

VIP® Gold for *Listeria*

AOAC Official Method 997.03

AOAC Performance Tested Method 060801

Part No: 60037-40 (40 tests)

General Description

VIP® Gold for *Listeria* is a single-step visual immunoassay for the detection of *Listeria* in food and environmental samples. Each device contains a proprietary reagent system, which forms a visually apparent antigen-antibody-chromogen complex if *Listeria* is present. The test is intended for use by laboratory personnel with appropriate microbiology training.

Kit Components

Each VIP® Gold for *Listeria* kit contains the following:

VIP® Gold for *Listeria* test devices

Equipment / Materials Required

Other necessary materials not provided include:

Media per Appendix A

Autoclave

Vortex mixer

Analytical balance, tolerance ± 0.2 g

Stomacher / Masticator machine

Stomacher-type bags with filter or equivalent

Incubator capable of maintaining 29–31 °C

Water bath capable of maintaining 95–105 °C or equivalent (e.g. autoclave with flowing steam, dry heater)

Micropipette(s) capable of delivering 0.1 mL and 1.0 mL

Sample Preparation

A. Test Portion Preparation & Enrichment

Two Step Enrichment Protocol (AOAC OMA 997.03) for meat, poultry, seafood, vegetables, dairy products, eggs, pasta, animal meal, nuts and environmental samples.

- a. **Food samples** Add 25 g test portion to 225 mL of modified Fraser Broth with lithium chloride (mFB+LiCl) (Appendix A). Stomach / masticate for 2 min and incubate 28 h (26–30 h) at 30 °C (29–31 °C).
- b. **Environmental samples** Add 60 mL of mFB+LiCl to sample bag containing environmental sponge sample. Ensure that the sponge is oriented horizontally in the sample bag. If using a swab, add environmental swab sample to 10 mL of mFB+LiCl. Mix well and incubate 28 h (26–30 h) at 30 °C (29–31 °C).
- c. Transfer 1 mL incubated mFB+LiCl to 9 mL Buffered *Listeria* Enrichment Broth (BLEB) (Appendix A). Mix thoroughly with vortex mixer. Incubate 24 h (22–26 h) at 30 °C (29–31 °C).

Single Step Enrichment Protocol (AOAC PTM 060801) for meat, poultry, seafood, vegetables, dairy products, and environmental samples.

- a. **Food samples** Add 25g test portion to 225 mL of Demi-Fraser Broth (DFB) (Appendix A). Stomach / masticate for 2 minutes and incubate 48 h (46–54 h) at 30 °C (29–31 °C).
- b. **Environmental samples** Add 100 mL of DFB to sample bag containing environmental sponge sample. Ensure that the sponge is oriented horizontally in the sample bag. If using a swab, add environmental swab sample to 10 mL of DFB. Mix well and incubate 48 h (46–54 h) at 30 °C (29–31 °C).

Note: Retain original BLEB or DFB tubes under refrigeration (2–8 °C). Use for confirmation of presumptive positive results.

B. Sample Inactivation

- a. Vortex mix incubated sample. Transfer 1.0 mL incubated BLEB or DFB to a clean test tube.
- b. Inactivate microorganisms at 100 °C (95–105 °C) for 5 min in a water bath, autoclave or dry heater.
- c. Cool tubes to 25–37 °C before testing. Tubes that have been inactivated can be stored for up to 4 days at 2–8 °C prior to testing.

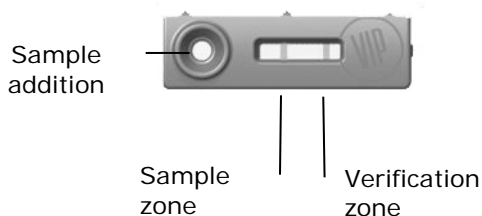
Test Procedure

- a. Open the sealed foil pouch containing the VIP® Gold devices and remove the sheet of test devices. Break away the necessary number of devices, one device for each test portion.
- b. VIP® Gold units may not be reused. Reseal unused VIP® Gold units in pouch containing desiccant. Store at room temperature (15–30 °C).
- c. Enriched broths should be equilibrated to 25–37 °C prior to running the test.
- d. Vortex mix contents. Transfer 0.1 mL of inactivated BLEB or DFB to sample addition well. Avoid transferring particulate matter to the device.
- e. Incubate device at room temperature (15–30 °C) for 10 min.

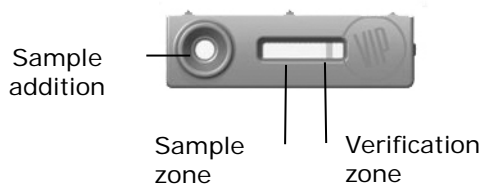
Results

Note: Examine test unit at 10 min. Beyond this time faint lines may develop because of non-specific color development and should be disregarded.

- a. Examine VIP® Gold unit for the presence of distinct detection lines in both test sample and test verification zone. Lines should be dark when contrasted with white background and should extend across the zone. Intensity of test sample and test verification lines may differ. Absence of a test verification line indicates an invalid test result. Contact Technical Services at (800) 245-0113.
- b. Test sample is considered **positive** when lines are present in the test sample zone and in the test verification zone.



- c. Test sample is considered **negative** when a line is present in the test verification zone and no line is seen in the test sample zone.



- d. Positive and negative control cultures should be run to familiarize the analyst with the results interpretation.

Confirmation

Presumptive positive samples should be confirmed from the retained BLEB or DFB tubes via either:

US FDA. 2003. *Bacteriological Analytical Manual* online, chapter 10.

<http://www.cfsan.fda.gov/~ebam/bam-toc.html>

USDA-FSIS. 2008. *Microbiology Laboratory Guidebook*, chapter 8.

http://www.fsis.usda.gov/science/microbiological_lab_guidebook/index.asp

Storage Conditions

Store VIP® Gold for *Listeria* kit components at room temperature (15–30 °C). **Do not refrigerate.**

Reseal unused VIP® Gold units in pouch with desiccant pack. The moisture indicator line on the desiccant pack must be blue.

Kit expiration is provided on the product pouch label.

Precautions

VIP® Gold for *Listeria* must be used as described herein. This product is not intended for human or veterinary use. Do not use test kit beyond expiration date indicated on the label. Do not mix reagents from different Assurance kit lots.

Avoid contact with eyes, skin, and clothing. Avoid swallowing or taking product internally.

Decontaminate materials by autoclave, bleach, etc., in accordance with good laboratory practices and in accordance with local, state and federal regulations. Waste may be contaminated with *Listeria* which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.

Pregnant women, elderly, and potentially immunocompromised individuals must be prohibited from laboratory rooms or areas where *L. monocytogenes* enrichment, isolation, and identification procedures are in progress. Although a properly sanitized laboratory area should not harbor *Listeria*, supervisors should use their own discretion in allowing these high-risk individuals into these areas.

Appendix A

Modified Fraser Broth with Lithium Chloride (mFB+LiCl)

Suspend 55 g of Fraser Broth Base (FB) in 1 L deionized water. Mix thoroughly until completely dissolved. Add 4 g lithium chloride (LiCl) and stir until completely dissolved. Autoclave at 121 °C for 15 min. Do not overheat. Do not add ferric ammonium citrate additive to broth. Do not use broth if precipitate forms. Alternately, prepare a 45% (w/v) LiCl solution by dissolving 45 g LiCl in enough deionized water to make a final volume of 100 mL. Filter sterilize through a 0.2 µm filter. Add 2 mL sterile LiCl solution to 225 mL of sterilized FB. If using commercially prepared 8 M LiCl solution, add 2.65 mL per 225 mL sterilized FB.

Buffered Listeria Enrichment Broth (BLEB)

Suspend 36.1 g Listeria Enrichment Broth in 1 L deionized water. Add 8.5g 3-3-(N-Morpholino) propanesulfonic acid (MOPS) free acid and 13.7g MOPS sodium salt. Mix thoroughly. Autoclave at 121 °C for 15 min.

Demi-Fraser Broth (DFB)

Suspend 55 g of Demi-Fraser Broth Base in 1 L deionized water. Mix thoroughly until completely dissolved. Autoclave at 121 °C for 15 min. Do not add ferric ammonium citrate additive to broth.

Manufacturing Entity

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BioControl Systems, Inc is an affiliate of Merck KGaA, Darmstadt, Germany.

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