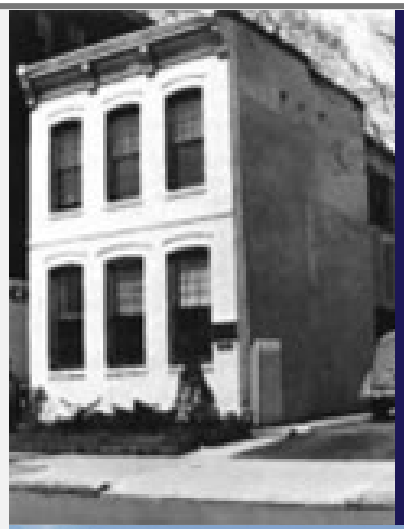
Improving the Detection of Shiga Toxin-Producing *Escherichia coli* (STEC)

Cara Wilder, Ph.D.
Technical Writer, ATCC
August 18, 2016

ATCC

About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one third with advanced degrees



Established partner to global researchers and scientists

Certification and Accreditation

ISO 9001:2008 Certification for quality management system

- Demonstrates commitment to quality products, customer service, and continued improvement

FM 516121

ISO 13485:2003 Certification for the design, development, production, testing, and distribution of medical devices

- Applies to synthetic molecular standards, the HIV surveillance kit, and other diagnostic and research kits

FM 610678

ISO Guide 34:2009 accreditation for production

- Applies to Certified Reference Materials (CRMs)

ISO/IEC 17025:2005 accreditation for testing

- Applies to all ATCC cultures, derivatives, and bioproducts tested in our laboratories

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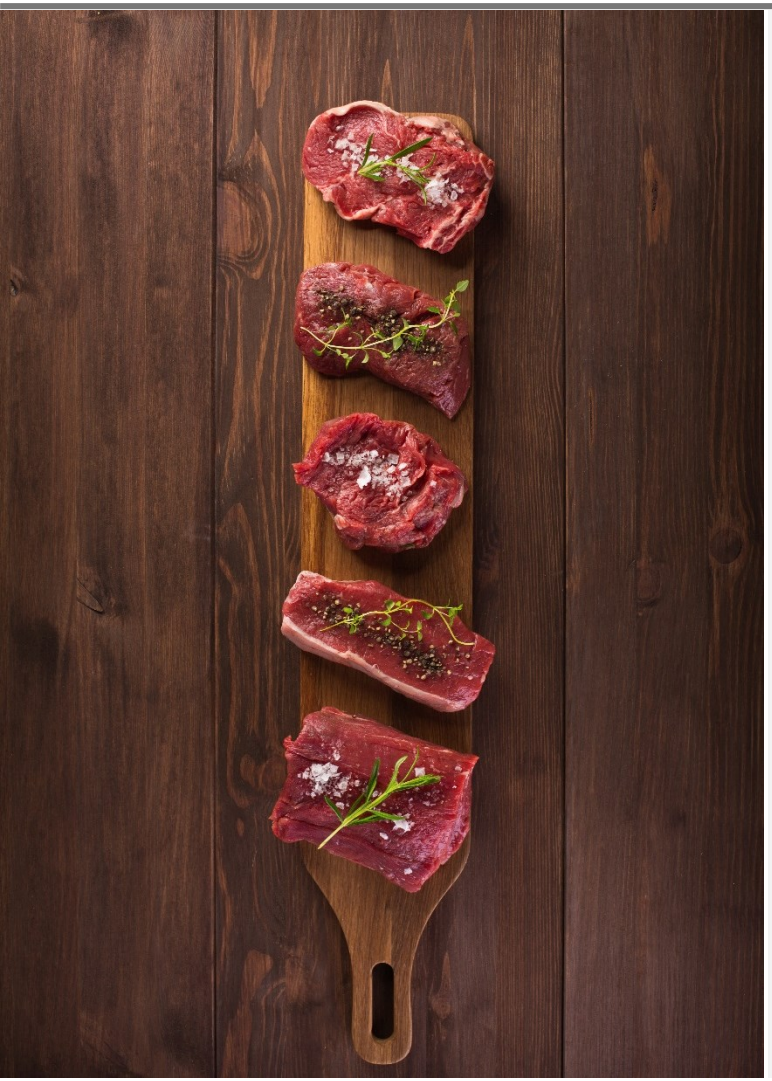
Outline



- Foodborne illnesses
- Shiga toxin-producing *Escherichia coli* (STEC)
- Importance of food testing controls
- Toxigenic, non-toxigenic, and reporter-labeled STEC strains available at ATCC

Foodborne Illnesses – A Common Problem

- Currently, there are more than **250 different foodborne diseases** described that are caused by microbes or chemical substances

- Food can become contaminated through a number of routes, including:
  - Contact with the intestinal contents of food animals
  - Processing with contaminated water
  - Introduced by food handlers
  - Cross-contamination from other raw products

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CDC. CDC and Food Safety.

Foodborne Illnesses – A Public Health Threat

- **1 in 6** Americans get sick every year from the consumption of contaminated food or beverages
- Globally, foodborne illnesses are estimated to affect **600 million people** and result in **hundreds of thousands** of deaths every year
- The CDC estimates that over **400,000** people are annually affected by antibiotic-resistant *Salmonella* or *Campylobacter* in the United States

Shigella image courtesy of CDC.

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WHO. Food Safety Fact Sheet. CDC. CDC and Food Safety.

Foodborne Illnesses – An Economic Burden

- Foodborne illnesses are estimated to cost the United States **$15.6 billion** annually
- The Grocery Manufacturer's Association reports that recalls from the past five years have had an estimated financial impact of **$10-30 million** per recall
- The CDC estimates that reducing foodborne infection by 10% would prevent **5 million** Americans from getting sick each year

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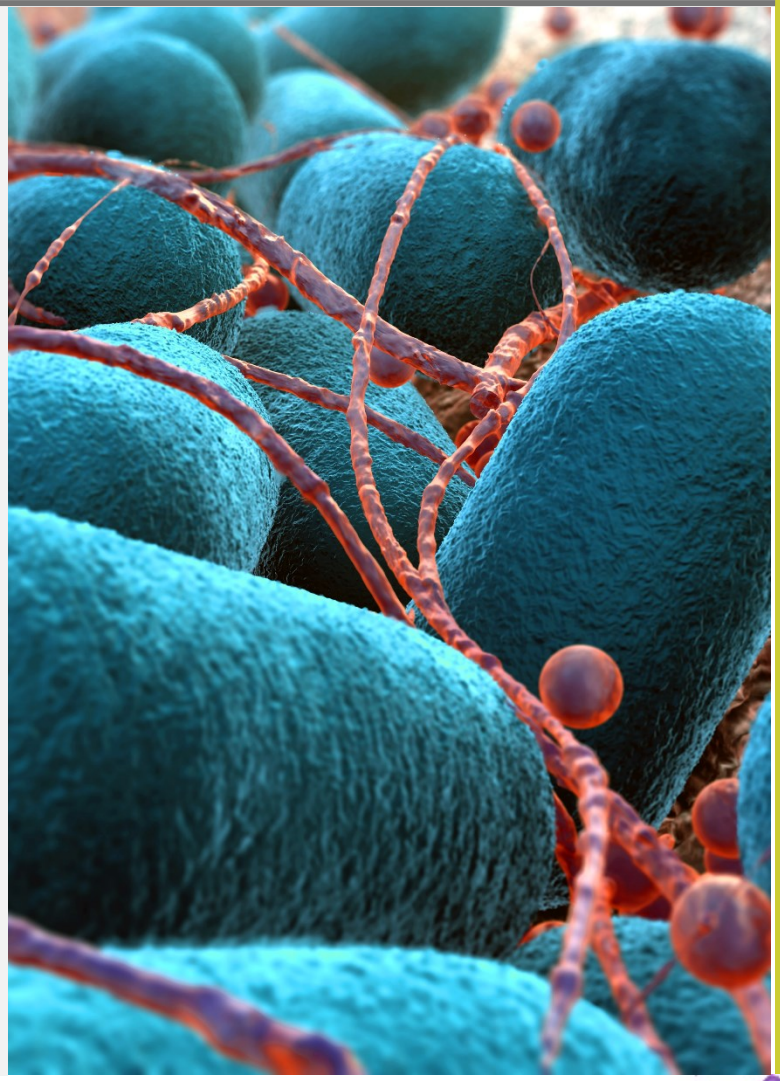
CDC Foundation. Business Pulse – Food Safety.

Escherichia coli

- *Escherichia coli* are Gram-negative facultative anaerobes that grown in the intestinal tract of animals

- Most strains of *E. coli* are harmless, but pathogenic strains do exist
  - Diarrhea
  - Urinary tract infection
  - Respiratory illness
  - Bloodstream infection



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Pathogenic *E. coli*

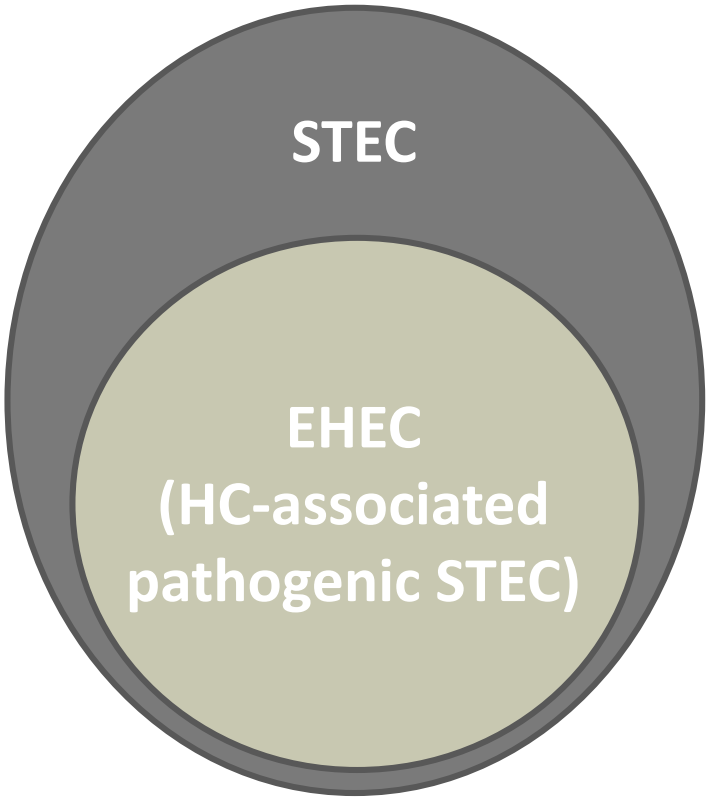
- Characterized by major surface antigens
  - Somatic (O) antigen } Serogroup
  - Flagellar (H) antigen } Serotype

- There are six pathotypes:
  - Enterotoxigenic *E. coli* (ETEC)
  - Enteroaggregative *E. coli* (EAEC)
  - Enteropathogenic *E. coli* (EPEC)
  - Enteroinvasive *E. coli* (EIEC)
  - Diffusely adherent *E. coli* (DAEC)
  - Enterohemorrhagic *E. coli* (EHEC)

Escherichia coli image courtesy of Elizabeth H. White, M.S.

Shiga Toxin-producing *E. coli* (STEC)

- Multiple names
  - Enterohemorrhagic *E. coli* (EHEC)
  - Verocytotoxin-producing *E. coli* (VTEC)
  - Shiga toxin-producing *E. coli* (STEC)
- >400 serotypes (O:H types) of STEC associated with human disease
- *E. coli* O157:H7 is the prototypical STEC



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Miko A, *et al.* Front Cell Infect Microbiol 4: 78, 2014.

Shiga Toxin-producing *E. coli* (STEC)

Estimated **265,000** foodborne associated illnesses and **3,600** hospitalizations in the United States each year

- *E. coli* O157:H7 accounts for about **36%** of STEC infections
- **~5-10%** of diagnosed infections develop into hemolytic uremic syndrome, a life-threatening complication that can cause permanent health damage

E. coli image courtesy of David Gregory & Debbie Marshall

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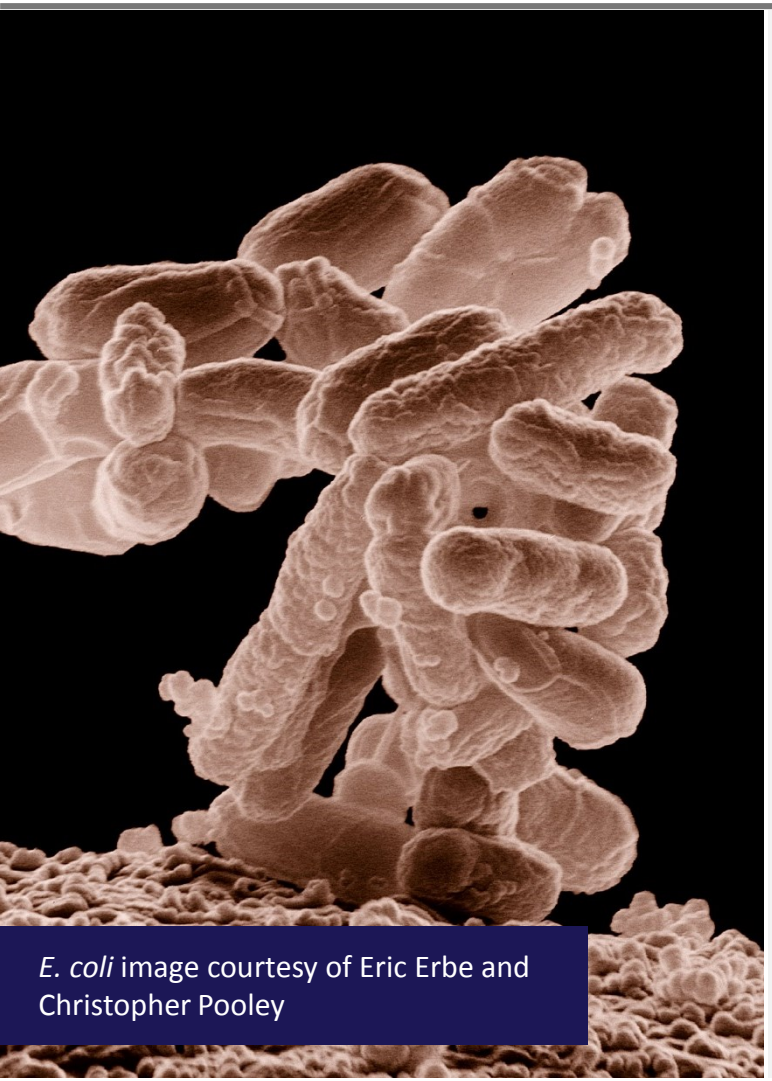
CDC. *E. coli*. General Information.

Shiga Toxin-producing *E. coli* (STEC)

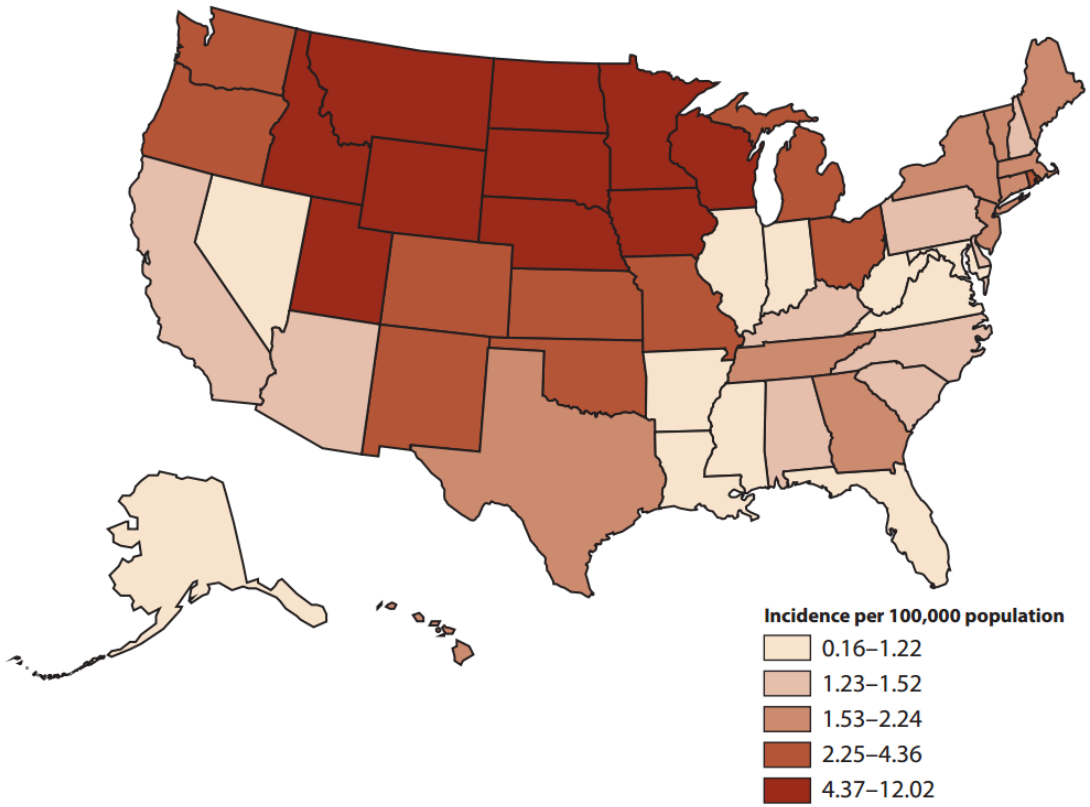


E. coli image courtesy of Eric Erbe and Christopher Pooley

Gene(s)	Virulence Factor
*stx*1, *stx*2	Shiga toxin
eae	Intimin
hlyA	Enterohemolysin
katP	Bifunctional catalase peroxidase
espP	Secreted serine protease
etpD cluster	Type II secretion pathway
Novel adhesion factors	Saa, Iha, Eaf1, LPF

Prevalence of STEC Strains

Incidence rate of STEC infection reported to CDC, United States, 2012 (n=6,506)



Laboratory-confirmed STEC infections reported to CDC, by serogroup, United States, 2012 (n=4756)		
Serogroup	**Number Reported**	**Percent**
O157	2460	51.7%
O26	655	13.8%
O103	568	11.9%
O111	365	7.7%
O121	163	3.4%
O45	120	2.5%
O145	109	2.3%
O118	38	0.8%
O186	28	0.6%
O5	24	0.5%
All others	226	4.8%

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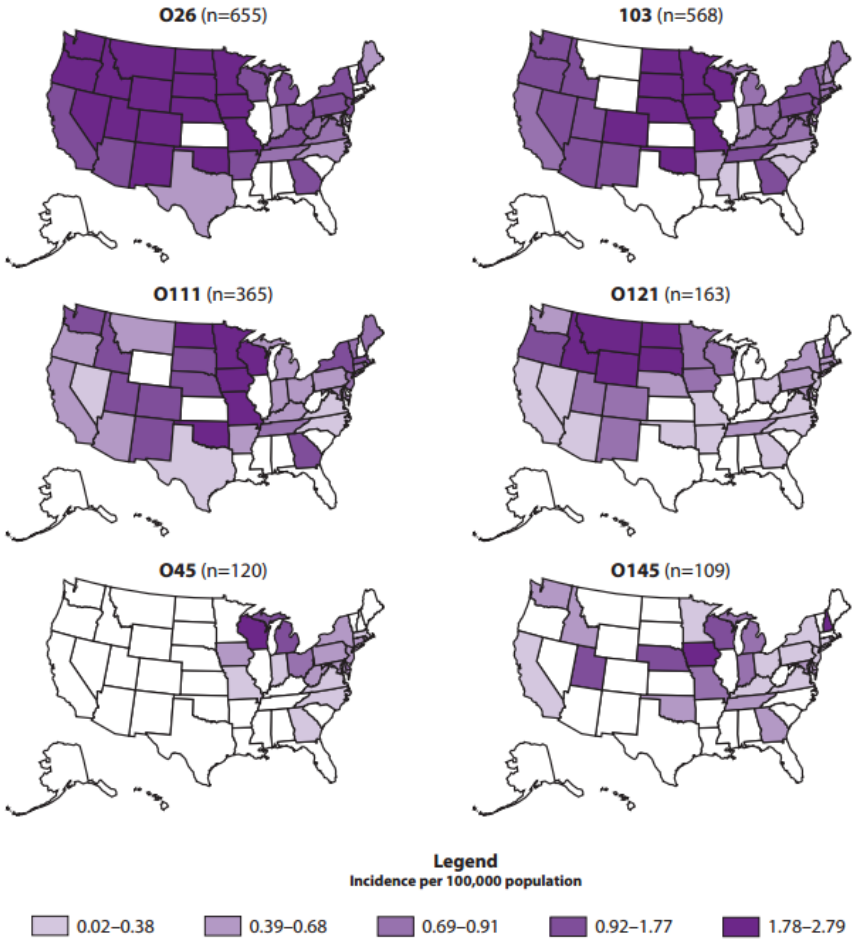
Image and information adapted from National Enteric Disease Surveillance: Shiga toxin-producing *Escherichia coli* (STEC) Annual Summary, 2012

Prevalence of STEC Strains

Incidence rate of laboratory-confirmed top 6 non-O157 STEC serogroups reported to CDC, United States, 2012 (n=1,980)



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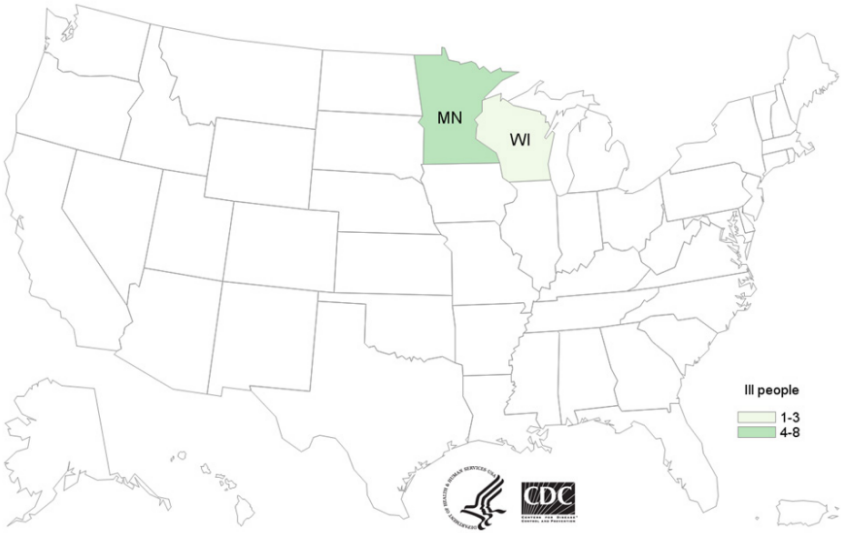
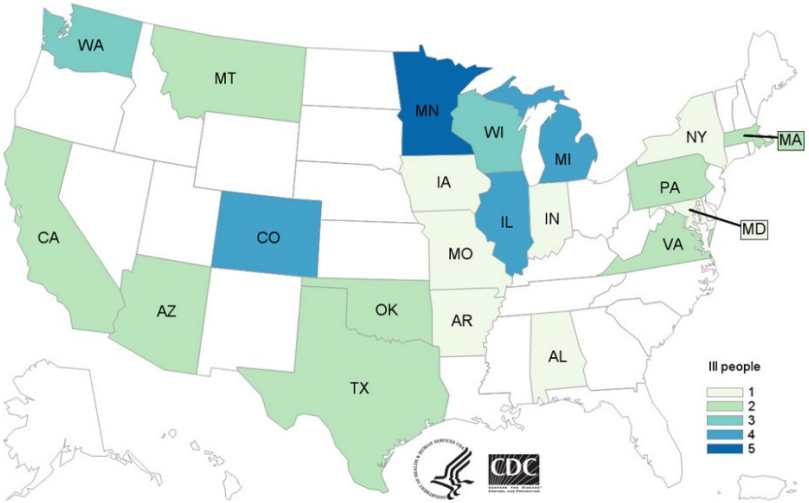
Recent STEC Outbreaks - 2016

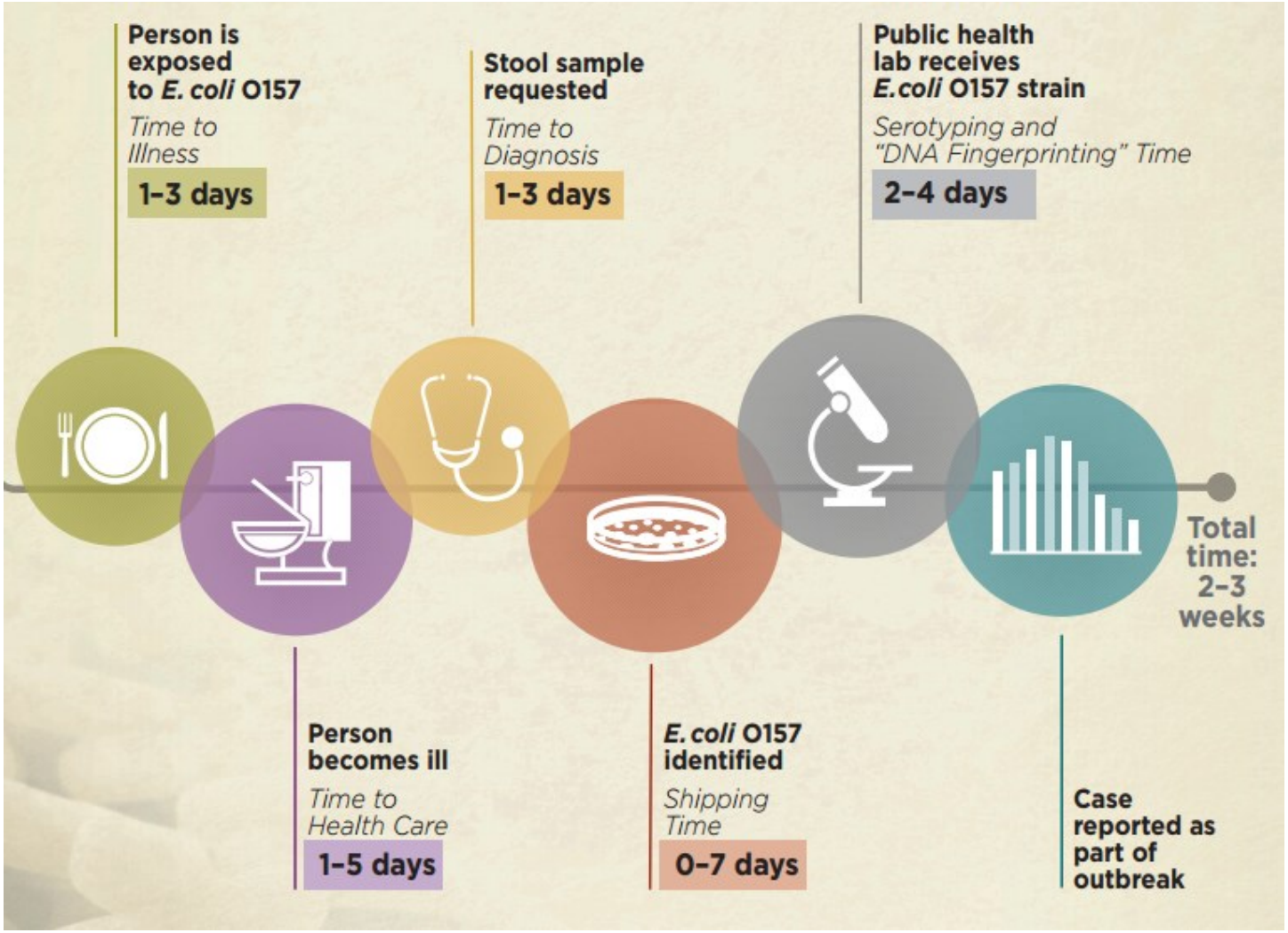




Multistate outbreak of STEC O157 infections linked to alfalfa sprouts as of March 22, 2016	
Case count	11
States	2
Deaths	0
Hospitalizations	2

Multistate outbreak of STEC O121 infections linked to flour as of July 25, 2016	
Case count	46
States	21
Deaths	0
Hospitalizations	13

Timeline for Reporting Cases



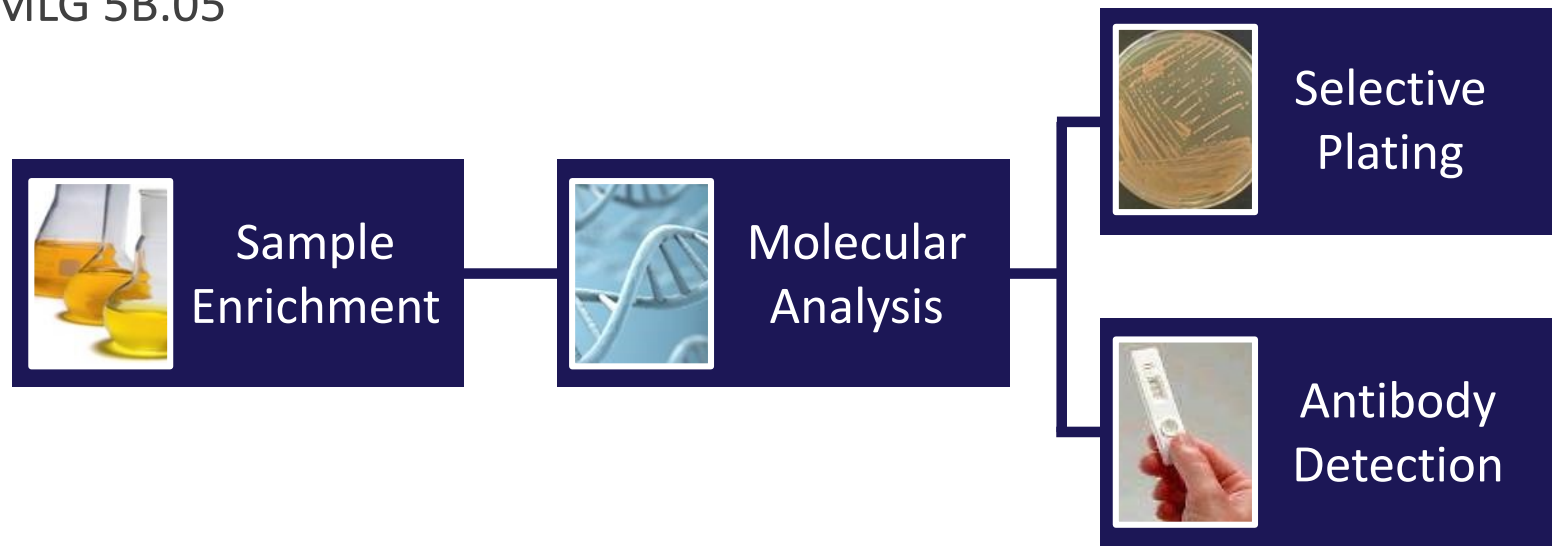
CDC. Timeline for Reporting Cases of *E. coli* O157 Infection.

Updated Food Safety Regulations

- In 2011, the United States Department of Agriculture (USDA) announced that it was going to ban the sale of any non-intact raw beef trimmings tainted with the Big-Six serogroups
- USDA Guidance:

  Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products

  MLG 5B.05



Importance of Food Testing Controls

- Reference materials are needed to evaluate products, raw materials, and associated equipment for microbial contamination from farm to fork
  - Quality control testing procedures
  - Process validation
  - Development of novel testing methods
- Protect consumer safety
- Protect brand reputation and prevent costly recalls

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ATCC Food Testing Reference Strains

- High-quality reference strains are needed for the routine testing of food products
- ATCC strains are backed by meticulous laboratory procedures to ensure viability, identity, and purity
- ATCC reference strains are frequently cited in published laboratory methods used by industry:
  - AOAC International
  - US Food and Drug Administration (BAM)
  - British Standards Institution
  - International Organization for Standardization (ISO)
  - Japanese Industrial Standards (JIS)

Search for strains at www.atcc.org/food

Image of *Salmonella* courtesy of CDC.

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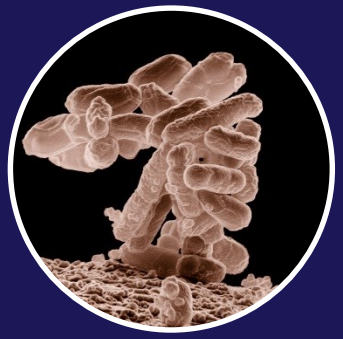
ATCC Food Testing Resources

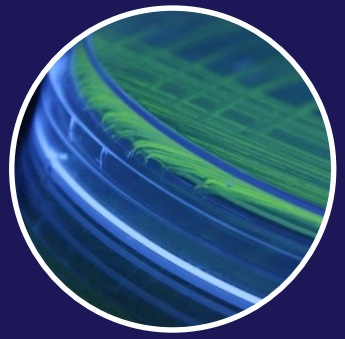
Reference Strains and Nucleic Acids

www.atcc.org/Food



Big-Six STEC DNA and Strains Panels

www.atcc.org/MP



Reporter-Labeled Strains

www.atcc.org/Reporters

ATCC Food Testing Solutions

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ATCC STEC Strains and Microbial Panels

- ATCC offers 50+ STEC strains representing *E. coli* O157 and the Big-Six
- Non-toxigenic strains are available and can be shipped internationally
- Strains were validated for serotype and the presence or absence of Shiga toxin (*stx1* and *stx2*) and intimin (*eaeA*) genes

ATCC®	Description	Number of Vials	BSL
MP-9™	Big-Six *Escherichia coli* Strains Panel	6	2
MP-10™	Big-Six *Escherichia coli* Genomic DNA Panel	6	1
MP-20™	Big-Six Non-Toxigenic *Escherichia coli* Strains Panel	6	2
MP-21™	Big-Six *Escherichia coli* NanoLuc® Strains Panel	6	2

ATCC

Big-Six *E. coli* Strains Panel

- Comprises six of the Big-Six non-O157 STEC serogroup strains
- Strains are tested for serotype and the presence of the Shiga toxin (*stx1* and *stx2*) and intimin (*eaeA*) genes

ATCC® MP-9™

ATCC® No.	Strain	Serotype	Virulence Genes	Isolation
BAA-2196™	2003-3014	O26:H11	*stx*1+/*stx*2+/*eae*+	Michigan
BAA-2193™	2000-3039	O45:H2	*stx*1+/*stx*2-/*eae*+	Main
BAA-2215™	2006-3008	O103:H11	*stx*1+/*stx*2-/*eae*+	Idaho
BAA-2440™	O111	O111	*stx*1+/*stx*2+/*eae*+	Unknown
BAA-2219™	2002-3211	O121:H19	*stx*1-/*stx*2+/*eae*+	Virginia
BAA-2192™	99-3311	O145	*stx*1+/*stx*2+/*eae*+	South Dakota

ATCC

Big-Six *E. coli* Genomic DNA Panel

- Comprises six of the Big-Six non-O157 STEC serogroups genomic DNA
- Parent strain for each preparation was tested for serotype and the presence of the Shiga toxin (*stx1* and *stx2*) and intimin (*eaeA*) genes

ATCC® MP-10™

ATCC® No.	Strain	Serotype	Virulence Genes	Isolation
BAA-2196D-5™	2003-3014	O26:H11	*stx*1+/*stx*2+/*eae*+	Michigan
BAA-2193D-5™	2000-3039	O45:H2	*stx*1+/*stx*2-/*eae*+	Main
BAA-2215D-5™	2006-3008	O103:H11	*stx*1+/*stx*2-/*eae*+	Idaho
BAA-2440D-5™	O111	O111	*stx*1+/*stx*2+/*eae*+	Unknown
BAA-2219D-5™	2002-3211	O121:H19	*stx*1-/*stx*2+/*eae*+	Virginia
BAA-2192D-5™	99-3311	O145	*stx*1+/*stx*2+/*eae*+	South Dakota

ATCC

Quantitative STEC Nucleic Acids

- Genomic DNA quantitated using Droplet Digital™ PCR
- Ideal for use in assay development and validation, and monitoring of day-to-day test variation and lot-to-lot performance of molecular-based assays
- Quantitative format allows for the generation of a standard curve for qPCR

ATCC® No.	Strain	Serotype	Virulence Genes	Isolation
BAA-2196DQ™	2003-3014	O26:H11	*stx*1+/*stx*2+/*eae*+	Michigan
BAA-2193DQ™	2000-3039	O45:H2	*stx*1+/*stx*2-/*eae*+	Main
BAA-2215DQ™	2006-3008	O103:H11	*stx*1+/*stx*2-/*eae*+	Idaho
BAA-2440DQ™	O111	O111	*stx*1+/*stx*2+/*eae*+	Unknown
BAA-2192DQ™	99-3311	O145	*stx*1+/*stx*2+/*eae*+	South Dakota
BAA-2326DQ™	TY-2482	O104:H4	*stx*2+/*aggR*+	Germany

ATCC

Non-toxigenic Big-Six non-O157 *E. coli*

- Comprises six non-toxigenic strains representing the Big-Six serogroups
- Each strain has been tested by ATCC for serotype and the presence/absence of the Shiga toxin (*stx1* and *stx2*) and intimin (*eaeA*) genes
- Can be shipped internationally

ATCC® MP-20™

ATCC® No.	Strain	Serotype	Virulence Genes
29552™	CDC 3250-76*	O111a,111b:K58:H21	*stx*1-/*stx*2-/*eae*-
BAA-2190™	98-3306	O121	*stx*1-/*stx*2-/*eae*-
BAA-2212™	2003-3055	O26:H4	*stx*1-/*stx*2-/*eae*-
BAA-2214™	2005-3342	O103	*stx*1-/*stx*2-/*eae*-
BAA-2216™	99-3071	O145:H34	*stx*1-/*stx*2-/*eae*+
BAA-2649™	04-3529	O45:H10	*stx*1-/*stx*2-/*eae*-

*Positive for the CVD432 and *aggR* genes, markers for Enteroaggregative *E. coli*

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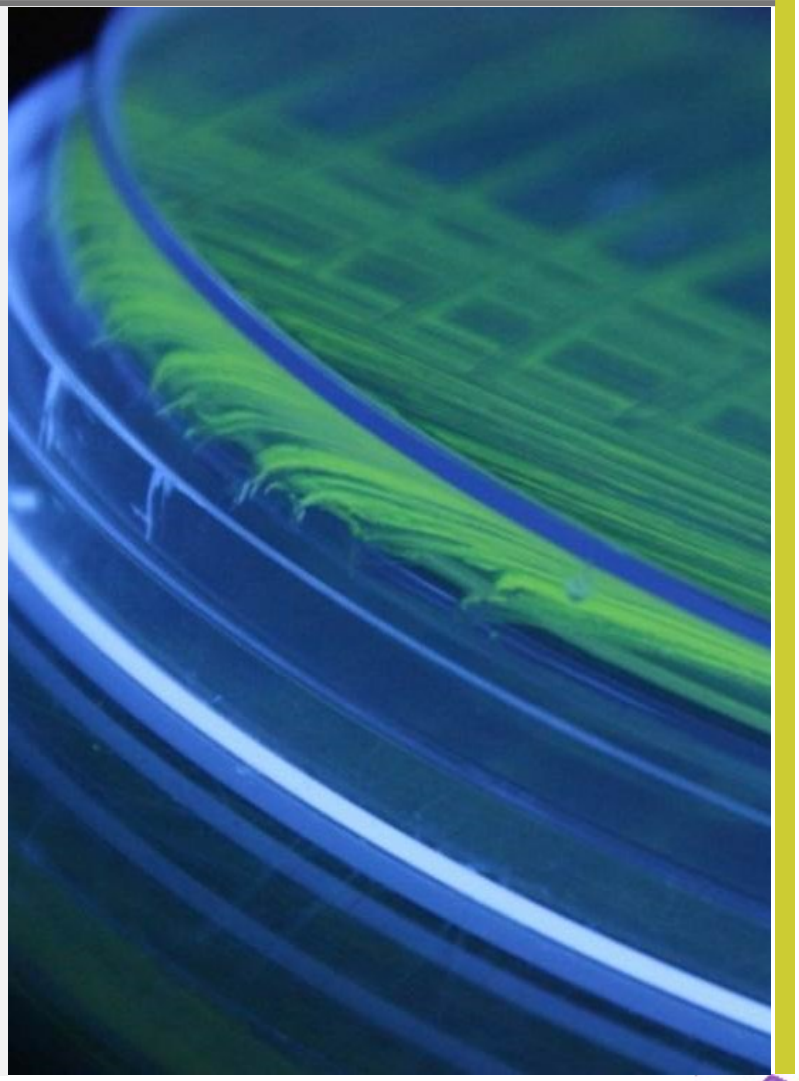
Reporter-labeled Strains for Food Testing

- Use of bacterial strains as positive controls in testing protocols is not widely practiced for fear of cross-contaminating samples
- Control strains with unique, easily detectable traits that distinguish positive control strains from actual food contaminants are needed
- Initial studies demonstrated the use of GFP- and firefly luciferase-labeled positive controls in monitoring microbial growth, survival, and colonization under various conditions



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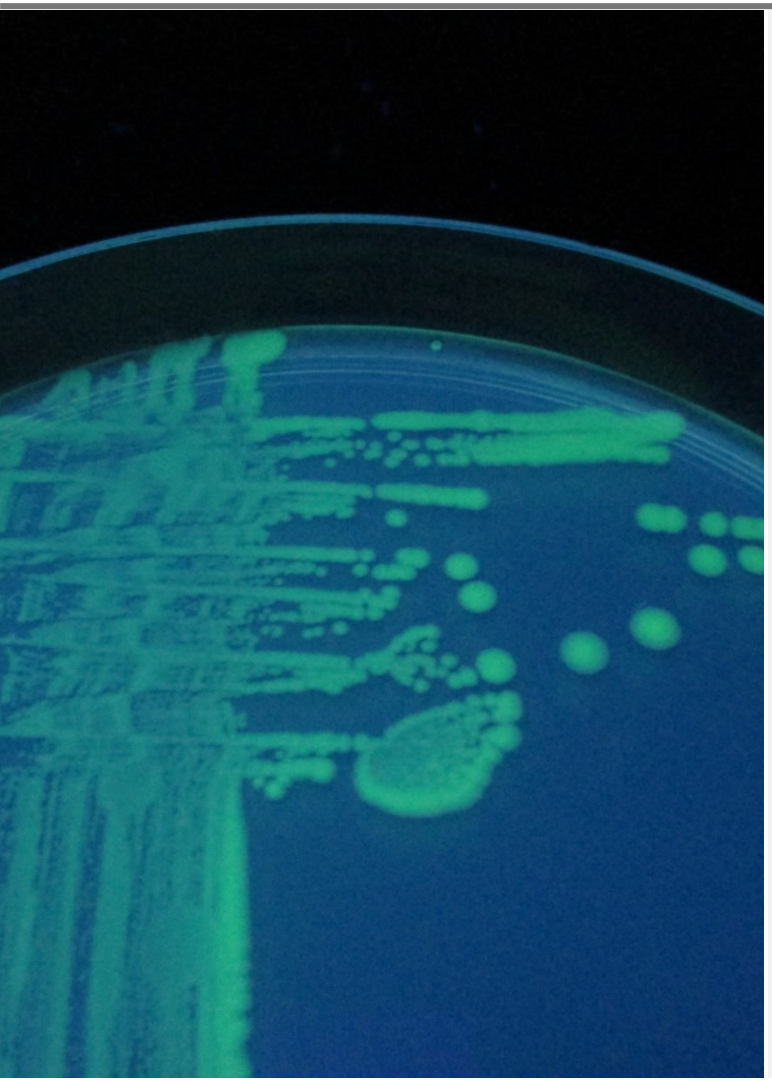
Fratamico PM *et al.* J Food Protect 60(10): 1167-1173, 1997.

GFP Strains for Food Testing



- Created using a synthetic GFP construct that does not have licensing fees
- Easily detected using a UV wand or transillumintor
- No additional substrates or buffers required

ATCC® No.*	Serotype	Virulence Genes
BAA-2196GFP™	O26:H11	*stx*1+/*stx*2+/*eae*+
BAA-2215GFP™	O103:H11	*stx*1+/*stx*2-/*eae*+
BAA-2209GFP™	O111	*stx*1+/*stx*2+/*eae*+
BAA-2219GFP™	O121:H19	*stx*1-/*stx*2+/*eae*+
35150GFP™	O157:H7	*stx*1+/*stx*2+/*eae*+
51657GFP™	O157:H7	*stx*1+/*stx*2+/*eae*+

*Strains will be available October 2016

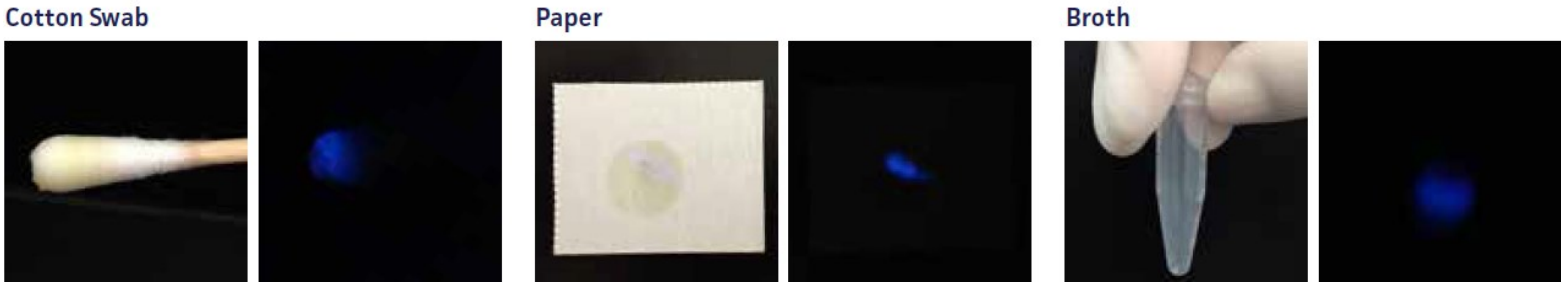
ATCC

Big-Six *Escherichia coli* NanoLuc® Strains Panel

- Comprises six luciferase-labeled Big-Six non-O157 STEC serogroups
- Intensely bright reporter that produces a glow response
- Portable system; does not require additional instrumentation

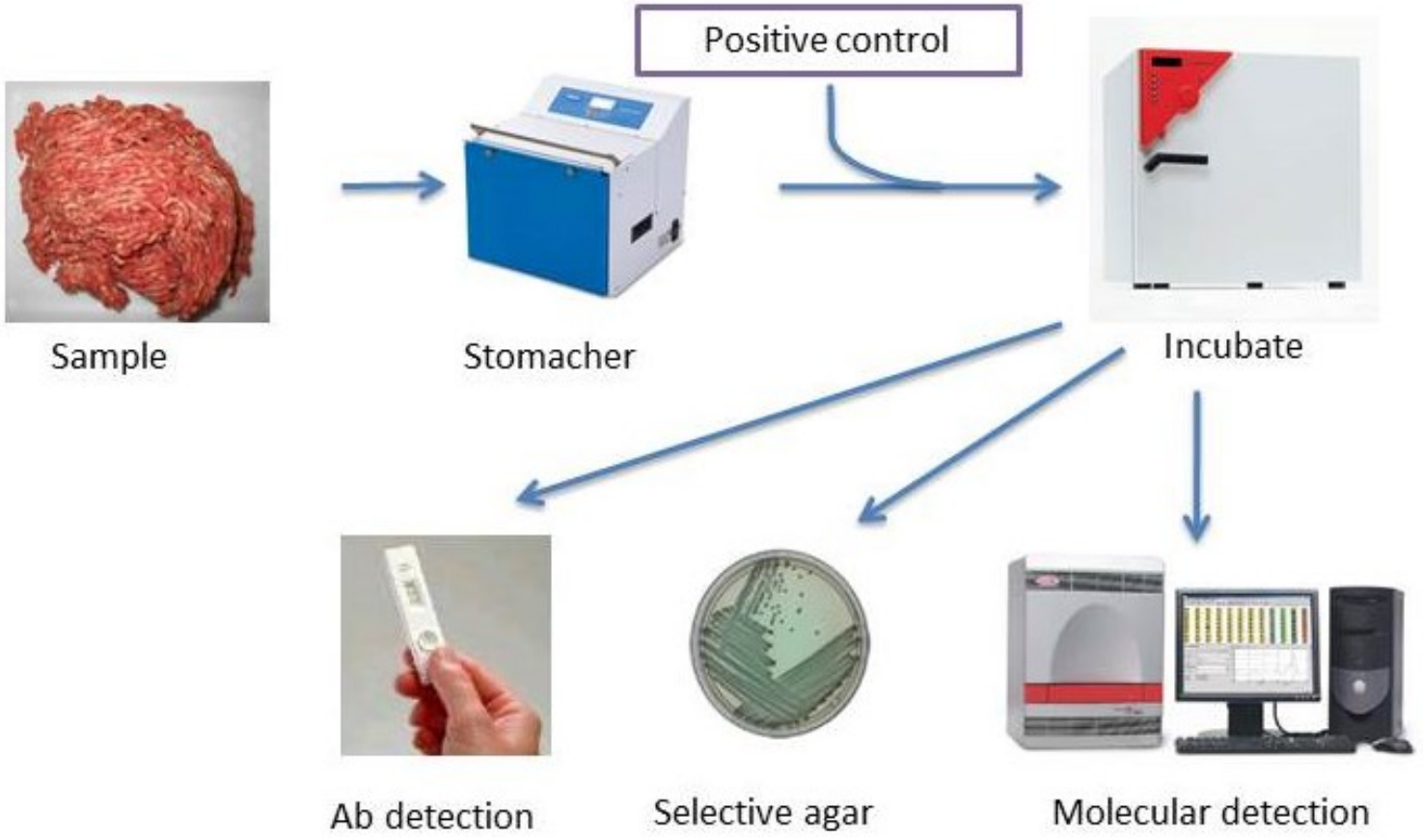
ATCC® MP-21™

ATCC® No.	Serotype	Virulence Genes
BAA-2580-PACK™	O26:H11	*stx*1+/*stx*2+/*eae*+
BAA-2581-PACK™	O45:H2	*stx*1+/*stx*2-/*eae*+
BAA-2582-PACK™	O103:H111	*stx*1+/*stx*2-/*eae*+
BAA-2583-PACK™	O111	*stx*1+/*stx*2-/*eae*+
BAA-2584-PACK™	O121:H19	*stx*1-/*stx*2+/*eae*+
BAA-2585-PACK™	O145	*stx*1-/*stx*2+/*eae*+



Detection of STEC Strains in Meat

Labeled controls rule out cross-contamination of samples with the control strain



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Non-pathogenic *E. coli* Surrogate Indicators Panel

- Non-toxigenic, can be used in BSL 1 environments
- Recommended by the USDA FSIS for safely measuring changes in microbial counts during validation studies
- Validation applications include beef carcass intervention, beef processing, and selected antimicrobial treatments for *E. coli* O157:H7 or *S. enterica*

ATCC® MP-26™

ATCC® No.	Strain	O Type	H Type	Isolation
BAA-1427™	P1	-	4	Cattle hides
BAA-1428™	P3	154	-	Cattle hides
BAA-1429™	P8	-	34	Cattle hides
BAA-1430™	P14	85	34	Cattle hides
BAA-1431™	P68	106	12	Cattle hides

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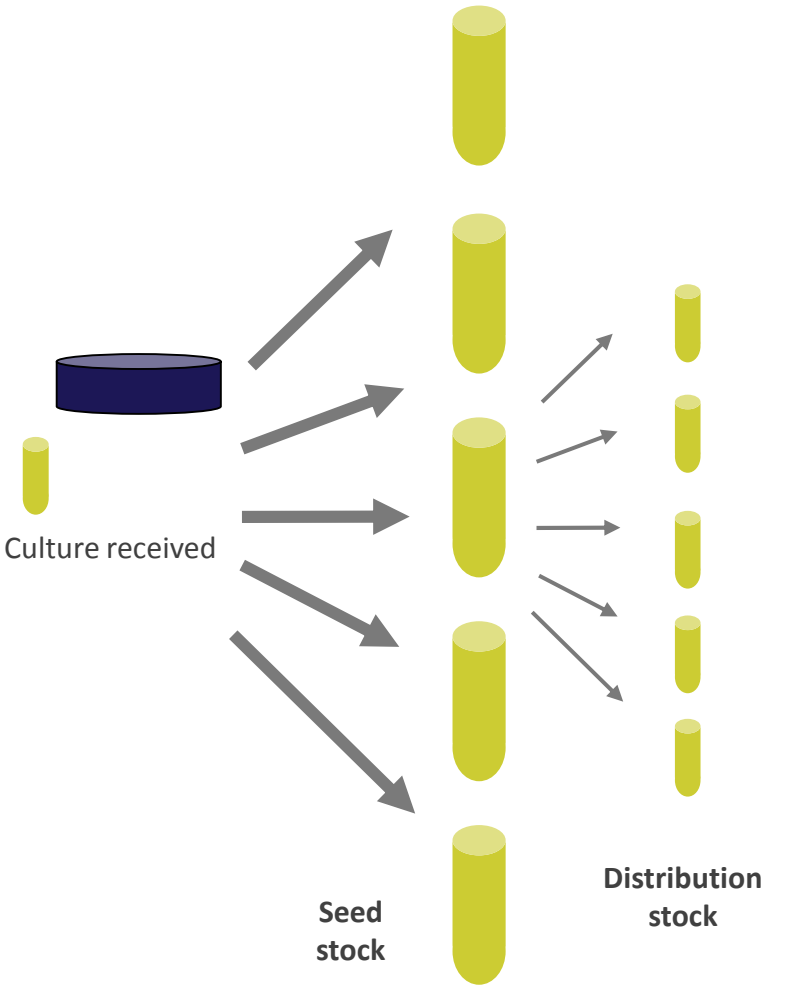
Production



- Preserved cultures remain as close as possible to the original culture
- Seed stock is archived for future replenishment
- Distribution stock are used for distribution
- Authentication compares:
  - Seed, Distribution, Initial culture

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Microbial Strain Authentication

ATCC uses a variety of methods to ensure identity, viability, and purity

- Phenotypic analysis
- Genotypic analysis
- Functional analysis

No single method of identification is sufficient

STEC Authentication at ATCC

- Bacterial identification
  - VITEK® 2, VITEK® MS, API® Strips, 16S rRNA sequencing
- Molecular characterization
  - PCR (*stx*1, *stx*2, and *eaeA*)
- Serotype identification
  - Agglutination
  - Immunoprecipitation assay

Conclusion

- Foodborne illnesses pose a significant health and economic burden associated with severe illness and death, healthcare cost, productivity losses, product recalls, disease surveillance, and outbreak response
- STEC strains cause a number of foodborne illnesses and related hospitalizations
- STEC O157 and the Big-Six serogroups have been frequently associated with foodborne outbreaks
- ATCC offers a number of STEC strains, nucleic acids, microbial panels, and reporter-labeled strains that support food safety testing
- ATCC STEC strains are highly characterized and authenticated, and have been evaluated for serotype and the presence/absence of relevant virulence genes

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Disclaimers

Thank you for joining today!

Register for more ATCC "*Excellence in Research*" webinars, or watch recorded webinars, at www.atcc.org/webinars

September 15, 2016

12:00 PM ET

Brian Cantwell, Ph.D., *Scientist*, ATCC

Finding your perfect match – Evolving technologies for bacterial strain typing

September 22, 2016

12:00 PM ET

Fang Tian, Ph.D., *Lead Scientist*, ATCC

Cindy Long, *Product Line Business Manager*, ATCC

ATCC quantitative nucleic acids – Empowering molecular-based assay development

Please email additional questions to: tech@atcc.org

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