

1-2 Test®

AOAC Official Method 989.13

1-2 Test Tray of 12 10107-12

1-2 Test Kit of 72 10107-72

General Description

The 1-2 Test is a rapid qualitative method for the detection of motile *Salmonella* in foods, food ingredients and environmental samples. It is based on the observation of *Salmonella* immobilized in motility medium by polyvalent H (flagellar) antibodies. Immobilization of motile *Salmonella* results in development of well-defined band of cells (ImmunoBand™).

Using the 1-2 Test

A. Sample Preparation and Enrichment

Pre-Enrichment

Prepare samples according to procedures described in the current edition of the Bacteriological Analytical Manual (BAM). Incubate 24 ± 2 h at $35\text{--}37^\circ\text{C}$.

For all samples with exception of raw flesh and highly contaminated products proceed to B. Test Procedure.

For raw flesh and highly contaminated products

Selective Enrichment

Transfer 1 mL incubated pre-enrichment mixture to 9 mL iodine-activated tetrathionate brilliant green broth (TBG). Gently vortex mixture. Incubate 6–8 h in a $42^\circ\text{C} \pm 0.5^\circ$ water bath.

B. Test Procedure

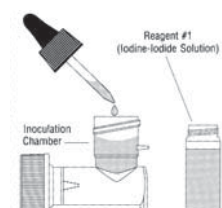
Each step of the preparation sequence can be performed on an individual unit or simultaneously on multiple units. Sample numbers can be recorded 1) on lower half of motility chamber but must not interfere with reading results, or 2) on flat surface of white cap.

a. Addition of Iodine-Iodide Solution

For all samples with exception of raw flesh and highly contaminated products

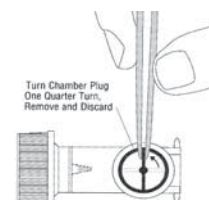
Position 1-2 Test unit with black cap UP and remove cap. Add 1 drop of Reagent #1 (Iodine-Iodide Solution) to inoculation chamber. Replace cap and gently shake unit to mix.

NOTE: This step is not performed for raw flesh and highly contaminated products because tetrathionate broth incubation is conducted in a test tube.



b. Chamber Plug Removal

Reposition 1-2 Test unit with black cap UP and remove cap. Remove chamber plug with sterile forceps and discard plug. If chamber plug is not removed, bacteria will be unable to move from inoculation chamber to motility chamber.



a. Inoculation

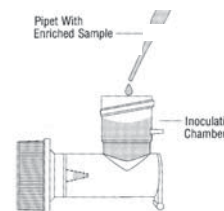
Prior to inoculation, be sure that enrichment broth containing sample is well-mixed.

For all samples with exception of raw flesh and highly contaminated products

Transfer 0.1 mL enriched sample into inoculation chamber.

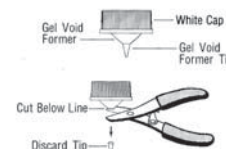
For raw flesh and highly contaminated products

Transfer 1.5 mL of tetrathionate broth from 42 °C incubation to the previously emptied inoculation chamber.



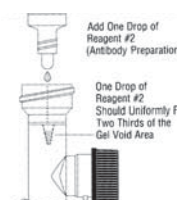
b. Gel Void Former Tip Removal

Position 1-2 Test unit with white cap UP and remove cap. Snip off tip of gel void former with shears and discard tip. The cut should be made at point where tip meets base of gel void former.



c. Addition of Antibody Preparation

Add 1 drop of Reagent #2 (Antibody Preparation) to gel void in motility chamber. Replace white cap. One drop of Reagent #2 should uniformly fill lower two-thirds of gel void. This can be determined by observing blue antibody solution in void. Do not add more than 1 drop.



d. Incubation

Place inoculated 1-2 Test units in incubator with white cap UP. Incubate units for 14 h to 30 h at 35–37 °C.

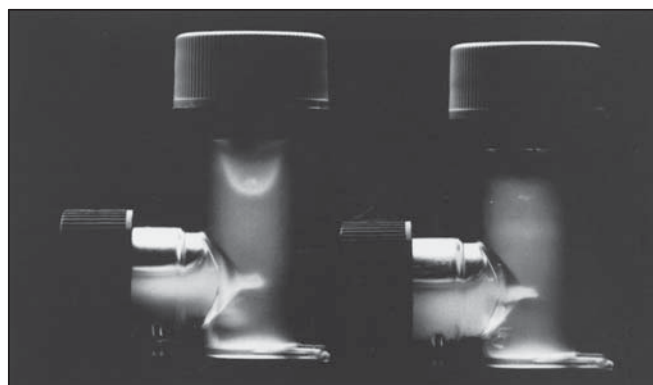
1-2 Test units may be incubated individually or while held in incubation tray.

B. Reading and Interpretation of Results

A desktop light is strongly recommended for reading results. With white cap UP, hold unit next to a strong light. Carefully observe motility chamber gel from all sides by rotating 1-2 Test unit back and forth through 360° turn in front of light source.

a. Positive Results

A presumptive positive test is indicated by the presence of a white band (ImmunoBand) that is U-shaped or meniscus-shaped. The band may be fully formed or more distinct on one side of gel, and is seen in upper half of motility chamber gel. A positive test indicates that the sample presumptively contains *Salmonella*. If not confirming immediately, store positive units under refrigeration (2–8 °C).



Positive 1-2 Test indicated by presence of a U- shaped ImmunoBand

Negative 1-2 Test indicated by absence of ImmunoBand

b. Negative Results

If no band is seen after incubating the test unit for at least 14 h, the test is negative. Negative units may show uniform turbidity throughout Motility Chamber as a result of movement of bacteria throughout the gel.

C. Confirmation

Confirm all presumptive positive samples as described in the AOAC International reference procedure. Isolate *Salmonella* from the tetrathionate broth in the inoculation chamber.

D. Positive and Negative Controls

Controls may be run with test samples. Use one 1-2 Test unit for each positive and negative control.

Positive Controls: Inoculate a known isolate of motile *Salmonella* into a 10 mL non-selective broth and incubate overnight at 35–37 °C. After incubation is complete, inoculate 0.1 mL of turbid broth culture into a 1-2 Test unit. Follow procedure in "Using the 1-2 Test".

Negative Controls: Inoculate a known isolate of a motile enteric bacterium (e.g. *Klebsiella spp.*) into a 10 mL non-selective broth and incubate overnight at 35–37 °C. After incubation is complete, inoculate 0.1 mL of turbid culture into a 1-2 Test unit. Follow procedure in "Using the 1-2 Test".

E. Storage Conditions

Refrigerate on arrival at 2–8 °C (36–46 °F)

DO NOT FREEZE.

F. Disposal

1-2 Test units are not reusable. Inoculated 1-2 Test units may contain pathogenic organisms and should be handled using appropriate aseptic technique. All caps must be kept tightly secured. After use all units must be steam sterilized at 121 °C for at least 30 minutes.

NOTE: Spanish and French translations available upon request.

Manufacturing Entity

BioControl Systems, Inc, 12822 SE 32nd St, Bellevue, WA 98005, USA.

BioControl Systems, Inc is an affiliate of Merck KGaA, Darmstadt, Germany.



Example of a meniscus shaped ImmunoBand



Example of an asymmetrical ImmunoBand



Example of a right-sided asymmetrical ImmunoBand



Example of a left-sided asymmetrical ImmunoBand

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