AOAC Official Method 999.09
VIP for *Salmonella* for the Detection of Motile and Non-Motile *Salmonella* in All Foods

First Action 1999

(Applicable to identification of *Salmonella* in all foods.)

Caution: *See* Appendix B, safety notes on handling microorganisms. Decontaminate all spent media and equipment used in test prior to disposal of media or re-use of equipment.

See Table **999.09A** for the results of the interlaboratory study supporting the acceptance of the method.

A. Principle

In visual immunoprecipitate (VIP) assay, proprietary antibodies, with a high specificity to *Salmonella* antigens, are bound to chromogenic carrier and, separately, to solid support matrix. Reagents are incorporated in test units and produce visually discernable reaction in presence of *Salmonella* spp. During initial hydration of test unit, *Salmonella* reacts with antibody-chromogen complex. Antigen antibody-chromogen complex is formed, which flows across lateral flow membrane and is subsequently bound by antibody immobilized on membrane. Positive reaction is indicated by presence of detection line positioned across the solid support in test window. Proper test completion is indicated by another line formed in test verification window. Absence of line in test verification window invalidates test.

B. Apparatus

(**a**) *Incubator.*—Maintaining 35–37°C.

(**b**) *Water baths.*—Maintaining 42 ± 0.2°C and one maintaining 100°C. Alternatively flowing steam autoclave set at 121°C can be used.

(**c**) *Syringe.*—With 0.2 μm or smaller porosity filter.

(**d**) *Micropipets.*—Accurately dispensing 0.1 mL.

(**e**) *Top loading balance.*—For weighing test portions.

(**f**) *Vortex mixer.*—For mixing contents of tubes.

C. Media and Reagents

(**a**) *Extraction reagent 1.*—Aqueous solution containing 1.1% sodium dodecyl sulfate.

(**b**) *Extraction reagent 2.*—Aqueous solution containing 1.1% cetyl trimethylammonium bromide.

(**c**) *VIP unit.*—One per test (available as VIP for *Salmonella* Assay from BioControl Systems, Inc., 12822 SE 32nd St, Bellevue, WA 98005, USA).

(**d**) *Buffered peptone water (BPW).*—Suspend 20 g commercial BPW in 1 L deionized H_2O. Stir until completely dissolved. Autoclave broth 15 min at 121°C. Final pH is 7.2 ± 0.2.

(**e**) *Buffered peptone water with novobiocin.*—Suspend 20 g dehydrated buffered peptone water in 1 L deionized water. Mix thoroughly. Dispense in 225 mL aliquots for food test portions. Autoclave at 121°C for 15 min. On day of use, add 4 mL 0.1% novobiocin solution, (**n**), to 225 mL BPW. Final pH is 7.2 ± 0.2.

(**f**) *Brilliant green dye water.*—Add 2 mL 1% brilliant green dye solution, (**k**), per 1 L sterilized water.

(**g**) *Reconstituted nonfat dry milk with brilliant green dye.*—Dissolve 100 g nonfat dry milk in 1 L deionized water. Mix thoroughly. Autoclave at 121°C for 15 min. Cool and add 2 mL 1% brilliant greeen dye solution, (**k**), per 1 L nonfat dry milk.

(**h**) *Brain heart infusion broth with enrichment supplement containing Oxyrase® (BHI + O).*—Suspend 37 g commercial BHI in 1 L deionized water. Mix thoroughly. Autoclave broth at 121°C for 15 min. On day of use, add 1 mL enrichment supplement containing Oxyrase® to each 225 mL bottle after test portion has been added and mixing is complete. Final pH is 7.4 ± 0.2.

(**i**) *Rappaport–Vassiliadis R10 medium (RV).*—Suspend 26.6 g commercial Rappaport–Vassiliadis R10 medium in 1 L deionized water and heat gently to dissolve. Dispense in 10 mL aliquots and sterilize by autoclaving at 116°C for 15 min. Final pH is 5.1 ± 0.2.

(**j**) *5X Rappaport–Vassiliadis R10 medium (5X RV).*—Suspend 133 g commercial Rappaport–Vassiliadis R10 medium in 1 L deionized water and heat gently to dissolve. Dispense in 5 mL aliquots in 25 × 150 mm test tubes and sterilize by autoclaving at 116°C for 15 min.

(**k**) *Tetrathionate broth (TT).*—Suspend 46 g commercial TT broth in 1 L deionized water. Mix thoroughly. Heat with agitation and boil for 1 min to completely dissolve powder. *Do not autoclave*. Cool to below 45°C. Prepare brilliant green dye solution by dissolving 1 g dye in sterile water and diluting to 100 mL. Add 1 mL 1% brilliant green dye solution to TT broth. Dispense in 10 mL aliquots in sterile test tubes and store at 4–8°C. Prepare I–KI solution by dissolving 6 g I and 5 g KI in 20 mL sterile water. On day of use, add 0.2 mL I–KI solution to each 10 mL tube to be used. Final pH is 8.4 ± 0.2.

(**l**) *5X Tetrathionate broth (5X TT).*—Suspend 230 g commercial TT broth into 1 L deionized water. Mix thoroughly. Heat with agitation and boil for 1 min to completely dissolve powder. *Do not autoclave.* Cool to 45°C and add 5 mL 1% brilliant green dye solution [*see* (**k**)]. Dispense in 5 mL aliquots in 25 × 150 mm sterile test tubes. On day of use, add 0.5 mL I–KI solution [*see* (**k**)] to each 5 mL tube to be used.

(**m**) *Trypticase soy broth + 2,4,dinitrophenol + 0.1% novobiocin (TSB + DNP + n).*—Suspend 30 g commercial TSB and 0.1 g DNP in 1 L deionized water. Mix thoroughly. Warm gently until the media is dissolved. Dispense in 10 mL aliquots and autoclave at 121°C for 15 min. On day of use, add 0.1 mL 0.1% novobiocin solution to each 10 mL tube prior to transfer of selective enrichment. Final pH is 7.3 ± 0.2.

(**n**) *0.1% Novobiocin solution.*—Suspend 0.1 g novobiocin sodium salt in 100 mL deionized water. Filter sterilize using a 0.2 μm or smaller porosity filter attached to a syringe. Solution is stable up to 60 days when stored in dark bottle at 2–8°C.

Items (**a**)–(**c**) are available as VIP for *Salmonella*, Visual Immunoprecipitate Assay for Detection of *Salmonella* from BioControl Systems, Inc., 12822 SE 32nd St, Bellevue, WA 98005, USA.

Item (**e**) is available as enrichment supplement containing Oxyrase® from BioControl Systems, Inc.

D. General Instructions

VIP units should be stored in foil pouch with desiccant at ambient temperature in cool dark place. After use, discard units into appropriate decontamination container and sterilize before disposal. Do not re-use VIP units and do not use after expiration date.

Perform positive and negative control cultures to become familiar with interpretation of results.

Table 999.09A Interlaboratory study results for detection of motile and non-motile *Salmonella* spp. in foods by VIP for *Salmonella*

Test sample	Level	MPN/g	Total No. of test portions	Test portions positive			χ^{2b}	Sensitivity rate[c]		Incidence of false negatives among total positive test portions, %[d]		Specificity rate[e]	Incidence of false positives among total negative test portions, %[f]	Agreement between VIP and OMA methods, %[g]
				VIP										
				Pres.[a]	Conf.[a]	OMA		VIP	OMA	VIP	OMA	VIP	VIP	
Ice cream	Low	0.009	75	29	18	16	0.1	66.7	59.3	33.3	40.7	92.3	7.7	73
	High	0.092	75	73	73	74	0.0	97.3	98.7	2.7	1.3	100	0.0	96
	Control	<0.003	75	7	0	0	—[h]	—	—	—	—	—	—	—
Liquid milk	Low	0.009	80	54	52	51	0.0	77.6	76.1	22.4	23.9	98.4	1.6	61
	High	0.231	80	79	79	80	0.0	98.8	100	1.2	0.0	100	0.0	99
	Control	<0.003	80	6	0	0	—	—	—	—	—	—	—	—
Milk chocolate	Low	0.023	75	19	18	13	0.6	65.5	48.3	34.5	51.7	99.3	0.7	67
	High	0.933	75	72	72	74	0.3	96.0	98.7	4.0	1.3	100	0.0	95
	Control	<0.003	71	2	0	0	—	—	—	—	—	—	—	—
Dried egg powder	Low	0.023	75	35	35	46	2.7	59.3	78.0	40.7	22.0	100	0.0	51
	High	0.933	75	72	72	75	1.3	96.0	100	4.0	0.0	100	0.0	96
	Control	<0.003	75	0	0	0	—	—	—	—	—	—	—	—
Raw ground chicken	Lot 1	0.933	85	84	84	85	0.0	98.8	100	1.2	0.0	100	0.0	99
	Lot 2	0.092	85	59	59	48	2.7	81.9	66.7	18.1	33.3	100	0.0	56
Raw ground pork	Low	0.004	75	26	24	22	0.0	61.0	56.1	39.0	43.9	98.6	1.4	55
	High	0.231	75	73	72	74	0.3	96.0	100	4.0	0.0	98.7	1.3	95
	Control	<0.003	75	8	0	0	—	—	—	—	—	—	—	—

[a] Pres. = presumptive data, conf. = culturally confirmed data.

[b] χ^2, as defined by McNemar is $(|a-b|-1)^2/(a+b)$ where a = test portions positive by VIP and negative by OMA, and b = test portions negative by VIP and positive by OMA. A χ^2 value > 3.84 indicates significance at $p < 0.05$.

[c] Sensitivity rate is defined as total number of analyzed positive test portions among "known" positive test portions/lab divided by total number of "known" positive test portions/lab, where "known" positive is defined as test portions confirmed positive by the reference method.

[d] Incidence of false negatives is 100 – sensitivity rate. Low number of total confirmed positives will result in high false negative data.

[e] Specificity rate is defined as total number of analyzed negative test portions among "known" negative test portions/lab divided by total number of "known" negative test portions/lab, where "known" negative is defined as test portions confirmed negative by the reference method and negative controls.

[f] Incidence of false positives is 100 – specificity rate.

[g] Rate reflects number of confirmed determinations that were equivalent between VIP and OMA.

[h] Satistical analysis not applicable. Methods gave equivalent results.

Table 999.09B

Food type	Pre-enrichment broth
Nonfat dried milk	Brilliant green water
Liquid egg products	Trypticase soy broth (TSB)
Raw foods	Buffered peptone water + novobiocin (BPW + n)
Chocolate based products	Nonfat dried milk + brilliant green
Dried powder processed products	Brain heart infusion broth + enrichment supplement containing Oxyrase® (BHI + O)
All other foods	Buffered peptone water (BPW)

E. Enrichment

(**a**) *Pre-enrichment (see Table **999.09B**).*—(*1*) *Foods with a low microbial load (excluding dried powder foods).*—Add 25 g test portion to 225 mL appropriate pre-enrichment broth, pre-warmed to 35–37°C. Mix thoroughly according to BAM/OMA, current edition. Incubate 6–8 h at 35–37°C. (*2*) *Foods with a low microbial load (dried powder foods).*—Add 25 g test portion to 225 mL pre-warmed BHI broth. Mix thoroughly according to BAM/OMA, current edition. Add 1 mL enrichment supplement containing Oxyrase® (BHI + O). Mix well by swirling. Incubate 6–8 h at 35–37°C. (*3*) *Foods with a high microbial load.*—Add 25 g test portion to 225 mL BPW. Add 4 mL 0.1% novobiocin solution. Mix thoroughly according to BAM/OMA, current edition. Incubate 18–26 h at 35–37°C.

(**b**) *Selective enrichment.*—(*1*) *Foods with a low microbial load (excluding dried powder foods).*—Transfer 25 mL pre-enrichment broth to 5 mL 5X RV medium and transfer another 25 mL to 5 mL 5X TT broth. Vortex mix thoroughly. Incubate in water bath 16–24 h at 42°C. (*2*) *Foods with a low microbial load (dried powder foods).*—Transfer 25 mL BHI + O broth to 5 mL 5X TT broth. Vortex mix thoroughly. Incubate in water bath 16–24 h at 42°C. (*3*) *Foods with a high microbial load.*—Transfer 0.1 mL pre-enrichment broth to 10 mL RV medium and transfer another 1.0 mL to 10 mL TT broth. Vortex mix thoroughly. Incubate in water bath 16–24 h at 42°C.

(**c**) *Post enrichment.*—Following selective enrichment, transfer and combine 0.5 mL TT broth and 0.5 mL RV medium into a single tube containing 10 mL pre-warmed TSB + DNP + n broth. For dried powder foods, transfer 1 mL TT into 10 mL pre-warmed TSB + DNP + n. Vortex mix thoroughly. Incubate all foods with a low microbial load 6–8 h at 35–37°C. Incubate raw foods or foods with a high microbial load in water bath 5–8 h at 42°C.

(**d**) *Extraction.*—Pre-warm extraction reagents by placing bottles in hot water for 10 min. Swirl each bottle gently to thoroughly mix. Add 0.1 mL extraction reagent 1 and 0.1 mL extraction reagent 2 to empty tube. Vortex mix incubated TSB + DNP + n broth tube and transfer 1.0 mL to tube with extraction reagents. Vortex mix thoroughly. Inactivate microorganisms by autoclaving at 121°C for 10 min. Cool tubes to 25–37°C before testing. Inactivated tubes can be stored at 4–8°C up to 4 days prior to testing.

F. VIP Procedure

Open sealed pouch and remove required number of VIP units. Use 1 unit per test. Do not re-use VIP units. Carefully reseal unused VIP units in pouch containing desiccant and store at ambient temperature in a cool dark place.

Transfer 0.1 mL inactivated test suspension to test well and incubate 10 min at ambient temperature. Proceed directly to **G**.

G. Reading and Interpreting Results

(*Note*: Examine device immediately after 10 min incubation. If readings are taken after 10 min, faint lines may develop because of nonspecific color development and should be disregarded.)

Examine VIP unit for presence of distinct detection lines in test and test verification windows. Lines should be dark when contrasted with white background and should extend across window. Intensity of test and test verification lines may differ. Test is valid as long as line is present in test verification window.

If no line is present in test verification window, test is invalid. Autoclave spent VIP units 15 min at 121°C prior to discarding.

H. Confirmation of Positive VIP Samples

Presumptive positive samples must be confirmed culturally as described in **967.26** (*see* 17.9.02), **967.27** (*see* 17.9.03), and **967.28** (*see* 17.9.07).

Reference: *J. AOAC Int.* **83**, 889(2000).

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