EZ-Check *Listeria monocytogenes* Kit

User Guide

Test for the real-time PCR detection of *Listeria monocytogenes* in food and environmental samples

Catalog #12018858

For In-Vitro Laboratory Use Only

Table of Contents

Section 1
Revision History

Release date	Version number	Change
February 2026	Bulletin 5151 Ver A	New document

Section 2
Introduction

Conventional bacteriological methods are often long and tedious. In comparison, EZ-Check *Listeria monocytogenes* is a simple and rapid qualitative test, allowing the detection of specific DNA sequences unique to *Listeria monocytogenes* found in environmental samples and food products. Using real-time polymerase chain reaction (PCR), *Listeria* spp.-specific DNA sequences are amplified and detected simultaneously by means of fluorescent probes. 96 tests can be processed at one time, with a minimized risk of contamination and an easy-to-use procedure. The intended users of this kit are trained laboratory personnel who are performing tests to detect *L. monocytogenes*. The use of this test allows results to be obtained within a few hours following enrichment of a sample.

Section 3
The EZ-Check *Listeria monocytogenes* Technology

The EZ-Check *Listeria monocytogenes* Kit must be used with EZ-Check Lysis Kit (catalog #12018075).

The EZ-Check *Listeria monocytogenes* Kit is based on gene amplification and detection by real-time PCR.
The kit's ready-to-use lyophilized PCR reagents contain oligonucleotides (primers and probes) specific for *Listeria monocytogenes*, as well as DNA polymerase and nucleotides. Detection and data analysis are optimized for use with a Bio-Rad real-time PCR instrument, such as the CFX Opus 96, CFX Opus Deepwell, CFX96 Touch Deep Well and CFX Duet Real Time PCR Detection systems.

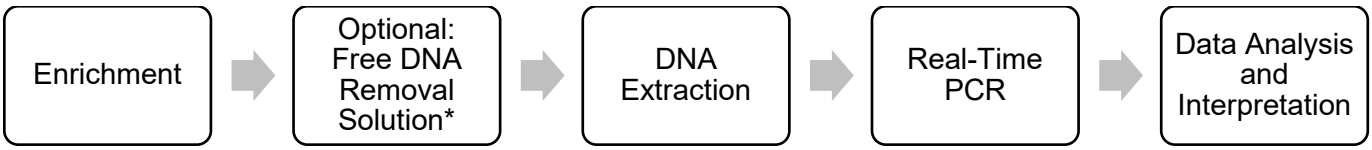
PCR is a powerful technique used to generate many copies of target DNA. During the PCR reaction, several cycles of heating and cooling allow DNA denaturation by heat followed by primers annealing to the target region. The DNA polymerase then uses these primers and deoxynucleotide triphosphates (dNTPs) 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 the DNA during the amplification step by hybridizing to the amplicons. These probes are linked to a fluorophore that fluoresces only when hybridized to the target sequence. FAM is the fluorophore linked to the probe hybridizing to the *Listeria monocytogenes* specific DNA sequence. In the absence of target DNA, no fluorescence will be detected. As the number of amplicons increases with each round of

amplification, fluorescence intensity also increases. The optical module measures this fluorescence at the annealing step during each PCR cycle while the associated software plots the fluorescence intensity versus number of cycles.

A synthetic DNA internal control is included in the reaction mix to validate any possible negative results. This control is amplified with a specific probe at the same time as the *Listeria monocytogenes* target DNA sequence and is detected by a second fluorophore.

This test allows the qualitative detection of *Listeria monocytogenes* in select food products and environmental samples after an enrichment step. It includes the following five main steps:



* Please refer to the user guide of the Free DNA Removal Solution (document #10000253856) for conditions of use.

Section 4
Kit Components

The EZ-Check *Listeria monocytogenes* Kit contains sufficient reagents for 96 tests.

Reagent ID	Item	Description	Quantity
RPS	EZ-Check *Listeria monocytogenes* ReadyPCR Strips*	Ready-to-use PCR reagent	12 strips of 8 wells
CAP	EZ-Check Optical Caps	Optical caps	12 strips of 8 caps
NC	EZ-Check Negative Control	PCR negative control	1 tube, 1.0 mL
PC	EZ-Check *Listeria monocytogenes* Positive Control	PCR positive control	1 tube, 0.6 mL

* EZ-Check *Listeria monocytogenes* ReadyPCR strips can be identified by dark blue tabs on each side of the PCR strips. *Listeria monocytogenes* dark blue color indicators are also visible on the kit box packaging and the CFX Maestro IDE software.

Section 5
Shelf Life and Storage

- The kit is shipped cold
- Store kits at 2–8°C
- Do not freeze
- Do not use reagents beyond the expiration date printed on the labels
- After first opening, ReadyPCR Strips sealed in the pouch with desiccant and stored at 2-8°C remain usable for up to 3 months

Section 6
Materials Required but Not Supplied

Equipment and Software

- Specific for DNA extraction: Heater-shaker* capable of maintaining 95–100°C and/or 37 ± 2°C, with a mixing speed of at least 1,300 rpm (for example, catalog #3594995 and catalog #12022466)
- Bio-Rad real-time PCR system* for example, the CFX Opus 96 (catalog #17007991) or CFX Opus Deepwell (catalog #17007992) or CFX Duet (catalog #17010275) Real-Time PCR Systems
- CFX Maestro Software, Industrial Diagnostic Edition, version 4.0 (catalog #3593893)
- iQ-Check Prep System (optional)* (catalog #3594911)
- Adjustable multichannel pipette 20-200 µL (catalog #17010702) and/or single pipette 20-200 µL (catalog #17010259)
- EZ-Check Lysis Rack (catalog #12019781)
- EZ-Check PCR Frame (catalog #12019771)
- Lab paddle blender for homogenizing test samples
- Incubator for sample microbiological enrichment

Note: We recommend using an uninterrupted power supply (UPS) with the thermal cycler and iQ-Check Prep Systems.

*Contact Bio-Rad Technical Support for information on recommended instruments.

Supplies

- EZ-Check Lysis Kit (catalog #12018075, 96 tests)
- Enrichment medium: *Listeria* Special Broth II (LSB II)
  - catalog #12017463, 225 mL x 6 bottles
  - catalog #12017388, dehydrated, 500 g
  - catalog #12017378, dehydrated, 5 kg
- RAPID'*L.mono* Agar (catalog #3563694, 90 mm x 20 dishes; 3555294, kit for 190 mL agar plus 2 supplements; 3564293, dehydrated, 500 g; 3564294, supplement 1; 3564746, supplement 2)
- RAPID'*Listeria* spp. Agar (catalog #3564744, dehydrated, 500 g; 3564745, supplement 1; 3564746, supplement 2)
- AL (Agar *Listeria* according to Ottaviani and Agosti) Agar (catalog #3563695, 90 mm x 20 dishes; 3564046, dehydrated, 500 g; 3564041, supplement 1; 3564042, supplement 2)
- Free DNA Removal Solution (catalog #3594970)
- iQ-Check Purification Reagent (catalog #12012383)
- Deep well microplate (catalog #3594900)
- Compatible sterile filter pipette tips adaptable to 5-50 µL or 20-200 µL pipettes (for example, catalog #17010688)
- EZ-Check Lysis Storage Caps (catalog #12022880)
- PCR capping tool (catalog #ECT2000)
- Filter bags
- Distilled sterile water
- Bleach, 5% and surface decontaminant
- Powder-free gloves

Supplies for iQ-Check Prep System

- 2 mL tubes for Free DNA Removal Solution (catalog #17010255)
- Deep well microplates (catalog #3594900)
- Conductive filter tips (catalog #12022468, 300 µL x 5760)
- 60 mL dilution container (catalog #12014473)

Section 7
Safety Precautions and Recommendations for Best Results

- Disclaimer: For our standard terms and conditions of sale, including any disclaimers, please visit https://www.bio-rad.com/en-us/terms-conditions

- This User Guide and the ones of associated reagents, instruments and software, must be read entirely prior to using the method

- This test must be performed by trained personnel

- The assay components are non-hazardous. All materials should be used following good laboratory practices. Materials and reagents must be discarded according to appropriate waste procedures used in the laboratory, and in accordance with local, state, and federal regulations. *L. monocytogenes* are Bio-Safety Level 2 organisms. All samples should be handled as potentially infectious and proper personal protective equipment should be utilized while handling samples, enrichments, or assay components. Pregnant women and immune-compromised individuals are advised to not carry out this test. Because this procedure detects pathogenic microorganisms and/or their metabolic products, care should be taken to avoid ingestion or inhalation of potentially infectious aerosols or contact with the skin. Laboratory personnel should follow appropriate laboratory safety precautions and have access to associated resources

- The quality of results depends on strict compliance with Good Laboratory Practices (for example, the EN ISO 7218 standard), especially concerning microbiology and PCR:
    - Never circulate laboratory equipment (pipets, tubes, etc.) from one workstation to another
    - Always use a positive control and a negative control for each series of amplification reactions
    - Do not use reagents after their expiration date
    - Vortex and spin down positive and negative controls from the kit before using them to ensure homogeneity
    - Periodically verify the accuracy and precision of pipets, as well as correct functioning of the instruments
    - Change gloves often, especially if you suspect they are contaminated
    - Clean workspaces periodically with 5% bleach and other decontaminating agents
    - Use powder-free gloves and avoid fingerprints and writing on tube caps. Both will interfere with data acquisition
- It is strongly advised to follow the general requirements described in the standard EN ISO 22174 (Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food pathogens — General requirements and definitions
    - Consult the Safety Data Sheet for additional information and local regulations for disposal
- After unsealing the ReadyPCR strips, ensure that the strips are placed in the CFX instrument within 4 hr. Once the lyophilized PCR reagent is hydrated, the resulting PCR mix is stable at 18-25°C
- When running the CFX Real time PCR system, always balance the tube strips to ensure the heated lid applies even pressure across the block.
- It is always recommended to perform fit-for-purpose or matrix verification studies prior to using this method
- Handle EZ-Check reagents as potentially biohazardous material under at least Biosafety Level 2 containment

Section 8
Protocol

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

A. Sample Enrichment

Equilibrate enrichment broths to room temperature (20–25ºC) before use.

Thaw frozen samples completely before use.

The use of an enrichment bag with incorporated filter is highly recommended.

The following table outlines the different protocols that can be used, depending on the application and the scope of the validation.

Enriched samples can be stored at 2-8°C for 72 hr before performing the EZ-Check lysis step.

AOAC		
Scope (Matrices) [1,2]	**Sample Preparation**	**Enrichment**
Raw milk, raw milk cheese, frozen chicken nuggets, deli ham, salami, deli turkey, smoked salmon, frozen cooked shrimp, fresh cut cantaloupe, frozen blanched green peas, egg salad, hummus, deli salad, pasteurized whole liquid egg, process water	• Homogenize 25 g or mL of sample for 1 min in 225 mL LSB II	Incubate 18–26 hr at 37 ± 1°C
Mexican cheese, ice cream, cheddar cheese, beef hot dogs, turkey hot dogs, raw fermented sausage, bagged salad, frozen blanched vegetable blend	• Homogenize 125 g of sample for 1 min in 1,125 mL LSB II	Incubate 18–26 hr at 37 ± 1°C
Stainless steel, sealed concrete, rubber environmental surfaces	• Moisten swabs (1 mL) and sponges (10 mL) with a neutralizing broth that does not contain aryl sulfonate complex • For surfaces being analyzed with swabs, sample a 1 x 1″ (2.54 x 2.54 cm) area • For surfaces being analyzed with sponges, sample a 4 x 4″ (10.16 x 10.16 cm) area • Add enough LSB II to cover the swab (10 mL) or sponge (60 mL) • Homogenize swab samples in the tube by shaking vigorously or vortexing for 30 sec • Homogenize sponge samples prior incubation by stomaching or hand massaging for 30 sec	Incubate 18–24 hr at 37 ± 1°C

[1] Validation includes direct streak to RAPID'*L. mono*, RAPID'*Listeria* spp., or AL Agar plates.
[2] If the sample exhibit PCR inhibition, proceed with a retest or a 1 in 10 dilution in sterile distilled water. If PCR inhibition is still present, use the iQ-Check Purification Reagent Treatment or a 1 in 50 dilution sterile distilled water (additional verification testing required).

B. Free DNA Removal Treatment (optional step)

The Free DNA Removal Solution provides an ideal way to remove free DNA.

1. Dispense 10 µL of activated* Free DNA Removal Solution into as many wells of an empty deep well microplate as there are samples to be analyzed.

2. Add 100 µL of decanted enriched sample per well.

   Note: To avoid matrix debris or fat layers, pipette just below the fat layer and above any debris at the bottom of the tube.

3. Incubate the deep well microplate in the heater-shaker WITHOUT shaking at 37 ± 2°C for 15-30 min

4. Proceed to the EZ-Check Lysis protocol using 25 µL of treated enriched sample with the EZ-Check Lysis Kit.

* For more information, refer to the Free DNA Removal User Guide (document #100000253856).

C. iQ-Check Prep System (optional)

The iQ-Check Prep System is used to automate the EZ-Check workflow including optional Free DNA Removal Treatment, DNA extraction and real-time PCR plate setup.

1. Open CFX Maestro IDE software and select the "EZ Lis. spp" assay protocol from the menu.

2. Assign sample types to each well of the plate view by selecting Unknown, Positive Control, or Negative Control.

3. Configure the required options for each well (ex: FDRS, dilution).

4. Click "Send to the iQ-Check Prep" to export the worklist.

5. Transfer a minimum of 500 µL of enriched sample into the appropriate well of a deep well plate or into tubes according to the plate layout.

6. Open the iQ-Check Prep Software on the instrument computer.

7. Load the worklist transferred in step 4.

8. Follow instructions to manually load consumables (tips, plates), reagents and prepared samples.

9. Start the run.

10. After the run is complete, remove the PCR plate and hermetically seal the strips with the EZ-Check Optical Caps.

11. Place the EZ-Check PCR Frame in the thermal cycler. Be sure to place the plate with well A1 in the upper left corner and close the instrument lid.

For more information, refer to the CFX Maestro Industrial Diagnostic Edition and iQ-Check Prep System User Guides.

D. DNA Extraction

General recommendations:

Use a dedicated area, equipment and consumables to perform this step.

Turn on the heater-shaker to preheat before starting the test. Set it to 95–100°C.

In general, avoid shaking the enrichment bag and collecting large fragments of food debris. For food samples with a fatty layer, collect the sample just below this layer.

For best results, allow the lysis strips to cool undisturbed before pipetting the DNA extract.

EZ-Check Lysis Protocol

1. Place the appropriate number of EZ-Check Lysis tubes (8 tubes/strip) in the EZ-Check Lysis Rack. Ensure the tubes are fully inserted in the EZ-Check Lysis Rack.

2. Gently tap the EZ-Check Lysis Rack on the bench to draw down the lysis reagent.

3. Peel off carefully the foil seal from the lysis tubes.

Note: The EZ-Check Lysis reagent may show droplets on the inner side of the well. This has no effect on extraction quality.

4. Add 25 µL of enriched sample to the lysis tubes. If Free DNA Removal Solution is used, add 25 µL of treated enriched samples to the lysis tubes.

5. Incubate lysis strips in the heater-shaker under agitation at 1,300 rpm for 15–20 min at 95–100°C.

6. Cool the lysis tubes by placing the EZ-Check Lysis Rack on the bench at room temperature undisturbed for 10-30 min.

At this step the protocol can be paused and resumed later.

- The DNA extract can be stored sealed for 7 days at 2-8°C directly in the EZ-Check Lysis tubes using the EZ-Check Lysis Storage Caps
- For frozen storage (-25°C, -15°C), the DNA extract must be transferred to a separate tube and can be stored sealed for up to 30 days (outside the scope of AOAC validation)

E. Real-Time PCR

Instrument and Software Setup

For instrument and software setup, follow instructions in the CFX Maestro, Industrial Diagnostic Edition (IDE) software user guide.

PCR Detection Preparation

1. Open the EZ-Check *Listeria monocytogenes* ReadyPCR Strips pouch.

2. Remove the required total number of strips (number of samples plus two controls) from the foil pouch.

   Note: Properly reseal unused strips in the pouch with the desiccant inside.

3. Place the ReadyPCR Strips on the EZ-Check PCR Frame making sure the strips are securely clipped to the frame. It is recommended to gently tap the EZ-Check PCR Frame on the counter to ensure beads are at the bottom of the PCR wells.

4. Carefully peel off the foil seal from each individual strip and visually check for the presence of one PCR bead per well. Once open, the ReadyPCR strips are stable up to 4 hr before being rehydrated.

5. Transfer 25 µL of DNA extract carefully along the inside wall of each well of the ReadyPCR Strips according to the plate setup. Do not aspirate the DNA extract from the bottom of the lysis tube to avoid transferring PCR inhibitors in the PCR well.

   Note: An effervescence reaction will indicate that the sample has been properly added to the PCR reagent.

6. Optional: If the sample exhibits PCR inhibition, proceed with a retest or a 1 in 10 dilution in sterile distilled water. If PCR inhibition is still present, use the iQ-Check Purification Reagent treatment or a 1 in 50 dilution sterile distilled water (additional verification testing required).

7. For negative and positive controls, transfer 25 µL of the EZ-Check Negative Control (NC) and EZ-Check *Listeria monocytogenes* Positive Control (PC) carefully along the inside wall of the corresponding ReadyPCR wells.

   Note: Rehydrated PCR beads with DNA extract or EZ-Check controls are stable up to 3 hr at room temperature.

8. Hermetically seal the wells of the PCR strips with the EZ-Check Optical Caps.

9. Place the EZ-Check PCR Frame in the thermal cycler. Be sure to place the plate with the A1 well at the upper left corner and close the instrument lid.

Laboratory Control Preparation (optional step)

Laboratory controls are treated as food samples. They are used to validate the full EZ-Check workflow. Extracted DNA from the laboratory controls is placed on the PCR plate and used as PCR plate controls as a replacement of the EZ-Check Negative and Positive Controls. To be valid, the laboratory negative control must not contain *Listeria monocytogenes* DNA. To be valid, the laboratory positive control must contain *Listeria monocytogenes* DNA amplified with Cq values between 26 and 36. See CFX Maestro Industrial Diagnostic Edition (IDE) Software User Guide (document #10000241897).

The end-user is responsible for establishing and implementing appropriate laboratory controls.

The validity and reliability of EZ-Check *Listeria monocytogenes* results are dependent on the correct verification, validation and preparation of these user-prepared controls.

Run PCR

To start the PCR run, follow instructions CFX Maestro, IDE software user manual.

F. Data Analysis

Data can be analyzed directly at the end of the PCR run or later by opening the stored data file. Follow instructions in the CFX Maestro, IDE software user manual 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).

CFX Maestro IDE software allows complete automated analysis for Bio-Rad real-time PCR detection systems. A verification of the typical characteristics of the amplification curves should be performed prior to releasing results. Contact your Bio-Rad technical support team if additional support is required.

Controls

Verify the positive and negative controls before interpreting sample results.

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

	Listeria monocytogenes Detection (FAM channel)	Internal Control Detection (HEX channel)
Negative control	Cq = N/A*	28 ≤ Cq ≤ 40
Positive control	26 ≤ Cq ≤ 36	Not relevant

* 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.

If results of negative and positive controls differ from those in the table above (invalid control), repeat the run and analysis described in D. Real-Time PCR and E. Data Analysis in Section 7 Protocol.

Samples

A **positive** EZ-Check *Listeria monocytogenes* PCR test must show a typical amplification curve and a Cq value ≥10 for the FAM fluorophore.

- If the Cq value for both channels is below 10, verify that the raw data curve is a regular amplification curve (with a flat baseline followed by a rapid exponential increase of

fluorescence and then a flattening out). If the curve seems correct, it may be considered a positive *Listeria monocytogenes* test

If there is no Cq value (Cq = N/A) for FAM, or if the curve is not a typical amplification curve, the internal control for that sample must be analyzed.

- This sample is considered a **negative** *Listeria monocytogenes* sample if there is no Cq value in the FAM channel and the internal control has a Cq value ≥28
- 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 (using 10 µL of DNA extract, perform a 1 in10 dilution in distilled sterile water, and then test 25 µL of the dilution) and the PCR repeated
- Should the Cq 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 test results is summarized in the following table:

L. monocytogenes Detection (FAM channel)	Internal Control Detection (HEX channel)	Interpretation
Cq ≥ 10	N/A*	Positive
Cq = N/A*	Cq ≥ 28	Negative
Cq = N/A*	Cq = N/A*	Inhibition**

* 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.

** When both target and internal control detection give a Cq value = N/A, the DNA extract must be diluted 1 in10 and tested again.

An invalid interpretation can be given when validation criteria are not met. Check the raw data and proceed as if the sample was inhibited.

Section 9
Confirmation of Positive Results

In the context of AOAC validation, a positive EZ-Check *Listeria monocytogenes* result should be considered presumptive positive and confirmed according to an appropriate reference method (for example, MLG, BAM, ISO, MFHPB, AOAC SMPR, etc.). Alternatively, streak 10 µL of enrichment broth directly to RAPID'*Listeria* spp Agar, RAPID'*L.mono* Agar or AL Agar and incubate for 24 ± 2 hr at 37 ± 1°C. The presence of characteristic *Listeria* spp. colonies should be biochemically confirmed for the presence of *Listeria* spp. Refer to the confirmation method

described in the RAPID'*Listeria* spp Agar user guide (document #10000127437) or RAPID'*L.mono*. Agar user guide (document #10000167776) or AL Agar user guide (document #10000245369).

In the case of discrepant results between the EZ-Check *Listeria monocytogenes*. Kit and any of the confirmation options listed above, follow the necessary steps to ensure valid results.

Section 10
Confirmation of Single Colonies Using EZ-Check Kit

EZ-Check *Listeria monocytogenes* Kit may also be used for confirming single isolated *Listeria monocytogenes* colonies on agar plates (outside the scope of AOAC Validation). Please refer to the user guide of the RAPID'*L. mono* Agar (document #10000127436), RAPID'*Listeria* spp. Agar (document #10000127437), or AL Agar (document #10000245369) for conditions of use.

1. Pick an isolated colony from a selective or nonselective agar plate with a toothpick, sterile loop, or other adapted consumable (for example, a pipet tip).

2. Resuspend the colony in 100 µL distilled sterile water in a microcentrifuge tube. Homogenize using a vortexer.

3. Add 25 µL of the suspension directly to the PCR mix bead or to the EZ-Check Lysis solution and follow the EZ-Check *Listeria monocytogenes* protocol for data and result interpretation.

Section 11
Test Performance and Validations

The EZ-Check *Listeria monocytogenes* Kit is specific for the detection of *Listeria monocytogenes* species.

AOAC Validation

The EZ-Check *Listeria monocytogenes* Kit is validated by the AOAC Research Institute under PTM 012605 for the detection of *Listeria monocytogenes* in raw milk[1, 2] (25 mL), raw milk cheese[2] (25 g), Mexican soft cheese[2] (125 g), ice cream[2] (125 g), cheddar cheese[2] (125 g), beef hot dogs[3] (125 g), turkey hot dogs[3] (125 g), raw fermented sausage[3] (125 g), frozen chicken nuggets[3] (25 g), deli ham[1, 3] (25 g), salami[3] (25 g), deli turkey[3] (25 g), smoked salmon[1, 2] (25 g), frozen cooked shrimp[2] (25 g), fresh cut cantaloupe[1, 2] (25 g), bagged salad[2] (125 g), frozen vegetable blend[2] (125 g), frozen green peas[2] (25 g), egg salad[2] (25 g), hummus[1, 2] (25 g), deli salad[2] (25 g), whole liquid egg[1, 2] (25 g), stainless steel surface[2] (4″ x 4″, sponge), sealed concrete surface (4″ x 4″, sponge), rubber surface (1″ x 1″, swab), and process water (25 mL). A positive result with the EZ-Check Kit is considered presumptive, and it is recommended it be confirmed following the recommendation in Section 9 above. The "EZ L. mono" APF, the use of the Free DNA Removal Solution, and the use of CFX96 Touch Deep Well, CFX Opus Deepwell and CFX Duet Real-Time PCR Systems are validated for all samples. The associated software is CFX Maestro IDE Software (version 4.0 and later). The iQ-Check Prep System is included in the scope of the AOAC validation.

[1] Tested against ISO 11290-1:2017 reference method
[2] Tested against FDA BAM Ch. 10 reference method
[3] Tested against USDA MLG 8.15 reference method

Section 12
References

AOAC Official Method 993.12-1996(1999). *Listeria monocytogenes* in milk and dairy products. Selective enrichment and isolation method

ISO 7218:2007. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations

ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1: Detection method

ISO 16140-2:2016. Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

ISO 22174. Microbiology of food and animal feeding stuffs - Polymerase chain reaction (PCR) for the detection of food-borne pathogens - General requirements and definitions

United States Department of Agriculture, Food Safety and Inspection Service (2025). Microbiology Laboratory Guidebook. Chapter 8.15: Isolation and Identification of *Listeria monocytogenes* and *Listeria* spp. from Ready-to-Eat Meat, Poultry, Siluriformes (Catfish), Egg Products, and Environmental Samples. https://www.fsis.usda.gov/sites/default/files/media_file/documents/MLG-8.15.pdf, accessed January 26, 2026

United States Food and Drug Administration (2022). Bacteriological Analytical Manual. Chapter 10: Detection of *Listeria monocytogenes* in foods and environmental samples, and enumeration of *Listeria monocytogenes* in foods. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-10-detection-listeria-monocytogenes-foods-and-environmental-samples-and-enumeration, accessed January 26, 2026

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