TRANSIA® PLATE

Listeria monocytogenes

LI0601 – Kit certified NF VALIDATION-
Ref. TRA 02/11 – 03/08

BIOCONTROL
Results. Right now.

INTENDED USE

TRANSIA® PLATE *Listeria monocytogenes* is intended to be used for the detection of *Listeria monocytogenes* in food products and environmental samples, without any cross reactivity to *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welshimeri* and *L. grayi*.

This test has been certified by AFNOR Certification for any product intended for human consumption and environmental samples.

ASSAY PRINCIPLE

TRANSIA PLATE *Listeria monocytogenes* is based on a two-step sandwich type ELISA (Enzyme Linked Immuno Sorbent Assay). The solid support of the reaction is a microtiter plate with divisible strips coated with antibodies specific for *L.monocytogenes**.

*Patented technology

MATERIALS

Kit Composition

- Microtiter plate with divisible strips, 96 wells (8 wells x 12 strips) – 1 pc
- Lid for the microtiter plate – 1 pc
- Negative Control: Fraser Broth – ready-to-use – 1 x 10 mL
- Positive Control: *L. monocytogenes* – ready-to-use – 1 x 6 mL
- Washing Buffer – 20 X concentrate – 1 x 60 mL
- Conjugate (antibodies conjugated to peroxidase) – ready to use – 1 x 16 mL
- Substrate (Urea H_2O_2) – ready-to-use – 1 x 57 mL
- Chromogen (TMB) – ready-to-use – 1 x 57 mL
- Stop Solution (H_2SO_4) – ready-to-use – 1 x 57 mL

Equipment required, but not provided

- For sample and reagent preparation
- Balance and weighing containers
- Homogenizer according to ISO 7218 (i.e. stomacher)
- Stomacher bags, preferably with a filter or Erlenmeyer flasks (500 mL)
- Tubes (20 mL) for subcultures
- Air incubator at 30 °C ± 1 °C
- Air incubator at 37 °C ± 1 °C
- Vortex mixer
- Test tubes (5 or 10 mL) resistant to 100 °C
- Water bath at 95 – 100 °C (boiling water)

For the immunoenzymatic test

- Micropipette 100 – 1000 µL
- Multipette with 2.5 and 5 mL combitips
- Air incubator at 37 °C ± 1°C
- Wash bottle or preferably an automatic microplate washer
- Absorbent paper
- Microtiter plate reader (with a 450 nm or 450/595 nm filter)

For confirmation of samples:

- Air incubator at 37 °C ± 1°C

Reagents required but not provided

For sample preparation:

- Distilled water
- Half Fraser Broth and its supplement. References given as an example: Biokar BK133HA and BS03008 (supplement); BioRad 3564604 and 3564616 (supplement)
- L-PALCAM Broth and its supplement (for pre-enrichment of raw meats). References given as an example: Merck Millipore 1108230500 and 1121220010 (supplement)
- Fraser Broth and its full supplement (for subcultures). References given as an example: Merck Millipore 1103980500 and 1000920010 (supplement)

For confirmation of positive results

- Selective agar plates for isolation of Listeria (such as Palcam, or a chromogenic agar according to Ottaviani et Agosti or RAPID' L. mono agar from BioRad

- Non selective agar plates such as Trypticase Soy agar with Yeast Extract
- Sheep blood agar plates
- Listeria biochemical identification gallery

STORAGE CONDITIONS

The kit components should be stored at 2 – 8 °C. The kit expiry date is shown on the box label.

SAFETY

Good laboratory practice (refer to EN ISO 7218) should be employed when using this kit. Safety clothing should be worn and skin contact with reagents avoided. Do not ingest.

Material and safety data sheets are available on request.

Contaminated material should be disposed according to local, state and federal regulations.

TEST PROCEDURE

PREPARATION OF REAGENTS

Important: Remove the reagents from the box. Allow the components to come to room temperature (15 – 30 °C) for at least one hour before using.

Have all reagents and samples ready for use so that the various materials can be added to the wells without delay.

Shake each vial manually or with vortex mixer before use.

Do not interchange reagents between kits with different batch numbers.

Preparation of Half Fraser, Fraser and L-PALCAM broths and plating media

Follow the manufacturer's instructions for preparation.

Dilution of Washing Buffer 20X

This buffer can be prepared in advance or during the first incubation step. See Assay Procedure, Step 4.

1. Dilute the provided Washing Buffer (twenty-fold concentrate, 20X) in distilled water to final 1X solution. Example: Add 60 mL of 20X Washing Buffer to 1140 mL of distilled water. If excess, record preparation date on container.

2. Mix and fill the washing device.

3. The prepared 1X Washing Buffer solution may be stored at room temperature (15 – 30 °C) for up to 1 month, or at 2 – 8 °C for up to 3 months.

Mixing of the Chromogen and the Substrate

The mixture of Chromogen and Substrate cannot be prepared more than 2 h in advance due to the potential loss of activity. See Asay Procedure, Step 6.

Mix 60 µL of the substrate and 60 µL of the Chromogen for each well used. Example: for 10 wells, mix 600 µL of substrate and 600 µL of Chromogen.

PREPARATION OF SAMPLES

Note that there are **four** Options for preparing samples.
Please review these Options prior to sample preparations.

OPTION A

For all products other than raw meat products or raw dairy products. Use only if screening for *L. monocytogenes*):

1. Homogenize X** g or X mL of the sample with 9X mL of Half Fraser broth in the stomacher.

2. Incubate at 30 °C ± 1 °C for 20 – 26 h.

3. After incubation, mix and inoculate 0.25 mL of culture in 10 mL full Fraser broth.

4. Incubate at 37 °C ± 1 °C for 24 – 26 h.

5. Heat 1 to 2 mL of enrichment medium for 20 min in a boiling water bath, then cool to room temperature. Store the unheated selective enrichment medium tubes for confirmation of positive ELISA results.

6. If the test cannot be done immediately after the 22 - 26 hours of Fraser Broth incubation, it can be stored for up to 72 hours at 2 – 8 °C before heat inactivation and then performing the ELISA test. The feasibility of storage was verified during the study done for NF VALIDATION certification.

OPTION B

For all products other than raw meat products or raw dairy products. Use this option if preliminary screening is for *Listeria* spp. using the Transia Plate *Listeria* kit.

1. Homogenize X** g or X mL of sample with 9X mL of Half Fraser broth in the stomacher.

2. Incubate at 30 °C ± 1 °C for 20 – 26 h.

3. After incubation, mix and inoculate 0.25 mL of culture in 10 mL full Fraser broth.

4. Incubate at 30 °C ± 1 °C for 22 – 26 h.

5. Extend incubation at 37 °C ± 1 °C for 16 – 24 h.

6. Heat 1 to 2 mL of enrichment medium for 20 min in a boiling water bath, then cool to room temperature. Store the unheated selective enrichment medium tubes for confirmation of positive ELISA results.

7. If the test cannot be done immediately after the 22 - 26 hours of Fraser Broth incubation, tubes can be stored for up to 72 hours at 2 – 8 °C before heat inactivation and then performing the ELISA test. The feasibility of storage was verified during the study done for NF VALIDATION certification.

OPTION C

For all raw meat products or raw dairy products:

1. Homogenize X** g or X mL of sample with 9X mL of Half Fraser broth in the stomacher.

2. Incubate at 30 °C ± 1 °C for 20 – 26 h.

3. After incubation, mix and inoculate 0.25 mL of culture in 10 mL full Fraser broth.

4. Incubate at 37 °C ± 1 °C for 40 – 48 h.

5. Heat 1 to 2 mL of enrichment medium for 20 min in a boiling water bath, then cool to room temperature. Store the unheated selective enrichment medium tubes for confirmation of positive ELISA results.

6. If the test cannot be done immediately after the 22 - 26 hours of Fraser Broth incubation, tubes can be stored for up to 72 hours at 2 – 8 °C before heat inactivation and then performing the ELISA test. The feasibility of storage was verified during the study done for NF VALIDATION certification.

OPTION D

For all raw meat products. This is faster than Option C and uses a different primary enrichment.

1. Homogenize X** g or X mL of sample with 9X mL of L- PALCAM broth in the stomacher.

2. Incubate at 37 °C ± 1 °C for 23 – 25 h.

3. After incubation, mix and inoculate 0.1 mL of culture in 10 mL full Fraser broth.

4. Incubate at 37 °C ± 1 °C for 22 – 26 h.

5. Heat 1 to 2 mL of enrichment medium for 20 min in a boiling water bath, then cool to room temperature. Store the unheated selective enrichment medium tubes for confirmation of positive ELISA results.

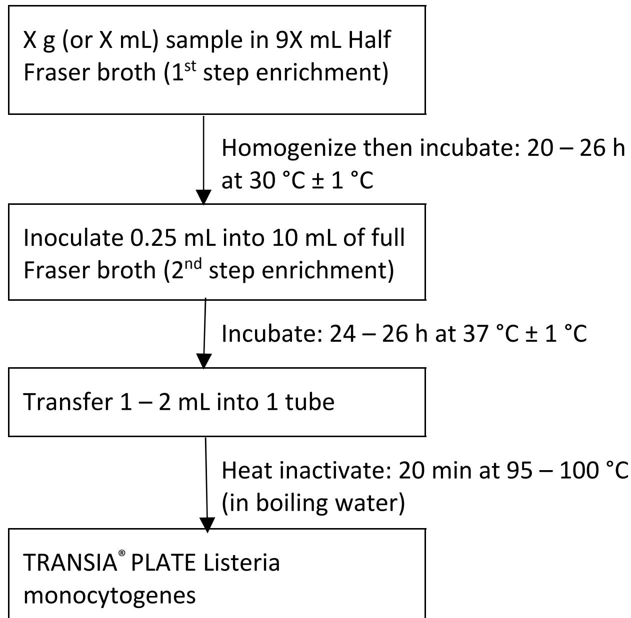
6. If the test cannot be done immediately after the 22 - 26 hours of Fraser Broth incubation, tubes can be stored for up to 72 hours at 2 – 8 °C before heat inactivation and then performing the ELISA test. The feasibility of storage was verified during the study done for NF VALIDATION certification.

(**) Tests portions weighing more than 25 g do not have NF Validation designation.

SAMPLE PREPARATION FLOW CHARTS

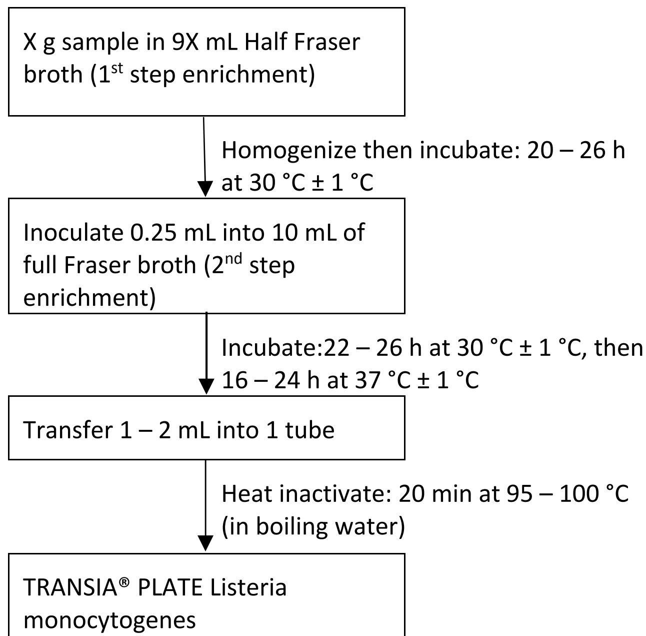
OPTION A

For all products other than raw meat products or raw dairy products. Use only if screening for *L. monocytogenes*).



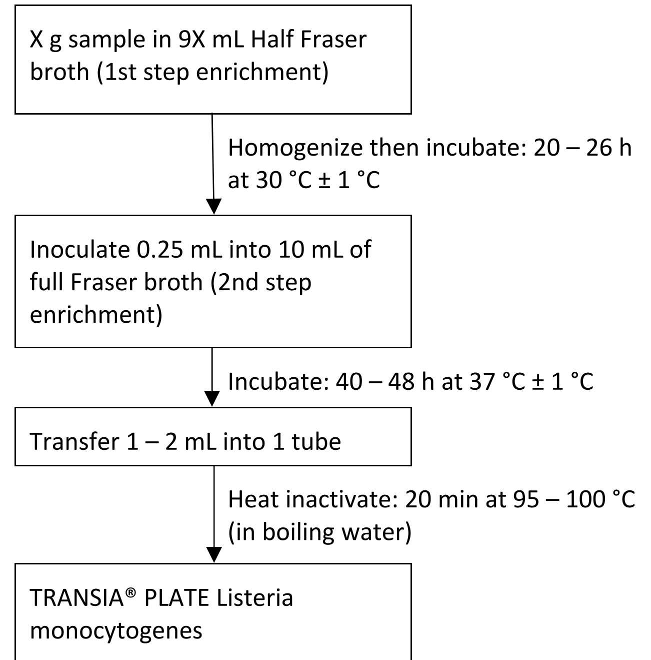
OPTION B

For all products other than raw meat products or raw dairy products. Use this option if preliminary screening is for *Listeria* spp. using the Transia Plate *Listeria* kit.



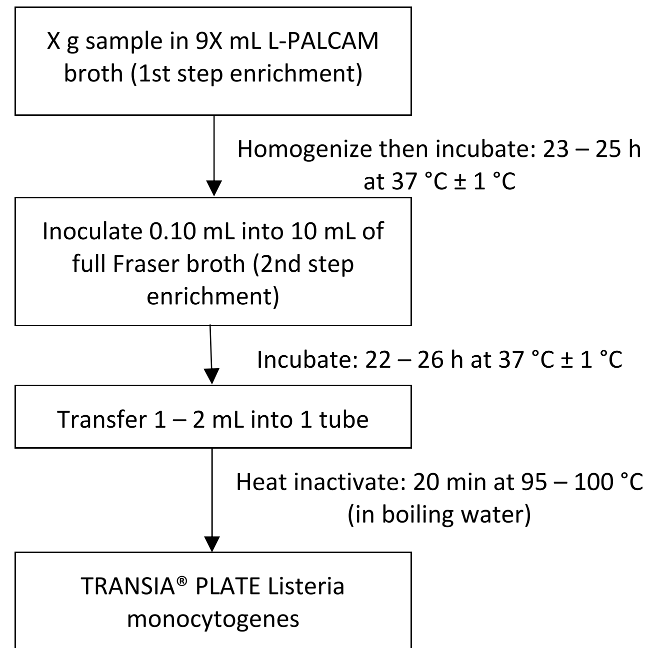
OPTION C

For all raw meat products or raw dairy products:



OPTION D

For all raw meat products. This is faster than Option C and uses a different primary enrichment.



Interpretation of Results

Validation of the test

The optical density of the positive control, OD (PC), must be greater than or equal to 1.00.

The optical density of the negative control, OD (NC), must be less than or equal to 0.20.

The test is validated if the optical densities of the controls meet the conditions described above.

Positive Threshold

Calculate the positive threshold as the average of the negative controls plus 0.10:

$$\text{Positive threshold} = \frac{\text{OD (NC1)} + \text{OD (NC2)}}{2} + 0.10$$

Negative Threshold

Calculate the negative threshold as the positive threshold multiplied by 0.9.

Negative threshold = positive threshold x 0.9

Positive Samples

A sample is considered positive for *L. monocytogenes* if the OD is greater than or equal to the positive threshold.

Negative Samples

The sample is considered negative for *L. monocytogenes* if the OD is less than the negative threshold.

Doubtful Samples

The sample is considered doubtful if the optical density is less than the positive threshold and greater than or equal to the negative threshold. This result must be confirmed by isolation of *L. monocytogenes* strains by streaking onto selective media plates using the second selective enrichment medium, followed by biochemical identification (see Confirmation of Positive Results).

Confirmation of Positive Results

Within the scope of NF Validation, any positive result must be confirmed

1. Use classical tests as described in the methods standardized by the CEN, ISO (including the purification step). First perform isolation starting from the selective medium used (Fraser broth) for enrichment.

2. Starting from the selective medium used (Fraser broth) for enrichment, isolate on chromogenic *Listeria* agar according to Ottavianni & Agosti or on RAPID'*L.mono* agar. The presence of characteristic colonies of *L. monocytogenes* on these agars is enough to confirm a positive result.

3. By using any other certified NF VALIDATION method that is essentially different from the present method. The detection protocol of the second validated method used for confirmation must be adhered to as a whole; that is, all steps prior to the intermediate step that leads to confirmation must be common to the two methods (example: common enrichment with the same medium). The two validated methods (one used in detection and the other used in confirmation) must therefore have a common origin.

In the case of conflicting results (presumptive positive by TRANSIA PLATE *Listeria monocytogenes*, but not confirmed, by the tests described in the methods above), the laboratory must use adequate validated means to check the validity of the result obtained.

We therefore strongly recommend using additional isolation media that use other biochemical characteristics of *L. monocytogenes*.

Performance characteristics

Detection Limit

The study performed within the scope of the NF Validation designation in 2007 demonstrated that the relative detection level of the method is between 0.2 and 2.8 cells/25 g of sample when all matrices and protocols are taken together.

Specificity

The study performed in 2007 within the scope of the NF Validation designation demonstrated specificity of the test for *L.monocytogenes*, except for a few strains of *L. ivanovii*.

Special application

The TRANSIA PLATE *Listeria monocytogenes* kit was also validated with TRANSIA Elisamatic (Gemini, Thunderbolt) and TRANSIA 4U automated equipment. The protocols are available on request. For further information regarding use of the kit in the automatic system, see the relevant User Manual.

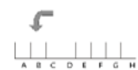
Warning

The reader used to interpret the results of the TRANSIA PLATE *Listeria monocytogenes* assay should be routinely calibrated and maintained as specified by the equipment manufacturer and in accordance with the laboratory's quality program.

Assay Procedure

Check that all reagents and samples are ready and at room temperature before they are used so as not to delay the distribution of the reagents into the microplate wells. Shake each bottle (manually or using a vortex) before use.

The rinsing step is very important. When rinsing, direct a strong stream of wash solution toward the bottom of the wells. Clean the microplate cover before each use.

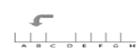


1. Place the necessary number of wells on the plate: 2 wells for the negative control, 1 well for the positive control, and 1 well per sample to be analyzed. Return the unused strips to their original package that also contains the dehydrating packet and seal it hermetically. Write the position of the controls and samples on the worksheet.

Sample and controls

2. Distribute 100 µL of the controls and samples into the assigned wells.

Conjugate

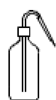


3. Distribute 100 µL of Conjugate into each well using a multipette. Be very careful to not touch the edges of the wells with the tip of the pipette. Mix the contents of the wells with a gentle circular motion. Cover the plate with the plate cover.

2 h

37 ± 2°C

4. Incubate at 37 ± 2 °C for 2 h (± 15 min). Prepare the Rinsing Buffer (see Preparation of Reagents). Just before the end of the incubation period, prepare the Substrate/ Chromogen solution (see Preparation of Reagents).



5 washes

5. Hold the plate firmly and empty its contents with a quick motion of the wrist. Rinse keeping Rinsing Buffer in the wells for 5 to 10 sec. Remove the buffer by turning the microplate upside-down, then drain it on absorbent paper by turning it upside-down and tapping it sharply several times. Repeat this rinsing procedure a total of 5 times.

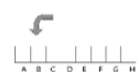
Substrate/Chromogen mixture

6. Distribute 100 µL of Substrate/Chromogen mixture into each well using a multipette and cover the microplate. Discard unused solution. Note: The Chromogen and the Substrate may be added without prior mixing. Add 50 µL of Substrate, then 50 µL of Chromogen into each well.

30 min at RT

7. Let incubate at room temperature (18 – 25 °C) for 30 min (± 5 min).

Stop Solution



8. Add 50 µL of Stop Solution into each well in the same order the Substrate/Chromogen mixture was added. Mix the contents of the wells so that the color change is complete: the blue solution will change to yellow.

9. Read the optical densities at 450/595 nm or 450 nm using a microplate reader (perform blank reading on air).

NF VALIDATION certificate granted by AFNOR Certification Mark to TRANSIA PLATE Listeria monocytogenes Validation as an alternative method for analysis for all products for human consumption and environmental samples, as compared to the reference method described in EN ISO 11290-1/A1:2004 according to the EN ISO 16140 standard.

For more information about the NF VALIDATION, please refer to the certificate available at www.afnor-validation.org or contact BioControl Systems. For more information about the NF VALIDATION certification's expiration date, please refer to the certificate TRA 02/08 - 03/01 available at http://nf-validation.afnor.org/en.

TRA 02/08 – 03/01
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

http://nf-validation.afnor.org/en (in English)

Product Warranty

Biocontrol Systems, Inc. (BCS) warrants this product to be free from defects in materials and workmanship, when stored under labeled conditions and used as intended until the expiration date stated on the package. BCS agrees during the applicable warranty period to replace all defective products after return to BCS. BCS shall not have obligation under this Limited Warranty to make replacements which result, in whole or in part, from negligence of the Buyer, or from improper use of the products, or use of the product in a manner for which it was not indicated. Buyer shall notify BCS of any products which it believes to be defective during the warranty period. At BCS option, such products shall be returned to BCS, transportation and insurance prepaid. BCS shall replace any such product found to be defective, at no charge. Should BCS examination not disclose any defect covered by the foregoing warranty, BCS shall so advise Buyers and dispose of the product in accordance with Buyer's instruction.

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