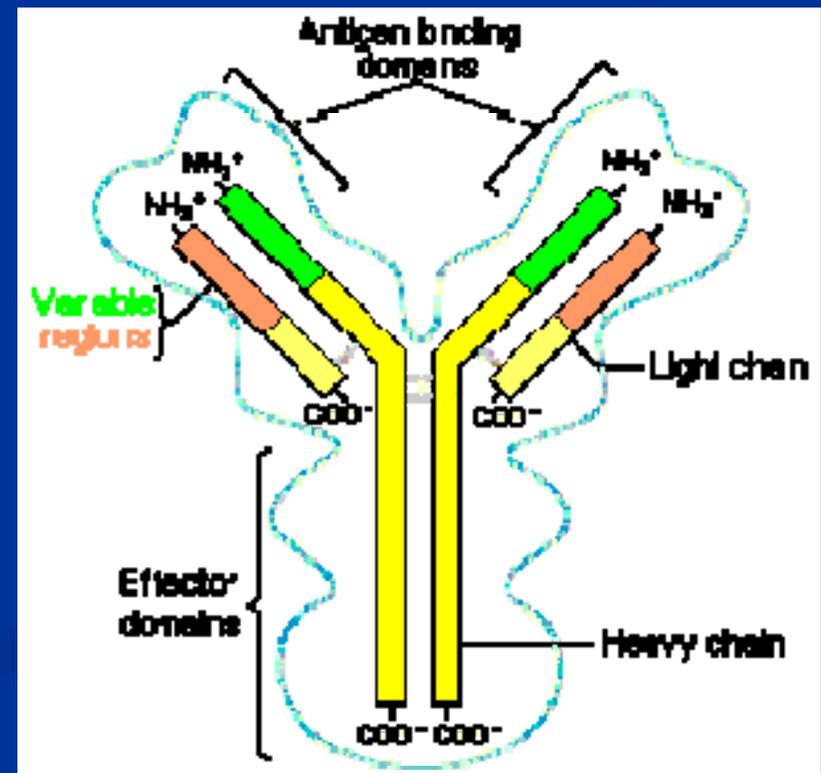
- Up to 96 independently programmable reaction sites
- Results as little as 30 minutes
- Multiplex assays
- Can determine concentration of unknown sample

Real Time PCR of *Vibrio parahaemolyticus* (TDH) in Oysters



Antibody-Based Methods

- Antibodies specific to surface proteins, flagella, or toxins
- Identifies to species level
 - Polyclonal antibodies recognize multiple proteins
 - Monoclonal antibodies recognize single protein



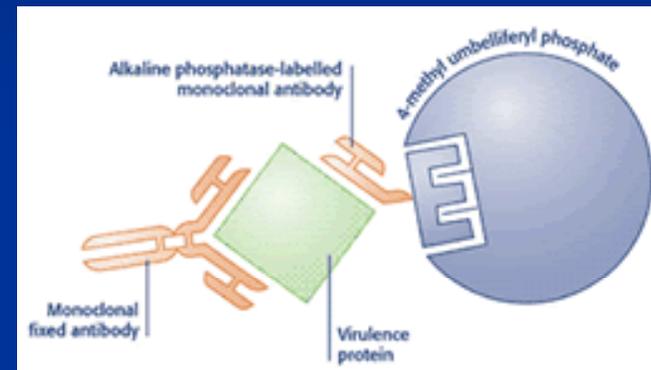
Assurance EIA® (Biocontrol)

- Enzyme-linked immunoassay
- 96-well microtiter plate with break-apart wells
- Tests available for *Listeria*, *Campylobacter* and *Salmonella*
- AOAC® Official MethodSM



Immunofluorescence

- Antibodies coupled to fluorescent dyes
- Positive cells will fluoresce under a fluorescence microscope
- Mini VIDAS[®] (bioMérieux Vitek, Inc., Hazelwood, MO) AOAC[®] Official MethodSM



mini VIDAS[®]

Latex Agglutination

- Antibodies are coupled to latex microspheres
- Visible clumping or agglutination confirms a positive reaction
- Tests available for *Salmonella*, *Shigella*, *Campylobacter*, and *E. coli*

Immunoprecipitate Detection

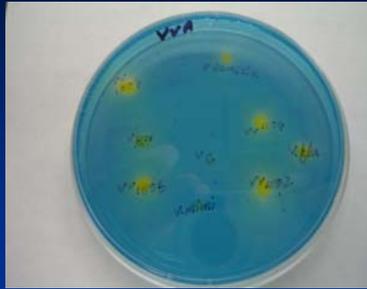
- Utilizes a chromatographic matrix with antibodies conjugated to a material that precipitates such as colored latex particles or colloidal gold
- Developed for several foodborne pathogens (*Salmonella*, *L. monocytogenes* and *Campylobacter*)
- Extremely rapid as short as 10 minutes
- VIP® (Biocontrol) AOAC® Official MethodSM
- Singlepath® (Merck, Darmstadt, Germany)



Immunoblot

- **Direct plating using antibodies for identification**
 - **Overlay colonies with membrane and treat with antibodies**
 - **Can determine the amount of bacteria in a food sample**
- **Results within 3 hours**
- **Methods can be developed for most bacteria**

Direct Colony Immunoblot



- Plate different species on TCBS, VVA and mCPC plates
- Incubate the plates
- Place the membrane on colonies
- Colony lifting
- Air drying the membrane
- Wash with PBS/ tween 20



- Block with BSA (30 min)
- Wash with PBS/ tween 20
- Add Primary antibody (1hr)
- Wash with PBS/ tween 20
- Add Secondary antibody (1hr)
- Wash with PBS/ tween 20
- Color development solution

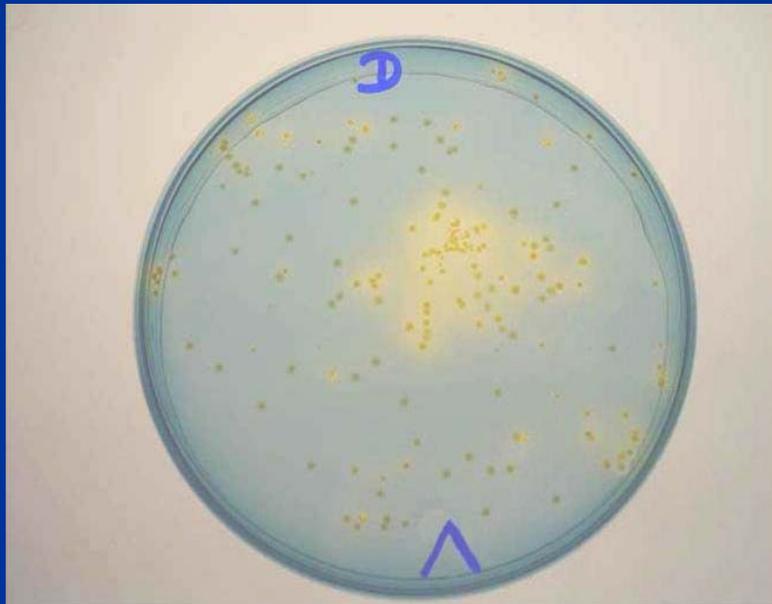
Sensitivity of Direct Colony Immunoblot to *Vibrio* species



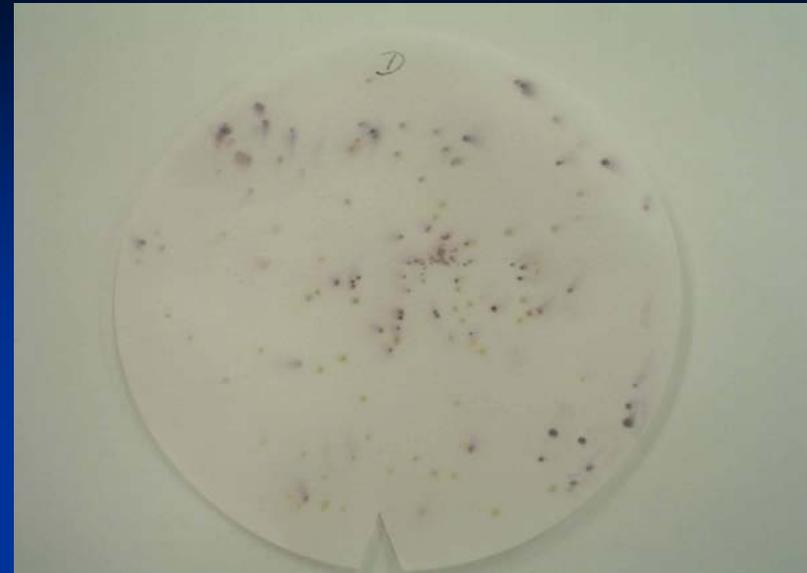
V. fluvialis, *V. mimicus*, *V. damsela*, *V. alginolyticus*, *V. cholerae*,
V. parahaemolyticus, *V. vulnificus*

Comparison of DNA probe method with Direct Colony immunoblot

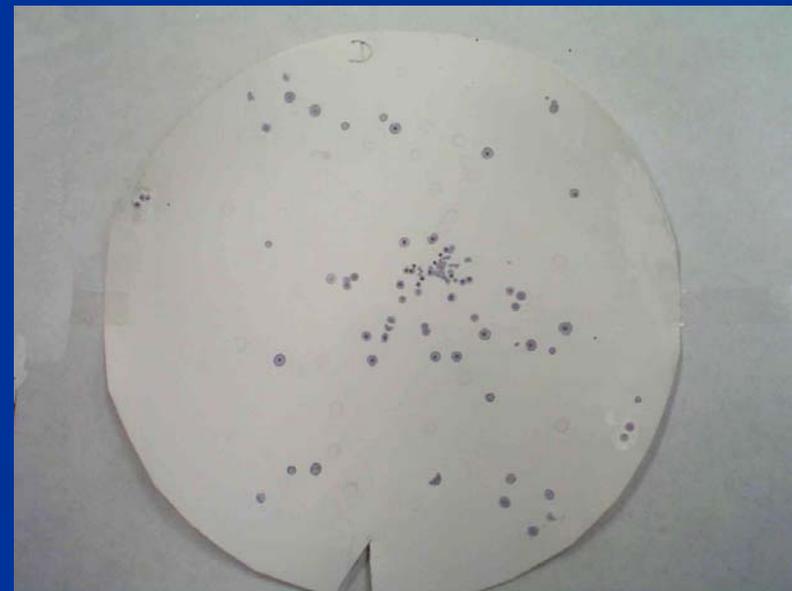
VVA plate



DNA probe



Direct colony immunoblot



Enumeration of *V. vulnificus* by direct colony immunoblot and DNA probe methods inoculated into different challenge levels of *V. parahaemolyticus*

Number of cells in sample		Enumeration of <i>V. vulnificus</i>	
<i>V. vulnificus</i>	<i>V. parahaemolyticus</i>	Direct colony blot	DNA probe
Log CFU/ml			
TNTC	4.50	2.02 ± 0.19	2.01 ± 0.17
2.16	3.55	2.14 ± 0.19	2.07 ± 0.28
2.06	2.27	2.10 ± 0.09	2.09 ± 0.09
1.99	1.37	2.17 ± 0.10	2.18 ± 0.16
2.04	0	2.15 ± 0.21	2.10 ± 0.19

Dehydrated Media Minikits

- These kits contain dehydrated media
- The media becomes hydrated when the pure bacterial culture is added.
- Each test strip contains wells with differential media for a specific microorganism.
 - Biochemical Test Strips by API Systems, bioMerieux Vitek, Inc., Hazelwood, MO, AOAC® Official MethodSM
 - MicroID (Remel, Lenexa, KS) AOAC® Official MethodSM

