I. Intended Use

The Biomerica *Listeria monocytogenes* test is a lateral flow immunoassay for the rapid determination of the presence of *Listeria monocytogenes* in human stool samples. The test is intended for professional use only.

II. Summary and Explanation

Listeria monocytogenes is a rod-shaped gram-positive aerobic to facultative anaerobic bacterium belonging to the pathogenic genus *Listeria*. In 2001 it was included in the list of nationally notifiable diseases in the United States[1], where 1,600 cases and 260 fatalities are reported annually. The occurrence of *Listeria monocytogenes* is not as much as other gastrointestinal pathogens but it is nevertheless a considerable risk to public health particularly to pregnant women, neonates and immunocompromised people. The transmission of the bacteria occurs through consumption of contaminated processed dairy products (milk, cheese, etc.), fresh produce (cantaloupe, cabbage, etc.), meat, meat products and even seafood. *Listeria monocytogenes* infection is known as Listeriosis. When not promptly diagnosed and treated Listeriosis can become systemic and lead to severe complications such as meningitis, multiple cerebral abscesses and abortion in pregnant women[2]. Thus, reliable and rapid testing methods of *Listeria monocytogenes* are of utmost importance to safeguard public health and to avoid its resulting economic burden.

The methods of detection of *Listeria monocytogenes* were initially by culture and biochemical confirmation[4]. These involve selective media enrichment, plating, morphological and biochemical characterization. Though these methods remain to be the gold standard the processes are tedious, cumbersome and time consuming. These led to the advent of reliable and more rapid techniques such as the antibody-based Enzyme-Linked Immunosorbent Assay (ELISA)[4-6] and molecular testings[4-6-7] (PCR, RT-PCR, restriction enzyme analysis, DNA sequencing). For epidemiological purposes where strain identification is needed the latter is the method of choice. Future trends[4,8-9] are even extending the applications to DNA microarrays and oligonucleotide-based microarrays. However, to date, ELISA is the more cost-effective and the more commonly utilized method and is therefore performed in more laboratories. Immunoassay techniques when done in an immunochromatrographic platform become even more affordable and can be used even in the point-of-care setting.

III. Principle of the Procedure

The *Listeria monocytogenes* test utilizes the principle of non-competitive immunoassay. If *Listeria monocytogenes* are present in the sample at concentrations above the detection level, a *Listeria monocytogenes* specific antibody conjugated to colloidal gold binds to the antigen forming an antigen-antibody conjugate complex. An antibody which is immobilized in the Test region ("T") of the membrane is reactive to another epitope of the *Listeria monocytogenes*. The capture antibody will then react with the antigen-antibody conjugate complex. The captured complex will appear as a pink color band on the membrane. A distinct green color band will always appear at the Control region ("C") indicating that the test is functioning properly.

IV. Materials Provided

1. Test device in foil pouch
2. Stool collection vial with buffer
3. Instruction manual

V. Materials Required but Not Provided

Timer and Gloves

VI. Warnings and Precautions

Safety Precautions: Human stool should be handled as if capable of transmitting infectious agents. It is recommended that these specimens be handled using established good laboratory working practices. The test should be discarded in a proper biohazard container after testing.

For *in vitro* diagnostic use: Do not use the kit beyond the expiration date printed on the outside of the foil pouch. Discard all used test devices into a proper biohazard container. The test should remain in the sealed pouch until use. Do not use the test if pouch is damaged. The test must be carried out within 2 hours of opening the sealed pouch.

VII. Kit Storage

The *Listeria monocytogenes* tests can be stored refrigerated or at room temperature (2-30°C). The test is usable until the expiration date stamped on the foil pouch.

VIII. Quality Control

Although the *Listeria monocytogenes* test contains an internal quality control (green color band in the Control region) good laboratory practice recommends the daily use of an outside control to ensure proper kit performance. Quality control samples should be tested according to the quality control requirements established by your laboratory.

IX. Test Procedure

Pre-treatment of the collected stool samples (see illustration 1):
Unscrew the cap of the provided stool collection vial filled with buffer. Using the stick on the cap collect about a pea size (250 mg) of solid sample. Place the stick back into the vial and close it. For liquid sample, aspirate about 250 µL into the vial and close it. Shake the vial thoroughly until the sample is dispersed well in buffer Use a separate specimen collection vial for each sample being tested.

Test Procedure (see illustration 2)
Always allow the test devices, stool samples and stool collection vials to reach room temperature (15-30ºC) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the *Listeria monocytogenes* device from its sealed pouch and use it as soon as possible.
2. Shake the specimen collection vial to ensure good sample dispersion. Break off the tip of the vial.
3. Dispense exactly 4 drops or 100 µL into the specimen well (S). Start the timer. Use a separate device for each sampl.e
4. Read the result at **10 minutes** after dispensing the sample.

Illustration 1

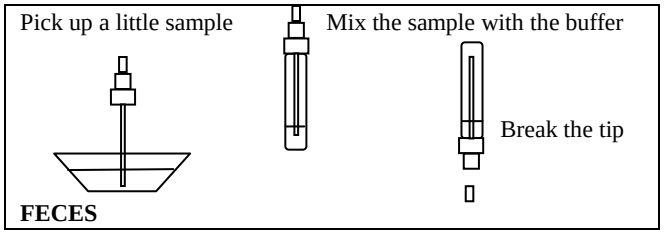
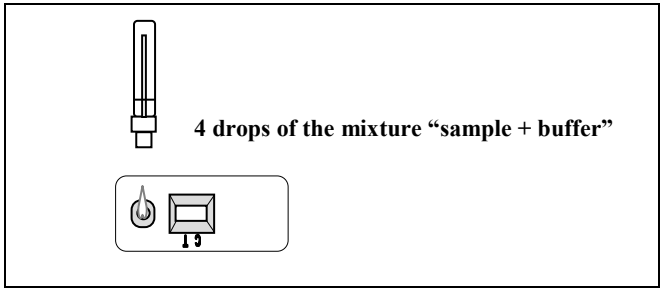
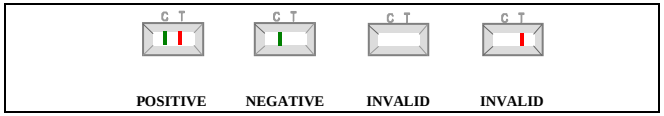


Illustration 2



X. Interpretation of Results

Illustration 3



POSITIVE: Two lines appear across the central window, in the Test region (a pink test line in the region marked with the letter T) and in the Control region (a green control line in the region marked with the letter C).

NEGATIVE: Only one band appears in the Control region (a green control line in the region marked with the letter C).

INVALID: A total absence of the control line regardless of the appearance of the test line. Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the test materials are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit and contact your local supplier.

The intensity of the pink colored band in the test line region (T) will vary depending on the concentration of antigens in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.

Internal procedural controls are included in the test:
- A green line appearing in the control region (C) confirms sufficient specimen volume and correct procedural technique.

XI. Performance Characteristics

Detection limit

The detection limit test is $6.25x10^4$ bacteria/mL.

Sensitivity and specificity

Thirty-two samples were studied using the Biomerica Listeria Test. Results of 99% sensitivity and >96% specificity were obtained. The results of all samples were confirmed by Merck Singlepath® L'mono.

The use of a mouse monoclonal antibody in *Listeria* Device ensures high degree of specificity for the detection of these bacteria. The antibodies used in this test recognize *Listeria* epitopes found in stool of patients, as well as in preparations from the bacteria cultures in vitro.

Cross-reactivity

A study was performed to determine the cross reactivity of the Biomerica Listeria Test. No cross reactivity was found to common intestinal pathogens, other organisms and substances present in feces *(H. pylori, Escherichia coli O157:H7, Astrovirus, Rotavirus, Adenovirus, Campylobacter, Salmonella).*

XII. Limitations of the Test

1. The test is a qualitative method for stool samples only. It is not meant for quantitative analysis.
2. Excessive amount of the sample applied to the specimen well can make the background appear dark which may lead to a difficult interpretation of the result. In such a case, the sample should be diluted and the test repeated.
3. If a negative result is obtained and clinical symptoms persist, additional testing using other clinical methods and/or seeing a physician is recommended. A negative result does not preclude the possibility of *Listeria monocytogenes* infection.
4. Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

XIII. References

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9. M Doumith, C Jacquet, V Goulet, C Oggioni, F Van Loock, C Buchriser, P Martin, Use of DNA arrays for the analysis of outbreak-related strains of *Listeria monocytogenes*, Intl J med Microbiol 2006 296 p 559-562

XIV. Ordering information

Ordering Information:

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EZ-LISTERIA

One-Step *Listeria monocytogenes* Rapid Test

January 2012

REF **1008**

Immunoassay for the qualitative determination of *Listeria monocytogenes* in human stool specimen

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FOR PROFESSIONAL USE ONLY

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