



RapidChek[®] SELECT[™] Salmonella Enteritidis Test System



Part #: 7000220, 7000221, 7000222

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 SELECT S. Enteritidis Test Kit is designed to detect *Salmonella* Enteritidis (including other Group D1 serovars) in chicken house drag swabs, egg pool samples and various chicken rinse samples. The test kit permits the presumptive detection and identification of the target pathogen in 32 to 48 hours, dependent on sample type, when present at levels of 1 *Salmonella* organism per sample. The intended user is a laboratory worker with the appropriate microbiological training in aseptic technique and the handling of pathogenic bacteria.

Principle of the Assay

This immunoassay test uses a double antibody sandwich format. It utilizes an antibody specific for *Salmonella* Group D1 including *S. Enteritidis*. This antibody is sprayed and immobilized on the surface of a membrane comprising a "test line". A second antibody reagent, also recognizing the *Salmonella* Enteritidis 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* Enteritidis and moves with the liquid sample onto the test membrane. The sample passes through the test line where the immobilized *Salmonella* Enteritidis antibody captures the antigen-antibody-gold complex,

causing the formation of an antibody-antigen "sandwich" and development of red color at the test line. Antibody-antigen sandwiches are not formed in the absence of the *Salmonella* Group D1 including *S. Enteritidis*, 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 Kits

7000220

<u>Description</u>	<u>Quantity</u>
RapidChek SELECT SE Test Strips	50
Transfer pipettes (100 µL)	50
Test Tubes	50
Package Insert/s	

7000221

<u>Description</u>	<u>Quantity</u>
RapidChek SELECT Primary Media	500g
RapidChek SELECT Primary Supplement	250mL
RapidChek SELECT Secondary Media	4x10g

7000222

<u>Description</u>	<u>Quantity</u>
RapidChek SELECT SE Test Strips	250
Transfer pipettes (100 µL)	250
Test Tubes	250
RapidChek SELECT Primary Media	500g
RapidChek SELECT Supplement	250mL
RapidChek SELECT Secondary Media	4x10g
Package Insert/s	

Storage of Reagents

The RapidChek SELECT S. Enteritidis Test Kit should be stored at room temperature (15-30°C) with one exception. **The RapidChek SELECT Primary Supplement should be**



kept refrigerated (2-8 °C). The RapidChek SELECT S. Enteritidis 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
Incubator capable of maintaining $42 \pm 2^\circ\text{C}$

RapidChek Media Preparation and Sample Enrichment-Drag Swab and Egg Pool Samples

A. Primary Media Preparation (1 L)

1. Weigh 20.0 ± 0.2 g of RapidChek SELECT Primary Media and add to 1 liter of deionized water. Shake vigorously until the media is dissolved.
2. Autoclave for 15 minutes at 121°C or filter sterilize (pore size of $0.2\mu\text{m}$) the rehydrated media.

Note: Autoclaved media may be stored for up to 4 weeks at 2-8 °C or room temperature. Filter sterilized media may be stored for up to 2 weeks at 2-8 °C.

3. Alternatively, rehydrate media in one liter of sterile, deionized water. In this manner, rehydrated media should be used within 3 hours of preparation. For best results, use the media as soon as it is prepared.
4. **Just prior to use:** Add 10 mL of supplement per 1 liter of base media. Shake to mix. Use within 3 hours of preparation.

Note: Do not autoclave the media after the supplement has been added.

B. Secondary Media Preparation (100 mL)

1. Weigh 7.4 ± 0.2 g of RapidChek SELECT Secondary Media and add to 100 mL of deionized water. Shake vigorously until the media is dissolved.
2. Bring to a boil while stirring. After boiling, the media can be stored for 2 weeks at 2-8 °C.

3. **Alternative Method:** Instead of boiling the media, add 7.4 grams of media to 100 mL of sterile, deionized water. In this manner, rehydrated media should be used within 3 hours of preparation. For best results, use the media as soon as it is prepared.

Note: The RapidChek SELECT Secondary Media is turbid, light green and presents a white precipitate.

Sample Enrichment

A. Chicken House Drag Swab Samples

1. Add 100 mL of pre-warmed ($42 \pm 2^\circ\text{C}$) supplemented RapidChek SELECT Primary media to the sample bag containing the drag swab.
2. Place the sample bag into a Stomacher device and stomach for 30 seconds or hand massage the bottom of the bag.
3. Close the bag loosely and incubate for 16-22 hours at $42 \pm 2^\circ\text{C}$.
4. Transfer 0.2 mL of the enriched sample to a tube containing 2 mL of RapidChek SELECT Secondary Media.
5. Lightly cover the tubes and return to the 42°C incubator and incubate for an additional 16-22 hours. After incubation gently shake the tubes.
6. Proceed to RapidChek SELECT S. Enteritidis Detection Procedure.

B. Egg Pool Samples- As described in the FDA's Federal Register 21 CFR Parts 16 and 118

1. Disinfect eggs with a 3:1 solution of 70% alcohol to iodine/potassium iodide solution.
2. Prepare 50 egg pools containing 20 eggs each.
3. Add 200 mL of supplemented RapidChek SELECT Primary Media to each pool of 20 eggs.
4. Incubate the pools of eggs at room temperature ($20-24^\circ\text{C}$) for 40-48 hours.
7. After incubation, transfer 0.1 mL of the enriched sample to a tube containing 1 mL of RapidChek SELECT Secondary Media.
8. Lightly cover the tubes and incubate for an additional 6-8 hours at 42°C .
9. After incubation gently shake the tubes.
10. Proceed to RapidChek SELECT S. Enteritidis detection procedure.



RapidChek Media Preparation and Sample Enrichment-Chicken Rinse Samples

A. Primary Media Preparation (1 L)

1. Weigh $40.0 \pm 0.2\text{g}$ of RapidChek SELECT Primary Media and add to 1 liter of deionized water. Shake vigorously until the media is dissolved.
2. Autoclave for 15 minutes at 121°C or filter sterilize (pore size of $0.2 \mu\text{m}$) the rehydrated media.

Note: Autoclaved media may be stored for up to 4 weeks at $2-8^\circ\text{C}$ or room temperature. Filter sterilized media may be stored for up to 2 weeks at $2-8^\circ\text{C}$.

3. Alternatively, rehydrate media in one liter of sterile, deionized water. In this manner, rehydrated media should be used within 3 hours of preparation. For best results, use the media as soon as it is prepared.
4. Just prior to use: Add 20 mL of supplement per 1 liter of base media. Shake to mix. Use within 3 hours of preparation.

Note: Do not autoclave the media after the supplement has been added.

B. Secondary Media Preparation (100 mL)

1. Weigh $7.4 \pm 0.2\text{g}$ of RapidChek SELECT Secondary Media and add to 100 mL of deionized water. Shake vigorously until the media is dissolved.
2. Bring to a boil while stirring. After boiling, the media can be stored for 2 weeks at $2-8^\circ\text{C}$.
3. **Alternative Method:** Instead of boiling the media, add 7.4 grams of media to 100 mL of sterile, deionized water. In this manner, rehydrated media should be used within 3 hours of preparation. For best results, use the media as soon as it is prepared.
4. Dispense 1 mL per sample tested of the prepared RapidChek SELECT Secondary Media into the supplied tubes. One tube is needed per enrichment tested.

Sample Enrichment

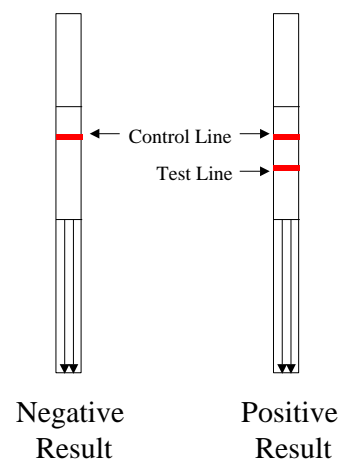
1. Add carcass sample to a large Stomacher bag.
2. As per the recommended USDA-FSIS method, add 400 mL of Buffered-peptone water (BPW) into the cavity of the bird and shake for 1 min.

3. Transfer 30 mL of the rinsate to a separate stomacher bag.
4. Add 30 mL of 2X RapidChek SELECT Primary Media with 2X Supplement to the 30 mL of the rinsate.
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 16-22 hours at $42 \pm 2^\circ\text{C}$.
7. Transfer 0.1 mL of enriched sample to a tube containing 1.0 mL of RapidChek SELECT Secondary Media.
8. Lightly cover the tubes and return to the 42°C incubator and incubate for an additional 16-22 hours. After incubation gently shake the tubes.
9. Proceed to RapidChek SELECT S. Enteritidis detection procedure.

RapidChek SELECT S. Enteritidis Detection Procedure

1. Remove the required number of test strips from the canister.
2. Insert the strip with arrows facing down into the tube.
3. Let the strip develop for 10 minutes.
4. The appearance of one red line (control) on the strip indicates a negative result.
5. 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 *S. Enteritidis* or another Group D1 serovar.

If at 10 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for *S. Enteritidis*. 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

Chicken House Drag Swab Samples: All presumptive positive results must be taken through an immunomagnetic separation (IMS) procedure prior to cultural confirmation. Please see insert of the IMS confirmation kit (Part Number 7000225) for details of the protocol. Enriched samples used in the RapidChek Test Procedure can be used for the IMS protocol followed by cultural confirmation. For confirmation procedures see the following:

- (1) RapidChek CONFIRM SE IMS Kit Package Insert
- (2) Environmental Sampling and Detection of *Salmonella* in Poultry Houses, October 2008, US FDA
<http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/ucm114716.htm>

Note: The RapidChek SELECT Salmonella Enteritidis assay is a screening test designed to detect SE antigens present on the bacterial cell surface. These antigens are present in inactivated (or killed) SE vaccines. In the unusual event the antigens are present in high concentrations in the environment they could be detected by the assay. Therefore, Romer Labs recommends that environmental samples are collected prior to vaccination of a poultry house.

Egg Pool Samples: Presumptive positive results must be confirmed by FDA Bacteriological Analytical Manual (BAM) Chapter 5 *Salmonella* method for the detection of *S. Enteritidis* in shell eggs. Enriched samples used in the RapidChek Test Procedure can be used for this

confirmation. For confirmation procedures see the following:

- (1) FDA/BAM - *Salmonella* (Chapter 5) In: US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Bacteriological Analytical Manual
<http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM070149>

Raw Chicken Rinse Samples:

Presumptive positive results must be confirmed by the USDA/FSIS MLG method for carcass rinse samples. Enriched samples used in the RapidChek Test Procedure can be used for this confirmation. Details can be found in the following reference:

- (1) FSIS/USDA/MLG –Microbiological Laboratory Guidelines Chapter 4: Isolation and identification of *Salmonella* from meat, poultry and egg products
http://www.fsis.usda.gov/PDF/MLG_4_05.pdf

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. 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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