VIP® Gold for *Salmonella*

Part No: 60038-40 (40 tests)

There are two validated methods that can be followed:

AOAC Official Method of Analysis 999.09

AOAC Performance Tested Method 031801

General Description

VIP® Gold for *Salmonella* is a single-step visual immunoassay for the detection of motile and non-motile *Salmonella* species in select food and environmental samples. Each device contains a proprietary reagent system, which forms a visually apparent antigen-antibody-chromogen complex if *Salmonella* is present. The test is intended for use by laboratory personnel with appropriate microbiology training.

Kit Components

Each VIP® Gold for *Salmonella* kit contains the following:

VIP® Gold for *Salmonella* test devices

Extraction Reagents 1 and 2

Equipment / Materials Required

Other necessary materials not provided include:

Media per Appendix A or B

Vortex mixer (vortexer)

Analytical balance, tolerance ± 0.2 g (capacity 500 g)

Stomacher / Masticator machine

Stomacher-type bags with filter or equivalent

Incubator capable of maintaining 35–37 °C

Incubator capable of maintaining 41.5–42.5 °C

Water bath capable of maintaining 41.5–42.5 °C

Micropipette(s) with 0.1 mL, 0.5 mL and 1.0 mL capacity

13 x 100 mm glass tubes with caps

AOAC OFFICIAL METHOD OF ANALYSIS 999.09

SAMPLE PREPARATION

Pre-Enrichment

a. **Low Microbial Foods** Add 25 g test portion to 225 mL of prewarmed appropriate pre-enrichment broth (Appendix A), stomach / masticate for 2 minutes and Incubate 6–8 h at 35–37 °C.

b. **Dried Powder Processed Foods** Add 25 g test portion to 225 mL of prewarmed Brain Heart Infusion broth + 1 mL enrichment supplement containing Oxyrase® (BHI+O) (Appendix A) and incubate 6–8 h at 35–3 °C.

c. **High Microbial Foods** Add 25 g test portion to 225 mL of Buffered Peptone Water + novobiocin (BPW+n) (Appendix A), stomach / masticate for 2 minutes and incubate 18–26 h at 35–37 °C.

Selective Enrichment

a. **Low Microbial Foods** Transfer 25 mL pre-enriched broth to 5 mL of 5X concentrate Rappaport-Vassiliadis R10 Broth (RV) in a 25 x 150 mm test tube and transfer another 25 mL broth to 5 mL of 5X concentrate Tetrathionate Broth (TT) (Appendix A). Mix thoroughly with vortexer. Incubate 16–24 h at 41.5–42.5 °C in a water bath.

b. **Dried Powder Processed Foods** Transfer 25 mL BHI+O to 5 mL of 5X TT broth. Mix thoroughly with vortexer. Incubate 16–24 h at 41.5–42.5 °C in a water bath.

c. **High Microbial Foods** Transfer 0.1 mL pre-enriched broth to 10 mL RV broth and transfer another 1.0 mL to 10 mL TT broth. Mix thoroughly with vortexer. Incubate 16–24 h at 41.5–42.5 °C in a water bath.

Post Enrichment

a. **Low Microbial Foods** Transfer and combine 0.5 mL of each paired TT and RV broths into a single tube of 10 mL *prewarmed* Trypticase Soy Broth with 2,4-dinitrophenol and novobiocin (TSB+DNP+n) (Appendix A). Mix thoroughly with vortexer. Incubate 6–8 h at 35–37 °C.

b. **Dried Powder Processed Foods** Transfer 1 mL TT into *prewarmed* 10 mL TSB+DNP+n. Mix thoroughly with vortexer. Incubate 6–8 h at 35–37 °C.

c. **High Microbial Foods** Transfer and combine 0.5 mL of each paired TT and RV broths into a single tube of 10 mL *prewarmed* TSB+DNP+n. Mix thoroughly with vortexer. Incubate for 5–8 h at 41.5–42.5 °C in a water bath.

Note: Retain original TSB+DNP+n broth tubes under refrigeration (2–8 °C). Use for confirmation of presumptive positive results.

PROCEED TO SAMPLE INACTIVATION

AOAC Performance Tested method 031801

SAMPLE PREPARATION

Enrichment for environmental surfaces and processed foods (select food)

a. For **ready-to-eat meat and poultry**, add **25 g test portion or 325 g test portion** to 225 mL or 1.3 L respectively *prewarmed* mEHEC (see Appendix B). Stomach/masticate for 2 minutes and incubate 22–30 h at 41.5–42.5 °C for 25 g samples and 24-32 h at 41.5–42.5 °C for 325 g samples.

b. For **environmental surfaces,** pre-moisten sterile dehydrated sponges with 10 mL D/E (Dey/Engley) Broth or Letheen Broth. Hydrate sterile swab by soaking in D/E or Letheen broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of *prewarmed* mEHEC, respectively. Mix well and incubate 20–28 h at 41.5–42.5 °C.

Pre-enrichment for raw and unprocessed foods (select foods) and poultry rinsate

c. For poultry rinsate, add 30 mL poultry rinsate to 30 mL of *prewarmed* mEHEC with novobiocin (mEHEC+n, see Appendix B). Mix well and incubate 20–28 h at 41.5–42.5 °C.

d. For **raw vegetables, nuts, meats and fresh pasta** add 25 g or 375 g test portion to 225 mL or 1.5 L respectively of *prewarmed* mEHEC with novobiocin (mEHEC+n), see Appendix B. Stomach / masticate for 2 minutes and incubate 20–28 h at 41.5–42.5 °C for 25 g samples and 24–32 h at 41.5–42.5 °C for 375 g samples.

Selective Enrichment for raw and unprocessed foods and poultry rinsate

a. **Raw and unprocessed foods.** Transfer 0.1 mL pre-enriched broth to 10 mL of *prewarmed* Tryptic Soy Broth with Novobiocin (TSB+n, Appendix B). Vortex to mix well. Incubate TSB + n for 6–24 h at 41.5–42.5 °C (preferably in a water bath).

PROCEED TO SAMPLE INACTIVATION

Sample Inactivation

a. Prewarm extraction reagents by placing bottles in hot water for 10 min. swirl each bottle gently to thoroughly mix.

b. Add 0.1 mL Extraction Reagent 1 and 0.1 mL Extraction Reagent 2 to an empty 13 x 100 mm tube.

c. Homogenize enriched sample and transfer 1.0 mL final enrichment broth to tube with extraction reagents. Cap tube. Vortex thoroughly.

d. **For Official Method of Analysis ONLY:**

Inactivate microorganisms by autoclaving at 121 °C for 10 min.

e. **For Performance Tested Method ONLY:**

Inactivate samples in a boiling water bath at 95–100 °C for 10 min.

Alternatively, inactivate microorganisms by autoclaving at 100–110 °C for 10 min.

f. Cool tubes to 15–30 °C before testing. Tubes that have been inactivated can be stored for up to 4 days at 2–8 °C prior to testing.

Test Procedure

a. Open the sealed foil pouch containing the VIP® Gold devices and remove the sheet of test devices. Break away the necessary number of devices, one device for each test portion.

b. Reseal unused VIP® Gold units in pouch containing desiccant. Store at room temperature (15–30 °C).

c. Enriched broths should be equilibrated to 15–30 °C prior to running the test.

d. Vortex contents. Transfer 0.1 mL of inactivated mEHEC (surfaces or processed foods) or 0.1 mL of inactivated TSB+n (raw or unprocessed foods and poultry rinsate) to sample addition well. Avoid transferring particulate matter to the device.

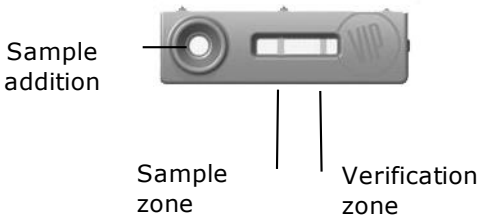
e. Incubate device at room temperature (15–30 °C) for 10–15 min. VIP® Gold devices may not be reused.

Results

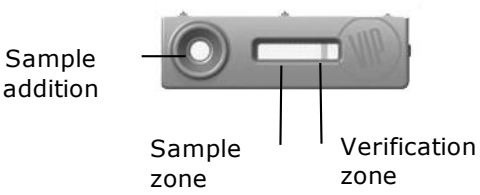
Note: Examine test unit at 10–15 min. Beyond this time, faint lines may develop because of non-specific color development and should be disregarded.

a. Examine VIP® Gold test for the presence of distinct detection lines in both test sample and test verification zone. Lines should be dark when contrasted with white background and should extend across the zone. Intensity of test sample and test verification lines may differ. Absence of a test verification line indicates an invalid test result. Contact Technical Services at (800) 245-0113.

b. Test sample is considered **positive** when lines are present in the test sample zone and in the test verification zone.



c. Test sample is considered **negative** when a line is present in the test verification zone and no line is seen in the test sample zone.



d. Positive and negative control cultures should be run to familiarize the analyst with the results interpretation.

Confirmation

Presumptive positive samples should be confirmed from the retained post enrichment broths via either:

US FDA BAM (*Bacteriological Analytical Manual*), Chapter 5, *Salmonella*:

http://www.fda.gov/Food/FoodScienceResearch/ LaboratoryMethods/ucm070149.htm

USDA FSIS MLG (*Microbiology Laboratory Guidebook*), Chapter 4.09:
http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERES

Storage Conditions

Store VIP® Gold for *Salmonella* kit components at room temperature (15–30 °C). Do not refrigerate.

Reseal unused VIP® Gold devices in pouch with desiccant pack. The moisture indicator line on the desiccant pack must be blue.

Kit expiration is provided on the product pouch label.

Precautions

VIP® Gold for *Salmonella* must be used as described herein. This product is not intended for human or veterinary use. Do not use test kit beyond expiration date indicated on the label.

Avoid contact with eyes, skin, and clothing. Avoid swallowing or taking product internally.

Decontaminate materials by autoclave, bleach, etc., in accordance with good laboratory practices and in accordance with local, state and federal regulations. Waste may be contaminated with *Salmonella* which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.

APPENDIX A– AOAC OMA 999.09 Media Preparation

Food Type	**Pre-Enrichment Broth**
Nonfat dry milk	Brilliant green dye water
Liquid egg products	Trypticase Soy Broth
Non-powder chocolate based products	Reconstituted nonfat dry milk + brilliant green dye solution
Orange juice	Universal Pre-enrichment Broth
Dried powdered processed products	Brain Heart Infusion + Oxyrase
All other low microbial foods	Buffered Peptone Water
High microbial foods	Buffered Peptone Water + novobiocin

Brain Heart Infusion Broth + enrichment supplements containing Oxyrase (BHI+O)

Suspend 37 g of Brain Heart Infusion broth in 1 L of deionized water. Mix thoroughly. Autoclave at 121 °C for 15 min. On day of use, after sample addition and blending, add 1 mL of enrichment supplement containing Oxyrase to each 225 mL aliquot. Mix gently.

Brilliant green dye water

Add 2 mL 1% brilliant green dye solution per 1 L sterile deionized water.

Reconstituted nonfat dry milk + brilliant green dye

Dissolve 100 g nonfat dry milk in 1 L deionized water. Mix thoroughly. Autoclave at 121 °C for 15 min. Cool and add 2.0 mL 1% brilliant green dye solution. Mix thoroughly.

Buffered Peptone Water + novobiocin (BPW+n)

Suspend 20 g of dehydrated Buffered Peptone Water in in 1 L of deionized water. Mix thoroughly. Dispense 225 mL aliquots for food samples. Autoclave at 121 °C for 15 min. On day of use, add 4 mL 0.1% novobiocin solution to 225 mL BPW.

5X Tetrathionate Broth (5X TT)

Suspend 230 g of dehydrated Tetrathionate Broth Base in 1 L of deionized water. Mix thoroughly. Heat with agitation and boil for one min. DO NOT AUTOCLAVE. Cool to below 45 °C and add 5 mL 1% brilliant green dye solution. Mix thoroughly. Dispense 5 mL aliquots into sterile test tubes. Store in the refrigerator (2–8 °C) if the media will not be used within 12 h. On day of use, add 0.5 mL of iodine-iodide solution to each 5 mL test tube.

Trypticase Soy Broth + 2,4-dinitrophenol + novobiocin (TSB+DNP+n)

Dissolve 30 g of dehydrated Trypticase Soy Broth and 0.1 g of 2,4-dintrophenol in 1 L of deionized water. Mix thoroughly. Dispense in 10 mL aliquots. Autoclave at 121 °C for 15 min. On day of use, add 0.1 mL 0.1% novobiocin solution per 10 mL tube.

5X Rappaport-Vassiliadis R10 broth (5X RV)

Suspend 133 g of dehydrated Rappaport-Vassiliadis Broth in 1 L of deionized water. Mix thoroughly. Dispense in 5 mL aliquots into large test tubes (25 x 150 mm). Autoclave at 116 °C for 15 min.

1% Brilliant green dye solution

Dissolve 1.0 g brilliant green dye in 100 mL sterile deionized water. DO NOT AUTOCLAVE.

Iodine-Iodide solution

Dissolve 6 g resublimed iodine and 5 g potassium iodide in 20 mL sterile deionized water. DO NOT AUTOCLAVE. Store in dark at 2–8 °C.

Novobiocin solution (1.0%)

Dissolve 1.0 g novobiocin (sodium salt) in 100 mL sterile deionized water. DO NOT AUTOCLAVE.

APPENDIX B– AOAC Performance Tested Method 031801

Media Preparation

mEHEC® medium

Enrichment Media recipes for processed foods (select food) and environmental surfaces (mEHEC)

a. For 25 g sample, prewarm 225 mL sterile deionized water at 42 °C overnight. On day of use, aseptically transfer 7.1 g of BioControl mEHEC powder into the prewarmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.

b. For 325 g sample, prewarm 1300 mL sterile deionized water at 42 °C overnight. On day of use, aseptically transfer 41.1 g of mEHEC into the prewarmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.

c. For 375 g sample, prewarm 1500 mL sterile deionized water at 42 °C overnight. On day of use, aseptically transfer 47.3 g of mEHEC powder into the prewarmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.

Alternatively, mEHEC media can be prepared in advance and autoclaved. Add 31.6 g media per liter of deionized water. Stir to dissolve the powder, dispense into desired volume and autoclave at 121 °C for 15 min. Media must be prewarmed to 42 °C overnight prior to sample addition.

Enrichment Media recipes for raw and unprocessed foods (select food) (mEHEC+n)

a. For 25 g samples, follow the directions in (a) above and also add 0.9 mL of 0.45% novobiocin solution.

b. For 375 g samples, follow the directions in (c) above and also add 6.0 mL of 0.45% novobiocin solution.

c. Alternatively, follow the directions in (d) above and add 4 mL of 0.45% novobiocin solution per liter of mEHEC medium. DO NOT AUTOCLAVE media containing novobiocin solution.

Trypticase Soy Broth + novobiocin (TSB+n)

Dissolve 30 g of dehydrated Trypticase Soy Broth in 1 L of deionized water. Mix thoroughly. Dispense in 10 mL aliquots. Autoclave at 121 °C for 15 min. On day of use, add 40 µL 0.45% novobiocin solution per 10 mL tube.

Novobiocin solution (0.45%)

Dissolve 0.45 g novobiocin (sodium salt) in 100 mL sterile deionized water. DO NOT AUTOCLAVE.

Manufacturing Entity

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