Hygiena™
Lateral Flow System

Listeria Test Kit
Part ASY2031

Contents

Listeria test strips – 45

Package insert

Intended Use

Hygiena™ Lateral Flow System *Listeria* tests have been designed to detect *Listeria* species in a variety of ready-to-eat foods (deli turkey, pepperoni, hot dogs, roast beef, potato salads), dairy (ice cream, soft cheese, milk), fish products (cooked shrimp and smoked fish) and on environmental surfaces (including rubber, painted concrete and stainless steel). The test permits presumptive detection and identification of the target pathogen when present at levels of one *Listeria* organism per 25 grams of sample. The test provides a simplified process with results in a minimum of 40 hours. *Listeria* test strips are designed to be used by trained technicians who follow good microbiology laboratory practices. Although the test strips are easy to use, the protocol involves the use of potentially hazardous microorganisms so appropriate safety practices must be observed.

Field of use: Data obtained from the Hygiena™ Lateral Flow System should not be used for human diagnostic or human treatment purposes. This product is not approved by the United States Food and Drug Administration or any other U.S or non-U.S. regulatory agency for use in human diagnostics or treatment. The Hygiena™ Lateral Flow System should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program. Not approved for clinical diagnosis. Use only for research and development, including quality assurance and quality control testing, under supervision of technically qualified persons. Read the Limitation of Warranty and Liability before using product.

Principle of the Method

This immunoassay test uses a double antibody sandwich format. An antibody that specific to *Listeria* is sprayed and immobilized in a test line on the surface of a membrane. A second antibody reagent, also recognizing *Listeria* and labeled with colloidal gold, is contained within a reagent pad upstream from the test line on the membrane.

As liquid sample moves by capillary action through the reagent pad, the antibody-gold reagent specifically binds to *Listeria* and moves with the sample into the test membrane. Moving up the test membrane, the sample passes through the test line, where the immobilized *Listeria* antibody captures the *Listeria*-antibody-gold complex, forming an antibody-*Listeria* sandwich. The test line then develops a red color. In the absence of *Listeria*, no antibody-*Listeria* sandwich is formed, and the test line does not turn red.

Reagents immobilized at the control line capture excess gold reagent passing through the test line. This causes the control line to develop a red color, which indicates that the test flowed correctly on the strip.

Therefore, a single (control) line on the membrane indicates a negative sample, and two (sample and control) lines indicate a positive sample.

Storage and Shelf Life

Store the Hygiena™ Lateral Flow System *Listeria* test strips at room temperature (15-30°C) in the supplied canister, which contains a desiccant liner. Storage conditions higher than room temperature may adversely affect performance of the test strip.

After opening the canister, take care to re-seal the lid so that the strips are protected from moisture. A humidity indicator included in each canister displays blue dots, which turn pink if the strips are exposed to moisture. Follow suggestions on the indicator if the dots turn pink.

Do not use test strips after the expiration date on the canister label.

Required Materials

Hygiena™ Lateral Flow System *Listeria* Test Kit
(PN ASY2031 – 45 test strips per kit)

Hygiena™ Lateral Flow System *Listeria* Media
(PN MED2008 – 500 g Base and 10 g Supplement)

Balance - 25 to 1000 grams with 0.2 g sensitivity

Stomacher with filtered bags
(Seward Stomacher® 400 Circulator or equivalent)

Incubator capable of maintaining 30 ± 0.5°C

Plastic test tubes (12 x 75 mm) with rack

Water bath capable of reaching 100°C or hotplate with stainless steel/Pyrex dish (40 cm x 15 cm)

Pipettes (400 µL) for transfers

For environmental samples:
Sponges – cellulose, non-bactericidal
Swabs – sterile, cotton-tipped
D/E neutralizing broth

Listeria Test Protocol

1. Prepare enrichment broth

1.1 Sterilize one liter of water either by autoclaving or filtering (pore size 0.2 µm) into a sterile container. Equilibrate to 20-30°C.

1.2 Add 53.0 ± 0.2 grams of Hygiena™ Lateral Flow System *Listeria* Media Base and 1.0 ± 0.05 grams of Hygiena™ Lateral Flow System *Listeria* Media Supplement to the sterilized water. Shake vigorously until the media is dissolved. Use immediately. Final pH should be 7 ± 0.2.

Note: Use enrichment broth within 5 hours of preparation (room temperature) or 24 hours at 4°C. For best results, use the broth as soon as it is prepared.

Alternative enrichment broth preparation

A1. Add 53.0 ± 0.2 grams of Hygiena™ Lateral Flow System *Listeria* Media Base to 1 liter of room temperature distilled water. Mix at 20-30°C until completely dissolved.

A2. Autoclave base broth at 121°C for 15 minutes, then allow it to cool to room temperature.

A3. Just prior to use, add 1.0 ± 0.05 grams of Hygiena™ Lateral Flow System *Listeria* Media Supplement to the base broth.

Prepared Base broth can be stored at 4°C for up to two weeks. After refrigeration, broth should be equilibrated to 20-30°C before use. Do not autoclave the broth after Supplement has been added

2. Collect samples

As part of good laboratory practice, we recommend that you run two positive controls (Listeria species and Listeria monocytogenes) and a negative control with your samples.

Food

2.1.1 Add 25 grams of sample to a sterile Stomacher bag.

Note: If polypropylene bottles are used for sample enrichment instead of Stomacher bags, the bottles should be lined with a disposable plastic bag to eliminate potential protein carryover, which will produce erroneous results.

2.1.2 Add 225 mL of prepared enrichment broth (Base plus Supplement at 30°C) to the Stomacher bag containing the sample.

Sponges

2.2.1 Add 100 mL of prepared enrichment broth (Base plus Supplement at 30°C) to a sterile bag.

2.2.2 If not pre-moistened, moisten 7.5 x 4 cm sponge with 10 ml D/E neutralizing broth.

2.2.3 Swipe a 4-inch square surface by wiping the sponge backward and forward for 30 seconds.

2.2.4 Place sponge in bag of prepared enrichment broth, seal and transport at room temperature to the laboratory.

Swabs

2.3.1 Add 10 mL of prepared enrichment broth (Base plus Supplement at 30°C) to a sterile bag.

2.3.2 If not pre-moistened, moisten sterile cotton-tipped swab with D/E neutralizing broth.

2.3.3 Swipe a 1-inch square surface by rubbing the swab backward and forward for 30 seconds.

2.3.4 Place swab in bag of prepared enrichment broth, seal and transport at room temperature to the laboratory.

3. Enrich samples

Place the sample bag into a Stomacher device and stomach for 30 seconds.

3.1 Close the bag loosely and incubate for 40 hours at 30 ± 0.5°C.

Note: Sample bags should be closed loosely to allow air exchange during sample enrichment and optimize pathogen growth and antigenic expression.

4. Boil enriched samples

4.1 Arrange and mark test tubes for each sample in a rack.

4.2 Transfer 400 µL of enriched sample to a plastic tube.

4.3 Place rack of tubes into a boiling water bath (100°C) for at least 5 and no more than 15 minutes.

4.4 Remove rack of tubes from water bath and allow to cool to room temperature.

5. Test samples

5.1 Remove the required number of test strips from the canister. Do not remove the label on the strip.

5.2 Insert the strip with **arrows facing downwards** into the tube.

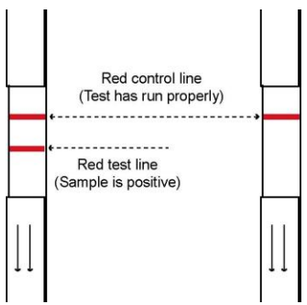
5.3 Allow the strip to develop for **10 minutes**, then check the results as follows:

a. At least one line, the control line, should always develop. A red line in this position indicates that the strip is functioning properly.

b. If at 10 minutes the test strip only shows a clearly visible control line, then the sample is negative for *Listeria*.

c. A red line appearing below the control line is the test line and indicates a positive result. If the test strip displays two (2) red lines, the test is complete and the sample is positive for *Listeria*

d. If no control line develops within 10 minutes, the test is invalid and needs to be repeated.



Note: Test strip results should always be interpreted after 10 minutes. Test strips interpreted after 20 minutes are invalid.

6. Confirm positive results

Use enriched samples **prior to boiling** to confirm presumptive positive results according to the following standard confirmation methods:

- Use the USDA-FSIS protocol to confirm roast beef, deli turkey, hot dogs, pepperoni, and all environmental samples.

  USDA-FSIS – *Isolation and Identification of* Listeria monocytogenes *from red meat, poultry, egg and environmental samples* (Chapter 8).

- Use the FDA protocol to confirm ricotta cheese, smoked fish, cooked shrimp, whole milk, ice cream, and potato salad.

  FDA-BAM – *Detection and Enumeration of* Listeria (Chapter 10).

Disposal

Decontaminate used test strips, pipettes and enrichments by autoclaving or according to your site practices. Ensure all biohazardous waste is disposed of according to local, regional and national regulations.

Precautions

Listeria monocytogenes is a significant human pathogen. Immunocompromised individuals and pregnant women should not be in the vicinity of samples being enriched or tested for *Listeria* as they represent particularly susceptible populations. Extreme care should be used in handling samples, enriched media and used test strips that could potentially contain this pathogen.

Validation

The Hygiena™ Lateral Flow System *Listeria* test has been validated to detect one *Listeria* cell per 25 grams of sample. The applicable sample matrices are ready-to-eat foods and environmental samples. Please call 302-695-5300 or visit www.hygiena.com for more information.

Samples of this test kit model were independently evaluated by the AOAC Research Institute and were found to perform to the producer's specifications as stated in the test kit's descriptive insert. The producer certifies this kit conforms in all respects to the specifications originally evaluated by the AOAC Research Institute as detailed in the Performance Tested Certificate number 080501.

Note: Although this test system is capable of detecting target pathogen present in enrichment media at the detection level sensitivity of the test strip, the successful detection of the target pathogen in a specific food matrix is dependent upon the ability of the target pathogen to adequately reach the test's detection level in the enrichment media. This ability may be influenced by a variety of factors, including but not limited to competitive flora, sample matrix, sample size and condition of the target pathogen.

Technical Assistance

For questions or comments, please contact your local distributor. You can also call 302-695-5300 in the U.S. or email diagnostics.support@hygiena.com.

Limitation of Warranty and Liability

NOTICE: READ THIS LIMITATION OF WARRANTY AND LIABILITY BEFORE USING THE HYGIENA™ LATERAL FLOW SYSTEM. If the terms are not acceptable, notify Hygiena immediately and arrangements will be made for return of the unused test strips and/or media to Hygiena and for the refund of the purchase price, less shipping costs. USE OF HYGIENA™ LATERAL FLOW SYSTEM TEST STRIPS AND/OR MEDIA CONSTITUTES AN ACCEPTANCE OF ALL TERMS AND/OR CONDITIONS OF THIS LIMITATION OF WARRANTY AND/OR LIABILITY. Any additional or different terms in User's purchase form(s) are material alterations and hereby rejected.

Hygiena warrants that the Hygiena™ Lateral Flow System test strips and/or media will be free of defects in materials and workmanship when used in accordance with the applicable instructions to the expiration date marked on the product label. Application protocols published by Hygiena are intended as guidelines; each User is expected to validate the applicability of each protocol to their individual applications.

HYGIENA MAKES NO OTHER WARRANTY, EITHER EXPRESSED OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING.

The sole obligation of Hygiena with respect to the foregoing warranties shall be, at its option, to either replace or to refund the purchase price of the Product(s) that proves defective in materials or workmanship within the warranty period, provided the User notifies Hygiena promptly of any such defect.

The accuracy of the Hygiena™ Lateral Flow System can be affected by factors over which Hygiena has no control, including, without limitation, the use of the test strips and/or media in a manner that is contrary to the conditions of use, the procedures or the instructions specified by Hygiena. Because of the large number of factors over which Hygiena has no control, Hygiena makes no promise or guarantee of the accuracy of results obtained from the use of the Hygiena™ Lateral Flow System. In particular, Hygiena disclaims any warranty or liability and assumes no responsibility whatever for the failure of the Hygiena™ Lateral Flow System due, in whole or in part, to User's failure to (a) properly maintain equipment, (b) maintain specified operating or storage conditions, (c) follow the specified instructions, or (d) use the proper microbiological techniques consistent with the standard of care accepted in the industry for the proper collection, storage, handling and preparation of the sample.

Externally caused failures, such as improper sample preparation, improper storage or loading of reagents, electrical outages, or out-of-specification environmental conditions are not covered under this warranty. Circumstances beyond the reasonable control of Hygiena, including fire, explosions, accidents, flood, labor trouble or shortage, war, act of or authorized by any government, inability to obtain suitable material, equipment, fuel, power or transportation, or acts of God are not covered under this warranty.

The Hygiena™ Lateral Flow System is designed to test only for the presence of the target organisms specified in the particular assay. Hygiena™ Lateral Flow System has been tested against many, but not all, strains of the target within the sample types specified in the package insert. Hygiena, therefore, cannot and does not make any representation or warranty that the Hygiena™ Lateral Flow System is capable of detecting every bacterium in the target genus, serotype, or species in any sample source. Accordingly, the Hygiena™ Lateral Flow System should not be used as the sole test for the release of User's products, nor should it be used as the sole basis for determining the safety of User's products.

USER ASSUMES ALL RISKS IN USING THE HYGIENA™ LATERAL FLOW SYSTEM. HYGIENA OR ITS AFFILIATES, SUPPLIERS, DISTRIBUTORS, ITS LICENSORS OR REPRESENTATIVES SHALL HAVE NO LIABILITY TO BUYER OR TO ANY OTHER PERSON OR ENTITY FOR ANY INDIRECT, INCIDENTAL, SPECIAL, OR CONSEQUENTIAL DAMAGES WHATSOEVER, INCLUDING, BUT NOT LIMITED TO, LOSS OF REVENUE OR PROFIT, LOST OR DAMAGED DATA OR OTHER COMMERCIAL OR ECONOMIC LOSS EVEN IF CAUSED BY THE NEGLIGENCE OF HYGIENA OR ITS AFFILIATES, SUPPLIERS, DISTRIBUTORS, ITS LICENSORS OR REPRESENTATIVES AND/OR IF HYGIENA HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, AND/OR IF THEY ARE FORESEEABLE.

Not an insurer: By selling Hygiena™ Lateral Flow System products or services, Hygiena or its representatives do not become an insurer of User's business. Hygiena or its representatives do not and cannot know all of the potential consequences to User's business of a failure of the Hygiena™ Lateral Flow System to perform as expected. That is why Hygiena does not agree to be responsible for User's incidental or consequential business losses in the event the Hygiena™ Lateral Flow System fails to perform as expected. For the same reason, Hygiena has limited its liability to the cost of the product. For additional protection against the risk of loss, User should consult an insurance broker and buy insurance suitable to the risks of its particular business.