

VIP® Gold for EHEC

Part No: 60036-40 (40 tests)

General Description

VIP® Gold for EHEC is a single-step visual immunoassay for the detection of *E. coli* O157:H7 in a variety of foods including dairy products, meats and poultry, fruits and vegetables, nutmeats, seafood, pasta, and liquid eggs using an 18 - 28 h enrichment protocol. It is applicable to detection of *E. coli* O157:H7 in raw and cooked beef and vegetables using an 8 h enrichment protocol. Each device contains a proprietary reagent system, which forms a visually apparent antigen-antibody-chromogen complex if *E. coli* O157:H7 is present. The test is intended for use by laboratory personnel with appropriate microbiology training.

Kit Components

Each VIP Gold for EHEC kit contains the following:

VIP Gold for EHEC test devices

Equipment / Materials Required

Other necessary materials not provided include:

Media per Appendix A

Autoclave

Vortex mixer

Analytical balance, tolerance ± 0.2 g

Stomacher / Masticator machine

Stomacher-type bags with filter or equivalent

Incubator(s) capable of maintaining 35 - 37 °C and 41.5 - 42.5 °C

Micropipette(s) capable of delivering 0.1 mL and 1.0 mL

Water bath capable of maintaining 95 - 105 °C or equivalent (e.g. autoclave with flowing steam, dry heater)

13 x 100 test tubes and caps

Sample Preparation

AOAC® OFFICIAL METHOD 996.09

Approved matrices include: dairy products, meats and poultry, fruits and vegetables, nutmeats, seafood, pasta, and liquid eggs using the mTSB enrichment protocol. Raw and cooked beef and vegetables are approved for use with the mEHEC® enrichment protocol.

A. Test Portion Preparation & Enrichment

- a. **8 hour enrichment in mEHEC** (beef and vegetables) Add 25 g test portion to 225 mL mEHEC media, prewarmed to 41.5 - 42.5 °C (Appendix A). Stomach / masticate for 2 minutes and incubate 8 h at 41.5 - 42.5 °C.
- b. **18 - 28 hour enrichment in mTSB+n** Add 25 g test portion to 225 mL modified Trypticase Soy Broth with novobiocin (mTSB+n). For raw beef enrich with mTSB+n with casamino acids and for vegetables, enrich with modified Trypticase Soy Broth with cefixime, cefsulodin and vancomycin (mTSB+CCV) (Appendix A). Stomach / masticate for 2 minutes and incubate 18 - 28 h at 35 - 37 °C. Incubate beef samples 18 - 28h at 41.5 - 42.5 °C.

If larger test portion sizes are analyzed, proportionately increase enrichment media volume to maintain 1:9 ratio.

Note: for viscous test portions add 2.25 - 4.5 mL of steamed Triton X-100 per 225 mL of enrichment broth at the time of test portion addition and prior to incubation.

Note: Retain original enriched broths under refrigeration (2 - 8 °C). Use for confirmation of presumptive positive results

- c. Proceed to **SAMPLE INACTIVATION**.

HEALTH CANADA METHOD MFLP-87

Approved matrices are raw meat using mEHEC® enrichment protocol and pasteurized juices using the mTSB enrichment protocol.

A. Test Portion Preparation & Enrichment

a. mEHEC Enrichment (raw meats)

For 375 g sample, add test portion to 1,500 mL of mEHEC media, prewarmed to 41.5-42.5 °C (Appendix A). For sample size 65 g up to 374 g, maintain the ratio of 1 portion sample to 4 portions mEHEC media. Stomach / masticate for 2 minutes and incubate 10 hours at 41.5-42.5 °C.

- b. **mTSB Enrichment (pasteurized juices)** Add 25 mL test portion to 225 mL modified Trypticase Soy Broth with novobiocin (mTSB+n), prewarmed to 41.5-42.5 °C (Appendix A). Stomach / masticate for 2 minutes and incubate 18 - 28 h at 41.5-42.5°C.

If larger test portion sizes are analyzed, proportionately increase enrichment media volume to maintain 1:9 ratio. It is recommended that analytical units greater than 25 mL be incubated for minimum 22–24 hours.

Note: Retain original enriched broths under refrigeration (2 - 8 °C) up to 3 days. Use for confirmation of presumptive positive results.

- c. Proceed to **SAMPLE INACTIVATION**.

Sample Inactivation

Applicable to mEHEC enrichment only.

- a. Gently mix the enriched test portion and allow food particles to settle.
- b. Transfer 4.0 mL of enriched broth to a test tube.
- c. Inactivate microorganisms at 95 - 105 °C for 10 min in a water bath, autoclave or dry heater.
- d. 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. VIP Gold units may not be reused. Reseal unused VIP Gold units in pouch containing desiccant. Store at room temperature (15 - 30 °C).
- c. Enriched broths should be equilibrated to 25 - 37 °C prior to running the test.
- d. Do not mix inactivated test broths from the 10 h (raw meat) protocol. Shake to mix the 18 h (pasteurized juices) enrichment to ensure sample homogeneity. Transfer 0.1 mL of inactivated test broth for 8 hour protocol or 0.1 mL of enriched broth for 18 - 28 hour protocol to sample addition well. Avoid transferring particulate matter to the device.

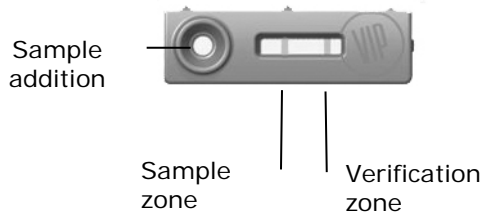
Note: Sample particles may affect flow. It is recommended that filter tips to be used when pipetting the sample.

- e. Incubate device at room temperature (15 - 30 °C) for:
 - a. 15 - 20 min for the 10 h raw meat protocol
 - b. 10 min for the 18 h pasteurized juices protocol

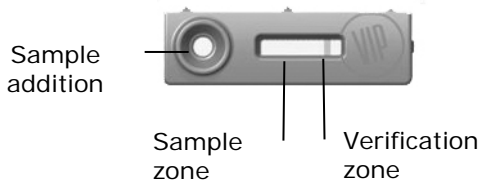
Results

Note: Examine VIP® Gold unit at specified time. Beyond this time faint lines may develop because of non-specific color development and should be disregarded.

- a. Examine VIP Gold unit 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

- a. US FDA. 2014. Bacteriological Analytical Manual online, chapter 4.
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm>
- b. USDA-FSIS. 2015. *Microbiology Laboratory Guidebook*, chapter 5.
<http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook>
- c. Health Canada Microbiological Methods Committee. 2014. MFHPB-10.
<http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php>

Storage Conditions

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

Reseal unused VIP Gold units 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 EHEC 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 *E. coli* O157:H7 which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.

Appendix A (PREPARATION OF MEDIA)

mEHEC Broth

On day of use, aseptically transfer 7.1 g of mEHEC® media in 225 mL of sterile deionized water prewarmed to 41.5 - 42.5 °C. Gently mix to dissolve the powder. Use prepared medium within 6 h (keep media warmed to 41.5 - 42.5 °C).

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 minutes. Media must be fully prewarmed to 41.5 - 42.5 °C prior to sample addition.

Modified Trypticase Soy Broth + novobiocin (mTSB+n)

Suspend 33 g of mTSB in 1 L of deionized water. Mix thoroughly until completely dissolved. Alternatively, mTSB can be prepared according to the following formula

mTSB Formula per Liter

Trypticase Soy Broth	30.0 g
Bile Salts No. 3	1.5 g
Dipotassium phosphate	1.5 g

Autoclave at 121 °C for 15 min. Cool to room temperature.

On day of use add 1.0 mL 0.45% novobiocin solution per 225 mL mTSB.

Modified Trypticase Soy Broth + novobiocin with casamino acids

Prepare mTSB as described above. Add 10 g of casamino acids (casein acid hydrolysate) per liter of media prior to autoclaving. Mix thoroughly until completely dissolved. Autoclave at 121 °C for 15 min. Cool to room temperature. On day of use add 1.0 mL 0.45% novobiocin solution per 225 mL mTSB with casamino acids.

Modified Trypticase Soy Broth + cefixime, cefsulodin and vancomycin (mTSB+CCV)

On day of use add 0.1 mL cefixime solution, 2.0 mL cefsulodin solution and 2.0 mL vancomycin solution to 1 L mTSB prepared as described above (do not add novobiocin).

0.45% Novobiocin solution

Dissolve 0.45 g novobiocin (sodium salt) in 100 mL sterile deionized water. DO NOT AUTOCLAVE. Store in dark at 2 - 8 °C.

Cefixime solution

Dissolve 0.05 g cefixime in 400 mL of sterile deionized water.
DO NOT AUTOCLAVE.
Store in dark at 2 - 8 °C.

Cefsulodin solution

Dissolve 0.2 g cefsulodin sodium salt hydrate in 40 mL of sterile deionized water. DO NOT AUTOCLAVE.
Store in dark at 2 - 8 °C.

Vancomycin solution

Dissolve 0.16 g vancomycin hydrochloride in 40 mL of sterile deionized water. DO NOT AUTOCLAVE.
Store in dark at 2 - 8 °C.

Manufacturing Entity

BioControl Systems, Inc, 12822 SE 32nd St, Bellevue, WA 98005, USA.
BioControl Systems, Inc is an affiliate of Merck KGaA, Darmstadt, Germany.

Warranty

BioControl Systems, Inc. (BCS) warrants this product to be free from defects in materials and workmanship, when stored under labeled conditions and used as intended until the expiration date stated on the package. BCS agrees during the applicable warranty period to replace all defective products after return to BCS. BCS shall not have obligation under this Limited Warranty to make replacements which result, in whole or in part, from negligence of the Buyer, or from improper use of the products, or use of the product in a manner for which it was not indicated. Buyer shall notify BCS of any products which it believes to be defective during the warranty period. At BCS option, such products shall be returned to BCS, transportation and insurance prepaid. BCS shall replace any such product found to be defective, at no charge. Should BCS examination not disclose any defect covered by the foregoing warranty, BCS shall so advise Buyers and dispose of the product in accordance with Buyer's instructions.

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