P-5911

BioSign™ Salmonella

Rapid *Salmonella* Antigen Detection Test

For *In Vitro* Use Only

Simple One-Step Immunoassay for the Qualitative Detection of Salmonella Antigen in Enriched Cultures

PBM

Catalog No.	BSP-501	35 Test Kit
	BSP-501-10	10 Test Kit

Intended Use

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

Summary and Principle of Procedure

Salmonella is a sporeless gram-negative rod shaped bacteria.[1] It is a pathogenic microorganism widely found among both domestic and wild animal populations. *Salmonella* causes two kinds of clinical illnesses: food poisoning symptom complex and enteric fever. *Salmonella* infection is one of the main causes of foodborne illness. Worldwide, the number of cases is steadily increasing.[2] Unlike classical methods for screening which require tedious pipetting or washing steps to produce results, the BioSign™ Salmonella test requires one simple step.

Principle

Sample material is enriched in specially defined broths to favor the growth of Salmonella. Enrichment procedures based on the international standard ISO 6579:1990 are recommended.[3] A recommended Salmonella enrichment protocol is depicted in Figure 1. Modifications of this protocol are possible. (More information is available upon request.)

The test device contains a dye pad impregnated with anti-Salmonella antibody-dye conjugate and a membrane strip, upon which anti-Salmonella antibody is immobilized on the membrane in the Test area. After proper enrichment, the post-enriched broth (Figure 1) is added to the Sample well using a transfer pipette and is allowed to soak in. If Salmonella antigens are present in the specimen, they will react with the conjugate dye, which in turn migrates on the membrane and binds to the immobilized antibody on the membrane in the Test area, to generate a colored band at the Test position (**T**) in the result window. The result is read in 5–10 minutes: one line at the Control position (**C**) indicates the absence of Salmonella, two lines at the Control position **(C)** and at Test position **(T**) in the result window indicate the presence of Salmonella. A line at the Control position (**C**) always forms to indicate the test worked properly.

Reagents

Materials Provided

The **BioSign™ Salmonella** test kit contains all the reagents necessary to perform the tests.

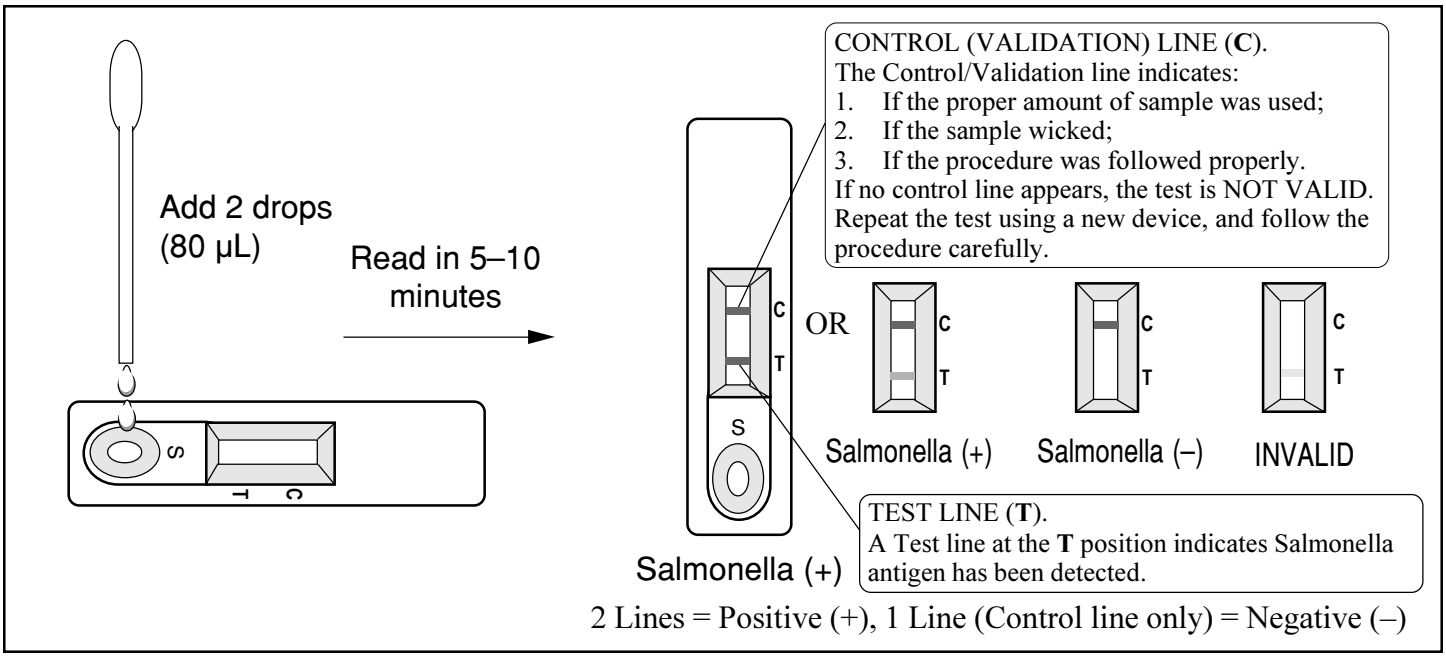
- Each **BioSign™** test device contains a membrane strip coated with anti-*Salmonella* antibody and a pad impregnated with anti-*Salmonella* antibody-dye conjugate in a protein matrix containing 0.1% sodium azide.
- Transfer Pipettes
- Instruction Insert

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™ Salmonella** test should be performed at room temperature.
- S*almonella* 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™ Salmonella** 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™ Salmonella** test kit should be stored at 2-30°C (36-86°F) in its sealed pouch. The storage conditions and stability dating given were established under these conditions.

Sample Culture and Preparation

Enrich the sample according to the following protocol.

Figure 1. Recommended *Salmonella* enrichment protocol based on ISO 6579:1990.

PRE-ENRICHMENT
25 g sample in 225 mL pre-enrichment broth (BPW or BPW+BG)†
(If necessary, Stomacher for 3 minutes)
↓
Incubate overnight (16-24 hrs) at 37°C
↓
SELECTIVE ENRICHMENT
Transfer 0.1 mL pre-enriched broth to 10 mL tube
Rappaport-Vassiliadis Broth
↓
Incubate 16-24 hrs (overnight) at 42°C
↓
POST-ENRICHMENT
Transfer 0.1 mL selective enriched broth to 10 mL tube
Buffered Peptone Water
↓
Incubate 6-8 hrs at 37°C
↓
DETECTION
Add 2 drops of the post-enriched culture to the Sample well of the **BioSign™ Salmonella** test

†BPW+BG = Buffered Peptone Water supplemented with 0.01% Brilliant Green (W/V); addition advised when high concentrations of competitive Gram-positive bacteria are present.

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

Other culture methods may be acceptable; after validation of the method, a culture sample obtained by another method may be assayed with the BioSign Salmonella test as described.

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 sample to the Sample well of the device and watching for the appearance of colored lines in the result window.

Test Protocol

1. Enrich *Salmonella* according to the recommended enrichment protocol (Figure 1) or approved equivalent.
2. Dispense 2 drops (80 µL) of the post-enriched culture solution into the Sample well (S) using a transfer pipette.
3. Read the result in 5 minutes, but no later than 10 minutes after adding sample.

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., *Salmonella* 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., *Salmonella* 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™ Salmonella** 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 *Salmonella* in enriched samples of food or feed origin. Performance with clinical samples (e.g. feces, blood, tissue) has not been validated.
- The **BioSign™ Salmonella** test has been tested by an independent laboratory and gives a positive result with the most commonly isolated foodborne *Salmonella* isolates. Sporadic false positive results can occur due to cross-reactions with certain rare species of *Citrobacter freundii*.
- 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, 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 anytime there is any question concerning the validity of results obtained. For information on how to obtain controls, contact PBM's Technical Services.

Process 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

The performance of **BioSign™ Salmonella** was compared with the traditional ISO 6579 method. Total of 64 samples were tested with the traditional ISO method and **BioSign™ Salmonella**. The results are summarized in Table 1.

Table 1.

		BioSign™ Salmonella (+)	(–)	Total
ISO method	(+)	41	4	45
	(–)	4	60	64

Relative Sensitivity : 91.8%
Relative Specificity : 94.1%
Overall Accuracy : 92.7%

The sensitivity of the **BioSign™ Salmonella** test was examined by testing serial dilutions of the various *Salmonella* strains. Test results show that **BioSign™ Salmonella** test can detect 5 x 10^5–5 x 10^6 cells/mL, depending on the *Salmonella* strain. The test exhibits decreased sensitivity with some *Salmonella* species, such as *arizonae, cerro, kedougou,* and *giessen.*

Reproducibility

The reproducibility of the test results of **BioSign™ Salmonella** 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 resultsobtained at these three sites with these controls demonstrated 100% agreement with each other.

Specificity

The specificity of the **BioSign™ Salmonella** test was determined by testing *Salmonella* species from the American Type Culture Collection. The list of detected species is given in Table 2.

Table 2. Specificity: *Salmonella* species detected by BioSign™ Salmonella

abortus-equi	*maartensdijk*
abortus-ovís	*maastricht*
adelaide	*menden*
anatum	*minnesota*
arizonae	*montevideo*
arizonae	*muenchen*
ssp.hinshawii	*newington*
bareilly	*newport*
berta	*ngili*
bredeney	*ochsenzoll*
breukelen	*oranienburg*
california	*panama*
cerro	*paratyphi-A*
chester	*paratyphi-A*
choleraesuis	*ssp.durazzo*
choleraesuis	*parera*
ssp.kunzendorf	*phoenix*
cubana	*pomona*
derby	*potsdam*
dublin	*pullorum*
enteritidis	*putten*
essen	*reading*
etterbeek	*rubislaw*
florida	*saintpaul*
gallinarum	*salamae*
gaminara	*sandiego*
give	*schalkwijk*
harmelen	*schottmuelleri*
heerlen	*senftenberg*
heidelberg	*simsbury*
hilversum	*sloterdijk*
hirschfeldii	*stanley*
hoograven	*tallahassee*
illinois	*tennessee*
inverness	*thompson*
javiana	*typhi*
kahla	*typhimurium*
kentucky	*typhisuis*
kirkee	*urbana*
kitenge	*vellore*
london	*worthington*
maarssen	*zwickau*

References

1. Farmer III, JJ and Kelly MT. Enterobacteriaceae. Manual of Clinical Microbiology, p. 360-383 (1991).
2. Jay JM. Foodborne gastroenteritis caused by *Salmonella, Shigella* and *Escherichia.* in "Modern food Microbiology" AVI Book p. 553-582 (1992).
3. Iso 6579. Microbiology—General guidance on methods for the detection of *Salmonella,* International Organization for Standardization, 2nd edition (1990).

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