I. Intended Use

The Biomerica *Salmonella* test is a lateral flow immunoassay for the rapid determination of the presence of *Salmonella species* in human stool samples. The test is intended for professional use only.

II. Summary and Explanation

The genus *Salmonella* contains only two species, *Salmonella enterica* (including six subspecies) and *Salmonella bongori*. The genus is also further subdivided into approximately 2,500 serovars (or serotypes). *Salmonella* species are the known causative agent of Salmonellosis. People afflicted with the illness develop diarrhea, fever and abdominal cramps 12 to 72 hours after infection. The symptoms can last from 4 days to 1 week. Over 40,000 cases of Salmonellosis are reported each year in the United States alone[1]. Even though most infected people recover without treatment, about 400 patients die each year due to acute Salmonellosis. *Salmonella* species are also related to Reiter's syndrome which is a disorder that is characterized by pain in the joints, irritation of the eyes and painful urination. Though recovery from Salmonella species can be fast, Reiter's syndrome can last for years and can lead to chronic arthritis. The detection of *Salmonella* species depends upon techniques, blood culture, sub-typing using polymerase chain reaction (PCR) [2-3], pulsed-filed gel electrophoresis[4] and enzyme linked immunosorbent assay (ELISA) [5]. A traditional method like blood culture coupled with microscopy can be limited by its lack diagnostic specificity. An advanced molecular method such as PCR is highly specific and the serotype can be identified, but is can be hampered by other particles present in the sample matrix. ELISA is an alternative but can also suffer from antigenic cross-reactivity. Since each method has its own advantages and disadvantages, other considerations are turn-around time, equipment and technician requirement. With these factors considered a good method of choice are immunochromatographic tests[6].

Immunochromatographic tests offer the potential of a rapid, one-step, stand alone device that can detect *Salmonella* species with the same sensitivity and specificity as a classical immunoassay.

III. Principle of the Procedure

The *Salmonella* species test utilizes the principle of non-competitive immunoassay. If *Salmonella* is present in the sample at concentrations above the detection level, a *Salmonella* species specific antibody conjugated to colloidal gold binds to the antigen forming an antigen-antibody conjugate complex. An antibody which is immobilized in the Test region ("T") of the membrane is reactive to another epitope of the *Salmonella* species. The capture antibody will then react with the antigen-antibody conjugate complex. The captured complex will appear as a pink color band on the membrane. A distinct green color band will always appear at the Control region ("C") indicating that the test is functioning properly.

IV. Materials Provided

1. Test device in foil pouch
2. Stool collection vial with buffer
3. Instruction manual

V. Materials Required but Not Provided

Timer and Gloves

VI. Warnings and Precautions

Safety Precautions: Human stool should be handled as if capable of transmitting infectious agents. It is recommended that these specimens be handled using established good laboratory working practices. The test should be discarded in a proper biohazard container after testing.

For *in vitro* diagnostic use: Do not use the kit beyond the expiration date printed on the outside of the foil pouch. Discard all used test devices into a proper biohazard container. The test should remain in the sealed pouch until use. Do not use the test if pouch is damaged. The test must be carried out within 2 hours of opening the sealed pouch.

VII. Kit Storage

The *Salmonella* species tests can be stored refrigerated or at room temperature (2-30°C). The test is usable until the expiration date stamped on the foil pouch.

VIII. Quality Control

Although the *Salmonella* species test contains an internal quality control (green color band in the Control region) good laboratory practice recommends the daily use of an outside control to ensure proper kit performance. Quality control samples should be tested according to the quality control requirements established by your laboratory.

IX. Test Procedure

Sample collection
Samples collected cannot be stored with preservatives or transport media. The samples can be stored refrigerated (2-4 °C) for 2 days prior to testing or frozen at -20°C for longer storage. Avoid freeze and thawing the samples. The samples need to be completely thawed, equilibrated to room temperature and mixed thoroughly before use.

Pre-treatment of the collected stool samples (see illustration 1):
Unscrew the cap of the provided stool collection vial filled with buffer. Using the stick on the cap collect about a pea size (250 mg) of solid sample. Place the stick back into the vial and close it. For liquid sample, aspirate about 250 µL into the vial and close it. Shake the vial thoroughly until the sample is dispersed well in its buffer Use a separate specimen collection vial for each sample being tested.

Test Procedure (see illustration 2)
Always allow the test devices, stool samples and stool collection vials to reach room temperature (15-30ºC) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the *Salmonella* species Device from its sealed pouch and use it as soon as possible.
2. Shake the specimen collection vial to assure good sample dispersion. Break off the tip of the vial.
3.. Dispense exactly 4 drops or 100 µL into the specimen well (S). Start the timer. Use a separate device for each sample
4. Read the result at **10 minutes** after dispensing the sample.

Illustration 1

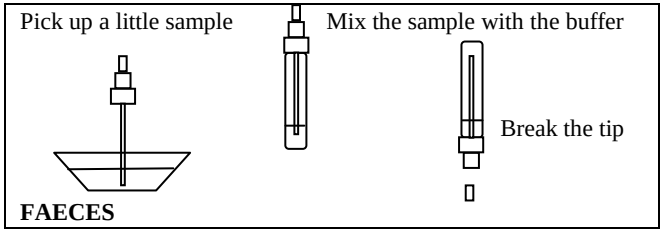
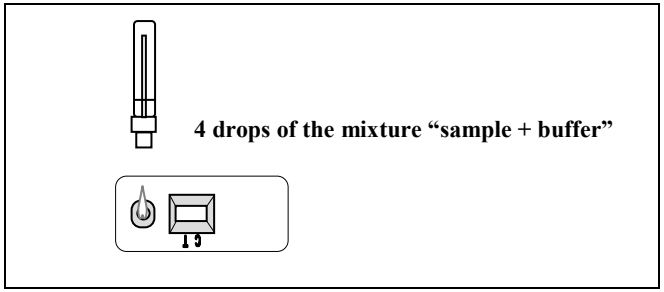
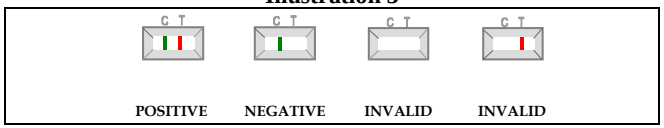


Illustration 2



X. Interpretation of Results

Illustration 3



POSITIVE: Two lines appear across the central window, in the Test region (a pink test line in the region marked with the letter T) and in the Control region (a green control line in the region marked with the letter C).

NEGATIVE: Only one band appears in the Control region (a green control line in the region marked with the letter C).

INVALID: A total absence of the control line regardless of the appearance of the test line. Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the test materials are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit and contact your local supplier.

The intensity of the pink colored band in the test line region (T) will vary depending on the concentration of antigens in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.

Internal procedural controls are included in the test:
- A green line appearing in the control region (C) confirms sufficient specimen volume and correct procedural technique.

XI. Performance Characteristics

Detection limit
The detection limit for the different serotypes is: *S. enteritidis* $1x10^4$bacteria/mL, *S. typhimurium* $1x10^4$ bacteria/mL and *S. typhi*: $1x10^7$bacteria/mL.

Sensitivity and specificity
Forty stool sample were studied using the Biomerica salmonella Test. Results of 99% sensitivity and >97% specificity were obtained. The results of all samples were confirmed by the Singlepath® Salmonella test.

The antibodies used in this test recognise *Salmonella* epitopes found in stool of patients, as well as in preparations from the bacteria cultures *in vitro*.

Cross-reactivity
A study was performed to determine the cross reactivity of the Biomerica *Salmonella* device. There is nocross reactivity with common intestinal pathogens, other organisms and substances occasionally present in feces: *H. pylori, Escherichia coli O157:H7, Listeria monocytogenes, Campylobacter*.

XII. Limitations of the Test

1. The test is a qualitative method for stool samples only. It is not meant for quantitative analysis.
2. Excessive amount of the sample applied to the specimen well can make the background appear dark which may lead to a difficult interpretation of the result. In such a case, the sample should be diluted and the test repeated.
3. If a negative result is obtained and clinical symptoms persist, additional testing using other clinical methods and/or seeing a physician is recommended. A negative result does not preclude the possibility of *Salmonella* species infection.
4. Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

XIII. References

1. CDC Salmonella Homepage http://www.cdc.gov/salmonella/general/
2. AR Jex, KK Stanley, W Lo, R Littman, JJ Verweij, BE Campbell, MJ Nolan, A Pangasa, MA Stevens, S Haydon, RB Gasser, Detection of pathogens in human faeces using an automated, robotic platform, Mol and Cel Prob, 2011 31(in Press 31Oct2011)
3. BHong Y, Lee MD, Hofacre CL, Maier M, White DG, Ayers S, Wang L, Berghaus R, Maurer J, A rapid screen of broth enrichments for Salmonella enteriditis, Hadar, Heidelberg and Typhymurium by using an allelotyping multiplex PCR that targets O- and H-antigen alleles, J Food Prot; 2009 72 (10) p 2198-2201
4. Hudson CR, Garcia M, Gast RK, Maurer JJ, Determination of close genetic relatedness of the major Salmonella entriditis phage types by pulsed-gel electrophoresis and DNA sequence analysis of several Salmonella virulence genes, Avian Dis; 2001(45) p 875-886
5. O siomaki, R Vuento, K Granfors, Serological diagnosis of Salmonella infections by enzyme immunoassay, The Lancet, 1989 333(8652) p1411-1414
6. Moongkarndi P, Rodpai E, Kanarat S, Evaluation of an immunochromatographic assay for rapid detection of salmonella enteric serovars Typhimurium and Enetiriditis, J Vet Diagno Invest; 2011 23(4) p 797-801

XIV. Ordering information

Ordering Information:

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IVD

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EZ-SALMONELLA

One-Step *Salmonella* species Rapid Test

January 2012

REF **1007**

Immunoassay for the qualitative determination of *Salmonella species* in human stool specimen

FOR *IN VITRO* DIAGNOSTIC USE ONLY

FOR PROFESSIONAL USE ONLY

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