iQ-Check® *Campylobacter*

Catalog #: 357-8135

User Guide

Test for the real-time PCR detection of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* in food and environmental samples

BIO-RAD

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I. INTRODUCTION

Campylobacter has emerged as the most frequent cause of gastroenteritis in man. *C. jejuni* and, to a lesser extent, *C. coli*, and *C. lari* are the species most commonly identified as causing infection. The bacteria are commensal in the intestinal tract of cattle, sheep, pigs and birds, and consequently foods of animal origin can become contaminated. The majority of *Campylobacter* infections are acquired through the consumption of contaminated water, raw and inadequately pasteurised milk, and undercooked meats, particularly poultry. The infectious dose of *Campylobacter* is thought to be as low as 500 bacteria.

Conventional bacteriological methods are often long and tedious. In comparison, iQ-Check *Campylobacter* is a simple and rapid qualitative test, allowing the detection of specific DNA sequences unique to *Campylobacter* found in food products. Using real-time polymerase chain reaction (PCR), *Campylobacter* specific DNA sequences are amplified and detected simultaneously by means of fluorescent probes. Up to 94 samples can be processed, with a minimized risk of contamination and an easy to use procedure. The use of this test allows results to be obtained within a few hours following pre-enrichment of a sample.

II. THE iQ-Check *Campylobacter* TECHNOLOGY

The iQ-Check *Campylobacter* kit is a test based on gene amplification and detection by real-time PCR. Ready-to-use PCR reagents contain DNA primers and DNA probes specific for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari*, as well as DNA polymerase and nucleotides. Detection and data analysis are optimized for use with a Bio-Rad real-time PCR system, such as the Chromo4™ System, the CFX96™ System and the MiniOpticon™.

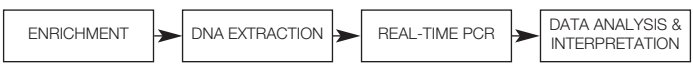
PCR is a powerful technique used to generate many copies of target DNA. During the PCR reaction, several cycles of heating and cooling allow DNA denaturation, by heat, followed by primers binding to the target region. The DNA polymerase then uses these primers and deoxynucleotide triphosphates (dNTPs)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

In real-time PCR, specific fluorescent probes are used to detect the 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 iQ-Check *Campylobacter* kits, FAM is the fluorophore linked to the probes hybridizing to the *Campylobacter* specific DNA sequences. 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 measures this fluorescence, whereas the associated software plots the fluorescence intensity versus number of cycles. This method allows a simple determination of the presence or absence of *Campylobacter jejuni*, *C. lari* or *C. coli* in a sample.

A synthetic DNA "internal control" is included in the reaction mix. This control is amplified with a specific probe at the same time as the *Campylobacter* target DNA sequences, and detected by a second fluorophore. It allows for the validation of any negative result.

This test allows the detection of *Campylobacter* in food products, including carcass rinses, and environmental samples previously enriched by culture (4 hr at 37°C then 20 hr at 41.5°C) in Bolton broth or in feces samples with no enrichment. It includes the following 4 main steps:



III. KIT COMPONENTS

Reference ID	Reagent	Quantity Provided
A	Lysis reagent	1 bottle (20 ml)
B	Fluorescent probes	1 tube (0.55 ml)
C	Amplification mix	1 tube (4.4 ml)
D	PCR negative control	1 tube (0.5 ml)
E	PCR positive control	1 tube (0.25 ml)

The iQ-Check *Campylobacter* kit contains sufficient reagents for 96 tests.

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

- Stomacher®, masticator or equivalent for homogenizing test samples.
- Incubator, capable of maintaining up to 42°C ± 1°C.
- Specific for extraction in 1.5 ml tube
  - Bench top centrifuge (max. 10,000-12,000 g).
  - Dry heat block (100°C ± 5°C).
- Specific for extraction in deepwell plate
  - Centrifuge with rotor for 96-wells plates (max. 2,250 g).
  - Dry heat block (100°C ± 5°C).
  - or agitator-incubator for deepwell plates, such as a "Thermomixer" (Eppendorf).
- Vortex apparatus.
- Magnetic stir plate.
- 20 µl, 200 µl and 1000 µl micropipettes.
- Combitip pipettes or equivalent repeat pipettors.
- Bio-Rad real-time PCR system, e.g. Chromo4, CFX96 or MiniOpticon Systems.

See real-time PCR system user guide for iQ-Check kits.

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

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

Supplies

- Pre-enrichment medium: supplemented or double strength blood free Bolton Broth (see appendix B).
- Buffered peptone water, (E.g. Bio-Rad cat.#:356-4684, 500 g; 355-4179, 225 ml x 6 bottles; 355-5789, 2.3 l x 5 bags; 355-5790, 5 l x 2 bags).
- Stomacher bag with incorporated filter.
- Specific for extraction in tube
  - 1.5 ml conical screwcap sterile tubes (E.g. Bio-Rad cat. #: 224-0110).
- Specific for extraction in deepwell plate
  - 1 ml deepwell plate, Bio-Rad cat.#: 359-0132.
  - Plastic sealing film, Bio-Rad cat.#: 359-0139.
  - Pre-pierced sealing film, such as "X-Pierce™ Sealing Films", Bio-Rad cat #: 360-0040, for North America only; cat #: 359-3977, x100.
- PCR plates, tubes, sealing tape and caps, see real-time PCR system user guide for iQ-Check kits.

- 1 ml and 10 ml pipettes.
- Sterile filter tips, adaptable to 20 µl, 200 µl and 1000 µl micropipettes.
- Tips for Combitip pipettes or equivalent repeat pipettors, sterile, individual package.
- 2 ml and 5 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 FOR BEST RESULTS

- This test must be performed by adequately trained personnel.
- Food samples and enrichment cultures must be handled and eliminated as potentially infectious material.
- 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.
  - Do not use reagents after their expiration date.
  - It is essential to use a positive control and a negative control for each series of amplification reactions.
  - 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 tubes. 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 all the protocol before starting the test.
Enrichment media must be at the appropriate incubation temperature before use.

A. Sample Enrichment or Sample Preparation

1 - Food sample enrichment

Enrichment media must be at the appropriate incubation temperature before use.

- Homogenize 25 g of sample in 225 ml of supplemented Bolton broth*, in a stomacher bag with incorporated filter.
- Incubate without shaking for **4 hours at 37°C** and transfer to **41.5°C ± 1°C for 20 hours** under microaerobic conditions.

* see appendix B for preparation of supplemented Bolton broth.

2 - Carcass rinse sample enrichment

- Rinse carcass in 400 ml buffered peptone water for one minute.
- Remove 30 ml and add to 30 ml double strength blood free Bolton enrichment broth (2x BF-BEB).
- Mix gently and incubate for 24 hours without shaking at 42 ± 1°C under microaerobic conditions.

3 - Carcass sponge sample enrichment

- After sponging the carcass, add 25 ml 2x BF-BEB to the sponge.
- Incubate for 24 hours without shaking at 42 ± 1°C under microaerobic conditions.

4 - Feces sample preparation

- Homogenize a w/v ratio of feces sample in supplemented Bolton broth* (for example a 10 g sample is added to 10 ml of Bolton broth), in a stomacher bag with incorporated filter.
- Allow to decant at room temperature for 10 minutes.

* see appendix B for preparation of supplemented Bolton broth.

B. DNA Extraction

General recommendations

- Before starting the test, turn on the heat block and set it to 95°C-100°C.
- In general, avoid shaking the enrichment bag and collecting large fragments of food debris. For food samples with a fatty supernatant, collect the sample just below this layer.
- Open tubes and wells carefully to avoid any possible cross-contaminations.
- Pipette the lysis reagent while it is stirring at medium speed on a magnetic stir plate, in order to keep in suspension and collect the resin.

1. Aliquote **100 µl of homogenized lysis reagent** to tubes or wells of a deepwell plate.

2. Add 100 µl of the decanted enriched sample.
   Mix by pipetting up and down and close the tube with caps or seal the deepwell plate with pre-pierced sealing film.

3. Incubate in the heat block at 95°C-100°C for 10 to 15 minutes or in the plate agitator-incubator for 15-20 minutes at 1,3000 rpm. A heating block is preferred for DNA extraction of feces samples.

4. Vortex at high speed (for tubes).

5. If using a deep-well plate, allow to cool down at room temperature.

6. Centrifuge at 10,000-12,000 g at least 2 minutes for tubes, centrifugation is not needed for deepwell plates.

The remaining supernatant can be stored for up to 1 year at -20°C. Before reusing the supernatant, always allow it to thaw, then centrifuge tubes at 10,000-12,000 g. for 5 minutes.

If you choose to temporarily stop the procedure, this is the recommended stopping point.

C. Real-time PCR

1. Instrument and software setup

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

2. PCR mix preparation

2.1 Prepare a PCR mix containing the amplification solution (reagent **C**) and the fluorescent probes (reagent **B**) according to the PCR mix calculation guide found in Appendix A. 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 and one negative control must be included in each PCR run.

2.2 After preparation, the PCR mix (reagent B + C) must be used immediately or is stable for **1 hour maximum at 2°C-8°C**.

2.3 Pipette **45 µl** of this PCR mix in each well according to your plate setup.

2.4 Add **5 µl** of sample or reagent **D** (negative control) or reagent **E** (positive control). 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).

2.5 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.

3. PCR Start

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

D. Data Analysis

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.

1. Interpreting Results

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

It is also possible to use the software iQ-Check Analysis (cat. # 359-3135) for an automated interpretation and report generation of all data (see iQ-Check Analysis user guide). Complete automated analysis are available with the Opticon Monitor™ Software for the Chromo4 system (see Chromo4 user guide for iQ-Check kits) or with the CFX Manager™ software Industrial Diagnostic Edition for the CFX96 and MiniOpticon systems (see CFX Manager IDE user guide).

1.1 Controls:

Before interpreting sample results, it is necessary to verify the positive and negative controls.

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

	***Campylobacter* detection (FAM)**	**Internal Control detection**
Negative control	Ct = N/A*	28 ≤ Ct ≤ 40
Positive control	26 ≤ Ct ≤ 36	Not significant

* The software indicates a Ct 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.

1.2 Samples:

A **positive** *Campylobacter* sample must have a Ct value ≥ 10 for the FAM fluorophore.

If the Ct value is below 10, verify that as raw data the curve is a regular amplification curve (with a flat base line, followed by a rapid increase of fluorescence and then a flattening out). If the curve seems correct, it may be considered a positive *Campylobacter* sample.

If there is no Ct value (Ct=N/A) for FAM, or 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** *Campylobacter* sample if there is no Ct value in FAM, and the internal control has a Ct ≥ 28.
- Should the internal control also not have a Ct value (Ct = 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 (see section VII. C, Real-time PCR).
- Should the Ct value for the internal control be < 28 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:

***Campylobacter* detection (FAM)**	**Internal control detection**	**Interpretation**
Ct ≥ 10	Not significant	Positive
Ct = N/A	Ct ≥ 28	Negative
Ct = N/A	Ct = N/A	Inhibition**

** When both *Campylobacter* and internal control detection give a Ct value = N/A, the sample must be tested again but diluted (1/10).

VIII. CONFIRMATION OF POSITIVE RESULTS

In the context of AOAC-RI validation, a positive iQ-Check *Campylobacter* result is considered presumptive positive and it is recommended it be confirmed according to the ISO 10272-1 Reference Method for raw ground chicken samples, or according to the USDA MLG standard method for the carcass rinses (available online at http://www.fsis.usda.gov/PDF/MLG_41_01.pdf).

IX. TEST PERFORMANCES AND VALIDATIONS

AOAC-RI VALIDATION

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 031209

iQ-Check *Campylobacter* is validated by AOAC-Research Institute under the Performance Tested Method Program for detection of *Campylobacter jejuni*, *C. coli* and *C. lari* in raw ground chicken, chicken carcass rinses and turkey sponges. 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: 031209.

X. REFERENCES

1 - Standard EN ISO 10272-1. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Campylobacter* spp. Part 1. Detection method. 2006.

2 - United States Department of Agriculture, Food Safety and Inspection Service. Microbiology Laboratory Guidebook – Chapter 41.01.Isolation, Identification and Enumeration of Campylobacter jejuni/coli/lari from Poultry Rinse and Sponge Samples. August 1, 2011 On line at http://www.fsis.usda.gov/PDF/MLG_41_01.pdf

APPENDIX A - PCR Mix Calculation Guide

To find the correct volumes to use when preparing the PCR mix, add the total number of samples and controls to be analyzed, and find the corresponding volumes of reagent B and reagent C in the table.

Total number of samples & controls	Probes Reagent B (µl)	Amplification mix Reagent C (µl)
1	5	40
2	11	86
3	16	130
4	22	173
5	27	216
6	32	259
7	38	302
8	43	346
9	49	389
10	54	432
11	59	475
12	65	518
13	70	562
14	76	605
15	81	648
16	86	691
17	92	734
18	97	778
19	103	821
20	108	864
21	113	907
22	119	950
23	124	994
24	130	1000
25	135	1100
26	140	1100
27	146	1200
28	151	1200
29	157	1300
30	162	1300
31	167	1300
32	173	1400
33	178	1400
34	184	1500
35	189	1500
36	194	1600
37	200	1600
38	205	1600
39	211	1700
40	216	1700
41	221	1800
42	227	1800
43	232	1900
44	238	1900
45	243	1900
46	248	2000
47	254	2000
48	259	2100

Total number of samples & controls	Probes Reagent B (µl)	Amplification mix Reagent C (µl)
49	265	2100
50	270	2200
51	275	2200
52	281	2200
53	286	2300
54	292	2300
55	297	2400
56	302	2400
57	308	2500
58	313	2500
59	319	2500
60	324	2600
61	329	2600
62	335	2700
63	340	2700
64	346	2800
65	351	2800
66	356	2900
67	362	2900
68	367	2900
69	373	3000
70	378	3000
71	383	3100
72	389	3100
73	394	3200
74	400	3200
75	405	3200
76	410	3300
77	416	3300
78	421	3400
79	427	3400
80	432	3500
81	437	3500
82	443	3500
83	448	3600
84	454	3600
85	459	3700
86	464	3700
87	470	3800
88	475	3800
89	481	3800
90	486	3900
91	491	3900
92	497	4000
93	502	4000
94	508	4100
95	513	4100
96	518	4100

APPENDIX B - Preparation of supplemented Bolton broth

- Suspend 27.6 g Bolton broth in 1 L of purified water.
- Autoclave for 15 min. at 121°C.
- Cool to 45-50°C.
- Aseptically add 50 ml saponin lysed defibrinated horse blood and the contents of 2 vials of Bolton Selective Supplement CVTC X131 (reconstituted with 5 ml of 50% ethanol).
- Mix well and dispense into sterile containers.

Example of references:

Bolton broth: IDG, Lab 135A
Defibrinated lysed horse blood: OXOID SR0048C
Supplement CVTC: IDG X131

Notice to purchaser: limited license

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,079,352, 5,789,224, 5,618,711, 6,127,155, 5,677,152 (claims 1 to 23 only) and 5,773,258 (claims 1 and 6 only), and claims outside the US corresponding to US Patent No. 4,889,818. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product solely in Food testing, Environmental testing, and Industrial microbiology, including reporting results of purchaser's activities for a fee or other commercial consideration, and also for the purchaser's own research. No right under any patent claim (such as the 5' Nuclease Process claims in US Patent Nos. 5,210,015 and 5,487,972) is conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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