SOLUS ONE *LISTERIA*

Immunoassay-Based Test System for the Detection of *Listeria* in Environmental Samples

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Product Code(s):	LIS1-0096; LIS1-0480
Certifying Body:	AOAC

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This method's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

This method has been evaluated in the AOAC® *Performance Tested Methods*℠ Program for the detection of *Listeria* spp. in stainless steel and polystyrene environmental surfaces. Solus One *Listeria* method has been compared to the FDA/BAM Chapter 10 reference methods, as applicable to matrices.
(AOAC® *Performance Tested*℠ License no. **051802**).

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1. INTRODUCTION

Solus One *Listeria* provides a negative or a presumptive positive result from a single enrichment step within 25 hours, including the assay time.

2. INTENDED USE

Solus One *Listeria* is for the next day detection of *Listeria* spp. in production environmental samples. The test method is easy to perform, however it requires laboratory facilities plus qualified and trained personnel. Basic training is recommended to first time users and is given by Solus Scientific Solutions Ltd.

Using the method includes compliance with Good Laboratory Practices (refer to EN ISO 7218).

3. REAGENTS PROVIDED

Most kit components are supplied stabilized and ready to use at working concentration with only the Washing Buffer concentrate requiring dilution. Each kit contains sufficient material for 1 (LIS1-0096) or 5 (LIS1-0480) x 93 determinations, plus controls. The kit expiry date is displayed on each product label.

Component	Appearance	Volume		Comments
		LIS1-0096	LIS1-0480	
Assay plate	96-well microplate with removable/ breakable strip format	1	5	Wells coated with antibodies against *Listeria* spp.
Negative control	Pale orange liquid. Green label.	3ml	10ml	Working concentration. Contains diluent with preservative.
Positive control	Black liquid. Red label.	3ml	10ml	Working concentration. Contains heat-killed *Listeria* in diluent with preservative.
Conjugate	Colorless/very pale straw-colored liquid. Orange label.	11ml	60ml	Working concentration. Contains horseradish peroxidase antibody conjugate in diluent with preservative.
Substrate	Colorless/very pale blue liquid. Blue label.	11ml	60ml	Working concentration. Contains 3, 3', 5, 5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilizers.
Stop solution	Colorless liquid. Silver label.	11ml	60ml	Working concentration. Contains 0.2M sulfuric acid.
Washing buffer concentrate	Colorless/yellow/orange liquid. White label.	10ml x 6	60ml x 5	Concentrated. Dilute before use.

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4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Refrigerator at 2-8°C	Pipettes and tips (1ml; 0.1ml)
Deionized or distilled (DI) water	Vortex mixer
SOLO+ medium pre-warmed to 30°C	Timer
Measuring cylinders for various volumes (e.g. 250 ml, 1L)	Incubator at 30±1°C
3ml transfer pipettes (sterile)	Incubator at 37±1°C
Sponge samplers or swabs soaked in suitable neutralizing buffer (e.g. Letheen broth or HiCap buffer)	Heating apparatus (e.g. heat block) capable of heating to 85-100°C
Tubes for sample boiling (e.g. 5ml polypropylene rimless tubes 12x75mm)	Dynex DS2 or Microplate washer and microplate reader with 450nm filter
	Autoclave for decontamination of samples

5. REAGENT PREPARATION

5.1 Wash Buffer:

Prepare the following in a clean vessel.

LIS1-0096	LIS1-0480
Add 10ml (1 bottle) of the washing buffer concentrate to 240ml DI water and swirl to mix.	Add 60ml (1 bottle) of the washing buffer concentrate to 1440ml DI water and swirl to mix.

5.2 Culture Broth (growth medium):

- Prepare SOLO+ medium following manufacturer's instructions. Allow to cool to ambient temperature (e.g. 18-25°C) before use.

6. SAMPLE PREPARATION AND ENRICHMENT

6.1 Environmental samples

- Sample the production environment surface with sampling device. Follow sampling device manufacturer's instructions for correct use, storage and transport.
- Warm SOLO+ to 30°C prior to use. Add 100ml SOLO+ to sponge sampler in a suitable bag. Add 10ml SOLO+ to a swab. Hand massage or mix by vortexing and incubate for 22-30 hours at 30±1°C.

Ensure that the bench processing time of samples is kept to a minimum and that transfer to 30°C incubator occurs as soon as possible. This is important to avoid extensive growth of competing organisms.

7. POST ENRICHMENT HEAT INACTIVATION

7.1. When the sample incubation period is completed, transfer 1-2ml aliquot (avoiding particulates) to a sample boiling tube (e.g. 5ml polypropylene tube).

7.2. Heat the aliquot to 85-100°C for 15-20 minutes in the tube. After heating, allow the sample to cool to ambient temperature (18-25°C). This may be accelerated by placing the tubes in cold tap water for ~5 minutes.

The non-heat-inactivated samples should be kept for verification until immunoassay results are obtained. These samples should be kept at 30±1°C if the test is to be carried out within 2 hours. If this is not possible, keep the broths for up to 72 hours at 2-8°C prior to the test.

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8. IMMUNOASSAY PROCEDURE

8.1. Take test kit from storage at least one hour before use to allow the components to reach ambient temperature (18-25°C). Determine the number of wells required for the test. Take required number of strips from the pouch and fit them to the frame provided. Unused strips should be returned to the pouch and stored at 2-8°C.

8.2. Prepare Wash Buffer as detailed in section 5.1 for the kit size being used.

8.3. Leave the first well in the strip empty to serve as a 'blank' for measuring the absorbance of the substrate.

8.4. Pipette 0.1ml of Negative Control (Green label) into the second well.

8.5. Pipette 0.1ml of Positive Control (Red label) into the third well.

8.6. Pipette 0.1ml of each heat-inactivated sample separately into consecutive wells in the strip. If there are wells left over at the end of a test strip the Positive or Negative Controls may be repeated. †

8.7. Incubate the plate (containing the strips) at 37±1°C for 60-65 minutes.

8.8. After incubation, aspirate the contents of the wells, removing as much of the liquid as possible. Wash the wells 5 times with wash buffer ensuring complete filling and emptying of the wells through each wash cycle. The washing technique is critical to assay performance, hence it is recommended to use a microplate washer instrument.

8.9. Pipette 0.1ml of Conjugate (Orange label) into all wells except the 'blank'.

8.10. Incubate the plate at 37±1°C for 30-35 minutes.

8.11. Repeat the wash cycles as detailed in section 8.8

8.12. Pipette 0.1ml of Substrate (Blue label) into all wells, including the 'blank' well.

8.13. Incubate the plate at ambient temperature (18-25°C) for 30-35 minutes in the dark.

8.14. After incubation, stop the reaction by adding 0.1ml of Stop Solution (Silver label) to all wells including the 'blank' well. The stop solution will cause any blue color in wells to change to yellow.

8.15. Read the optical densities of wells within 10 minutes in a plate reader using a 450nm filter. Before reading, inspect the wells for air bubbles and, if present, burst with a needle. The reader should be zeroed against the 'blank' well before the other wells are read. Do not use reference filter. The use of automatic ELISA equipment is preferred and should be set up and validated to this protocol.

† If using the Dynex instrumentation, care must be taken to avoid bubbles in the sample and reagent tubes, or films forming across the tube above the level of the liquid. It is essential to check that the system has successfully pipetted samples into the assay plate before proceeding.

9. INTERPRETATION OF RESULTS

Results are expressed as optical density (OD_{450}) measurements using a microplate reader.

Acceptance criteria:

Negative Control OD_{450}	< 0.075
Positive Control OD_{450}	> 0.500

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The value of the blanking well (usually A1 when processing manually) should always be subtracted from all other results. Should the value of Negative or Positive controls not meet these criteria, the test is not considered valid and must be performed again.

Samples with OD_{450} readings of < 0.200 are considered negative in which case the analysis is complete, the results may be reported and the corresponding non-heat-inactivated samples may be discarded following local regulations/ guidelines.

Sample with OD_{450} ≥ 0.200 are considered presumptive positive for *Listeria*. Presumptive positive results must be verified using a recognized culture method.

10. CONFIRMATION OF POSITIVE RESULTS FROM ONE *LISTERIA* IMMUNOASSAY

- All samples identified as positive must be confirmed using the conventional tests described in the standardized methods by the FDA (BAM), USDA or ISO. The confirmation step must start from the non-heat-inactivated samples stored at 30°C or 2-8°C.
- Streak 10µl onto appropriate selective agar plates. Incubate agars as specified by standard *Listeria* cultural protocol. If typical *Listeria* colonies are observed on the plates this is sufficient to confirm the sample is positive for *Listeria* spp. Additional tests such as biochemical identification galleries can be used if there is any doubt about the validity of the result or to identify the species involved.
- Only trained microbiologists should attempt confirmation.

11. KIT STORAGE AND EXPIRY

The kit and any unused kit components should be stored at 2-8°C. DO NOT FREEZE. The kit expiry date is displayed on the kit box plus all of the kit components within the box. Any unused diluted wash buffer can be stored for up to 10 days if kept at 2-8°C. Any unused microplate strips should be returned to the foil pouch with the desiccant sachet and the seal closed completely, then stored at 2-8°C.

12. SAFETY

While the procedures detailed are simple and easy to perform, they require laboratory facilities with qualified personnel trained for the handling of potentially pathogenic organisms. Training is recommended to first time users and is provided by Solus Scientific Solutions Ltd.

- The Stop Solution contains sulphuric acid which is corrosive. Wash immediately with large quantities of water if the solution comes into contact with skin or mucous membranes.

As a guide, the following precautions should be taken as a minimum:

- Protective clothing should be worn including lab coat, safety glasses, mask and gloves where appropriate.
- Do not pipette by mouth.
- Avoid contact with the skin.
- Do not eat, drink or apply cosmetics in the laboratory.
- Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes.

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13. PRECAUTIONS FOR OPTIMAL PERFORMANCE

- Reagents are provided at fixed working concentration. Optimum sensitivity and specificity will be reduced if reagents are modified or not stored under the recommended conditions.
- Do not mix different lots of reagents.
- Avoid microbial contamination of opened reagent bottles.
- Ensure that no cross contamination occurs between wells.
- It is essential for proper performance of the test that the enzyme-conjugated antibody is not allowed to contaminate other reagents and equipment.
- Ensure that kit components are not exposed to temperatures greater than 40°C.
- Solutions containing sodium azide should not be used for cleaning of equipment, especially washing devices (the peroxidase enzyme used in the kit is inactivated by sodium azide).
- Do not use for diagnostic purposes of medical specimens.

14. MSDS INFORMATION

Material safety data sheets (MSDS) are available for this test on request.

15. WARRANTY

Accurate results depend on the proper use of the kit by following the instructions for use carefully. If the kit fails to perform according to specification, please contact:

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Summary of changes

Change date	Issue Number	Change Summary
Jan2020	1	Rebranding and combining of Solus One Listeria – insert 29 – Issue 3 – 11/18 and Solus One Listeria – insert 23 – Issue 2 – 11/18 into a single document.

NOTE: Minor changes (e.g. formatting, grammar, correcting typographical errors) are not included in the summary of changes.

For more information visit www.solusscientific.com

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