TRANSIA® AG *Listeria*

AOAC Official Method 996.14

AOAC Performance Tested Method 060802

An enzyme immunoassay for the detection of *Listeria* in environmental samples, foods, and ingredients.

Intended Use

TRANSIA® AG for *Listeria* is an enzyme immunoassay that detects *Listeria* in environmental samples and a variety of foods including fish, fruit and fruit products, meat and meat products (red meats, pork, and poultry), pasta, dairy products, eggs and egg products. The test is intended for use by laboratory personnel with appropriate microbiology training.

Directions for Use

A. Test Portion Preparation & Enrichment

Two Step Enrichment Protocol

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 minutes and incubate 26–30 h at 29–31 °C.

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 26–30 h at 29–31 °C.

b. Transfer 1 mL incubated mFB+LiCl to 9 mL Buffered *Listeria* Enrichment Broth (BLEB) (Appendix A). Incubate 22–26 h at 29–31 °C.

Single Step Enrichment Protocol

a. **Food samples** Add 25 g test portion to 225 mL of Demi-Fraser Broth (DFB) (Appendix A). Stomach / masticate for 2 minutes and incubate 46–54 h at 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 46–54 h at 29–31 °C.

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

B. Sample Inactivation

a. Transfer 1.0 mL incubated BLEB or DFB to a clean test tube.

b. Inactivate microorganisms at 95–105 °C for 15 min.

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.

C. Reagent Preparation

a. Before beginning the assay, prepare reagents and allow all kit components to reach room temperature (15–25 °C).

Wash Solution preparation — add 1.0 mL of Wash Solution Concentrate to 100 mL of deionized water. This volume is sufficient to wash 48 wells. Wash Solution is stable for 30 days at room temperature (15–25 °C).

D. Sample Analysis

a. Fit required number of microwells into the holder. Reseal unused microwells in foil pouch. In addition to the sample wells, allow three extra wells for 2 Positive Controls and 1 Blank.

b. Inactivated samples should be equilibrated to 25–37 °C prior to running the test.

c. Vortex mix samples and Positive Control before pipetting. A new pipette tip must be used for each sample. Pipette 100 µL of inactivated sample appropriate wells. Also pipette 100 µL of **Positive Control** into the appropriate wells. LEAVE BLANK WELL EMPTY. Cover plate with plastic lid and incubate 30 min at 35–37 °C. Do not stack anything on top of the microwell holder during incubation. Do not agitate plate during any incubation step.

d. Following incubation, wash all wells three times according to the following procedure.

Washing Procedure — Completely remove contents of wells with a microwell washer. Immediately fill wells completely with 250 µL of wash solution. Repeat twice for a total of three aspiration/wash cycles per step.

Alternate Washing Procedure — Remove contents of wells by inverting and vigorously tapping plate. Completely fill each well with wash solution using a clean wash bottle. Repeat twice for a total of three aspiration/wash cycles per step.

Note: Effective washing is critical to obtain accurate data. Avoid overfilling wells to prevent antigen carryover to adjacent non-reactive wells. Avoid under filling wells to prevent ineffective washing.

e. Immediately following removal of third wash, gently mix and add 100 µL of Conjugate Solution to each well, including Positive Control and Blank wells. Cover and incubate 30 min at 35–37 °C.

f. Following incubation, wash all wells three times as described above.

g. Immediately following third wash, gently mix and add 100 µL of **Substrate** to each well, including Positive Control and Blank wells. Cover plate with plastic lid and incubate 30 min at 35–37 °C. After incubation, DO NOT WASH WELLS. Proceed directly to Reading Results.

E. Reading Results

To obtain valid results the microwell plate reader must be calibrated against the Blank well before reading samples and Controls.

a. If results are not read immediately, add 50 µL of Stop Solution to each well and read within 1 hour.

b. Fit microwell plate reader with 405 or 410 nm filter immediately following incubation.

c. Standardize the reader by reading Blank well and adjusting optical density (OD) to zero.

d. Read the absorbance or each well, starting with the two positive controls, then the sample wells.

Note: When the reader is standardized to the Blank well, certain samples may read less than zero OD (a negative reading). This is not uncommon and indicates a negative result.

F. Interpreting Results

Control Value — The Positive Control absorbance values should be $\geq$ 0.8 OD. Absorbance values that fall below this value may indicate problems with washing procedure.

Cutoff Value — Calculate the average absorbance value of the two Positive Control readings and multiply by 0.25 to establish the Cutoff Value:

$$\textbf{Cutoff Value} = \frac{(PC1 + PC2)\ x\ 0.25}{2}$$

PC1 and PC2 = Positive Control absorbance values (OD).

Note: Microwell plate reader linear range is variable depending on manufacturer's specifications. If PC is reported as "over" or numerical value exceeds 2.5, use 2.5 for Cutoff Value calculation purposes.

Positive Results — Samples with absorbance values greater than or equal to the Cutoff Value are positive.

Negative Results — Samples with absorbance values less than the Cutoff Value are negative.

G. Confirmation

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

a. US FDA. 2003. Bacteriological Analytical Manual online, chapter 10. http://www.cfsan.fda.gov/~ebam/bam-toc.html

b. USDA-FSIS. 2008. Microbiology Laboratory Guidebook, chapter 8. http://www.fsis.usda.gov/science/microbiological_lab_guidebook/index.asp

H. Storage

Store TRANSIA® AG for *Listeria* kit components in the refrigerator (2–8 °C). Store unused microwells in the sealed foil pouch with desiccant.

Prepared wash solution can be stored at room temperature (15–25 °C) for up to 30 days.
Kit expiration is provided on the product pouch label.

I. Disposal

Decontaminate materials by autoclave, bleach, etc., in accordance with good laboratory practices and in accordance with local, state and federal regulations.

J. Precautions

Do not use test kit beyond expiration date indicated on the product pouch label.

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.

K. Materials

Each TRANSIA® AG for *Listeria* kit contains the following:

- TRANSIA® AG for *Listeria* test wells
- Wash Solution Concentrate
- Substrate
- Conjugate
- Positive Control
- Stop Solution

Other necessary materials not provided include:

- Media per Appendix A
- Autoclave
- Vortex mixer
- Analytical Balance
- Stomacher / Masticator machine
- Stomacher-type bags with filter or equivalent
- Incubator capable of maintaining 29–31 °C and 35–37 °C
- Micropipette capable of delivering 100 µL and 1,000 µL
- Water bath or equivalent capable of maintaining 95–105 °C

Appendix A - Enrichment Media Recipes

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. Alternatively, 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 8.5 g 3-3- (N-Morpholino) propanesulfonic acid (MOPS) free acid and 13.7 g 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.

Mix thoroughly until completely dissolved. Autoclave at 121 °C for 15 min.

Do not add ferric ammonium citrate additive to broth.

Product Information

TRANSIA® AG for *Listeria* must be used as described herein.

This product is not intended for human or veterinary use. Avoid contact with eyes, skin, and clothing. Avoid swallowing or taking product internally. Do not use TRANSIA® AG for *Listeria* reagents that have expired. Do no mix reagents from different TRANSIA® AG kit lots.

Manufacturing Entity

BioControl Systems, Inc, 12822 SE 32nd St, Bellevue, WA 98005, USA.

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

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

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