RapidChek® *Listeria* species Food System

Part #: 7000171, 7000175, 7000176, 7000179, 7000181, 7000182

AOAC Approved Protocols:

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 020401

Intended Use

The RapidChek *Listeria* Lateral Flow Test Kit is designed to detect *Listeria* species in a variety of ready to eat foods, dairy products, fish products and environmental surfaces. The test kit permits the presumptive detection and identification of the target pathogen by a one step process in a minimum of 40 hours when the target pathogen is present at levels of one *Listeria* organism per 25 grams of sample.

Principle of the Assay

This immunoassay test uses a double antibody sandwich format. An antibody specific to *Listeria* is sprayed and immobilized in a line on the surface of a membrane comprising a "test line". 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 the sample moves by capillary action from the filter pad into the antibody–gold pad, the antibody–gold reagent specifically binds *Listeria* and moves with the liquid sample into the test membrane. The sample passes through the test line where the immobilized *Listeria* antibody captures the *Listeria*–antibody–gold complex, causing the formation of an antibody–*Listeria* "sandwich" and development of red color at the test line. Antibody–*Listeria* sandwiches are not formed in the absence of *Listeria*, resulting in no red color development at the test line. Reagents immobilized at the control line capture excess gold reagent passing through the test line. The presence of red color at the control line indicates that the test strip flowed correctly. Therefore, the presence of only one line (control line) on the membrane indicates a negative sample and the presence of two lines indicates a positive sample.

Content of Kits

7000171

Description	Quantity
RapidChek *Listeria* Test Strips	45
Transfer pipettes (400 µL)	45
Plastic tubes (12 x 75mm)	45
Package Insert/s	

7000175

Description	Quantity
RapidChek *Listeria* Test Strips	45
Transfer pipettes (400 µL)	45
Plastic tubes (12 x 75mm)	45
RapidChek *Listeria* Media	500g
RapidChek *Listeria* Supplement	10g
Package Insert/s	

7000176

Description	Quantity
RapidChek *Listeria* Media	500g
RapidChek *Listeria* Supplement	10g

7000179

Description	Quantity
RapidChek *Listeria* Media	5.3kg
RapidChek *Listeria* Supplement	100g

7000181

Description	Quantity
RapidChek *Listeria* Test Strips	450
RapidChek *Listeria* Media	5.3kg
RapidChek *Listeria* Supplement	100g
Package Insert/s	

7000182

Description	Quantity
RapidChek *Listeria* Test Strips	450
Package Insert/s	

Storage of Reagents

The RapidChek *Listeria* Media Supplement must be stored refrigerated (2-8°C). The RapidChek *Listeria* Test Kit (media and strips) should be stored at room temperature (15-30°C). The RapidChek *Listeria* test strips used in this kit must be kept in the canister with the humidity indicating card. The humidity indicating card should be blue in color. After opening the canister, care should be taken to re-seal the closure to protect the strips from moisture.

Materials Required but Not Supplied

Stomacher-type bags or equivalent
Stomacher machine (optional)
Plastic test tube rack (Fisher Scientific, dimensions 20 cm x 10 cm, holds 75 x 12mm tubes)
Hotplate or heating block (capable of reaching 100°C)
Incubator capable of maintaining 30 ± 2°C
Balance with an accuracy of ± 0.05 grams

Media Preparation and Sample Enrichment

A. Media Preparation, Not Autoclaved

1. Sterilize one liter of water either by autoclaving for 15 minutes at 121°C or filtration into a sterile container and equilibrate to 20-30°C.
2. Weigh 53.0 ± 0.2g of RapidChek *Listeria* Media and 1.0 ± 0.05g of RapidChek *Listeria* Media Supplement and add to the sterilized water. Shake vigorously until the media is completely mixed.
3. Rehydrated media should be used within 3 hours of preparation if stored at room temperature or within 24 hours if stored at 4°C. For best results, use the media as soon as it is prepared.

B. Alternative Option: Media Preparation, Autoclaved

1. Add 53.0 ± 0.2g of RapidChek *Listeria* Media to 1 liter of room temperature distilled water. Shake until completely dissolved.
2. Autoclave at 121°C for 15 minutes.
3. Allow the RapidChek *Listeria* Media to cool to room temperature. Just prior to use, add 1.0 ± 0.05g of RapidChek *Listeria* Supplement to the media base that has been equilibrated to 30°C.

Note: The media base (with or without supplement) can be stored at 4°C or room temperature for up to four weeks. After refrigeration, media should be equilibrated to 20-30°C before adding supplement and before use.

C. Sample Enrichment

1. Add 25 grams of the sample to be analyzed into a sterile Stomacher bag or equivalent.
2. Add 225 mL of the prepared RapidChek *Listeria* Media with Supplement to the Stomacher bag containing the sample.
3. Place the sample bag into a Stomacher device and stomach for 30 seconds or hand massage from the bottom of the bag.
4. Close the bag loosely and incubate for 40 up to 48 hours at 30 ± 2°C.
5. Proceed to the RapidChek *Listeria* Detection Procedure.

RapidChek *Listeria* Detection Procedure

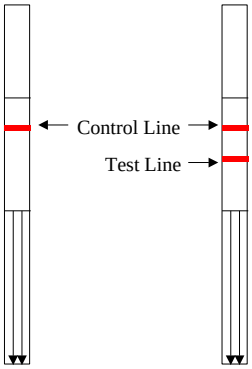
1. Take one transfer pipette from the bag (or utilize a calibrated pipette capable of dispensing 400 µL). Squeeze and hold the bubble on top of the pipette and place into the sample enrichment.
2. Release the bulb completely filling the barrel of the pipette.

Note: The bubble will not completely fill with solution.

3. After placing the correct number of supplied plastic tubes into a test tube rack, transfer an aliquot of enriched broth to each tube.
4. Place the rack of tubes into a boiling water bath (100°C) or heat block for 5 to 15 minutes.
5. Remove tubes after boiling and allow them to cool to room temperature prior to testing.
6. Remove the required number of test strips from the canister.

7. Insert the strip with **arrows facing down** into the tube.
8. Let the strip develop for 10 minutes.
9. The appearance of **one red line** (control) on the strip indicates a negative result.
10. The appearance of **two red lines** on the strip indicates a positive result.

Illustration of Positive and Negative Results



Negative Result | Positive Result

At least one line, the Control Line, should always develop. A red line in this position indicates that the strip is functioning properly. If the test strip displays 2 red lines, the test is complete and the sample is a presumptive positive for *Listeria* species.

If at 10 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for *Listeria* species. If no control line develops within 10 minutes, the test is invalid and needs to be repeated.

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

Confirmation

Presumptive positive results must be confirmed by the BAM or the USDA/FSIS Method for the detection of *Listeria*. It is recommended that roast beef, deli turkey, hot dogs, pepperoni and all environmental samples be confirmed using the USDA/FSIS Method. It is recommended that ricotta cheese, smoked fish, cooked shrimp, whole milk, ice cream and potato salad be confirmed using the FDA/BAM protocol.

Enriched media samples used in the RapidChek *Listeria* test procedure **prior to boiling** can be used for this confirmation. For the confirmation procedures see the following:

(1) FDA/BAM – Detection and Enumeration of Listeria monocytogenes (chapter 10) in US Food, Drug and Administration, Center for Food Safety and Applied Nutrition, Bacteriological Analytical Manual http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm071400.htm

(2) USDA/FSIS – *Isolation and Identification of* Listeria monocytogenes *from red meat, poultry, egg and environmental samples*. (Chapter 8, revision 8) in USDA-FSIS Microbiology Laboratory Guidebook http://www.fsis.usda.gov/PDF/MLG-8.pdf

For all procedures positive and negative controls for enrichment, detection and confirmation are recommended as part of Good Laboratory Practice.

Disposal

Decontaminate RapidChek test strips, pipettes and media by autoclave, bleach, etc., in accordance with local, state and federal regulations.

Positive Controls

Romer Labs recommends two controls for use with the RapidChek *Listeria* kit:

(1) For RapidChek complete enrichment/detection system – *L. monocytogenes* ATTC 19115, available from American Type Culture Collection at www.atcc.org

(2) For RapidChek Lateral Flow Strip – *Listeria*, genus specific positive control available from Kirkegaard Perry Laboratories at www.KPL.com

Product Shelf life

The expiration date for the product is displayed along with the part and lot number on the Product Label located on the re-sealable canister. The test strips have a 1 year shelf life from the date of manufacture under desiccated room temperature (15-30°C) conditions.

Contact customer service with any questions about product shelf life.

Precautions

1. *Listeria monocytogenes* is a significant human pathogen. Immuocompromised individuals, such as 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 which could potentially contain this pathogen. Ensure all biohazardous waste is disposed of appropriately.
2. 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.
3. Storage conditions higher than room temperature may adversely affect performance of the test strip.
4. Follow standard Good Microbiological Practices where appropriate.

Warranties and Liabilities

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