



RapidChek® *Listeria* NextDay™ Food and Environmental System



**Order #: 7000238, 7000248, 7000243, 7000244, 7000249, 7000239,
7000261, 7000262**

AOAC Approved Protocols:

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



Intended Use

The RapidChek *Listeria* NextDay Test System is designed to detect *Listeria* species on environmental surfaces and a variety of ready-to-eat foods and dairy products including hot dogs, frozen breaded chicken, and shredded Mexican cheese (25g and 125g), as well as roast beef, frozen meatballs, whole milk, ice cream, ricotta cheese and cheese powder (25 g). By monitoring for the presence of *Listeria* species, the kit can be used for the control and prevention of *Listeria monocytogenes* in food products. The test kit permits the presumptive detection and identification of the target pathogen in a minimum of 24 hours for environmental surfaces and 27 hours for most claimed food types when *Listeria* species is present at low levels. The kit is intended for use by qualified, properly-trained personnel in commercial, academic, and governmental organizations involved in the processing of food products and/or monitoring the safety of such products.

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

7000238

<u>Description</u>	<u>Quantity</u>
RapidChek <i>Listeria</i> Strips	45
Transfer pipettes (400 µL)	45
Plastic tubes (12 x 75mm)	45
Package Insert	1

7000248

<u>Description</u>	<u>Quantity</u>
RapidChek <i>Listeria</i> Strips	180
Transfer pipettes (400 µL)	180
Plastic tubes (12 x 75mm)	180
RapidChek <i>Listeria</i> NextDay Media	500g
Package Insert	1

7000243

<u>Description</u>	<u>Quantity</u>
RapidChek <i>Listeria</i> NextDay Media	500g

**7000244**

<u>Description</u>	<u>Quantity</u>
RapidChek <i>Listeria</i> NextDay Media	5 kg

7000249

<u>Description</u>	<u>Quantity</u>
RapidChek <i>Listeria</i> Strips	1,800
RapidChek <i>Listeria</i> NextDay Media	5 kg
Package Insert	1

7000239

<u>Description</u>	<u>Quantity</u>
RapidChek <i>Listeria</i> Strips	450
Package Insert	1

7000261

<u>Description</u>	<u>Quantity</u>
RapidChek <i>Listeria</i> Strips	45
RapidChek <i>Listeria</i> NextDay Media	500g
Package Insert	1

7000262

<u>Description</u>	<u>Quantity</u>
RapidChek <i>Listeria</i> Strips	450
RapidChek <i>Listeria</i> NextDay Media	5 kg
Package Insert	1

Storage of Reagents

The RapidChek *Listeria* NextDay 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

Environmental sampling sponge or swab
 Dey-Engley neutralizing broth (or equivalent)
 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 ± 0.1 gram

Media Preparation and Sample Enrichment**A. Media Preparation, Autoclaved**

1. Add 46.2 ± 0.2g of RapidChek *Listeria* NextDay Media to 1 liter of room temperature DI water. Shake until completely dissolved.
2. Autoclave at 121 °C for 15 minutes.

Note: The media base 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 use.

B. 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 mL of prepared RapidChek *Listeria* NextDay Media. 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 24 - 40 hours at 30 ± 2°C.
6. Proceed to the RapidChek detection procedure.

C. 25g Food Sample Enrichment

1. Add 25 grams of the sample to be analyzed into a sterile Stomacher bag or equivalent.
2. Add 225 mL of prepared RapidChek *Listeria* NextDay Media to the Stomacher bag containing the sample.
3. Place the sample bag into a Stomacher device and stomach for 30 seconds or hand massage the bottom of the bag.
4. Close the bag loosely and incubate for 27 - 48 hours at 30 ± 2°C. **Shredded cheese (25 g) requires a 48 h incubation.**
5. Proceed to the RapidChek detection procedure.

D. 125g Food Sample Enrichment

1. Add 125 grams of the sample to be analyzed into a sterile Stomacher bag or equivalent.
2. Add 500 mL of prepared RapidChek *Listeria* NextDay Media to the Stomacher bag containing the sample.



- Place the sample bag into a Stomacher device and stomach for 30 seconds or hand massage the bottom of the bag.
- Close the bag loosely and incubate for 27 - 48 hours at $30 \pm 2^\circ\text{C}$. **Hot dogs (125 g) require a 48 h incubation.**
- Proceed to the RapidChek detection procedure.

E. 125g Shredded Cheese Sample Enrichment

- Add 125 grams of the sample to be analyzed into a sterile Stomacher bag or equivalent.
- Add 500 mL of prepared RapidChek *Listeria* NextDay Media to the Stomacher bag containing the sample.
- Place the sample bag into a Stomacher device and stomach for 30 seconds or hand massage the bottom of the bag.
- Close the bag loosely and incubate for 22 - 26 hours at $30 \pm 2^\circ\text{C}$.
- Transfer 1 mL to 4 mL fresh RapidChek *Listeria* NextDay Media.
- Incubate for 18 - 22 hours at $30 \pm 2^\circ\text{C}$.
- Proceed to the RapidChek detection procedure.

RapidChek *Listeria* NextDay Detection Procedure

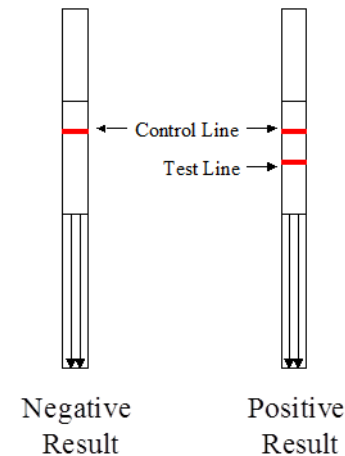
- After placing the correct number of supplied plastic tubes into a test tube rack, transfer an aliquot of enriched broth to each tube.
- Take one transfer pipette from the bag (or utilize a calibrated pipette capable of dispensing 400 μL). Squeeze and hold the bubble on top of the pipette and place into the sample enrichment. For 25g and 125g shredded cheese and 25g cheese powder samples pipette 1mL of sample into the test tube.
- Release the bulb completely filling the barrel of the pipette.

Note: The bubble will not completely fill with solution.

- Place the rack of tubes into a boiling water bath (100°C) or heat block for 5 to 15 minutes.
- Remove tubes after boiling and allow them to cool to room temperature prior to testing.
- Remove the required number of test strips from the canister.
- Insert the strip with **arrows facing down** into the tube.

- Let the strip develop for 10 minutes.
- The appearance of **one red line** (control) on the strip indicates a negative result.
- 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 according to the confirmation procedure outlined in the USDA-FSIS MLG 8.09 (*Isolation and Identification of Listeria monocytogenes from red meat, poultry, egg products, and environmental samples*) or the FDA's Bacteriological Analytical Manual (or BAM Chapter 10: Detection and Enumeration of *Listeria monocytogenes* in Foods). The website locations of the USDA-FSIS and the FDA BAM methods for the detection of *Listeria*:

1. <http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES>
2. <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm>

Enriched samples from the RapidChek *Listeria* NextDay test procedure **prior to boiling** should be used for confirmation. Streak a loop or a drop approximating 0.1 mL of the RapidChek *Listeria* NextDay media over the surface of a MOX and Agar *Listeria* Ottavani and Agosti plate (optional). Incubate the plates at $35 \pm 2^\circ\text{C}$ for 26 ± 2 hours.

Examine the RapidChek *Listeria* NextDay media-streaked MOX plate for colonies with morphology typical of *Listeria* spp. At 26 ± 2 hours, suspect colonies on MOX are typically small (ca. 1 mm) and are surrounded by a zone of darkening due to esculin hydrolysis. Typical colonies on Agar *Listeria* Ottavani and Agosti are blue to green and for *Listeria monocytogenes* and *Listeria ivanovii*, the colored colonies are surrounded by an opaque halo. If suspect colonies are present on MOX, transfer suspect colonies to horse blood overlay (HL) agar. If suspect colonies are present on ALOA, transfer suspect colonies to tryptic soy agar containing yeast extract (TSAYE). Incubate the streaked plates at $35 \pm 2^\circ\text{C}$ for 22 ± 4 hours.

If no suspect colonies are evident, re-incubate the MOX plate for an additional 26 ± 2 hours. If suspect colonies are present on MOX after this second incubation, streak for isolation on one or more HL agar plates. Incubate the streaked HL at $35 \pm 2^\circ\text{C}$ for 22 ± 4 hours.

After incubation, examine the HL plate(s) against backlight for translucent colonies surrounded by a small zone of β -hemolysis. If at least one suspect colony is clearly isolated, proceed to confirmatory testing. Hold all HL plates containing suspect colonies (room temperature or refrigeration) until confirmatory testing is complete.

If suspect colonies or β -hemolytic growth are present on HL, but not clearly isolated, re-streak representative suspect colonies/growth onto one or more fresh HL plates and incubate at $35 \pm 2^\circ\text{C}$ for 22 ± 4 hours.

Confirm ALL samples by biochemical confirmation using API *Listeria* identification system or equivalent reagents/methods that can differentiate among *Listeria* species.

If the manufacturer recommended method does not confirm the presumptive positive result, then the full reference confirmation should be conducted. For this, 0.1 mL of the RapidChek *Listeria* NextDay broth should be transferred to 10 mL of Fraser broth and the USDA-FSIS method (MLG 8.09) subsequently followed.

Disposal

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

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 ($15\text{-}30^\circ\text{C}$) conditions. Contact customer service with any questions about product shelf life.

Precautions

1. *Listeria monocytogenes* is a significant human pathogen. Immunocompromised 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. *Staphylococcus aureus* may produce a faint test signal when present at high levels.
3. 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.
4. Storage conditions higher than room temperature may adversely affect performance of the test strip.
5. Follow standard Good Microbiological Practices where appropriate.

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