GENE-UP® *Listeria* spp. LIS

Qualitative test for the detection of *Listeria* spp. in food and environmental samples. For microbiological control only

SUMMARY

Listeria spp. have been recognized as a cause of foodborne illness worldwide.[1] The current classification of the genus *Listeria* includes six species that are non-spore-forming, short, motile, Gram-positive rods. Several of these species are pathogenic to humans and animals.[2] *Listeria* can grow at wide temperature and pH ranges and can tolerate high concentrations of sodium chloride.[3,4] Because *Listeria* spp. are ubiquitous in soil, water and several animals intended for consumption,[5] *Listeria* has been isolated from various food products including dairy, meat, vegetables, and seafood, as well as from food processing plants.

In humans, *Listeria monocytogenes* causes listeriosis, which may include meningitis, septicemia, encephalitis, fetal loss and death. Groups at greatest risk include pregnant women, neonates, older adults and immunocompromised people.[4]

GENE-UP® *Listeria* spp. (LIS) is a real-time Polymerase Chain Reaction (PCR) assay for detection of *Listeria* (except *Listeria grayii)* in food and environmental samples.

PRINCIPLE AND EXPLANATION

The GENE-UP *Listeria* spp. kit is to be used with compatible PCR strip tubes (see equipment and consumables lists) in the GENE-UP Thermocycler. Each reaction vial in the GENE-UP *Listeria* spp. kit contains all of the necessary components for PCR, including sample-specific primers and probes and an internal amplification control.

The GENE-UP Thermocycler detects fluorescence at several wavelengths (channels) to allow for multi-target detection in the same reaction vessel. The fluorescent signal from a sample is recorded in channel 640, while the fluorescent signal for an internal amplification control is recorded in channel 705. The software automatically interprets the results for the internal amplification control and determines the sample result based on the outcome of the control.

Both the assay for the sample and the internal amplification control utilize dual Fluorescence Resonance Energy Transfer (FRET) hybridization probes. These probes consist of two different short oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the reaction cycle. The first probe for the sample assay is labeled at the 3' end with fluorescein; the second probe is labeled at the 5' end with LC Red 640. FRET occurs only after the two probes come in close proximity from hybridizing to the template DNA.

The resulting fluorescent signal from the FRET interaction, which forms a real-time amplification curve, is how the amplified target is detected by the GENE-UP Thermocycler. After the PCR cycling program finishes, the PCR product(s) are melted to determine the presence of the target DNA. The software uses both the real-time amplification curve and the melt peak to make a positive or negative call.

Internal amplification control

The internal amplification control, contained in the freeze-dried pellets, validates that the reaction conditions are sustainable for PCR to take place, thus validating a negative outcome for the sample. The internal amplification control is amplified by the same primer set but uses a different set of hybridization FRET probes to allow detection in the 705 channel.

COLOR DYES

Color dyes have been added to each reaction component. The sample lysis buffer is red in color, and the reagent PCR master mix is blue in color when rehydrated. When these two components are mixed in a given well on a PCR plate or strip, they form a purple color.

CONTENTS OF THE KIT

The GENE-UP® *Listeria* spp. kit contains the following:

Contents	Component	Description
6 pouches with 4 vials	REAG	**Reagent**: freeze-dried pellets (1 vial = 8 PCR reactions; 192 reactions total)
1 pouch with:		
2 vials	RecBUF	**Reconstitution buffer**: 2 x 600 µL reconstitution buffer (blue)
1 vial	–BUF	**Negative-control buffer**: 850 µL

The kit also includes two bags of Eppendorf MasterClear™ strip caps (12 x 8 each) and strip tubes (12 x 8 each).

Instructions for Use are provided in the kit or downloadable from www.biomerieux.com/techlib.

ADDITIONAL MATERIALS AND CONSUMABLES REQUIRED

The items below are NOT included with the reagent kits and are necessary for use of the GENE-UP *Listeria* spp. kit.

Equipment	Consumables
GENE-UP® Thermocycler (REF 414056)	GENE-UP® Lysis Kit (REF 414057)
Sample Manipulation:	**Sample Manipulation:**
Blender with paddles* • AES Chemunex Smasher® (REF AESAP1064) • easyMIX™ paddle blender (REF AESAP1068)	LPT broth • 6 x 225 mL (bioMérieux REF 410848) • 6 x 100 mL (bioMérieux REF 410846) • 20 x 10 mL (bioMérieux REF 410845) • 4x 3 liter (bioMérieux REF 410849) • 10 x 225 ml mini bag (bioMérieux REF 410847)
Vortex-Genie® Pulse	Sterile filter blender bags* • AES400P/50G Type P filter bag • 415180 SMASHER® XL Bag 2L (400)
Pipettes:	**Confirmation*:**
Adjustable, variable volume pipette: • 0.5 –10 µL (single or multichannel pipette) • Compatible with 5 µL, 20 µL and 45 µL	• ALOA® 120 x 90mm (REF AEB520079) • ALOA® 20 x 90mm (REF AEB520080) • Fraser Broth (REF 42072) • API® LIS (REF 10300)
Repeater pipette (optional)	
Centrifugation:	**Pipette Tips:**
Plate Centrifuge* • MPS1000™ Mini PCR Plate Spinner 110V (REF 419196) or • MPS1000™ Mini PCR Plate Spinner 230V (REF 414555)	Compatible sterile, filter pipette tips for 20 µL and 45 µL
Mini Tube Centrifuge (VWR REF 93000-196) or equivalent	10 µL Biotix filter pipette tip (REF 419194)

GENE-UP Accessories:	PCR Consumables†:
GENE-UP® PCR Tube Holder (REF 414573)	12 x 8 Eppendorf MasterClear™ strip caps (REF 89094-210)
GENE-UP® Lysis Tube Remover (REF 414469)	12 x 8 Eppendorf MasterClear™ strip tubes (REF 89094-212)
GENE-UP® Lysis Rack Adaptor (REF 414570)	
GENE-UP® Heavy Rack Holder (REF 414571)	
GENE-UP® Lysis Tube Holder (REF 414572)	

* Items listed are examples of materials that can be used.

†All required strip tubes and caps are included in the kit; additional strips and tubes must be purchased separately. It is not possible to use the 96-wells plates with the GENE-UP Thermocycler.

Standard supplies and equipment commonly found in a microbiology laboratory are not provided.

WARNINGS AND PRECAUTIONS

For microbiological control only

1. The GENE-UP Listeria spp. kit must be used with the GENE-UP Lysis Kit (REF 414057).
2. For professional use only.
3. Place the instrument in a room designed for microbiological analysis.
4. Comply with Good Laboratory Practice (e.g., standard ISO 7218).[6]
5. This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
6. Do not use reagents after the expiration date indicated on the label.
7. Visually inspect vials before testing. Do not use vials with evidence of damage, leakage, or deterioration.
8. Do not mix reagents (or disposables) from different lots.
9. Powder-free latex or nitrile gloves are recommended for all PCR steps.
10. Spills should be wiped up thoroughly after treatment with bleach or a nucleic acid degradation solution. See the GENE-UP user manual for information on cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
11. The equipment and accessories should be regularly cleaned and decontaminated.
12 **NEVER** remove the caps from the lysis tubes.
13. Do not try to remove the strip tube caps once they have been sealed to the PCR strip tubes.

Listeria is a Gram-positive, non-spore-forming, aerobic bacillus organism.[7,8] Care must be taken when handling samples that may contain *Listeria* spp.

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures.[7] While BSL-2 containment is suitable for all other *Listeria*, BSL-3 practices and equipment are recommended for activities likely to produce significant aerosols or for activities involving production quantities of this particular organism.

Laboratory personnel must be adequately trained to handle pathogens before being permitted to analyze samples for *Listeria* spp. Follow appropriate safety guidelines when handling potentially contaminated samples. Waste should be disposed of in compliance with local and national legislation.

Listeriosis can be life-threatening to certain vulnerable groups, such as pregnant women and those with compromised immune systems, and can sometimes be life-threatening to healthy individuals. Pregnant women and immune-compromised individuals should take special care when handling samples that are potentially contaminated with *Listeria.*

STORAGE CONDITIONS

1. Store the GENE-UP kit, at room temperature (15-25°C).
2. DO NOT REFRIGERATE.
3. Do not use the reagents beyond the expiration date printed on the kit box and/or label.
4. After opening the kit, check that the pouches are correctly sealed and undamaged. If not, do not use them.
5. If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.
6. Only remove the required number of vials from the pouch.
7. Once pouches are opened, freeze-dried pellets should be reconstituted and used within 30 days. Freeze-dried pellets should be stored in original sealed pouch (with lab adhesive or bag clip).
8. Once freeze-dried pellets are reconstituted, testing on the GENE-UP Thermocycler should be initiated as soon as possible. Storage conditions for vials are presented in the following table:

Storage Condition	Time
Ambient temperature	2 hours
+2-8°C	2 days
-20°C	8 days

SAMPLE PREPARATION

Allow the enrichment broths to reach room temperature (15-25°C) before use. Frozen samples must be thawed before use.[9]

AOAC RI approved protocols (No. 051603) and NF VALIDATION BIO 12/x-x/x protocols

In a blender bag plus filter, aseptically place the following:

Protocol	Outside NF VALIDATION	NF VALIDATION	AOAC PTM
Standard procedure for all human and animal food products and production environmental samples (excluding primary production samples)	• 25 g of sample • 225 mL of LPT broth • Mix using a paddle blender • Incubate at 37±1°C for 22-26 hours	-	• 25 g of sample • 225 mL LPT broth • Mix using a paddle blender • Incubate at 35±1°C for 22-26 hours **NOTE**: For certain matrices, it is recommended to follow the specific preparation techniques described in the appropriate standards.
Meat and dairy products	-	• 25 g of sample • 225 mL of LPT broth • Mix using a paddle blender • Incubate at 37±1°C for 22-26 hours	-
Large sample size of soft cheese samples and ready-to-eat food products	-	-	Protocol for 125 g: • 125 g of sample • 375 mL of LPT broth • Mix using a paddle blender • Incubate at 35±1°C for 24-28 hours
Environmental surface sponges	• 1 sponge • 100 mL of LPT broth • Mix manually for 1 minute • Incubate at 37±1°C for 18-24 hours	-	• 1 sponge • 100 mL of LPT broth • Mix manually for 1 minute • Incubate at 35±1°C for 18-24 hours
Environmental surface swabs	• 1 swab • 10 mL of LPT broth • Mix manually for 1 minute • Incubate at 37±1°C for 18-24 hours	-	• 1 swab • 10 mL of LPT broth • Mix manually for 1 minute • Incubate at 35±1°C for 18-24 hours

NOTE: For environmental samples, the collection device should first be dampened with a sterile diluent (e.g., LPT broth) containing if necessary a suitable neutralizing agent (e.g., Lecithin-Polysorbate-L.Histidine-Sodium thiosulfate mixture or Dey Engley Neutralizing buffer),

1. After incubation, manually mix the contents of the blender bag. Optionally, a sterile technique can be used to remove 1 mL of enriched sample and to place it in a pre-labeled microcentrifuge tube.
2. For PCR testing, continue to the “Lysis” section.
3. For confirmation of positive results, follow guidelines for continued enrichment steps in the “Confirmation of Positive Results” section.

NOTE: Do not discard the individual enriched samples until the analysis is complete and it has been confirmed that further testing is not required.

LYSIS

NOTE: Refer to GENE-UP Lysis Kit package insert (REF 414057) for general information about the kit.

1. Use the plate map created in the GENE-UP Routine software to determine the number of lysis tubes required from the GENE-UP Lysis Kit and place the correct number of lysis tubes in the GENE-UP Lysis Tube Holder. (If less than 8 tubes in a strip are required, the strips can be cut apart, and only the used tubes are placed in the GENE-UP Lysis Tube Holder.)

   CAUTION: Never open the lysis tubes. If a lysis tube opens or leaks, this should be considered a contamination event.
2. Place the GENE-UP Lysis Tube Holder on the GENE-UP Heavy Rack Holder.
3. Transfer 20 µL of sample into the lysis tube (buffer is red). Use the Plate Map from the GENE-UP Routine software to pipette each sample into the correct plate position.
4. Remove the GENE-UP Lysis Tube Holder from the GENE-UP Heavy Rack Holder.
5. Place the GENE-UP Lysis Tube Holder on the GENE-UP Lysis Rack Adaptor.
6. Run the bead beater at maximum speed for 5 minutes. The speed must be above 2000 rpm.
7. When lysis is complete, remove the GENE-UP Lysis Tube Holder from the GENE-UP Lysis Rack Adaptor.
8. Clip the GENE-UP Lysis Tube Holder into the GENE-UP Heavy Rack Holder, and proceed to Final Setup for PCR.

NOTE: Do not discard the individual enriched samples until the analysis is complete and further testing is not required. Enriched samples can be stored at 2-8°C for up to 72 hours. The lysate can be stored for up to 3 days at 2-8°C or -20±5°C for extended storage.

FINAL SETUP FOR PCR

Before beginning the procedure, put on a clean pair of powder-free latex or nitrile gloves.

1. Determine the number of samples to be tested and open a Freeze-dried reagent pouch. A single freeze-dried vial contains enough reagent to prepare 8 samples.
2. To ensure that the reagent pellet or pellet fragments are at or near the bottom of each vial, tap the reagent vial on the bench or centrifuge for 3 seconds. Carefully remove the rubber cap from the reagent vial. Do not discard the rubber cap.
3. Add 45 µL of the reconstitution buffer (blue) to the reagent vial without touching the pellet. Mix using a vortex (after closing the vial) to rehydrate the pellet. Centrifuge the tube or tap it on the bench to ensure all the liquid is on the bottom of the vial.
4. Place empty strip tubes onto the GENE-UP PCR Tube Holder following the plate map from the GENE-UP Routine software.
5. Pipe 5 µL of the blue rehydrated solution into a PCR strip tube in the GENE-UP PCR Tube Holder.

6. Using a 10 µL Biotix filter pipette tip, transfer 5 µL of lysed sample (red) in the appropriate PCR tube containing 5 µL of the blue rehydrated solution. To determine the appropriate plate position for each sample, refer to the Plate Map from the GENE-UP Routine software. When the sample is added to the PCR reagent, the solution will turn purple in color.

   NOTE: Do NOT force the pipette tip into the lysis tube. Do NOT agitate the lysate before aspirating the sample. The solid material must stay at the bottom of the tube.

   NOTE: Avoid pipetting beads and bubbles.

   NOTE: For dark samples, red coloration of the lysate may not be visible; there may also be no purple coloration of the final PCR solution.

   If using a multichannel pipette, perform the following steps:

   a. Ensure that the pipette is level when aspirating and dispensing
   b. Remove tips slowly in order to avoid pulling the caps off of the lysis tubes
   c. Visually check for the presence of lysate in the tips
   d. Visually check to confirm the absence of beads in the tips.
7. Place a strip cap on each strip tube.
8. Seal the strip caps onto each strip tube using the top piece (any of the four curved edges) of the GENE-UP Lysis Tube Remover Tool.
9. Place the GENE-UP PCR Tube Holder containing the PCR tubes in the plate centrifuge. Balance the centrifuge. Spin for 10 seconds.
10. The plate is now ready to be processed in the GENE-UP instrument and must be started within 15 minutes.

NOTE: The lysis tubes can be removed from the GENE-UP Lysis Tube Holder using the GENE-UP Lysis Tube Remover Tool. The GENE-UP Lysis Tube Holder is reusable, but the used lysis tubes should be disposed of in accordingly (see Waste Disposal section).

NOTE: Unused rehydrated PCR reagent should be stored according to the guidelines in the Storage Condition section. Reconstitution buffer can be saved to rehydrate the remaining reactions if fewer than 96 reactions were utilized.

NEGATIVE CONTROL PROCEDURE

Follow the same procedure in the Final Setup for PCR section, using 5 µL of negative control buffer instead of lysed sample (step 6).

START THE RUN AND VIEW THE RESULTS

Please refer to the appropriate GENE-UP user manual for instructions on how to start a run, view results, and use the GENE-UP Routine software.

RESULTS INTERPRETATION

Results are automatically interpreted once the PCR run is completed. The Routine software interprets data of both amplification and melting curves for each sample and gives a positive, negative, or invalid result as indicated in the following table.

Listeria **(640 nm)**	**Internal amplification control (705 nm)**	**Result**
+	+	+
+	-	+
-	+	-
-	-	Invalid

In case of an invalid result, proceed to the protocol in the "PCR Inhibition Protocol" section to remove inhibitions.

PCR INHIBITION PROTOCOL

In case of an inhibited result, dilute the lysate to 1:3 in the negative control buffer :

1. Transfer 10 µL of negative control buffer in an adapted microtube and add 5µL of lysate.
2. Follow the same procedure in the "Final Setup for PCR" section, using 5 µL of this dilution of lysate.

NOTE: It is recommended to retest in parallel the lysate without dilution.

CONFIRMATION OF POSITIVE RESULTS

Confirmation of positive results obtained using the AOAC RI approved protocols

All positive results must be confirmed according to the BAM[9] or MLG[10], or according to the following bioMérieux GENE-UP confirmation protocol. Confirmation must be performed using the enrichment broth (stored at 2-8°C for up to 72 hours).

1. Isolate the enrichment sample, and streak the sample on an ALOA agar plate; incubate at 35°±1°C for 48 ± 3 hours. The plate should be read after 24 and 48 hours (in case there are no typical colonies after 24 hours).
2. The presence of typical colonies confirms a positive result.
3. If identification of colonies is necessary, use an API® LIS strip to directly test isolated colonies (without a purification step).

In the event of discordant results such as a positive result with the GENE-UP test, or no confirmation using on a plate (untypical colonies), the laboratory must take the necessary steps to ensure that the results obtained are accurate. The following steps are recommended:

1. Transfer 100 µL of sample from the first enrichment to 10 mL Fraser broth for a second enrichment. Incubate at 35°±1°C for 24 ± 3 hours.
2. Isolate on an ALOA agar plate and incubate at 35°±1°C for 48 ± 3 hours. The plate should be read after 24 and 48 hours (in case there are no typical colonies after 24 hours).
3. The presence of typical colonies confirms a positive result.
4. If no typical *Listeria* colony is identified, repeat steps 1 and 2, using 500 µL from the first enrichment.

Confirmation of positive results obtained using the method certified NF VALIDATION and outside NF Validation

In the context of NF VALIDATION mark, all positive results obtained with GENE-UP® *Listeria* spp must be confirmed. If the confirmation is not initiated immediately after a positive GENE-UP test, store the enrichment broth at 2-8°C. Confirmation must be initiated within 72 hours following the end of incubation.

1. Isolate the enrichment sample, and streak the sample on an ALOA agar plate; incubate at 37±1°C for 48 ± 3 hours. The plate should be read after 24 and 48 hours (in case there are no typical colonies after 24 hours).
2. The presence of typical colonies confirms a positive result.
3. If identification of colonies is necessary, use an API® LIS strip to directly test isolated colonies (without a purification step).

In the event of discordant results such as a positive result with the GENE-UP test, or no confirmation using on a plate (untypical colonies), the laboratory must take the necessary steps to ensure that the results obtained are accurate. The following steps are recommended:

1. Transfer 100 µL of sample from the first enrichment to 10 mL Fraser for a second enrichment. Incubate at 37±1°C for 24 ± 3 hours.

2. Isolate on an ALOA agar plate and incubate at 37±1°C for 48 ± 3 hours. The plate should be read after 24 and 48 hours (in case there are no typical colonies after 24 hours).
3. The presence of typical colonies confirms a positive result.
4. If no typical *Listeria* colony is identified, repeat steps 1 and 2, using 500 µL from the first enrichment.

Confirmation could be performed using a method certified NF VALIDATION based on a different principle. The validated protocol of the second method should be followed entirely (e.g., enrichment duration

QUALITY CONTROL

External quality control can be performed using one *Listeria* strain. Add one isolated colony from a fresh and pure culture in 10 mL of LPT broth. Mix and incubate at 35-37°C for 18-24 hours. Dilute 1/100 the culture in LPT in order to obtain a suspension containing approximately 10^6 cells/mL of the strain.

Follow the protocol from Lysis steps to Confirmation of Positive Results sections. Check that the results obtained correspond to the characteristics of the tested strains.

NOTE: It is the responsibility of the user to perform Quality Control in accordance with any applicable local regulations.

LIMITATIONS OF THE PROCEDURE

The GENE-UP *Listeria* spp. kit has been evaluated on a large number of matrices. However, given the wide variety of products and manufacturing procedures, it is recommended to check that the composition of the matrices tested does not affect the reliability of GENE-UP results.

NOTE: *Listeria grayii* is not detected by the method.

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products. It is the responsibility of each laboratory to handle waste and effluents produced according to their type and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

The GENE-UP Listeria method has been validated and certified by the AOAC Research Institute as a Performance Tested Method (Certificate No. 051603) for the detection of Listeria in a variety of foods.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 060903

PERFORMANCE TESTED METHOD
Certified by AOAC Research Institute
www.aoac.org

The following matrices were included in the AOAC validation: Fresh spinach (25g), Fresh lettuce (25g), Deli ham (25g), Cooked shrimp (25g), Smoked salmon (25g), Beef hot dogs (25g), Vanilla ice cream (25g), Deli turkey (25g), Cooked chicken nuggets (25g), Mexican soft cheese (25g), Smoked salmon (125 g), and Stainless steel.

USE / VALIDATION STATEMENT

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AVAILABILITY

For technical assistance, contact your local bioMérieux representative.

INDEX OF SYMBOLS

Symbol	Meaning
	Catalogue number
	Legal Manufacturer
	Date of Manufacture
	Temperature limitation
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Keep away from sunlight
	Do not use if package is damaged
LIS	*Listeria* (spp.)

REVISION TABLE

This section contains a summary of changes made to the GENE-UP *Listeria* (spp.) Instructions for Use.

Revision Date	Revision Number	Change Type	Change Summary
2016-04	43-04320 B	Content Change	**Final Setup for PCR:** Clarification of steps. **Quality Control:** Clarification of process. **Sample Preparation:**- Addition of protocol information for 25 g using the method certified NF VALIDATION **Confirmation of Positive Results:** Addition of information using the method certified NF VALIDATION **Color Dyes**, **Results Interpretation, PCR Inhibition Protocol:** Addition of sections.
2015-10	43-04320 A	N/A	Creation of new document.

NOTE: Minor typographical, grammar, and formatting changes are not included in the revision history.

Change Type categories:

- **Correction =** Correction of documentation anomalies.
- **Content Change =** Implementation of new and modified (updated) intended use and performance characteristics.
- **Administrative =** Implementation of non-technical changes noticeable to the user.

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