



Testing Methodologies Applicable to Shiga Toxin-Producing Escherichia Coli (STEC)

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Shiga Toxin-Producing Escherichia Coli (STEC)



Enterohemorrhagic E. coli (EHEC)

- Gram-negative rods
- 400+ STEC serotypes (not all pathogenic)

How to define when a given strain is or is not pathogenic?

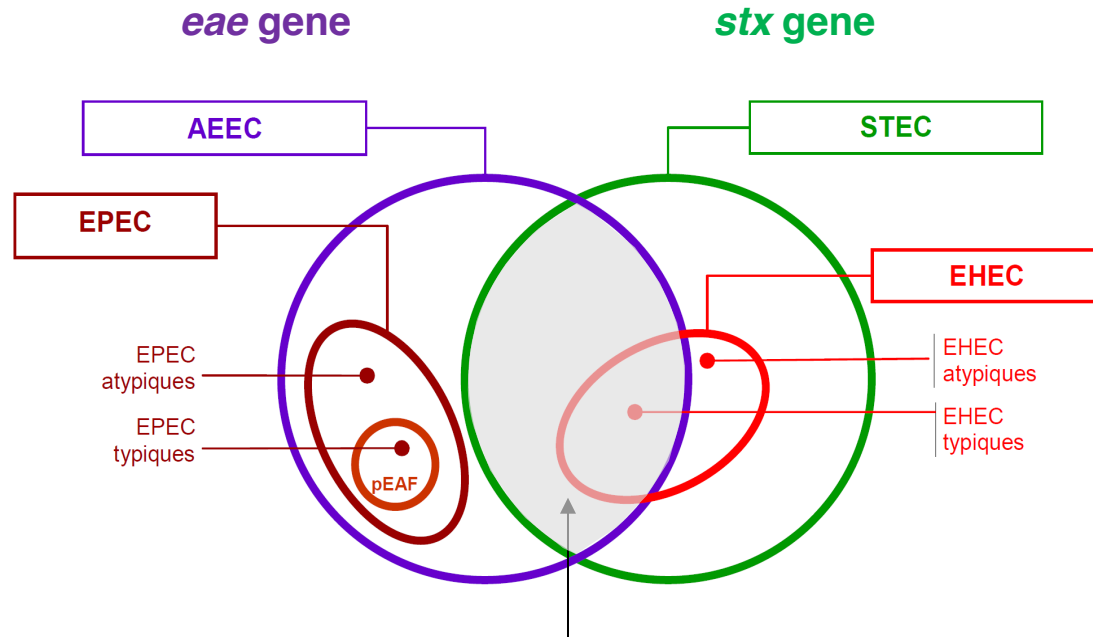
It is very difficult to distinguish STEC from other E. coli as the only phenotypic feature that identifies STEC is the production of the Shiga toxins



Strains with co-occurrence of 2 virulence factors:

Toxin (stx1/2) and Adhesion determinants (eae)

EHEC (Highly Pathogenic STEC)



Potentially pathogenic bacteria *stx* + and *eae* +

Leading cause of EHEC infections in humans → O157:H7 serotype

Most common non-O157 serogroups

→ O26, O103, O91, O146 and O145 (Europe)*

→ O26, O45, O103, O111, O121 and O145 (US)*

Food Matrices at Higher Risks?

Raw or undercooked meat, Raw milk products, Fruit & Vegetables (e.g. sprouts)

Table 4: Number of human cases, hospitalisations and deaths per implicated food vehicle category reported in strong evidence STEC food-borne outbreaks from 2012 to 2017

Implicated food vehicle category (number of reported strong evidence outbreaks; number of reporting countries)	Human cases	Hospitalisations	Deaths
Bovine meat and products thereof (15; 7)	143	76	0
Milk and dairy products^(a) (14; 8)	94	43	2
Tap water, including well water (8; 4)	75	7	0
Vegetables, fruit and products thereof^(b) (7; 3)	575	73	2
Pig meat and products thereof (2; 1)	6	2	0
Other or mixed red meat and products thereof (2; 2)	10	0	0
Sheep meat and products thereof (1; 1)	27	9	0
Unspecified meat ^(c) (1; 1)	2	1	0
Fish and seafood ^(d) (1; 1)	5	0	0
Herbs and spices (1; 1)	50	3	0
Total	987	214	4

(a): Includes all foods categorised under 'milk', 'dairy products (other than cheeses)' and 'cheese' from the Zoonoses Catalogue. In at least six outbreaks, the actual source was raw milk.

(b): Includes all foods categorised under 'fruit, berries and juices and other products thereof' and 'vegetables and juices and other products thereof' under the Zoonoses Catalogue.

(c): All foods categorised under 'meat and meat products' from the Zoonoses Catalogue.

(d): Includes all foods categorised under 'fish and fish products' and 'crustaceans, shellfish, molluscs and products thereof'.



STEC Current Testing Methods

STEC genome plasticity → Emerging strains

→ The detection of the stx gene(s) is the only true discriminant between STEC and other E. coli.

Current testing methods → High percentage of presumptive positive samples that could take up to 5 days for culture confirmation

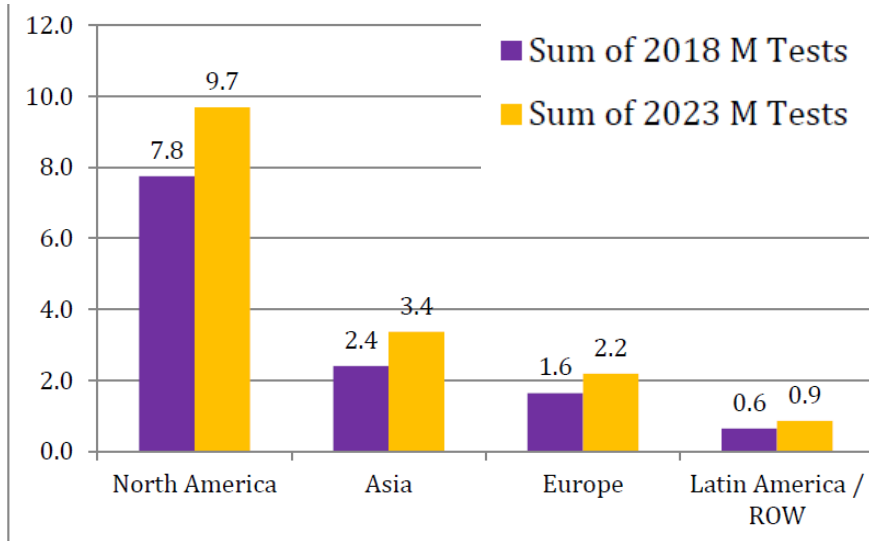
- **Immunological** methods, based on the detection of the Shiga toxins, provide **indirect evidence** of the presence of STEC
- Methods based on **PCR** are the **most appropriate** approaches to detect STEC in complex matrices.
- It is possible to identify accessory virulence features, such as the adhesion determinants (eae gene),
- It gives an indication of the presence of STEC strains considered to be more likely to cause severe disease in humans.

Pathogenic E.coli Confirmation Test Volume Rapid Methods

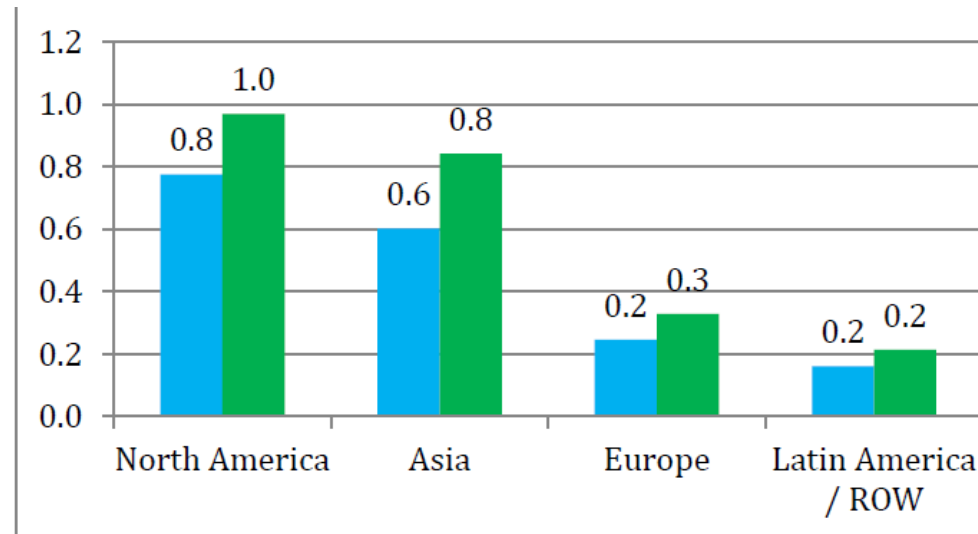
Globally, 41M tests are performed / 15M of these tests are performed using rapid methods (PCR, immunoassay, lateral flow, etc.)

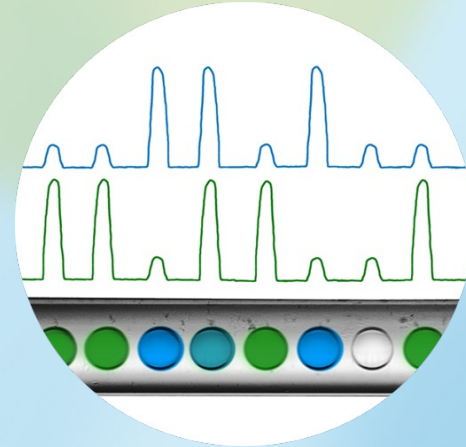
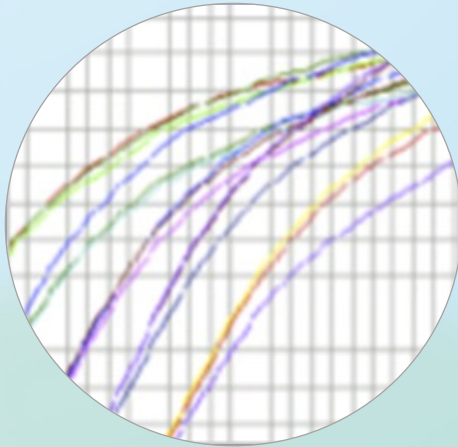
→ Approximately 6-8M tests for confirmation after rapid method screening

Pathogenic *E.coli* Test Volume – Rapid Methods



Pathogen *E.coli* Confirmation Test Volume – Rapid Methods





Bio-Rad solution for STEC detection in food



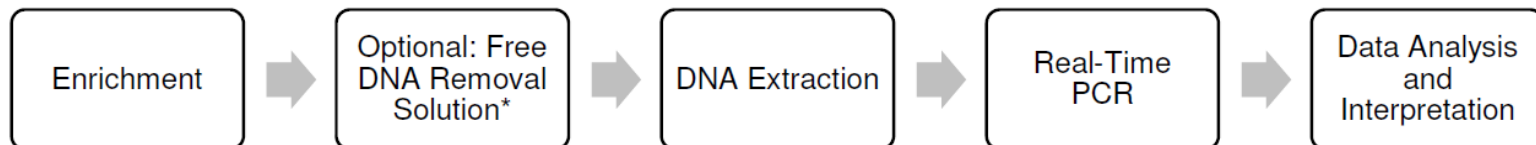
iQ-Check STEC solution by Bio-Rad

iQ-Check STEC VirX / iQ-Check STEC SerO



Description:

- Based on gene amplification and detection by real-time PCR
- Ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific for ***stx1/stx2*** and ***eae* virulence genes**, as well as DNA polymerase and nucleotides
- iQ-Check STEC SerO for **TOP 7 serogroups**
- Included a synthetic DNA internal control and positive and negative control
- Detection and data analysis are optimized for use with CFXOpus DW & CFX Manager IDE

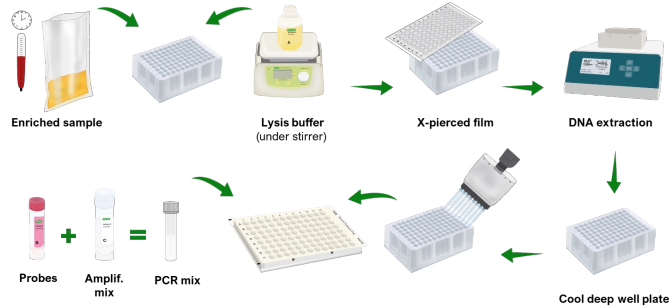


iQ-Check PCR Real Time STEC kit - Workflow



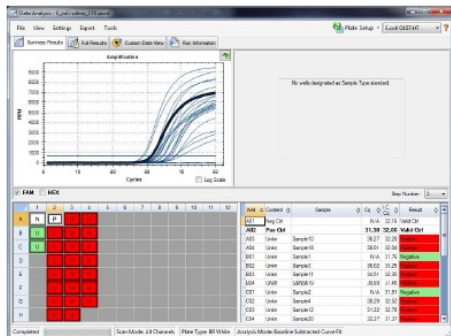
Enrichment:

- Different enrichment proposed for different matrices



Extraction and plate preparation step:

- Flexible solution: from low to high throughput
- One plate & one protocol for different pathogens
- Easy to use and implement in laboratories




Amplification step:

- System easy to install
- User friendly software: only on window to set up and run PCR
- All iQ-Check food test can be run on the same plate

International Validation

- AOAC, Microval, approved
- Screening step for the USDA MLG reference method Chapter 5C.02.



CERTIFICATION

AOAC Research Institute
Performance Tested MethodsSM

Certificate No.
031209

The AOAC Research Institute hereby certifies the method known as:

iQ-Check *Campylobacter* Real-Time PCR

Corporate Location
Bio-Rad Laboratories
2000 Alfred Nobel Drive
Hercules, CA 94547 USA

Manufacturing Location
Bio-Rad Laboratories
925 Alfred Nobel Drive
Hercules, CA 94547 USA

This method has been evaluated in the AOAC Research Institute *Performance Tested MethodsSM* Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested MethodsSM* certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date November 12, 2022
Expiration Date December 31, 2023


2275 Research Blvd., Ste. 300, Rockville, Maryland, USA. Telephone: +1-301-924-7077 Fax: +1-301-924-7089
Internet e-mail: aoacr@aoac.org * World Wide Web Site: <http://www.aoac.org>


iQ-Check STEC VirX Kit

User Guide

Test for the real-time PCR detection of virulence genes in Shiga toxin-producing *Escherichia coli*

Catalog # 579136





MICROVAL[®] nEn

CERTIFICATE OF COMPLIANCE

LRQA

hereby declares that the certification assessment has demonstrated that

iQ-Check STEC VirX Kit


Manufactured by: Bio-Rad Laboratories Supplied by: Bio-Rad


Method Comparison and Inter-laboratory study
Report for the
EN ISO 16140-2:2016 validation study of the
iQ-Check[™] STEC VirX Kit for the real-time PCR detection of
virulence genes in Shiga toxin-producing *Escherichia coli* in
raw meat products (excluding poultry) and raw dairy products

MicroVal study number	2021LR96
Method/Kit names	iQ-Check STEC VirX Kit
Report version	IMCS and ILS Report - Version 1 03 August 2022
Manufacturer	BIO-RAD 3 Boulevard Raymond Poincaré F-92430 Marnes-La-Coquette (France)
MicroVal Expert Laboratory	ADRIA Développement ZA Cneach Owen F-29196 Dumper Cedex (France)

This report consists of 102 pages, including 9 appendices.
Only copies including the entirety of this report are authorised.
Competencies of the laboratory are certified by COFRAC accreditation for the
analyses marked with the symbol*.

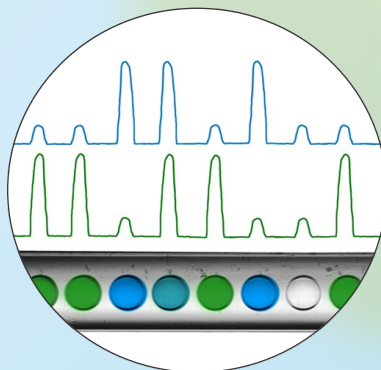
Standardised report -
Qualitative methods





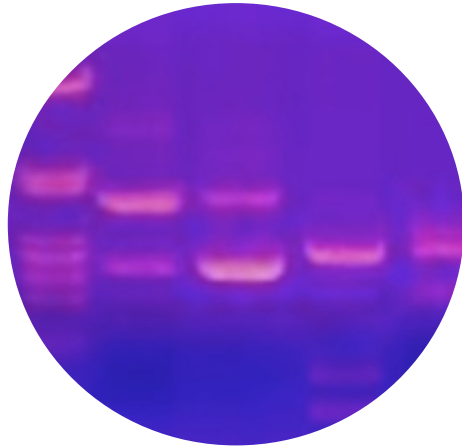
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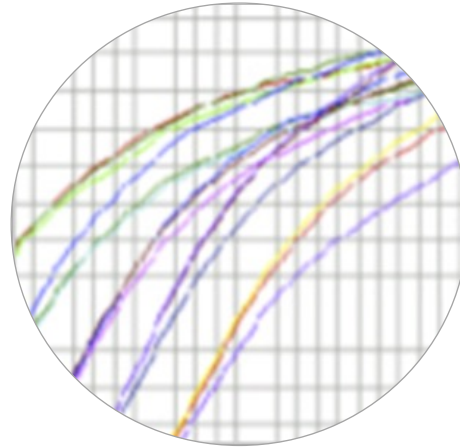
dd-PCR STEC solution by Bio-Rad

1st generation



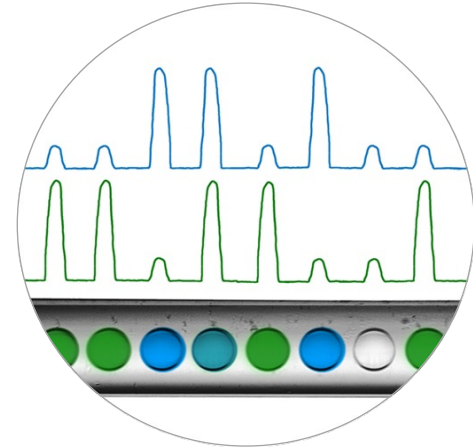
PCR
Qualitative

2nd generation



Real-time PCR
Relative Quantification

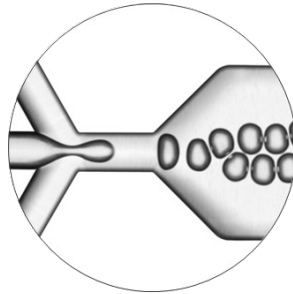
3rd generation



Droplet Digital PCR
Absolute Quantification

Droplet Digital PCR (ddPCR) – Power is Partitioning

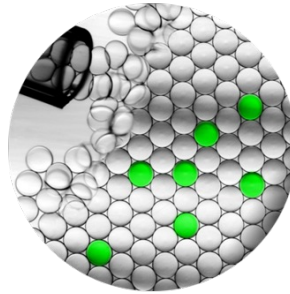
Division of the sample into droplets



Droplet Generator



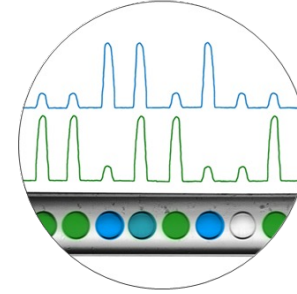
Amplification



CFX96 Touch Thermal cycler



Reading droplets

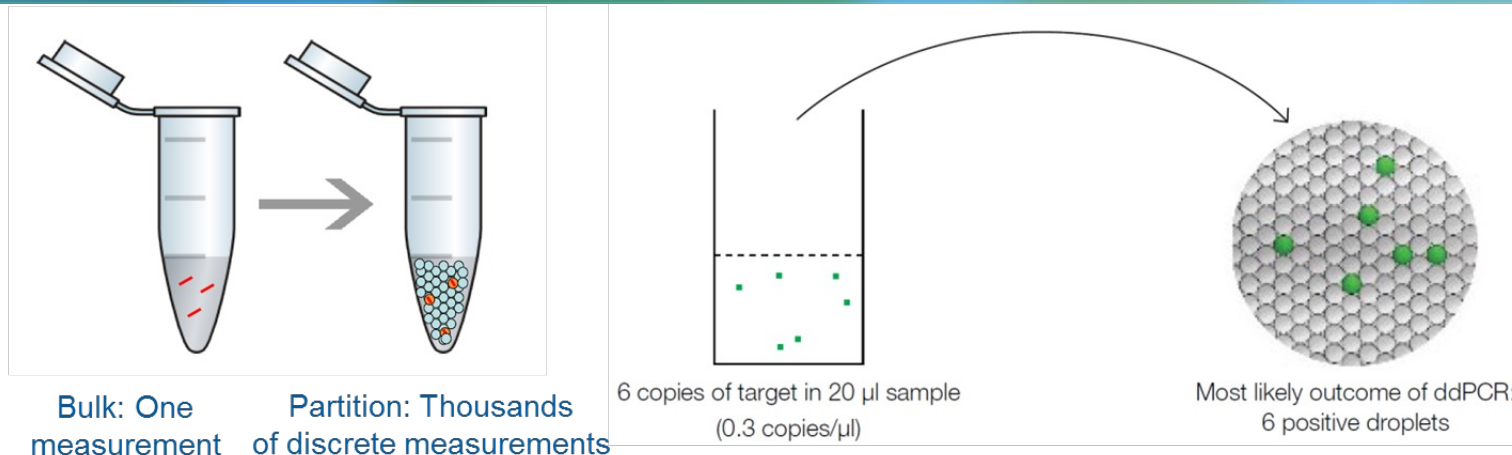


QX200 Droplet Reader



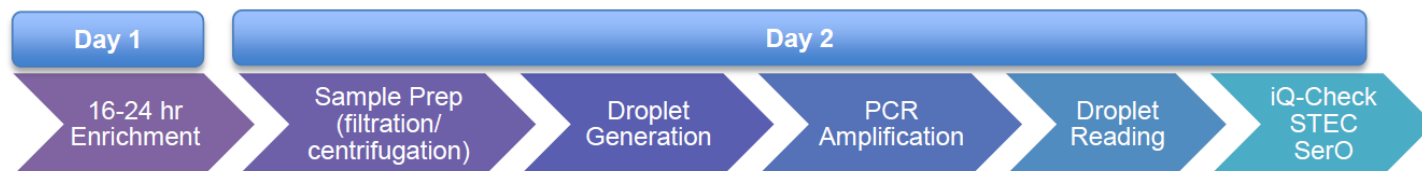
- Partition sample so **individual nucleic acids** are localized to separated containers (nanoliter droplets)
- PCR reactions are **independent, single amplification** events
- The number of negative/positive droplets is **directly related to the initial concentration** in the sample
- **Co-localization of genes** (ex. For STEC *stx* and *eae*)

ddPCR – Propositions

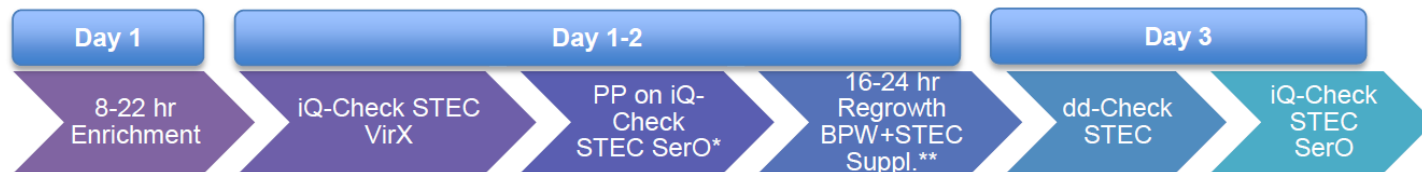


Bacterial isolation allowing colocalisation characterisation

- Screening Method



- Confirmatory Method



Summary

- Flexible solution: from low to high throughput
- Ready-to-use PCR reagents
- Specific for *stx1/stx2* and *eae* virulence genes and TOP 7 serogroup
- Solution adapted for different matrices
- Easy to use and implement in laboratories
- User friendly software and automated interpretation
- One plate & one protocol for different pathogens
- International Validation: AOAC, microval and USDA FSIS



Thank you for your attention!!!