iQ-Check® STEC SerO

Catalog #: 357-8140

User Guide

Test for the real-time PCR detection of 7 major serogroups in Shiga Toxin Producing *E. coli*

BIO-RAD

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I. INTRODUCTION

E. coli bacteria are normal flora in human and animal intestines and are usually harmless. However, some strains can cause diseases to humans. Among them, Shiga toxin-producing Escherichia coli (STEC) are known to be highly pathogenic to humans. They can lead to hemorrhagic colitis and hemolytic uremic syndrome (HUS). STECs are defined by the presence of the *stx1* or *stx2* (Shiga toxin genes) in their genome. The *eae* (intimin) gene is an additional virulence marker.

The most known of these STEC strains is *E. coli* O157:H7 for which there is a "zero tolerance" policy in North America. Such policy will be implemented by FSIS for non-O157 STEC as well, in the meat sector. Outbreaks are commonly associated with the consumption of raw meat, but also with dairy products and more recently with produce. In the context of the ISO or the FSIS guidelines, a sample positive for both *stx1/2* and *eae* targets will have to be tested for the presence of the 4 or 6 major *E. coli* serogroups : O26, O45, O103, O111, O121 and O145.

The iQ-Check STEC SerO kit, based on a multiplex real-time PCR system, allows the detection of these 6 major serogroups, plus *E. coli* O157:H7, in three wells, within few hours after the iQ-Check STEC VirX result.

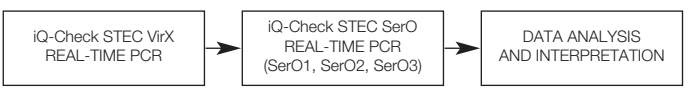
II. THE iQ-Check STEC TECHNOLOGY

PCR is a powerful technique used to generate many copies of target DNA. During the PCR reaction, several cycles of heating and cooling allow DNA denaturation, by heat, followed by primers annealing to the target region. The DNA polymerase then uses these primers and deoxynucleotide triphosphates (dNTPs) to extend the DNA, creating copies of the target DNA. These copies are called amplicons.

In real-time PCR, specific probes are used to detect DNA during the amplification, by hybridizing to the amplicons. These probes are linked to a fluorophore which fluoresces only when hybridized to the target sequence. In the absence of target DNA, no fluorescence will be detected. As the amount of amplicons increases with each round of amplification, fluorescence intensity also increases. During each PCR cycle, at the annealing step, the optical module or detector measures this fluorescence, whereas the associated software plots the fluorescence intensity versus number of cycles.

The iQ-Check STEC SerO kit is a multiplex real-time PCR test. Ready-to-use PCR reagents contain oligonucleotides (primers and fluorescent double strand probes) specific for the 6 STEC major serogroups and *E. coli* O157:H7 as well as DNA polymerase and nucleotides. To cover all theses 7 serogroups, three different multiplex systems, detecting each 2 or 3 targets, are included in the kit. In each system, two fluorophores are linked to probes hybridizing to the target DNA sequences. A synthetic DNA "internal control", included in the reaction mix, is amplified at the same time as the target DNA sequences, and detected by another probe labeled with a third fluorophore. It allows for the validation of any negative result. Detection and data analysis are optimized for use with a Bio-Rad real-time PCR instrument, such as the CFX96™ system.

This method allows a simple determination of the major STEC serogroups in all food products and environmental samples. It includes the following steps:



III. KIT COMPONENTS

The iQ-Check STEC SerO kit contains sufficient reagents to test up to 32 tests per PCR Test Group.

Reference ID	Reagent	Quantity Provided
B1	Fluorescent probes O157:H7and O111	1 tube (0.18 mL)
B2	Fluorescent probes O26, O103 and O145	1 tube (0.18 mL)
B3	Fluorescent probes O45 and O121	1 tube (0.18 mL)
C	Amplification mix	1 tube (1.65 mL)
D	PCR negative control	1 tube (0.5 mL)
E	PCR positive control	1 tube (0.25 mL)

IV. SHELF LIFE AND STORAGE

Once received, the kit must be stored between +2°C and +8°C. Reagents stored between +2°C and +8°C can be used until the expiration date indicated on the reagent tube.

V. MATERIAL REQUIRED BUT NOT SUPPLIED

Equipment

- Vortex apparatus.
- 20 µL, 200 µL and 1000 µL micropipettes.
- Combitip pipettes or equivalent repeat pipettors.
- Bio-Rad real-time PCR system (Industrial Diagnostic CFX96™ real-time PCR detection system, 96 wells).

* Contact Bio-Rad for detailed information on instruments recommended by our technical department.

Note: We recommend using a universal power source (UPS) with the thermal cycler.

Supplies

- PCR plates, tubes, sealing tape and caps, see real-time PCR system user guide for iQ-Check kits.
- Sterile filter tips, adaptable to 20 µL, 200 µL and 1000 µL micropipettes.
- Tips for Combitip pipettes or equivalent repeat pipettors, sterile, individual package.
- 1.5 mL or 2 mL sterile test tubes.
- Powder-free gloves.
- Distilled sterile water.
- Bleach 5%.
- Cleaning agent such as DNA AWAY® or RNase AWAY®.

VI. PRECAUTIONS AND RECOMMENDATIONS

- This test must be performed by adequately trained personnel.
- Cultures must be handled as potentially infectious material and eliminated according to local rules and regulations.
- All potentially infectious material should be autoclaved before disposal.
- The quality of results depends on strict compliance with the following Good Laboratory Practice (for example the EN ISO 7218 standard), especially concerning PCR:

- The laboratory equipment (pipettes, tubes, etc.) must not circulate from one work station to another.
- It is essential to use a positive control and a negative control for each series of amplification reactions.
- Do not use reagents after their expiration date.
- Vortex reagents from the kit before using them to ensure homogeneity.
- Periodically, verify the accuracy and precision of pipettes, as well as correct functioning of the instruments.
- Change gloves often, especially if you suspect they are contaminated.
- Clean work spaces periodically with at least 5% bleach and a decontaminating agent like DNA AWAY.
- Use powder-free gloves and avoid fingerprints and writing on caps of PCR tubes or plates. Both cases will interfere with data acquisition.

• It is strongly advised to follow the general requirements described in the standard EN ISO 22174:2005 "Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food pathogens – General requirements and definitions".

VII. PROTOCOL

It is strongly recommended to read the entire protocol before starting the test.

The test is performed on the same DNA extracted with the iQ-Check STEC VirX kit. The DNA will be tested in three wells.

A. Real-Time PCR

a. Instrument and Software Setup

For instrument and software setup, follow instructions in the real-time PCR system user guide for iQ-Check™ kits.

b. PCR Mix Preparation

1. Prepare 3 PCR mixes according to the PCR mix calculation guide found in Appendix. To find the correct volumes to use, add the total number of samples and controls to be analyzed, and find the corresponding volumes in the table. At least one positive (reagent E) and one negative control (reagent D) must be included in each PCR Test Group for the validation of each PCR mix.
   • PCR Test Group SerO1: mix amplification solution (**reagent C**) and fluorescent probes **B1**.

- PCR Test Group SerO2 : mix amplification solution (**reagent C**) and fluorescent probes **B2**.
- PCR Test Group SerO3 : mix amplification solution (**reagent C**) and fluorescent probes **B3**.

Note: Pay attention to correctly use probes B1 for the Test Group SerO1, the probes B2 for the test Group SerO2, probes B3 for the Test Group SerO3.

2. After preparation, the PCR mixes (reagent B + C) should be used immediately, or are stable for **1 hour maximum at +2°C - 8°C**.

3. Pipette **20 µL** of these PCR mixes in wells according to your plate setup (one mix for one PCR Test Group).

4. Add 5 µL of sample or **reagent D** (negative control) or **reagent E** (positive control) in the wells of each PCR Test Group. Do not vortex the sample before pipetting. Seal hermetically the wells of the plate or strips. It is important to avoid bubbles at the bottom of the wells by pipetting carefully. As an optional step to eliminate any bubbles, centrifuge the sealed PCR plate or the PCR strips (quick spin).

5. Place the plate or strips in the thermal cycler. Be sure to place the plate correctly: A1 well at the upper left corner. Close the reaction module.

c. PCR Start

To start the PCR run, follow instructions in the real-time PCR system user guide for iQ-Check kits.

B. Data Analysis and Results Interpretation

Data can be analyzed directly at the end of the PCR run or at a later time by opening the stored data file. Follow instructions in the corresponding real-time PCR system user guide for iQ-Check kits for opening data files and setting the data analysis parameters.

Interpreting Results

Once the data analysis parameters have been set, results are interpreted by analyzing the Cq values of each sample (the cycle at which the amplification curve crosses the threshold) in each PCR Test Group.

1. Controls

Before interpreting sample results, it is necessary to verify the positive and negative controls of each PCR Test Group.

For the PCR Test Group to be valid, the controls must have the following results, as summarized in the table below, otherwise the PCR reaction needs to be repeated.

	Target 1 (FAM)	**Target 2 (Cy5 channel)**	**Internal control detection (HEX channel)**
Test Group SerO1	O111	O157:H7	
Negative control	Cq = N/A*	Cq = N/A*	26 ≤ Cq ≤ 36
Positive control	26 ≤ Cq ≤ 36	26 ≤ Cq ≤ 36	Not significant
Test Group SerO2	O103 and O145	O26	
Negative control	Cq = N/A*	Cq = N/A*	26 ≤ Cq ≤ 36
Positive control	26 ≤ Cq ≤ 36	26 ≤ Cq ≤ 36	Not significant
Test Group SerO3	O45	O121	
Negative control	Cq = N/A*	Cq = N/A*	26 ≤ Cq ≤ 36
Positive control	26 ≤ Cq ≤ 36	26 ≤ Cq ≤ 36	Not significant

* The software indicates a Cq value of N/A (not applicable) when the fluorescence of a sample does not rise significantly above the background noise, and hence does not cross the threshold.

For each PCR Test Group, if results of negative and positive controls differ from those in the table above, it is necessary to repeat the PCR of the given Test Group.

2. Samples

A sample **positive** for a targeted serogroup must have a Cq value ≥ 10 in FAM or Cy5 channels.

- If the Cq value for FAM or Cy5 is below 10, verify that as raw data the curve is a regular amplification curve (with a flat base line, followed by a rapid exponential increase of fluorescence and then a flattening out). If the curve seems correct, it may be considered a sample positive for the targeted serogroup
- If there is no Cq value (Ct=N/A) for FAM or Cy5, or if the curve is not a typical amplification curve, the internal control for that sample must then be analyzed:

- This sample is considered as a **negative** for the targeted serogroup if there is no Cq value in FAM, or in Cy5, and the internal control has a Cq ≥ 26.
- Should the internal control also not have a Cq value (Cq = N/A), this probably indicates an inhibition of the PCR reaction. The sample needs to be diluted (perform a 1/10 dilution in distilled sterile water, using 10 µL of DNA extract, then use 5 µL of the dilution for amplification), and the PCR repeated.
- Should the Cq value for the internal control be < 26 it is not possible to interpret the result. Verify that the threshold was correctly placed, or that the curve as raw data is a regular amplification curve. If the curve does not have a characteristic shape, it will be necessary to repeat the PCR test.

Interpretation of sample results is summarized in the following table:

Target 1 detection (FAM)	Target 2 detection (Cy5 channel)	Internal control detection (HEX channel)	Interpretation
SerO1 (O111)	**SerO1 (O157:H7)**		
Cq ≥ 10	Cq ≥ 10	Not significant	Positive for O111 and O157:H7
Cq ≥ 10	Cq = N/A	Not significant	Positive for O111
Cq = N/A	Cq ≥ 10	Not significant	Positive for O157:H7
Cq = N/A	Cq = N/A	Cq > 26	Negative
Cq = N/A	Cq = N/A	Cq = N/A	Inhibition
SerO2 (O103 / O145)	**SerO2 (O26)**		
Cq ≥ 10	Cq ≥ 10	Not significant	Positive for O103 and/or O145 and O26
Cq ≥ 10	Cq = N/A	Not significant	Positive for O103 and/or O145
Cq = N/A	Cq ≥ 10	Not significant	Positive for O26
Cq = N/A	Cq = N/A	Cq > 26	Negative
Cq = N/A	Cq = N/A	Cq = N/A	Inhibition
SerO3 (O45)	**SerO3 (O121)**		
Cq ≥ 10	Cq ≥ 10	Not significant	Positive for O45 and O121
Cq ≥ 10	Cq = N/A	Not significant	Positive for O45
Cq = N/A	Cq ≥ 10	Not significant	Positive for O121
Cq = N/A	Cq = N/A	Cq > 26	Negative
Cq = N/A	Cq = N/A	Cq = N/A	Inhibition

VIII. TEST PERFORMANCES AND VALIDATIONS

iQ-Check STEC SerO is validated by AOAC Research Institute under the Performance Tested Method Program for detection of *Escherichia coli* O157:H7, O26, O45, O103, O111, O 121 and O145 in raw beef trim. A positive result with iQ-Check should be considered presumptive and it is recommended it be confirmed by standard reference methods. (See references 1 and 2, section X). Certificate number: 121203.

iQ-Check STEC SerO has been granted a No Objection Letter from the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) for raw beef trim.

IX. REFERENCES

1. Centers for Disease Control and Prevention. *Bacterial Foodborne and Diarrheal Disease National Case Surveillance. Annual Report, 2005*. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2007.

2. EFSA Journal 2009; 7(11):1366 [43 pp.]. Technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food (VTEC surveys on animals and food).

3. Food Safety and Inspection Service, Federal Register, Vol. 76, No. 182, 9 CFR Parts 416, 417, and 430, [Docket No. FSIS–2010–0023], Shiga Toxin-Producing *Escherichia coli* in Certain Raw Beef Products.

4. ISO/TS 13136:2011 (E), Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) belonging to O157, O111, O26, O103 and O145 serogroups - Qualitative Method.

5. United States Department of Agriculture Food Safety And Inspection Service, Office of Public Health Science, MLG 5B.01, Detection and Isolation of non-O157 Shiga-toxin Producing *Escherichia coli* (STEC) from Meat Products.

APPENDIX - PCR Mix Calculation Guide

To find the correct volumes to use when preparing the PCR mixes, add the total number of samples and controls to be analyzed on the PCR Test Group, and find the corresponding volumes of reagents B1, B2 or B3 and reagent C in the table.

Total number of samples & controls	Probes Reagent B1 or B2 or B3 (µL)	Amplification mix Reagent C (µL)
1	5	15
2	11	33
3	16	48
4	22	66
5	27	81
6	32	96
7	38	115
8	43	130
9	49	147
10	54	160
11	59	177
12	65	195
13	70	210
14	76	230
15	81	245
16	86	260
17	92	275
18	97	290
19	103	310
20	108	325
21	113	340
22	119	357
23	124	370
24	130	390
25	135	405
26	140	420
27	146	440
28	151	450
29	157	470
30	162	485
31	167	500
32	173	520

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NOTES

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