RapidChek® *Listeria* species Test System

Part #: 10001173, 10001361 10001364, 10001697 10001365, 10001699 10001700, 10001366 10001369, 10001370 10001372, 10001412

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RapidChek® *Listeria* species Test System

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 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.

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Content of Kits

10001361 (Test Kit)

Description	Quantity
RapidChek® *Listeria* Test Strips (10001173)	45
Transfer pipettes (400 µL)	45
Plastic tubes (12 x 75 mm)	45
Package Insert/s	

10001364 (Environmental System)

Description	Quantity
RapidChek® *Listeria* Test Strips (10001173)	90
Transfer pipettes (400 µL)	90
Plastic tubes (12 x 75 mm)	90
RapidChek® *Listeria* Media	500 g
RapidChek® *Listeria* Supplement	10 g
Package Insert/s	

10001697 (Environmental Media Pouch Kit)

Description	Quantity
RapidChek® *Listeria* Test Strips (10001173)	45
Transfer pipettes (400 µL)	45
Plastic tubes (12 x 75 mm)	45
Pre-weighed media pouches	3
Pre-weighed supplement pouches	3
Package Insert/s	

10001365 (Food System)

Description	Quantity
RapidChek® *Listeria* Test Strips (10001173)	45
Transfer pipettes (400 µL)	45
Plastic tubes (12 x 75 mm)	45
RapidChek® *Listeria* Media	500 g
RapidChek® *Listeria* Supplement	10 g
Package Insert/s	

RapidChek® *Listeria* species Test System

10001699 (Food Media Pouch Kit)

Description	Quantity
RapidChek® *Listeria* Test Strips (10001173)	45
Transfer pipettes (400 µL)	45
Plastic tubes (12 x 75 mm)	45
Pre-weighed media pouches	10
Pre-weighed supplement pouches	10
Package Insert/s	

10001700 (Food Sample Pack)

Description	Quantity
RapidChek® *Listeria* Test Strips (10001173)	5
Transfer pipettes (400 µL)	5
Plastic tubes (12 x 75 mm)	5
RapidChek® *Listeria* Media	63.6 g
RapidChek® *Listeria* Supplement	1.2 g
Package Insert/s	

10001366 (Media System)

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

10001369 (High Volume System)

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

10001370 (High Volume Environmental System)

Description	Quantity
RapidChek® *Listeria* Test Strips (10001173)	990
RapidChek® *Listeria* Media	5.3 kg
RapidChek® *Listeria* Supplement	100 g
Package Insert/s	

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10001372 (High Volume Test Kit)

Description	Quantity
RapidChek® *Listeria* Test Strips (10001173)	450
Package Insert	

10001412

Description	Quantity
RapidChek® *Listeria* Test Combs	384
tests Cluster Tubes	384
Cluster Tube Rack	1
Package Insert	

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 x 10 cm, holds 75 x 12 mm 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 ± 0.05 g 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 ± 0.2 g 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 ± 0.05 g 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/or before use.

C. Sample Enrichment, Sponge Samples

1. Pre-moisten a 7.5 x 4 cm cellulose, non-bactericidal sampling sponge with 10 mL of DE broth or another neutralizing buffer.
2. Sample a 4 inch square surface by wiping the sponge on the surface in a backward and forward motion for 30 seconds. Place the sponge into a sterile bag for either transportation to the lab or sample enrichment.
3. Add 60 – 100 mL of prepared RapidChek® *Listeria* Media with Supplement. Make sure to completely cover the sponge with media.
4. Place the sample bag into a Stomacher device and stomach for 30 seconds or hand massage the bottom of the bag.
5. Close the bag loosely and incubate for 40 up to 48 hours at 30 ± 2 °C.
6. Proceed to the RapidChek® *Listeria* detection procedure.

D. Sample Enrichment, Swab Samples

1. Pre-moisten a sterile, cotton tipped swab (Fisher Scientific) with DE broth or another neutralizing buffer.
2. Sample a one inch square of the surface by rubbing the swab in a backward and forward motion for 30 seconds.
3. Place the swab into a sterile bag or container for either transportation to the lab or sample enrichment.
4. Add 10 – 20 mL of RapidChek® *Listeria* Media with Supplement. Make sure that the media completely covers the tip of the swab.
5. Place the sample bag into a Stomacher device and stomach for 30 seconds or hand massage the bottom of the bag.
6. Close the bag loosely and incubate for 40 up to 48 hours at 30 ± 2 °C.
7. Proceed to the RapidChek® *Listeria* detection procedure.

E. Food Samples

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

RapidChek® *Listeria* Detection Procedure

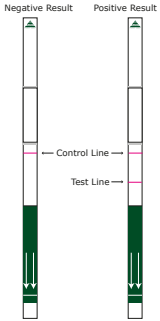
1. Take one transfer pipette from the bag (or utilize a calibrated pipette capable of dispensing 400 µL). Squeeze and hold the bubble on top of the pipette and place into the sample enrichment.
2. Release the bulb completely filling the barrel of the pipette.

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 (95 – 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



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 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 available from Sera Care at www.seracare.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.

Warranty and Liabilieties

The user assumes all risk in using Romer Labs products and services. Romer Labs will warrant that its products and services meet all quality control standards set by Romer Labs and Romer Labs will, at its option, repair or replace any product, components, or repeat services which prove to be defective in workmanship or material within product specific warranty periods or expiration dates and which our examination shall disclose to our satisfaction to be defective as such. This warranty is expressly in lieu of all other warranties, expressed or implied, as to description, quality, merchantability, fitness for any particular purpose, productiveness, or any other matter. Romer Labs shall be in no way responsible for the proper use of its products. Romer Labs hereby disclaims all other remedies, warranties, guarantees or liabilities, expressed or implied, arising by law or otherwise, and it shall have no liability for any lost profits or damage, direct, indirect or otherwise, to person or property, in connection with the use of any of its products or services. This warranty shall not be extended, altered or varied except by a written instrument signed by an authorized representative of Romer Labs.

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