STEC Detection and Characterization: Current and Future Methods

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Enteric Diseases Laboratory Branch

16th Annual PulseNet Update Meeting
28-30 August 2012
Atlanta, Georgia

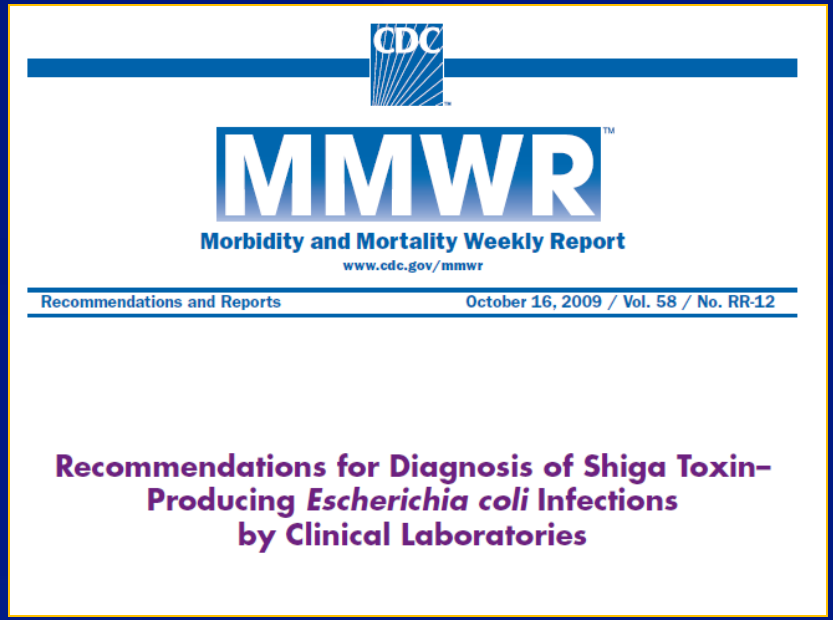
National Center for Emerging and Zoonotic Infectious Diseases
Division of Foodborne, Waterborne, and Environmental Diseases

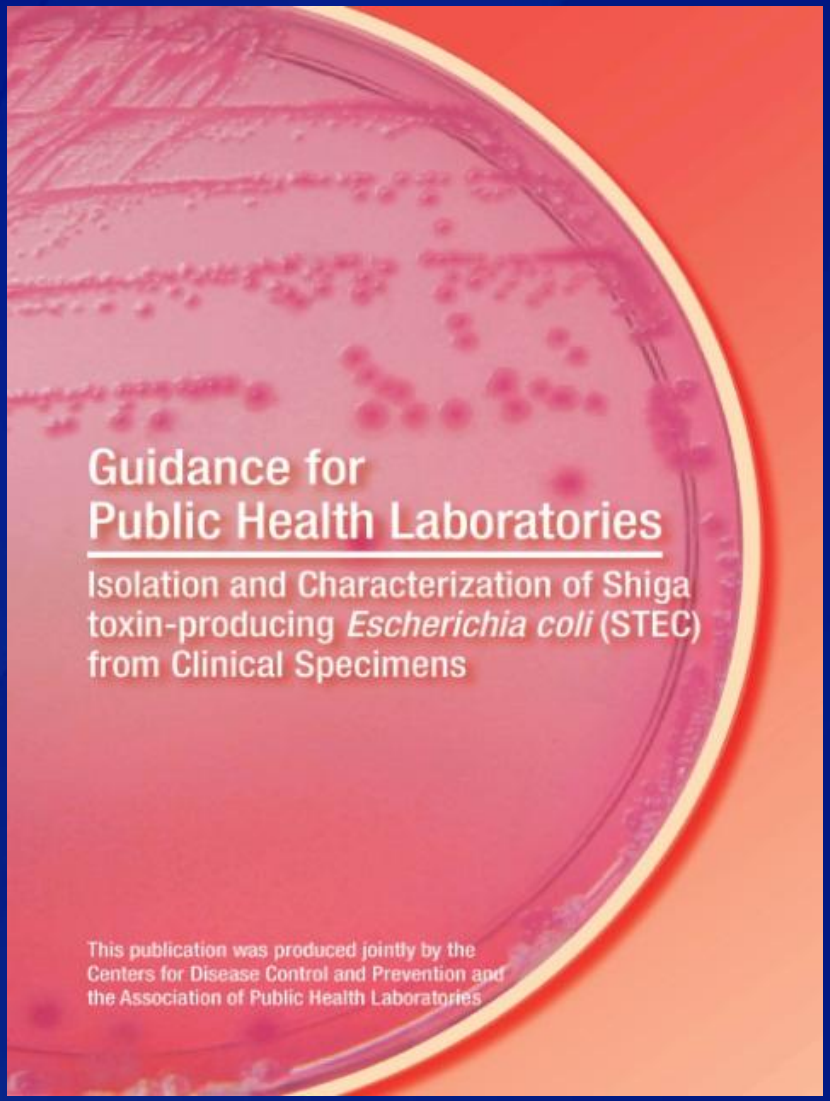
Objectives and Outline

- **Objectives**
  - Describe new technologies for non-culture based testing for STEC
  - Describe assays being used to characterize STEC isolates associated with foodborne illness
- **Outline**
  - Detection assays for STEC
  - Strain characterization assays
    - Virulence profiling
    - O:H antigen determination

Clinical laboratory recommendations, 2009

- Simultaneously culture all stools submitted from patients with acute community-acquired diarrhea or suspected HUS for O157 and assay for non-O157 STEC with a test that detects Shiga toxin
- Report and send *E. coli* O157 isolates and Stx+ broths to a public health laboratory as soon as possible



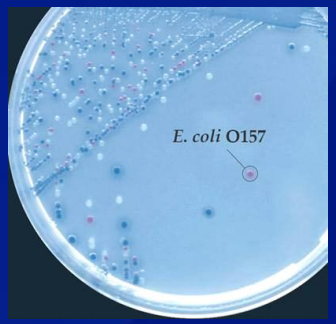


http://www.aphl.org/AboutAPHL/publications/Documents/FS_2012April_Guidance-for-PHLs-Isolation-and-Characterization-of-Shiga-Toxin-Producing-Escherichia-coli-STEC-from-Clinical.pdf)

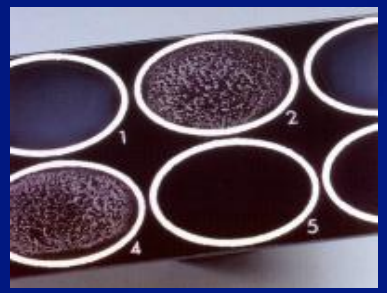
Clinical Diagnosis of STEC infection

Stool Specimen

Culture for O157 STEC



Streak to Selective/differential agar



Test in O157 latex reagent

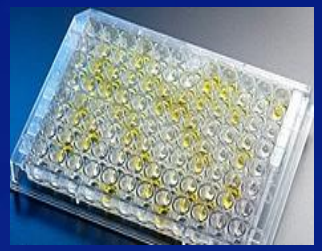
Test
- Shiga toxin or H7
- ID as *E. coli*

Send STEC O157 and positive broths to public health lab

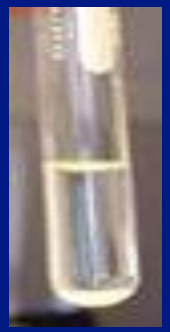
Culture for non-O157 STEC

Enrichment broth

16-24 hours

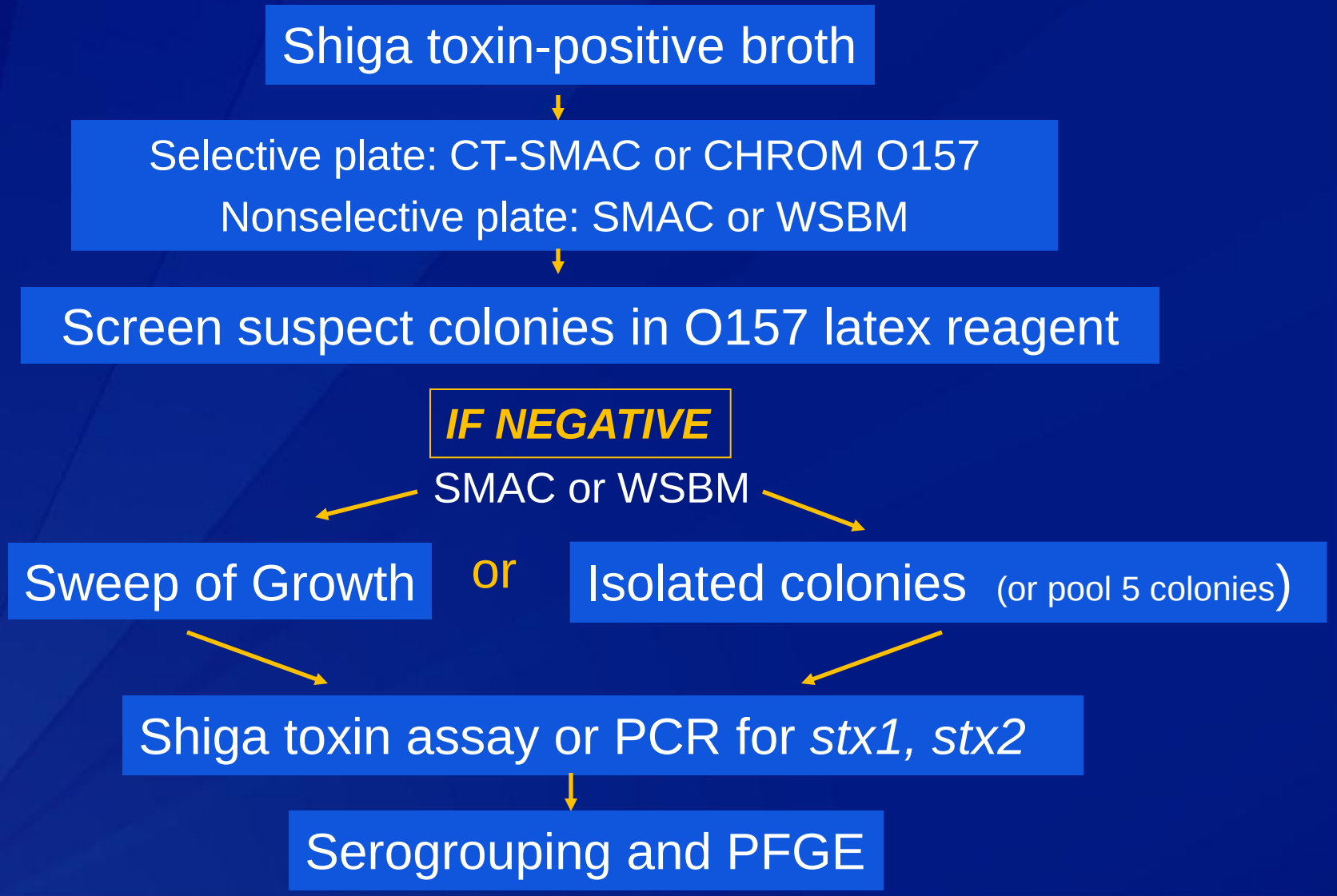


Shiga toxin or *stx* gene detection



Stx/*stx*+ broth

Isolation of STEC from Stx-positive broths by PHLs



Detection of STEC in Foods

http://www.fsis.usda.gov/PDF/MLG_5B_00.pdf

Sample enrichment

↓

Genomic DNA extraction

↓

TaqMan-based multiplex real-time PCR assay:

stx$_1$, *stx*$_2$, *eae* (intimin) and 16S rRNA

If positive ↓

O-antigen identification (real-time PCR)

↓

Immunomagnetic separation

↓

Selective plating → **confirmation**

MDBR
Medical Devices Business Review

DIATHERIX LABORATORIES

Diatherix Laboratories Rolls Out Gastrointestinal Test Panel

Published: 30-Mar-2010

Diatherix Laboratories has added a gastrointestinal (GI) panel to its lineup of sensitive, response diagnostic tests. Diatherix GI panel can detect and differentiate nine types of GI infections and helps physicians to identify severe, life-threatening infections in patients.

Detects 9 bacterial pathogens

- *Clostridium difficile*
- *Clostrodium difficile* toxin B
- *Campylobacter jejuni*
- *Escherichia coli O157*
- *Listeria monocytogenes*
- *Salmonella enterica*
- *Shigella flexneri*
- *Shigella sonnei*
- *Vibrio parahaemolyticus*

Cyptosporidium parvum

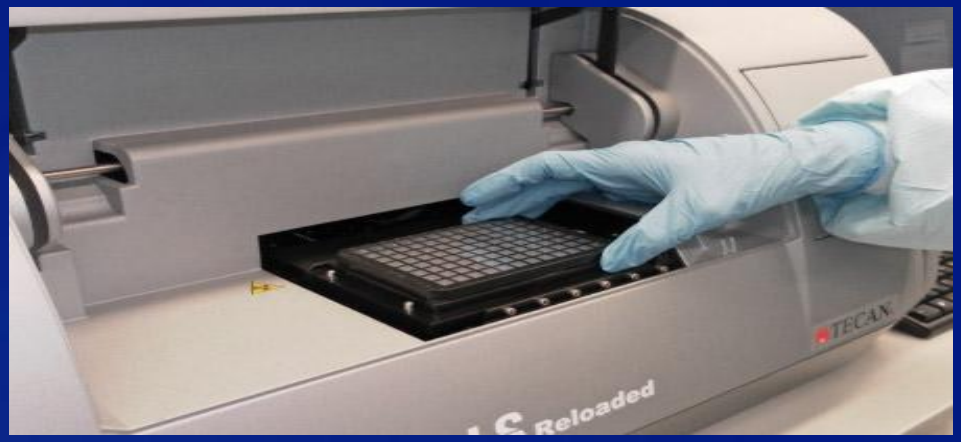
Giardia lamblia

Adenovirus 40, 41

601 Genome Way · Suite 4208 · Huntsville, Alabama 35806

Target-enriched Multiplex PCR (Tem-PCR)

- Nested PCR
- Internal primers have embedded “super primers sites”
- Biotinylated super primers “enrich” the amplicons through a second round of amplification adding a fluorescent tag
- amplified products are then detected with specific probes in a microarray hybridization platform



Luminex

- Detects *E. coli* O104
- Available in Europe; not yet in the US
- Tests for 15 bacteria, viruses and parasites in under 5 hours

NEW xTAG® Gastrointestinal Pathogen Panel (GPP)

The evolution of GI diagnosis

Now you can test for 15 key bacteria, viruses, and parasites – ***all in under 5 hours***

- xTAG® GPP is the first diagnostic to offer detection of 15 major gastrointestinal pathogens in a single test
- Results within 5 hours for timely and better patient care
- Fast turn-around time and multiplex testing mean better use of time and human resources

Distributed by

diax Via Toscana, 2 - 20090 Vignate Milano - tel +39.02.95.360.619 e mail : info@diax.it

Luminex
xTAG® GPP
Transforming GI diagnostics.

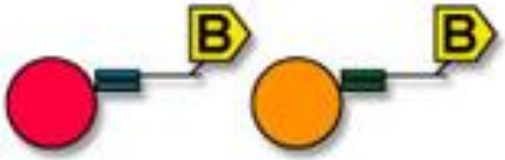
CE IVD For In Vitro Diagnostic Use. Products are region specific and may not be approved in some countries/regions. Please contact Luminex to obtain the appropriate information for your country of residence.

xTAG™ Chemistry

I. Multiplex PCR
A multiplexed PCR reaction is performed to amplify the regions of interest in the target human or infectious agent genes.

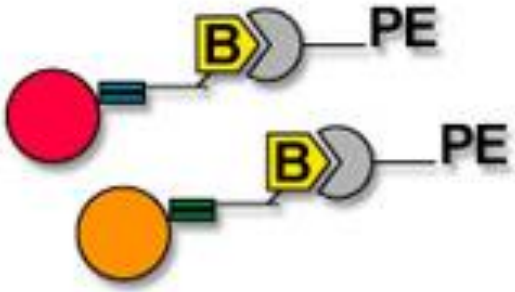
II. Exo/SAP treatment of PCR Product
The PCR reaction is treated to remove excess nucleotides and primers.

III. Multiplex ASPE/TSPE
The PCR reaction is then subjected to a primer extension step that is specific for the allele or the infectious agent that is being analyzed (Allele Specific Primer Extension (ASPE) or Target Specific Primer Extension (TSPE)). The 5' end of the ASPE or TSPE primers is attached to an xTAG universal tag sequence.



IV. Universal Array Sorting
The 5' universal tag sequence is hybridized to the complementary anti-tag sequence coupled to a particular xMAP bead set.

Wash (Remove in Streamlined)



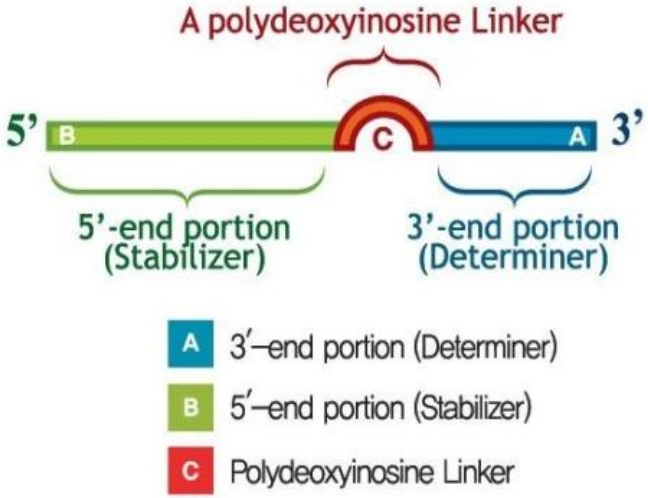
V. Detection
The hybridized beads are read by the Luminex System and results are analyzed by the data analysis software.

Luminex System Detection

IVD solutions through partnership

Mast Group

Seeplex®



Seeplex® System

Seeplex® is a breakthrough multiplexing PCR technology that enables a new standard in simultaneous multi-pathogen detection. Seegene applies its novel and proprietary Seeplex® system utilizing its DPO™ (Dual Priming Oligonucleotide) technology to create multi-pathogen tests delivering maximum specificity, reproducibility and sensitivity.

DPO™ Technology

DPO™ technology is a fundamental tool for blocking extension of non-specifically primed templates generating consistently high specificity. The strength and utility of this DPO™ technology can be successfully incorporated into molecular diagnostics systems such as multiplex diagnostics and SNP genotyping systems.

Automated Detection of PCR Products

Caliper LifeSciences

Caliper LabChip® Dx

MCE®-202 MultiNA

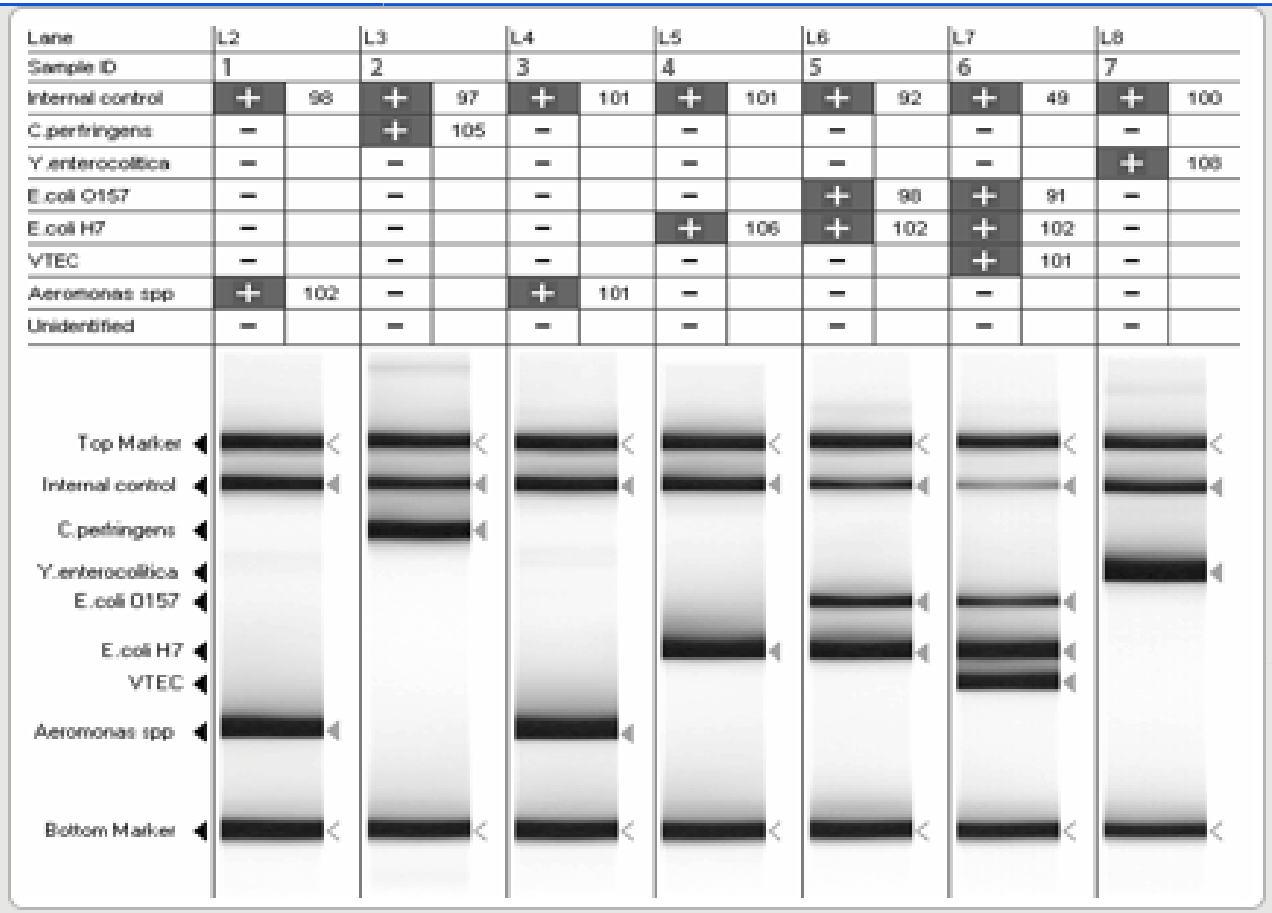
ScreenTape® System

Agilent Technologies

SHIMADZU
Solutions for Science
since 1875

Seegene Diarrhea ACE Detection for Stool
1 Viral and 2 Bacterial Panels (14 Agents in 6 hr)

Diarrhea-B2 ACE Detection



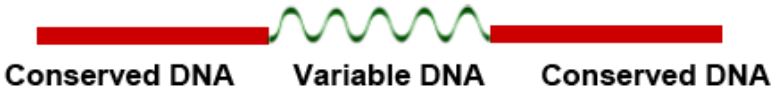
1	Aeromonas *spp*.
2	C. perfringens
3	Aeromonas *spp*.
4	E.coli : H7
5	E.coli : O157 E.coli : H7
6	*E.coli : O157* *E.coli : H7* VTEC
7	Y.enterocolitica 1~7: Clinical samples

PLEX-ID Instrument Overview

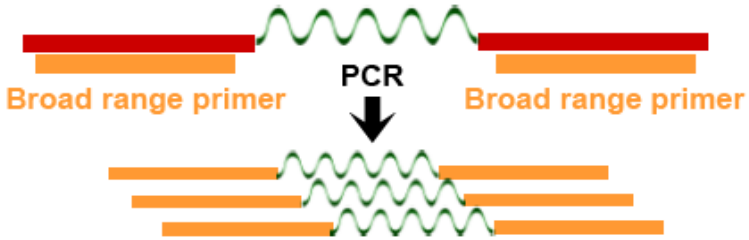
Desalting
ESI Mass Spec
Organism identification

Steps in Pathogen Detection with Ibis Technology

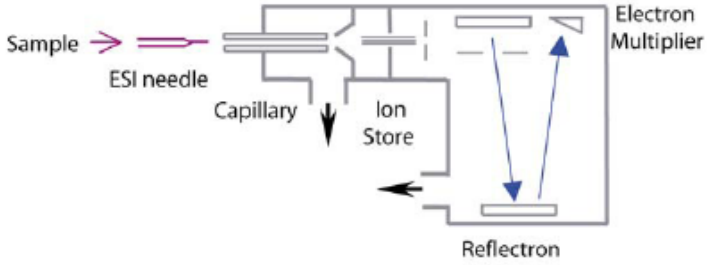
STEP 1 **Identify genomic regions for identification:**
Variable DNA sequences flanked by conserved sequences



STEP 2 **Amplify nucleic acids to measure:**
This can be tuned to on an assay by assay basis

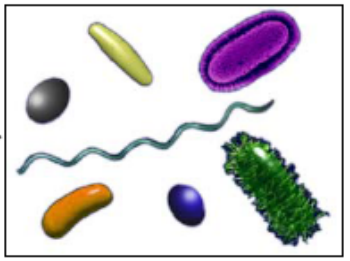


STEP 3 **Measure nucleic acid:**
ESI-TOF



STEP 4 **Identify the organisms:**
Base-composition signatures

As: 17
Gs: 30
Cs: 11
Ts: 61



Pall GeneDisc® System

- First technology to receive multiparametric validation from the AOAC Research Institute for the detection of non-O157 STEC in meat.
- Tests simultaneously for O157 STEC and four of the top six non-O157 STECs targeted by the USDA.
- Pathogenic *E.coli* O104:H4 test kit available.

VereID™ Biosystem

Lab-on-Chip Platform

PCR – Microarray Detection on a Single Chip

VereID™ Biosystem combines molecular biology, microfluidics and microelectronics to bring the future of diagnostics and surveillance to you today.

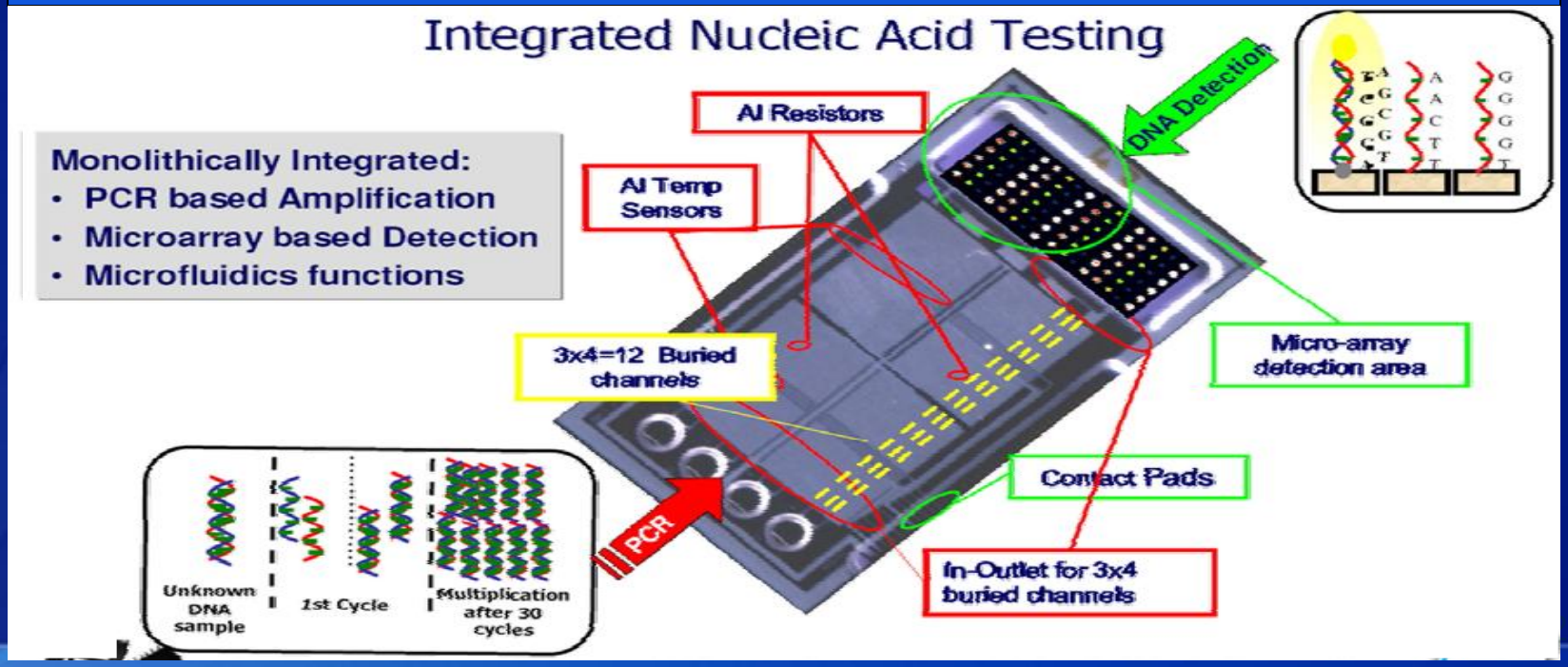
VereID™ Bio-System and VereChip™

Combining Molecular Biology, Microfluidics & Microelectronics

A Breakthrough Innovation with the Integration of Two Powerful Molecular Biological Technologies:

Polymerase Chain Reaction (amplification of DNA) and Microarray

- High accuracy and sensitivity
- Provides genetic information of infection



VereFoodborne™

VereFoodborne ™ will be able to detect, differentiate and ID all of the following diseases in ONE TEST

Bacteria

No.	Genus	Group/subgroup	Class
1	Vibrio	Vibrio cholerae	Gammaproteobacteria
2	Vibrio	Vibrio parahaemolyticus	
3	Staphylococcus	Staphylococcus aureus	Bacilli
4	Listeria	Listeria monocytogenes	
5	Bacillus	Bacillus cereus	
6	Clostridium	Clostridium perfringens (Welch)	Clostridia
7	Campylobacter	Campylobacter jejuni	Epsilon Proteobacteria
		Campylobacter coli	
		Campylobacter lari	
8	**Escherichia**	**Shiga toxin-producing Escherichia coli (e.g. E.coli O157)**	Gammaproteobacteria
9	Escherichia	Escherichia coli other spp.	
10	**Shigella**	**Shiga toxin-producing Shigella (e.g. Shigella dysenteriae)**	
11	Shigella	Shigella other spp. (e.g. Shigella flexneri, Shigella sonnei)	
12	Salmonella	Salmonella spp.	
13	Cronobacter	Cronobacter sakazakii	

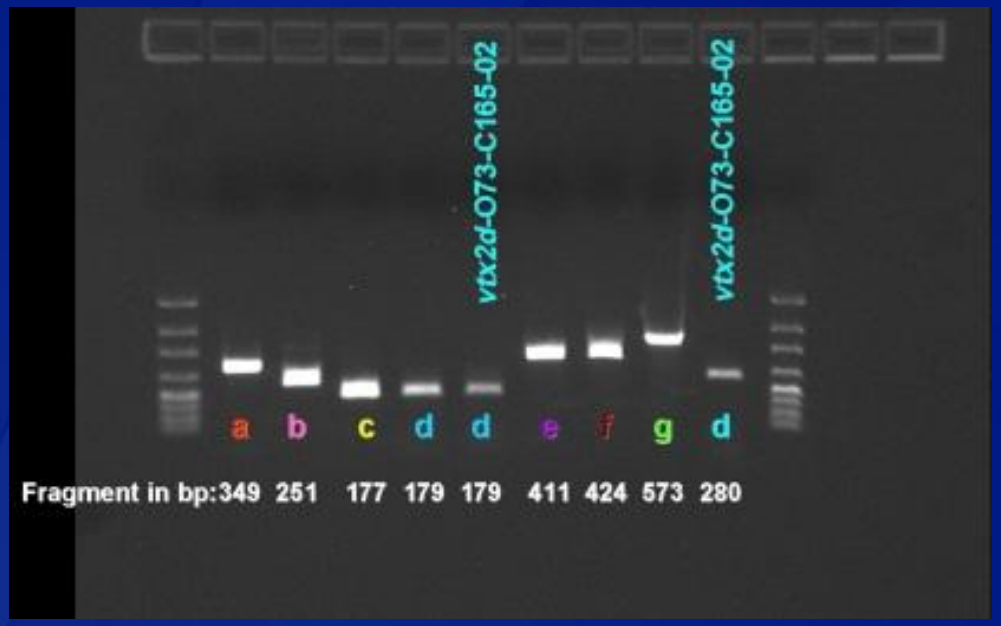
RNA Virus

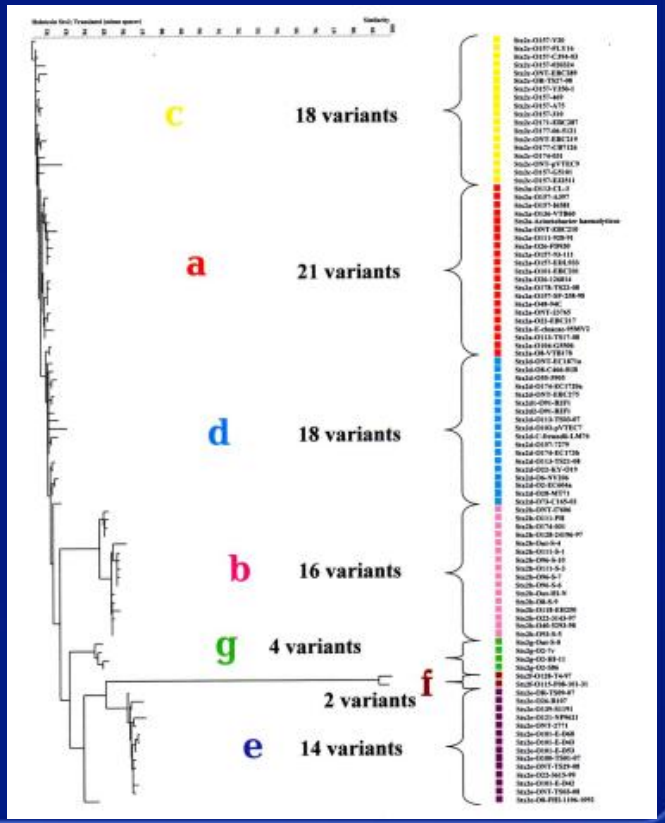
No.	Genus	Group/subgroup	Class
14	Norovirus	NLV Geno Group I	ssRNA positive-strand
15	Norovirus	NLV Geno Group II	ssRNA positive-strand
option 1	Rotavirus	Rotavirus A	dsRNA
option 2	Rotavirus	Rotavirus B var.1	
		Rotavirus B var.2	
option 3	Rotavirus	Rotavirus C	

International harmonization of Stx Nomenclature

- *Scheutz F et al.* A multi-center evaluation of a sequence-based protocol to subtype Shiga toxins and standardize Stx nomenclature
  http://jcm.asm.org/content/early/2012/06/28/JCM.00860-12.full.pdf+html
-

Conventional PCR Assays for how to detect different Stx subtype genes



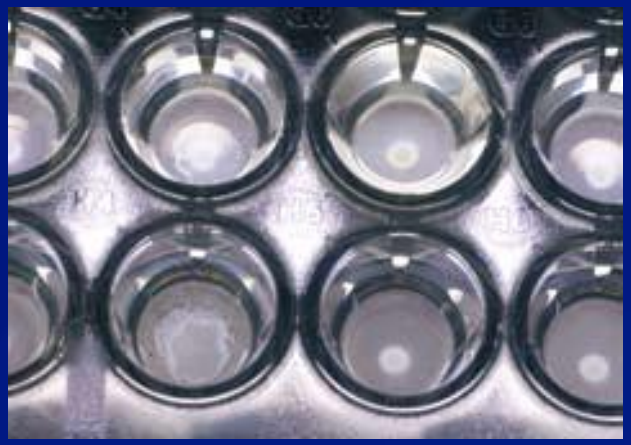


Escherichia coli serotyping

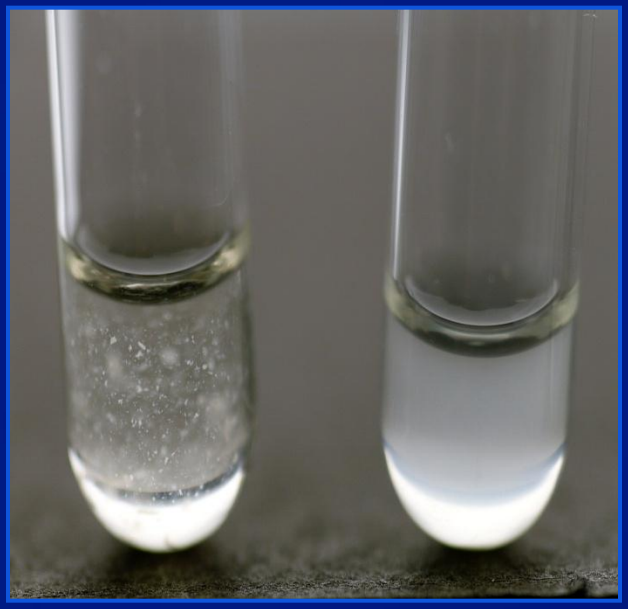
Staten Serum Institute
WHO Collaborating Center for Reference and Research on *Escherichia* and *Klebsiella*

- *187 O antigens and 56 H antigens*
- *> 200 different serotypes of STEC in humans and animals*

O antigen determination



H antigen determination



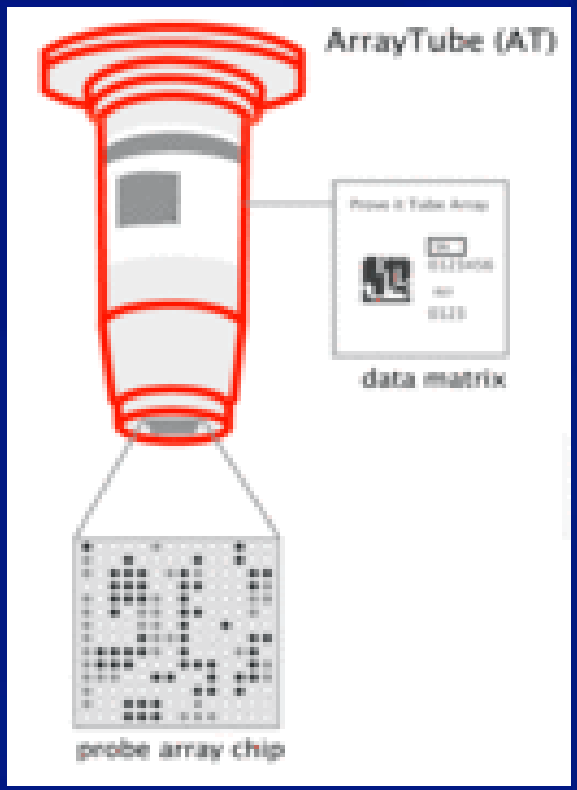
IDENTIBAC

E. coli Genotyping Kit Gene

Chip Version No.03 m (metal markers)

- Detects 92 virulence genes with 146 probes
- Identifies the virulence profile for all pathogenic types of *E. coli* and determines the underlying molecular cause of virulence
- Results obtained within 24 hours
- The AMR-ve Array detects 54 resistance genes in gram negative bacterial isolate.
- Launching O:H serotyping assay-

  Ballmer K *et al*. Fast DNA Serotyping of *Escherichia coli* by Use of an Oligonucleotide Microarray. J. Clin. Microbiol. 45:370-379, 2007.



IDENTIBAC

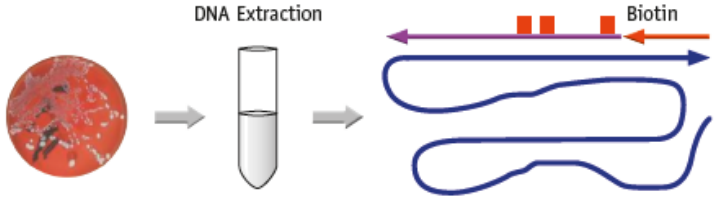
E. coli Genotyping Kit Gene

ASSAY PRINCIPLE

Following the extraction of genomic DNA the technique has the following basic stages:

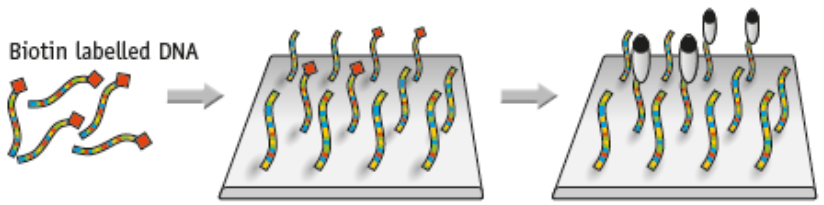
Amplification

The extracted RNA free genomic DNA is Biotin labelled and amplified in a linear multiplex PCR reaction.



Hybridisation

The resulting single stranded PCR product hybridises to the corresponding probes. The bound PCR product is detected using a horse-radish peroxidase – streptavidin conjugate, which converts the substrate [seramun green] into a coloured precipitate.



E. coli Genotyping Kit Gene

Detection + Analysis

The Reader (ArrayMate™ or ATR 03 Reader) enables the capture and visualisation of the array image. The presence of a dark spot indicates successful hybridisation. The software, supplied with the ArrayMate™ Reader (or ArrayTube™ Reader ATR 03), measures the signal intensity of each probe and determines which genes are present in the sample.

Analysis of the tubes by the ArrayMate™ Reader

AEM
Journals.ASM.org

Association of Nucleotide Polymorphisms within the O-Antigen Gene Cluster of *Escherichia coli* O26, O45, O103, O111, O121, and O145 with Serogroups and Genetic Subtypes

Keri N. Norman,[a] Nancy A. Strockbine,[b] and James L. Bono[a]

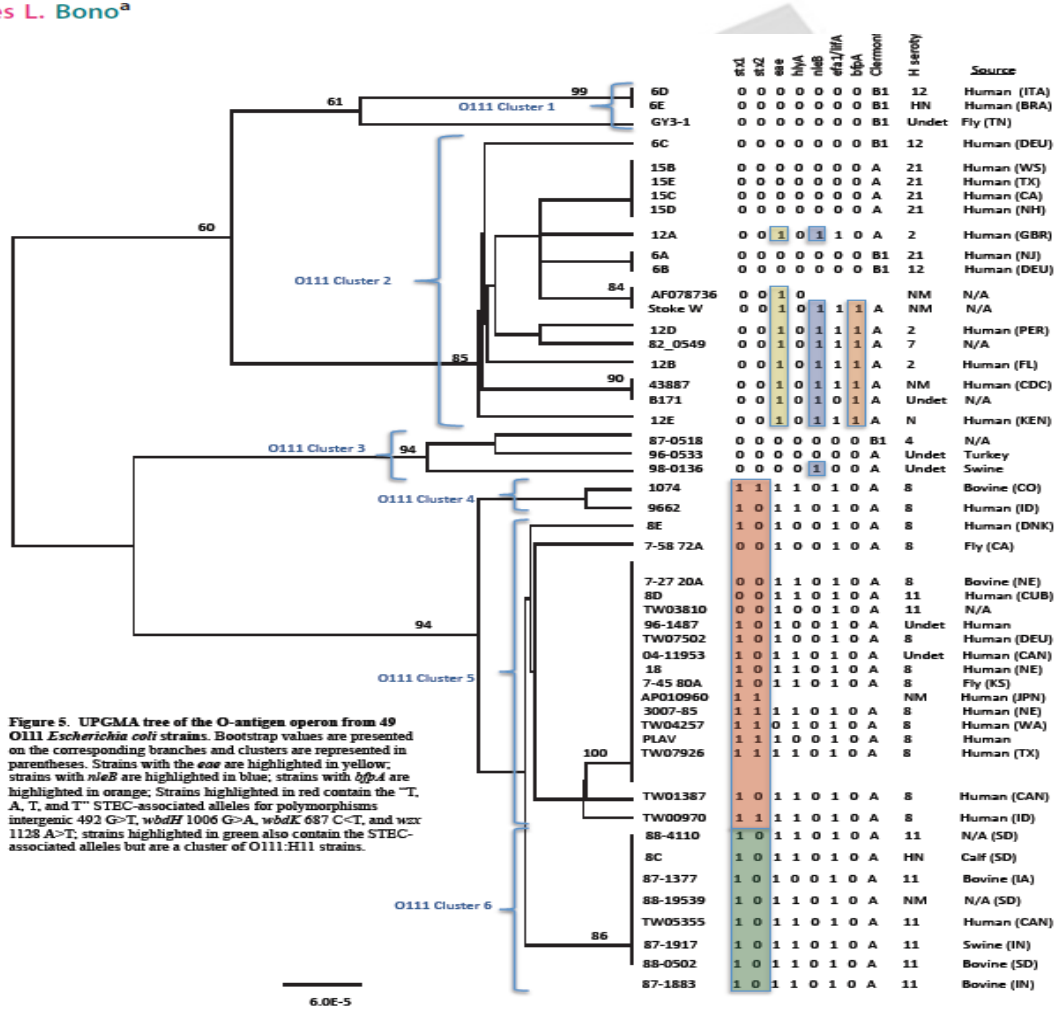


Figure 5. UPGMA tree of the O-antigen operon from 49 O111 *Escherichia coli* strains. Bootstrap values are presented on the corresponding branches and clusters are represented in parentheses. Strains with the *eae* are highlighted in yellow; strains with *nleB* are highlighted in blue; strains with *bfpA* are highlighted in orange; Strains highlighted in red contain the "T, A, T, and T" STEC-associated alleles for polymorphisms intergenic 492 G>T, *wbdH* 1006 G>A, *wbdK* 687 C<T, and *wzx* 1128 A>T; strains highlighted in green also contain the STEC-associated alleles but are a cluster of O111:H11 strains.

NeoSEEK™ System for Rapid Genomic Detection and Identification of STEC

- Provide next-day results on enrichment broths
- Multi-plex PCR identified 71 genetic markers with mass spectrometry-based detection of specific targets through "DNA bar codes"
- Includes O157, O26, O45, O103, O111, O121, and O145 STEC
- Detection of virulence factors and their subtypes

NEOGEN® CORPORATION

DNA Sequencing: Next Generation Diagnostics

life technologies™ | Semiconductor Sequencing for Life™ ion torrent

OPEN ACCESS Freely available online

PLoS one

Prospective Genomic Characterization of the German Enterohemorrhagic *Escherichia coli* O104:H4 Outbreak by Rapid Next Generation Sequencing Technology

Alexander Mellmann[1], Dag Harmsen[2]*, Craig A. Cummings[3], Emily B. Zentz[4], Shana R. Leopold[1], Alain Rico[5], Karola Prior[2], Rafael Szczepanowski[2], Yongmei Ji[3], Wenlan Zhang[1], Stephen F. McLaughlin[3], John K. Henkhaus[4], Benjamin Leopold[1], Martina Bielaszewska[1], Rita Prager[6], Pius M. Brzoska[3], Richard L. Moore[4], Simone Guenther[5], Jonathan M. Rothberg[7], Helge Karch[1]

1 Institute of Hygiene, University Münster, Münster, Germany, **2** Department of Periodontology, University Münster, Münster, Germany, **3** Life Technologies, Foster City, California, United States of America, **4** OpGen, Gaithersburg, Maryland, United States of America, **5** Life Technologies, Darmstadt, Germany, **6** Robert Koch Institute, Wernigerode Branch, Wernigerode, Germany, **7** Ion Torrent by Life Technologies, Guilford, Connecticut, United States of America

Abstract

An ongoing outbreak of exceptionally virulent Shiga toxin (Stx)-producing *Escherichia coli* O104:H4 centered in Germany, has caused over 830 cases of hemolytic uremic syndrome (HUS) and 46 deaths since May 2011. Serotype O104:H4, which has not been detected in animals, has rarely been associated with HUS in the past. To prospectively elucidate the unique characteristics of this strain in the early stages of this outbreak, we applied whole genome sequencing on the Life Technologies Ion Torrent PGM™ sequencer and Optical Mapping to characterize one outbreak isolate (LB226692) and a historic O104:H4 HUS isolate from 2001 (01-09591). Reference guided draft assemblies of both strains were completed with the newly introduced PGM™ within 62 hours. The HUS-associated strains both carried genes typically found in two types of

Mellmann A, Harmsen D, Cummings CA, Zentz EB, Leopold SR, et al. (2011) Prospective Genomic Characterization of the German Enterohemorrhagic ***Escherichia coli*** O104:H4 Outbreak by Rapid Next Generation Sequencing Technology. PLoS ONE 6(7): e22751. doi:10.1371/journal.pone.0022751

STEC assay formats for detection and characterization

- Automated conventional PCR
- Real-time PCR
- Liquid and solid microarrays
- Lab-on-a-Chip
- Mass spectrometry

Advantages and Challenges of Near and Next Generation Diagnostic Methods

Advantages:

- Rapid, actionable results provided to the physician
- Comprehensive testing for pathogens
- Increased efficiency
- Reduced cost

Challenges:

- Find ways to collect specimens so that follow-up testing can be performed on positive specimens to detect and control outbreaks and monitor antimicrobial susceptibility

Acknowledgements

- *CDC: Peter Gerner-Smidt, John Besser, Patti Fields, Evan Sowers, Devon Stripling, Haley Martin, Lauri Lindberg, Rebecca Lindsey, Cheryl Tarr, Deb Talkington, Cheryl Bopp, Kathy Greene, Kelly Hise, Steven Stroika, Efrain Ribot, Eija Trees, Rajal Mody, Hannah Gould, Patricia Griffin*
- *Statens Serum Institut: Flemming Scheutz*
- *USDA: Pina Fratamico, Jim Bono, Keri Norman*

Disclaimer:

"The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention and should not be construed to represent any agency determination or policy."