Specificity

The specificity of the **BioSign™ *E. coli O157*** test was determined by testing different bacterial strains (Table 2).

Table 2. Specificity: *E. coli O157* serotypes and other bacterial strains detected by BioSign™ *E. coli O157*

Strain	BioSign™ Result
Blank BPW	–
Citrobacter freundii	–
Enterobacter agglomerans	–
Enterobacter cloacae	–
E. coli ATCC (11229)	–
E. coli ATCC (11775)	–
E. coli O157:H7	+++
E. coli (SLR 56)	–
E. coli (SLR 57)	–
E. coli	–
E. coli O157:H45	+++
E. coli O157:H38	+++
E. coli O157:H19	+++
E. coli (O29:?)	–
E. coli O55:H6	–
Hafnia alvei	–
Klebsiella oxytoca	–
Proteus vulgaris	–
Pseudomonas aeruginosa	–
Salmonella typhimurium	–
Serratia liquefaciens	–
Shigella flexneri	–
Staphylococcus aureus	–
Streptococcus faecalis	–
Yersinia enterocolitica	–

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BioSign™ *E. coli O157*

Rapid *E. coli O157* Antigen Detection Test

For *In Vitro* Use Only

Simple One-Step Immunoassay for the Qualitative Detection of *E. coli O157* Antigen in Enriched Cultures

PBM

Catalog No.	BSP-503	35 Test Kit
	BSP-503-10	10 Test Kit

Intended Use

BioSign™ *E. coli O157* detects *E. coli O157* antigen in enriched cultures. The test is intended for use as an early indicator of *E. coli O157* pathogen contamination in food samples.

Summary and Principle of Procedure

E. coli O157 is currently the prominent serogroup associated with enterohaemorrhagic *Escherichia coli* infections. It is associated with hemorrhagic colitis as well as both hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. Unlike classical methods for screening which require tedious pipetting or washing steps to produce results, the **BioSign™ *E. coli O157*** test requires one simple step.

Principle

Sample material is enriched in specially defined broths to favor the growth of *E. coli O157*. Enrichment procedures based on international standards are recommended.[1,2,3,4] Modifications of this protocol are possible. The **BioSign™** test device contains a dye pad impregnated with anti-*E. coli O157* antibody-dye conjugate and a membrane strip, upon which anti-*E. coli O157* antibody is immobilized on the membrane in the Test area. After proper enrichment, the enriched broth is added to the sample well using a transfer pipette and is allowed to soak in. If *E. coli O157* antigens are present in the specimen, they will react with the conjugate dye, which binds to the immobilized antibody on the membrane, to generate a colored band at the Test position in the result window. The result is read in 5–10 minutes: one line only at the Control position (**C**) indicates the absence of *E. coli O157*; two lines at the Control position **(C)** and the Test position **(T)** indicate the presence of *E. coli O157*. A line at the Control position (**C**) always forms to indicate the test worked properly.

Reagents

Materials Provided

The **BioSign™ *E. coli O157*** test kit contains all the reagents necessary to perform the tests.

- Each **BioSign™** test device contains a membrane strip coated with anti-*E. coli O157* antibody and a pad impregnated with anti-*E. coli O157* antibody-dye conjugate in a protein matrix containing 0.1% sodium azide.
- Transfer Pipettes
- Instruction Insert

Materials Required but not Provided

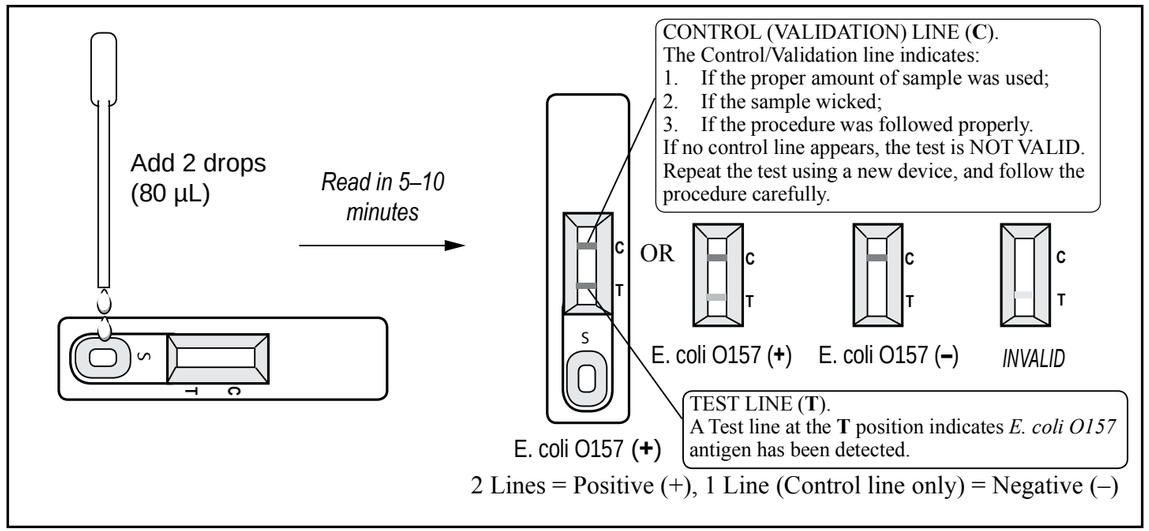
- Timer
- Enrichment broths
- Incubators or water baths capable of maintaining 37.0 ± 1.0°C or 42.0 ± 0.5°C
- Sterile glassware

Precautions

- The **BioSign™ *E. coli O157*** test should be performed at room temperature.
- *E. coli O157* screening and the use of this kit should be performed by persons with a basic knowledge of microbiology and associated hazards.
- Enriched material may contain high concentrations of pathogenic bacteria. Follow strict microbiological guidelines for working with biohazardous materials. Autoclave contaminated waste material before disposal.
- Do not smoke, eat or drink in areas where the specimens or kit reagents are handled.
- Wear disposable gloves while handling the kit reagents or specimens and wash hands thoroughly afterwards.
- All samples should be handled as if capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow standard procedures for proper disposal of specimens.
- The **BioSign™ *E. coli O157*** device should remain in its sealed pouch until ready for use.
- Do not use the test kit after the expiration date.

Storage and Stability

The **BioSign™ *E. coli O157*** test kit should be stored at 2-30°C (36-86°F) in its sealed pouch. The expiration date given was established under these conditions.



Sample Culture and Preparation

The following is the advised *E. coli O157* Enrichment Protocol based on research conducted at Campden and Corleywood Food Research Association and Milk Marketing Board.

SELECTIVE ENRICHMENT
25 g sample in 225 ml mEC+n*
↓
Incubate overnight (16–24 hrs at 37×C)
↓
DETECTION
Add 2 drops of the enriched culture to the Sample well of the **BioSign™ *E. coli O157*** test device

*mEC+n = Modified *E. coli* broth supplemented with novobiocin (Tryptone: 20.0 g/l, Bile salts No. 3: 1.12 g/l, Lactose: 5.0 g/l, K_2HPO_4 4.0: g/l, KH_2PO_4 1.5 g/l, NaCl: 5.0 g/l, novobiocin (sodium salt): 0.02 g/l)

Approximately 80 µL of enriched culture sample is required for each test.

Other culture methods may be used after validation of the method using the **BioSign *E. coli O157*** test.

Procedure

Procedural Notes

The instructions below must be followed to achieve optimal test results. Follow the assay procedure and always perform the test under carefully standardized conditions.

- If specimens, kit reagents or **BioSign™** devices have been stored in a refrigerator, allow them to reach room temperature before use.
- Do not open the foil pouch until you are ready to perform the test.
- Several tests may be run at one time.
- To avoid cross contamination, use a new transfer pipette for each specimen.
- Label the device with the sample name or control number.
- To add sample, allow the transfer pipette to fill with the enriched culture sample and add 2 drops into the Sample well, holding the pipette in a vertical position.
- After testing, dispose of the **BioSign™** device and transfer pipette following proper laboratory practices. Consider each material that comes into contact with specimen to be potentially infectious.

The test procedure consists of adding the enriched sample to the Sample well of the **BioSign™** device and watching for the appearance of colored lines in the result window.

Test Protocol

1. Enrich *E. coli O157* according to the recommended enrichment protocol or approved equivalent.
2. Dispense 2 drops (80 µL) of the enriched culture sample into the Sample well (**S**) using a transfer pipette.
3. Read the result in 5ï 10 minutes.

Interpretation of Results

Positive: Two Lines. The appearance of two reddish-purple lines—one at the Test position (**T**) and the other at the Control position (**C**)—indicates a positive test result; i.e., *E. coli O157* pathogens have been detected. The color intensity of the Test line may be weaker or stronger than that of the Control line.

*Note: The test result can be read as soon as a distinct purple color band appears at the Test position (**T**). The Test band will appear before the Control band in most of the strong positive cases. The Test band may appear after the Control band in weak positive cases, and the Control band may be darker than the Test band. The three possible positive cases, therefore, are:*

*a. Two strong colored bands at both the Test (**T**) and Control (**C**) positions.*
*b. One strong colored band at the Test position (**T**) and one light colored band at the Control position (**C**).*
*c. One light colored band at the Test position (**T**) and one strong colored band at the Control position (**C**).*

Negative: One Line. The appearance of only one reddish-purple line at the Control position (**C**) and no distinct line at the Test position (**T**) indicates the test result is negative (i.e., *E. coli O157* pathogens have not been detected).

Invalid: A distinct colored line should always appear at the Control position (**C**). The test is invalid if no line forms at the Control position (**C**). Such tests should be repeated with a new **BioSign™ *E. coli O157*** test device.

Limitations

- Proper enriched culture broth must be obtained for a qualitatively good test.
- This product is intended for use in the rapid detection of *E. coli O157* in enriched samples of food or feed origin. Performance with clinical samples such as feces, blood, or tissue has not been validated.
- There is a possibility that factors such as technical or procedural errors may interfere with the test and cause erroneous results.

User Quality Control

Quality Control: Control standards are not supplied with this kit; however, it is recommended that a control be tested as good laboratory testing practice. Before using a new kit with specimens, positive and negative controls should be tested to confirm the test procedure, and to verify the tests produce the expected Q.C. results. Q.C. specimens should also be run whenever there is any question concerning the validity of results obtained. For information on how to obtain controls, contact PBM's Technical Services.

Procedural Control: The Control line can be considered an internal procedural control. A distinct reddish-purple Control line should always appear if the test procedure is performed properly, an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the test reagents are working. If the Control line does not appear at the Control position (**C**), the test is invalid and a new test should be performed. If the problem persists, contact PBM for technical assistance.

A clear background in the result window is considered an internal negative procedural control. If the test has been performed correctly with adequate sample volume and the device is working properly, the background in the result window will be clear, providing a distinct result.

Performance Characteristics

Sensitivity

The sensitivity of the **BioSign™ *E. coli O157*** test was examined by testing serial dilutions of *E. coli O157* (Table 1). Test results show that **BioSign™ *E. coli O157*** test can detect 1.7 x 10^4 cells/mL or greater of *E. coli O157:H7* strain.

Table 1. Sensitivity: Levels of *E. coli* serotypes *O157:H7 and O157:H45* detected by BioSign™ *E. coli O157*

Strain	Concentration	BioSign™ Result
E. coli O157:H7	1.7 x 10^8	+++
	1.7 x 10^7	+++
	1.7 x 10^6	+++
	1.7 x 10^5	+
	1.7 x 10^4	+/–
	1.7 x 10^3	–
	Blank	–
E. coli O157:H45	2.7 x 10^8	+++
	2.7 x 10^7	++
	2.7 x 10^6	+
	2.7 x 10^5	–
	2.7 x 10^4	–
	2.7 x 10^3	–
	Blank	–

Reproducibility

The reproducibility of the **BioSign™ *E. coli O157*** was examined at three different sites using a total of 15 blind controls, consisting of 5 negative samples, 5 moderately positive samples, and 5 strongly positive samples. The results obtained at these three sites with these controls demonstrated 100% agreement with each other.