Listeria Antigen Detection (In Food)

Cat # 8324-3

INTENDED USE

The Diagnostic Automation, INC. *Listeria* assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for the presence of *Listeria* antigen.

SUMMARY

The genus *Listeria* is comprised of six species (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seegligeri* and *L. grayi*). Of these six species, only *L. monocytogenes* has been consistently associated with human illness although presence of the other species may indicate that contamination with *L. monocytogenes* is possible.

*L. monocytogenes* (LM) is widespread in nature and has been isolated from diverse sources such as soil, vegetation, water and marine sediments. Foodstuff's found contaminated with LM include raw lobster, raw and cooked shrimp, various vegetables (especially those grown organically) and raw and smoked fish. Lately, the greatest threat to the consumer has been the outbreaks of *Listeria* from ready-to-eat food products such as hot dogs and cold cuts.

The Diagnostic Automation, INC. *Listeria* ELISA is a rapid and reliable test, which significantly reduces the time required to screen foods for the presence of *Listeria*. The assay can detect LM, *L. innocua* and *L. welshimeri*. Primary enrichment cultures grown for 24 to 48 hours can be tested in less than one hour, allowing ELISA-negative product to be released sooner than with standard methods. Presumptively positive ELISA samples can be cultured further for confirmation by standard methods.
PRINCIPLE OF THE TEST
The Diagnostic Automation, INC. *Listeria* ELISA is a double antibody (sandwich) ELISA utilizing specific anti-*Listeria* antibodies coated to microwells. After addition of the sample and the enzyme conjugate, a positive reaction (indicating the presence of *Listeria* antigen) produces a deep blue color. Addition of the Stop Solution ends the assay and turns the blue color to yellow. The results may be read visually or with an ELISA reader.

REAGENTS
- Microwell test strips containing anti-*Listeria* polyclonal antibodies: 96 Test Wells
- Test strip holder: One (1)
- Reagent 1: One (1) bottle containing 11 ml
- Reagent 2: One (1) bottle containing 11 ml
- Reagent 3: One (1) bottle containing 11 ml
- Positive control: One (1) vial containing 1 ml of *Listeria* antigen in a buffered base.
- Negative control: One (1) vial containing 1 ml of buffered base.
- Chromogen: One (1) bottle containing 11 ml of chromogen tetramethylbenzidine (TMB).
- Wash Concentrate 20X: Three (3) bottles containing 25 ml of concentrated buffer and surfactant with preservative.
- Stop solution: One (1) bottle containing 11 ml of 1 M phosphoric acid.

Additional Materials Required:
- Stomacher (Tekmar stomacher lab-blender 400) or blender
- Shaking or similar incubator
- Microelisa plate reader capable of bichromatic readings at 450/620-650 nm (optional)
- Incubator, 30 °C
- Pipetter, 100 µl
- Disposable micropipette tips
- Microbiological media for preparation of necessary enrichment broth (see references)
- Appropriate containers for storage and disposal of materials potentially contaminated with infectious agents
- Data record sheets
- Disinfecting Solution
- 80 °C water bath

PRECAUTIONS
Do not use solutions if they precipitate or become cloudy. 
Exception: Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming. 
Do not add azides to the samples or any of the reagents. 
Some reagents contain a preservative. 
Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples. 
Though and complete washing steps is critical to proper performance of the test.

STORAGE CONDITIONS
Reagents, strips and bottled components: Store between 2 – 8 °C. 
Squeeze bottle containing diluted wash buffer may be stored at room temperature.
REAGENT PREPARATION

Wash Buffer - Remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

MEDIUM PREPARATION

1. Follow manufacturer’s directions. The kit has been validated for use with a first incubation using one-half strength Fraser’s broth followed by a second day incubation using whole strength Fraser’s. Test broth at 24 and 48 hours.

SAMPLE PREPARATION

1. Add 225 ml of broth to 25 g food product in a sterile stomacher bag or blender jar.
2. Stomach or blend sample and broth for 2 minutes.
3. Transfer aliquot of stomacher bag to one-half strength Fraser brother on shaker at 30 °C for 24 hours.
4. Transfer a sample into whole Fraser and incubate further at 30 °C for another 24 hours.
5. Remove a 1 ml aliquot from each sample and place in a separate clean screw top test tube. Place sample in an 80 °C water bath for 20 minutes to kill the culture. Cool sample and proceed to the test.

TEST PROCEDURE

1. Break off the required number of wells (number of samples plus 2) and place in strip holder.
2. Add 100 µl of the negative control to well #1 and 100 µl of the positive control to well #2.
3. Add 100 µl of the test sample to the appropriate well.
4. Incubate at room temperature (15 to 30 °C) for 30 minutes, then wash.*
5. Add 2 drops of Conjugate to each well.
6. Incubate for 30 minutes, then wash.
7. Add 2 drops of Chromogen to each well.
8. Incubate for 15 minutes.
9. Add 2 drops of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
10. Read results visually or at 450/620-650 nm.

* Each washing consists of dumping the contents of the wells into an appropriate container with disinfecting solution (e.g. 3% bleach in water) and using the diluted wash buffer to fill in each well, shaking out the contents and refilling the wells for a total of 3-5 times. Washings should be aggressive and plates should be slapped against absorbent paper after the last wash and before addition of the next reagent. Samples with sticky particulate matter may require more thorough washing than other samples. The potential exists for false positive results if the sample is not thoroughly washed from the well before addition of subsequent reagents.

Only one set of controls is required per run.
Read results within 4 hours from addition of Stop Solution.
All incubations are at room temperature (15-30 °C).

INTERPRETATION OF RESULTS - VISUAL

Positive: Any sample well that has obvious yellow color.
Negative: Any sample well that does not have obvious yellow color.
INTERPRETATION OF RESULTS – OD READINGS

Zero reader on air. Read all wells using a bichromatic reading with filters at 450nm and 620-650nm.

**Positive:** OD units equal to or greater than 0.08 units.
**Negative:** OD units less than 0.08 units.

QUALITY CONTROL

The Positive and Negative Controls must be run each time the assay is performed. For a valid run, the Negative Control must be below 0.08 ODs and the Positive Control greater than 1.0 OD units. If either Control is out of range, do not use the kit and contact Diagnostic Automation, INC. Technical Service at (818) 591-3030 or email at www.rapidtest.com.

**Problem:** Negative control has substantial color development.
**Correction:** Washings were insufficient. Repeat test with more vigorous washings.

REFERENCES


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<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
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<tr>
<td>2007-12-14</td>
<td>DA-Listeria Antigen Detection (In Food) 2009</td>
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