

17.9.18

AOAC Official Method 989.13
Motile *Salmonella* in All Foods
Immunodiffusion (1–2 TEST) Method
First Action 1989
Revised First Action 1994
Final Action 1998

(Applicable to the detection of motile *Salmonella* in all foods.)

Method detects presence of motile *Salmonella* in all foods. It is not confirmatory because polyvalent H (flagellar) antibodies used in test may cross-react with small percentage of non-*Salmonella*. Method does not detect nonmotile *Salmonella*.

If test is positive, enrichment broths from inoculation chamber of test unit must be streaked onto selective/differential agar media as in **967.26B** (see 17.9.02). Typical or suspicious colonies must be identified as in **967.26C** (see 17.9.02), **967.27** (see 17.9.03), and **967.28** (see 17.9.07).

See Tables **989.13A** and **B** for the results of the interlaboratory study supporting the acceptance of the method.

A. Principle

Detection of *Salmonella* is based on presence and observation of *Salmonella* immobilized in motility medium by polyvalent H (flagellar) antibodies. Figure **989.13** shows small disposable plastic device (1–2 TEST unit) which has 2 chambers. Enriched test portion is inoculated into smaller chamber. Larger motility chamber of unit contains peptone-based motility medium. Motility chamber is sealed with gel-former plug. Tip of this plug forms void in motility medium for addition of flagellar antibody preparation. For shipping, opening between 2 chambers is sealed with chamber plug, which is removed and discarded prior to addition of inoculum. *Salmonella* contained in tetrathionate broth move from this medium into motility medium to react with flagellar antibodies. Immobilization of motile *Salmonella* results in development of well defined immunoband of cells.

B. Reagents

(a) 1–2 TEST unit.—See Figure **989.13**.

Table 989.13A Interlaboratory study results for determination of *Salmonella* in all foods by immunodiffusion screening method

Results	Percent	95% Confidence range (approx.)
Agreement ^a	96.1	94.5–97.7
False negatives (BAM/AOAC) ^b	1.7	0.5–3.0
False negatives (immunodiffusion) ^c	3.6	1.8–5.5

Source: Original collaborative study, *J. Assoc. Off. Anal. Chem.* **72**, 300(1989).

^a Rate reflects number of test portions read identically between BAM/AOAC [*Bacteriological Analytical Manual* (1998), 8th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA] culture method and immunodiffusion method.

^b Rate reflects number of test portions positive by immunodiffusion method but determined as negative by BAM/AOAC culture method.

^c Rate reflects number of test portions positive by BAM/AOAC culture method but determined as negative by immunodiffusion method.

(b) *Iodine-iodide solution*.—0.21 g resublimed I₂ and 0.18 g KI/mL H₂O.

(c) *Antibody solution*.—Specific polyclonal flagellar and somatic antibodies to *Salmonella* serotypes.

(d) *Diagnostic reagents*.—Necessary for culture confirmation of presumptive positive 1–2 TEST results. See **967.25B** (see 17.9.01).

(e) *Tetrathionate brilliant green broth (TBG)*.—Suspend 5.0 g tryptose or proteose peptone, 1.0 g bile salts, 10.0 g CaCO₃, and 30.0 g Na₂S₂O₃ in 1 L H₂O, and mix well. Adjust pH to 7.4–7.6. Heat medium until it boils, then cool and store at 4°C. On day of use, add aseptically 1.0 mL 1% aqueous brilliant green dye solution and 20.0 mL iodine solution to 1 L medium, and mix well. Dispense 9 mL portions into 16 × 150 mm test tubes.

Items (a)–(c) are available as BioControl 1–2 TEST (BioControl Systems, Inc., 12822 SE 32nd St, Bellevue, WA 98005, USA). Store at 4–8°C.

Table 989.13B Interlaboratory study results for comparison of BAM culture and modified immunodiffusion methods for motile *Salmonella* in raw foods or foods with a high microbial load

Test sample	MPN, cells/g	False negatives ^a , %		Performance rates ^b , %				
		Immuno-diffusion	BAM culture	Sensitivity		Specificity		Agreement
				Immuno-diffusion	BAM culture	Immuno-diffusion	BAM culture	Modified immunodiffusion vs BAM culture
Raw shrimp	0.0	—	—	—	—	100	100	100
	0.009	9	0	91	100	—	—	98.5
	0.043	2	0	98	100	—	—	98.5
Animal meals	—	—	—	100	100	—	—	100
	0.009	8.3	5.6	91.7	94.4	—	—	93.8
	0.39	3.3	1.6	96.7	98.4	—	—	95.2
Raw poultry	0.009	4	5.4	96	94.6	—	—	90.7
	0.043	8.5	1.4	91.5	98.6	—	—	90.7
	0.093	14.9	13.4	85.1	86.6	—	—	74.3

^a False-negative percent calculated as 100 minus sensitivity rate.

^b Performance rates calculated according to procedure of McClure [*J. Assoc. Off. Anal. Chem.* **73**, 953(1990)].

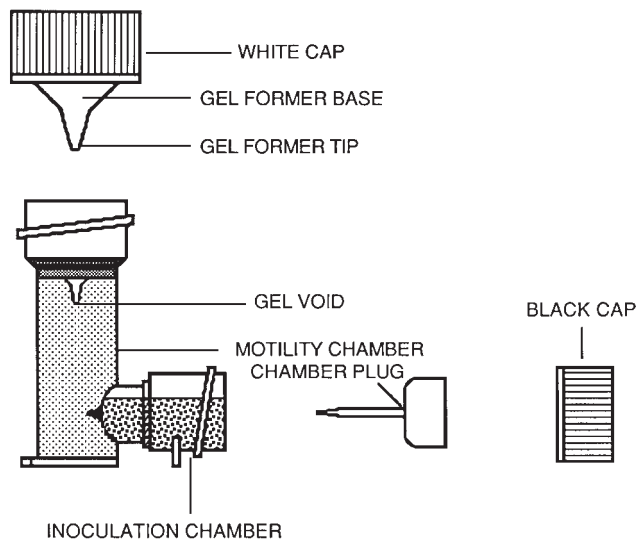


Figure 989.13—TEST unit for immunodiffusion screening method for motile *Salmonella*.

C. Preparation of Test Portion

(a) *Pre-enrichment*.—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella*. Methods used may vary with product and should be performed as indicated in *Bacteriological Analytical Manual*, current edition.

(b) *Selective enrichment*.—For raw foods or foods with a high microbial load only. Transfer 1 mL incubated pre-enrichment mixture to 9 mL iodine activated TBG, **B(e)**, shake gently, and incubate 6–8 h at $42 \pm 0.5^\circ\text{C}$.

D. General Instructions

Components and procedures of test kit have been standardized for use in 1–2 TEST procedures. Components or procedures other than those supplied by BioControl Systems, Inc., may yield unsatisfactory results, and should be pretested.

E. Immunodiffusion Detection

(a) *Test unit preparation and inoculation*.—Each test unit has 2 chambers: inoculation chamber and motility chamber (Figure 989.13). Each step of preparation sequence can be performed on individual unit or multiple units as needed. Test portion numbers can be recorded on lower portion of motility chamber but must not interfere with reading of results. Alternatively, test portion numbers may be recorded on flat surface of white cap. When cap is replaced, it must be screwed on tightly. (1) For all food types

except raw foods or foods with a high microbial load, position unit with black cap UP, and remove black cap. Add 1 drop of iodine–iodide solution, **B(b)**, to inoculation chamber. Replace black cap and gently shake unit to mix. (*Note*: Do not perform this step for raw foods or foods with a high microbial load because TBG incubation is conducted in test tube.) (2) With black cap off, remove chamber plug from inoculation chamber with sterile forceps and discard plug. If chamber plug is not removed, bacteria will be unable to move from inoculation chamber to motility chamber. (3) Be sure that broth containing test portion is well mixed prior to inoculation. Using pipet, transfer 0.1 mL pre-enriched test portion (except raw foods or foods with a high microbial load) from **C(a)** into inoculation chamber. For raw foods or foods with a high microbial load only, add 1.5 mL TBG from 42°C incubation **C(b)** to previously emptied inoculation chamber. Replace black cap. (4) Position unit with white cap UP and remove white cap. Snip off tip of gel-former plug with shears or clippers and discard tip. Cut should be made at point where tip meets base of plug. If tip of gel-former plug is not removed, antibody solution will be displaced from gel void where white cap is replaced. (5) Add 1 drop of antibody solution, **B(c)**, to gel void in motility chamber. Replace white cap. Antibody preparation should fill ca $\frac{2}{3}$ of gel void. This can be determined by observing blue antibody solution in gel void.

(b) *Incubation*.—Place inoculated unit in incubator with white cap UP. Incubate unit in shipper/incubation tray for 14–30 h at 35°C .

(c) *Reading positive results*.—With white cap UP, hold unit next to strong light. Desktop fluorescent light is recommended for reading test results. Carefully observe motility chamber gel by rotating unit back and forth through 360° turn in front of light source. Positive test is indicated by presence of white band that is U-shaped or meniscus-shaped. Band may be fully formed or may be more distinct on one side of gel, and is seen in upper half of motility chamber gel. Positive test indicates that test portion presumptively contains *Salmonella*. Positive test results must be confirmed by standard culture methods as in **F**.

(d) *Reading negative results*.—If no band is observed after at least 14 h incubation, test is negative. Negative units may show uniform turbidity throughout motility chamber as result of movement of bacteria in gel.

F. Confirmation of Positive Test Portions

Perform culture confirmation by using 3 mm loop to obtain inoculum from TBG in inoculation chamber and streaking HE, XLD, and BS plates. Identify typical or suspicious colonies from selective plates as in **967.26C** (see 17.9.02), **967.27** (see 17.9.03), and **967.28** (see 17.9.07).

Reference: *J. AOAC Int.* **78**, 59(1995).

Revised: June 2000