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 Salmonella Lateral Flow Test Kit (AOAC License Number 030301) is designed to detect Salmonella in a variety of foods including raw meat, poultry, dairy products, processed meats and fresh produce. The test kit permits the presumptive detection and identification of the target pathogen in 24 and 48 hours when present at levels of one Salmonella organism per 25 grams of sample.

Principle of the Assay
This immunoassay test uses a double antibody sandwich format. An antibody specific for Salmonella is sprayed and immobilized in a line on the surface of a membrane comprising a “test line”. A second antibody reagent, also recognizing the Salmonella 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 to Salmonella and moves with the liquid sample onto the test membrane. The sample passes through the test line where the immobilized Salmonella antibody captures the protein–antibody–gold complex, causing the formation of an antibody–protein “sandwich” and development of red color at the test line. Antibody–protein sandwiches are not formed in the absence of the Salmonella, 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 strip has 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.

Contents of Kit

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>RapidChek Salmonella Test Strips</td>
<td>50</td>
</tr>
<tr>
<td>Transfer pipettes (500 μL)</td>
<td>50</td>
</tr>
<tr>
<td>Test Tubes</td>
<td>50</td>
</tr>
<tr>
<td>Package Insert/s</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>RapidChek Salmonella Media</td>
<td>500g</td>
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</table>

Storage of Reagents
The RapidChek Salmonella Test Kit should be stored at room temperature (15-30°C). The RapidChek Salmonella test strips used in this kit should be kept in the plastic canister with the humidity indicating card. The humidity indicating card should be blue in color. After opening the canister, care should be taken to close the lid tightly to protect the test strips from moisture.

Materials Required but Not Supplied
Stomacher-type bags or equivalent
Stomacher machine or equivalent (optional)
Balance with an accuracy of ± 0.2 g.
For the 24 Hour RapidChek Procedure
RapidChek *Salmonella* Media, 500 g (7000167)
TT (Tetrathionate) Broth Base, Hajna (Becton Dickinson and Company, Microbiology Systems, Sparks, MD 21152)
Incubator capable of maintaining \(42 \pm 0.5^\circ\text{C}\)

For the 48-Hour RapidChek Procedure
Buffered peptone water (BPW), Lactose Broth (LB), Tryptic Soy Broth modified with ferrous sulfate or Universal Pre-enrichment Broth (UPB) and TT Broth Base, Hajna formulation or Tetrathionate Broth, original formulation
Incubators capable of maintaining \(35 \pm 1^\circ\text{C}\) and \(42 \pm 0.5^\circ\text{C}\)

**Media Preparation and Sample Enrichment**

For RapidChek Media System (24 or 48 hours)
A. Media Preparation
1. Sterilize one liter of water either by autoclaving or filtration (pore size of 0.2 \(\mu\text{m}\)) into a sterile container.
2. Equilibrate the sterilized water to \(42 \pm 0.5^\circ\text{C}\) in a water bath or incubator.
3. Weigh 27.0 \(\pm 0.2\) g of RapidChek *Salmonella* Media and add to the 1 liter of sterilized water. Shake vigorously until the media is dissolved.
4. Rehydrated media should be used within 3 hours of preparation. For best results, use the media as soon as it is prepared.

B. Alternative Option: Media Preparation, Autoclaved
1. Add 27.0 \(\pm 0.2\)g of RapidChek *Salmonella* Media to 1 liter of room temperature distilled water. Shake until completely dissolved.
2. Autoclave at 121\(^\circ\text{C}\) for 15 minutes.
3. Autoclaved, re-hydrated media should be used within four weeks if stored at 4\(^\circ\text{C}\).

C. Sample Enrichment
1. Add 25 g of the sample to be analyzed into a sterile Stomacher bag.
2. Add 225 mL of rehydrated RapidChek media (pre-warmed to 42\(^\circ\text{C}\)) 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 5 to 24 hours at 42 \(\pm 0.5^\circ\text{C}\).
5. After AT LEAST 5 hours, remove the bag from the incubator and vigorously mix contents by holding the top and the bottom of the stomacher bag tightly and shaking vigorously.
6. Transfer 1 mL of enrichment sample to a tube containing 10 mL of prepared TT Hajna broth (pre-warmed to 42\(^\circ\text{C}\)).
7. Return tube to the 42\(^\circ\text{C}\) incubator and incubate for 19 to 24 hours.
8. Proceed to RapidChek *Salmonella* detection procedure.

For Conventional Media System (48 hours)
A. Media Preparation
1. *Buffered peptone water:* add 20 g of media, which includes peptone (10.0 g), sodium chloride (5.0 g), disodium phosphate (3.5 g) and monosodium phosphate (1.5 g) to 1 L of purified water, mix thoroughly and autoclave at 121\(^\circ\text{C}\) for 15 min.
2. *Lactose broth:* add 13 g of media into 1 L of purified water, mix thoroughly and autoclave at 121\(^\circ\text{C}\) for 15 min.
3. *Tryptic Soy broth:* add 30 g of media and 35 mg of ferrous sulfate to 1 L of purified water, mix thoroughly and autoclave at 121\(^\circ\text{C}\) for 15 min.
4. *Universal Pre-enrichment broth:* add 38 g of media into 1 L of purified water, mix thoroughly and autoclave at 121\(^\circ\text{C}\) for 15 min.
5. *Tetrathionate broth (TT):* add 46 g of media into 1 L of purified water, mix thoroughly and bring to boil and then cool to below 50\(^\circ\text{C}\). Then add 20 mL/L of iodine solution (6 g iodine crystals, 5 g potassium iodine dissolved in 20 mL of distilled or deionized water) and 10 mL/L of Brilliant Green solution (0.1 g/100 mL of distilled or deionized water) dispense 10 mL per tube into sterile test tubes.
6. *TT Hajna:* add 91.5 g to 1 L of purified water, bring to boil and then cool to below 50\(^\circ\text{C}\). Then add 40 mL/L of iodine solution (which includes 5 g of iodine crystals and 8.0 g of potassium iodide in 40 mL of sterile water) and dispense into sterile tubes.
B. Sample Enrichment
1. Add 25 g of the sample to be analyzed into a sterile Stomacher bag.
2. Add 225 mL of either BPW, LB, mTSB or UPB to the Stomacher bag containing the sample. The type of primary enrichment broth utilized will be dictated by the food being tested. BPW is recommended for raw meat and poultry, LB is recommended for milk, processed meats and fresh produce, while mTSB is suggested for liquid eggs and UPB is used for fresh juices including orange juice. Incubate all broths for 24 hours at 35°C.
3. After incubation, remove the bag from the incubator and gently mix contents using a gentle swirling motion.
4. From BPW enrichment, transfer 0.5 mL to 10 mL of TT Hajna broth and incubate enrichment at 42 ± 0.5°C for 19-24 hours.
5. From all other primary enrichment broths, transfer 1.0 mL to 10 mL of TT broth and incubate enrichment at 42 ± 0.5°C for 19-24 hours.
6. After incubation, proceed to RapidChek Salmonella detection procedure.

RapidChek Salmonella Detection Procedure

1. Take one transfer pipette from the bag (or utilize calibrated pipette capable of dispensing 500 μL). Squeeze and hold the bubble on top of the pipette and place in the sample enrichment.
2. Release the bulb completely filling the barrel of the pipette.
   Note: The bubble will not completely fill with solution.
3. Transfer the aliquot of enriched broth to the plastic tubes supplied. Label each tube with the appropriate sample identification.
4. Remove the required number of test strips from the canister and insert the strip with arrows facing down into the tubes.
5. Let the strip develop for 10 minutes.
6. The appearance of one red line (control) on the strip indicates a negative result.
7. 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 a presumptively positive for Salmonella.

If at 10 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for Salmonella. 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 USDA-FSIS or Bacteriological Analytical Manual (BAM) methods for the detection of Salmonella. Enriched media samples used in the RapidChek Salmonella Test Procedure can be used for this confirmation. For confirmation procedures see the following:

(1) USDA-FSIS, MLG – 5th Edition, Chapter 4 entitled Isolation and identification of Salmonella from meat, poultry and egg products
(2) FDA/BAM - Salmonella (chapter 5) In: US Food, Drug and Administration , Center for Food Safety and Applied Nutrition, Bacteriological Analytical Manual
   http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm
Disposal

Decontaminate used 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 canister.

This product has 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. *Salmonella* is a human pathogen. Extreme care should be used in handling samples, enriched media and used test strips. 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. Do not use test strips beyond the expiration dating on the kit package label.
5. Follow standard Good Microbiological Practices where appropriate.

Warranties and Liabilities

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