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AOAC Official Method 2014.01
Salmonella in Selected Foods
3M™ Petrifilm™ *Salmonella* Express System
First Action 2014
Final Action 2017

[Applicable to detection of *Salmonella* spp. in raw ground beef (25 g), raw ground chicken (25 g), pasteurized liquid whole egg (100 g), raw ground pork (25 g), cooked chicken nuggets (325 g), frozen uncooked shrimp (25 g), fresh bunched spinach (25 g), dry dog food (375 g), and stainless steel. Not applicable to some lactose-positive *Salmonella* species.]

See Tables **2014.01A** and **B** for results of the interlaboratory study supporting acceptance of the method. *See* Appendix available on the *J. AOAC Int*. website for detailed tables of results of the collaborative study (http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac).

Caution: Do not use the 3M Petrifilm SALX System method in the diagnosis of conditions in humans or animals. To reduce the risks associated with exposure to chemicals and biohazards, perform pathogen testing in a properly equipped laboratory under the control of trained personnel. Always follow standard good laboratory safety practices (GLP), including proper containment procedures, and wearing appropriate protective apparel and eye protection while handling testing materials and test samples. Avoid direct contact with the contents of the enrichment medium and inoculated plates. Dispose of enrichment media and inoculated plates according to all applicable government regulatory regulations and applicable laboratory procedures. Wear appropriate protective apparel while handling the 3M Petrifilm SALX

Table 2014.01A. Summary of results for detection of *Salmonella* in raw ground beef (25 g)

Method[a]	3M Petrifilm *Salmonella* Express System with alternative confirmation			3M Petrifilm *Salmonella* Express System with traditional confirmation		
Inoculation level	Uninoculated	Low	High	Uninoculated	Low	High
Candidate presumptive positive/ total No. of samples analyzed	2/168	85/168	168/168	2/168	85/168	168/168
Candidate presumptive POD (CP)	0.01 (0.00, 0.04)	0.51 (0.43, 0.58)	1.00 (0.98, 1.00)	0.01 (0.00, 0.04)	0.51 (0.43, 0.58)	1.00 (0.98, 1.00)
s_r[b]	0.11 (0.10, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)	0.11 (0.10, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)
s_L[c]	0.00 (0.00, 0.04)	0.00 (0.00, 0.13)	0.00 (0.00, 0.15)	0.00 (0.00, 0.04)	0.00 (0.00, 0.13)	0.00 (0.00, 0.15)
s_R[d]	0.11 (0.10, 0.12)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)	0.11 (0.10, 0.12)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)
P-value[e]	0.5158	0.9341	1.0000	0.5158	0.9341	1.0000
Candidate confirmed positive/ total No. of samples analyzed	0/168	83/168	168/168	1/168	83/168	168/168
Candidate confirmed POD (CC)	0.00 (0.00, 0.02)	0.49 (0.42, 0.57)	1.00 (0.98, 1.00)	0.01 (0.00, 0.03)	0.49 (0.42, 0.57)	1.00 (0.98, 1.00)
s_r	0.00 (0.00, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)	0.08 (0.07, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)
s_L	0.00 (0.00, 0.15)	0.00 (0.00, 0.11)	0.00 (0.00, 0.15)	0.00 (0.00, 0.03)	0.00 (0.00, 0.11)	0.00 (0.00, 0.15)
s_R	0.00 (0.00, 0.21)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)	0.08 (0.07, 0.09)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)
P-value	1.0000	0.9757	1.0000	0.4418	0.9757	1.0000
Positive reference samples/ total No. of samples analyzed	0/168	86/168	167/168	0/168	86/168	167/168
Reference POD	0.00 (0.00, 0.02)	0.51 (0.43, 0.59)	0.99 (0.97, 1.00)	0.00 (0.00, 0.02)	0.51 (0.43, 0.59)	0.99 (0.97, 1.00)
s_r	0.00 (0.00, 0.15)	0.51 (0.46, 0.52)	0.08 (0.07, 0.15)	0.00 (0.00, 0.15)	0.51 (0.46, 0.52)	0.08 (0.07, 0.15)
s_L	0.00 (0.00, 0.15)	0.00 (0.00, 0.12)	0.00 (0.00, 0.03)	0.00 (0.00, 0.15)	0.00 (0.00, 0.12)	0.00 (0.00, 0.03)
s_R	0.00 (0.00, 0.21)	0.51 (0.47, 0.52)	0.08 (0.07, 0.09)	0.00 (0.00, 0.21)	0.51 (0.47, 0.52)	0.08 (0.07, 0.09)
P-value	1.0000	0.9695	0.4418	1.0000	0.9695	0.4418
dLPOD (candidate vs reference)[f]	0.00 (–0.02, 0.02)	–0.02 (–0.13, 0.09)	0.01 (–0.02, 0.03)	0.01 (–0.02, 0.03)	–0.02 (–0.13, 0.09)	0.01 (–0.02, 0.03)
dLPOD (candidate presumptive vs candidate confirmed)[f]	0.01 (–0.01, 0.04)	0.01 (–0.10, 0.12)	0.00 (–0.02, 0.02)	0.01 (–0.02, 0.04)	0.01 (–0.10, 0.12)	0.00 (–0.02, 0.02)

[a] Results include 95% confidence intervals.

[b] Repeatability standard deviation.

[c] Among-laboratory standard deviation.

[d] Reproducibility standard deviation.

[e] *P*-value = Homogeneity test of laboratory PODs.

[f] A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods.

Plate as some of the components may be considered allergenic and irritants to some individuals.

To reduce the risks associated with environmental contamination, follow current industry standards and local regulations for disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information. For questions about specific applications or procedures, visit www.3M.com/foodsafety or contact your local 3M representative or distributor. Review the policies recommend by the Centers for Disease Control and Prevention on dealing with pathogens (http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf).

A. Principle

The 3M Petrifilm SALX System is a chromogenic culture medium system that is intended for the rapid and specific detection and biochemical confirmation of *Salmonella* spp. from food and food process environmental samples. After enrichment in prewarmed 3M *Salmonella* Enrichment Base with 3M *Salmonella* Enrichment Supplement, the 3M Petrifilm SALX System provides presumptive positive results in as little as 40 h from low microbial background foods ($<10^4$ CFU/g) and 48 h from high microbial foods ($\geq 10^4$ CFU/g). The 3M Petrifilm SALX System does not specifically differentiate some lactose-positive *Salmonella* species (primarily *S. arizonae* and *S. diarizonae*) from other lactose-positive organisms. Refer to the 3M Petrifilm *Salmonella* Express System Instructions for Use for additional information.

B. Apparatus and Reagents

(**a**) *3M Petrifilm Salmonella Express Plate.*—Twenty-five plates/pouch (3M Food Safety, St. Paul, MN, USA).

(**b**) *3M Petrifilm Salmonella Express Confirmation Disk.*—Five disks/pouch (3M Food Safety).

(**c**) *3M Salmonella Enrichment Base.*—500 g or 2.5 kg/bottle (3M Food Safety).

Table 2014.01B. Summary of results for detection of *Salmonella* in dry dog food (375 g)

Method[a]	3M Petrifilm *Salmonella* Express System with alternative confirmation			3M Petrifilm *Salmonella* Express System with traditional confirmation		
Inoculation level	Uninoculated	Low	High	Uninoculated	Low	High
Candidate presumptive positive/ total No. of samples analyzed	0/144	82/144	142/144	0/144	82/144	142/144
Candidate presumptive POD (CP)	0.00 (0.00, 0.03)	0.57 (0.48, 0.66)	0.99 (0.95, 1.00)	0.00 (0.00, 0.03)	0.57 (0.48, 0.66)	0.99 (0.95, 1.00)
s_r[b]	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.12 (0.11, 0.16)	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.12 (0.11, 0.16)
s_L[c]	0.00 (0.00, 0.16)	0.08 (0.00, 0.24)	0.00 (0.00, 0.04)	0.00 (0.00, 0.16)	0.08 (0.00, 0.24)	0.00 (0.00, 0.04)
s_R[d]	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.12 (0.11, 0.13)	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.12 (0.11, 0.13)
P-value[e]	1.0000	0.2242	0.9861	1.0000	0.2242	0.9861
Candidate confirmed positive/ total No. of samples analyzed	0/144	81/144	141/144	0/144	82/144	141/144
Candidate confirmed POD (CC)	0.00 (0.00, 0.03)	0.56 (0.46, 0.66)	0.98 (0.94, 0.99)	0.00 (0.00, 0.03)	0.57 (0.48, 0.67)	0.98 (0.94, 0.99)
s_r	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.14 (0.12, 0.16)	0.00 (0.00, 0.16)	0.49 (0.43, 0.52)	0.14 (0.12, 0.16)
s_L	0.00 (0.00, 0.16)	0.10 (0.00, 0.26)	0.03 (0.00, 0.08)	0.00 (0.00, 0.16)	0.11 (0.00, 0.27)	0.03 (0.00, 0.08)
s_R	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.14 (0.13, 0.17)	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.14 (0.13, 0.17)
P-value	1.0000	0.1290	0.0976	1.0000	0.1114	0.0976
Positive reference samples/ total No. of samples analyzed	0/144	71/144	144/144	0/144	71/144	144/144
Reference POD	0.00 (0.00, 0.03)	0.49 (0.39, 0.59)	1.00 (0.97, 1.00)	0.00 (0.00, 0.03)	0.49 (0.39, 0.59)	1.00 (0.97, 1.00)
s_r	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.10 (0.00, 0.26)	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)	0.10 (0.00, 0.26)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.00 (0.00, 0.22)	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.00 (0.00, 0.22)
P-value	1.0000	0.1550	1.0000	1.0000	0.1550	1.0000
dLPOD (C vs R)[f]	0.00 (–0.03, 0.03)	0.07 (–0.07, 0.21)	–0.02 (–0.06, 0.01)	0.00 (–0.03, 0.03)	0.08 (–0.07, 0.22)	–0.02 (–0.06, 0.01)
dLPOD (CP vs CC)[f]	0.00 (–0.03, 0.03)	0.01 (–0.18, 0.22)	0.01 (–0.03, 0.05)	0.00 (–0.03, 0.03)	0.00 (–0.14, 0.14)	0.01 (–0.03, 0.05)

[a] Results include 95% confidence intervals.

[b] Repeatability standard deviation.

[c] Among-laboratory standard deviation.

[d] Reproducibility standard deviation.

[e] *P*-value = Homogeneity test of laboratory PODs.

[f] A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods.

Table 2014.01C. Sample matrix and enrichment scheme[a]

Sample matrix	Sample size, g	Enrichment broth volume, mL	Enrichment time, h	Secondary enrichment time, h
Raw ground beef (80% lean)	25	225	18–24	8–24
Raw ground chicken	25	225	18–24	8–24
Raw ground pork	25	225	18–24	8–24
Frozen uncooked shrimp	25	225	18–24	8–24
Fresh bunched spinach	25	225	18–24	24
Stainless steel; environmental sponges	1 Sponge (4 × 4 in.)	225	18–24	
Pasteurized liquid whole egg	100	900	18–24	
Cooked breaded chicken	325	2925	18–24	
Dry dog food	375	3375	18–24	

[a] AOAC RI Certificate No. 061301.

(**d**) *3M Salmonella Enrichment Supplement.*—1 g/vial (3M Food Safety).

(**e**) *3M Petrifilm Flat Spreader.*—Two spreaders/box (3M Food Safety).

(**f**) *3M Rappaport-Vassiliadis R10 (R-V R10) Broth.*—500 g/bottle (3M Food Safety).

(**g**) *Sterile diluents.*—Butterfield's Phosphate Diluent, distilled water, or reverse osmosis water.

(**h**) *Sterile 10 µL inoculation loop.*

(**i**) *Pipet.*—Capable of dispensing 2 mL.

(**j**) *Pipettor.*—Capable of dispensing 100 µL.

(**k**) *Sterile pipet tips.*—Capable of 100 µL.

(**l**) *Filter stomacher bags.*—Seward Laboratory Systems Inc. (Bohemia, NY, USA), or equivalent.

(**m**) *Stomacher.*—Seward Laboratory Systems Inc., or equivalent.

(**n**) *Permanent ultra-fine tipped marker.*—For circling presumptive positive colonies on the 3M Petrifilm *Salmonella* Express Plate.

(**o**) *Incubators.*—Capable of maintaining 41.5 ± 1°C.

(**p**) *Freezer.*—Capable of maintaining –10 to –20°C, for storing opened 3M Petrifilm *Salmonella* Express Plate pouches, hydrated 3M Petrifilm SALX Plates, and 3M Petrifilm SALX Plates after incubation.

(**q**) *Refrigerator.*—Capable of maintaining 2–8°C for storing unopened 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disk.

C. General Instructions

(**a**) Store 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks at 2–8°C. After opening the 3M Petrifilm SALX Plate pouches, seal the pouch and store at ambient temperature, less than 60% relative humidity (RH). Hydrated 3M Petrifilm SALX Plates can be stored up to 7 days at 2–8°C. Post-incubation 3M Petrifilm SALX Plates can be stored at –10 to –20°C for up to 3 days. Hydrate the 3M Petrifilm SALX Plates with 2.0 ± 0.1 mL sterile diluent. Do not allow the top film to close before dispensing the entire 2.0 mL volume. Gently roll down the top film onto the diluent to prevent trapping air bubbles. Place the 3M Petrifilm Flat Spreader on the center of the plate. Press gently on the center of the spreader to distribute the diluent evenly. Spread the diluent over the entire 3M Petrifilm SALX Plate. Remove the spreader and leave the 3M Petrifilm SALX Plate undisturbed for 1 min. Prior to use, place the plates on a flat surface for 1 h at room temperature (20–25°C/<60% RH) and protected from light to allow the gel to form. Hydrated plates can be stored at room temperature (20–25°C/<60% RH) protected from light for up to 8 h before use.

(**b**) Follow all instructions carefully. Failure to do so may lead to inaccurate results.

(**c**) After use, the enrichment medium and the 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information and local regulations for disposal.

D. Sample Enrichment

(*1*) Prewarm 3M *Salmonella* Enrichment Base with 3M *Salmonella* Enrichment Supplement (50 mg/L) to 41.5 ± 1°C.

(*2*) Aseptically combine the enrichment medium and sample following Table **2014.01C**. For all meat and highly particulate samples, the use of filter bags is recommended. Homogenize thoroughly for 2 min and incubate at 41.5 ± 1°C for 18–24 h.

(*a*) *Foods with high microbial backgrounds (≥10^4 CFU/g).*—Transfer 0.1 mL of the primary enrichment into 10.0 mL R-V R10 broth. Incubate for 8–24 h at 41.5 ± 1°C.

(*b*) *Foods with low microbial backgrounds (<10^4 CFU/g).*—Proceed to 3M Petrifilm SALX Plate preparation as described in **E**.

E. Preparation of the 3M Petrifilm Salmonella Express Plates

(*1*) Place the 3M Petrifilm SALX Plate on a flat, level surface.

(*2*) Use prescribed diluents to hydrate the 3M Petrifilm SALX Plates: Butterfield's Phosphate Diluent, distilled water, or reverse osmosis water.

(*3*) Lift the top film and with the pipet perpendicular dispense 2.0±0.1 mL sterile diluent onto the center of bottom film. Do not close the top film before dispensing the entire 2.0 mL volume.

(*4*) Gently roll down the top film onto the diluent to prevent trapping air bubbles.

(*5*) Place the 3M Petrifilm Flat Spreader on the center of the plate. Press gently on the center of the spreader to distribute the diluent evenly. Spread the diluent over the entire 3M Petrifilm SALX Plate growth area before the gel is formed. Do not slide the spreader across the film.

(*6*) Remove the spreader and leave the 3M Petrifilm SALX Plate undisturbed for at least 1 min.

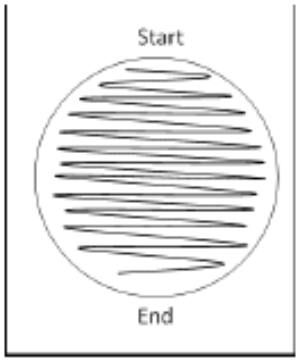

Figure 2014.01. Streaking pattern on the 3M Petrifilm SALX Plate.

(*7*) Place 3M Petrifilm SALX Plate on a flat surface for at least 1 h at room temperature (20–25°C/<60% RH), protected from light to allow the gel to form prior to use. Hydrated 3M Petrifilm SALX Plates can be stored at room temperature (20–25°C/<60% RH) for up to 8 h before use if protected from light.

(*8*) If hydrated plates are not used within 8 h, store in a sealed plastic bag, protected from light, and store at –20 to –10°C for up to 5 days.

F. 3M Petrifilm Salmonella Express Plate Inoculation

(*1*) Remove the enrichment medium from the incubator and agitate contents by hand.

(*2*) Use a sterile 10 µL loop (3 mm diameter) to withdraw each sample. Use a smooth loop (one that does not have jagged edges and is not distorted) to prevent the gel surface from breaking.

(*3*) Open the 3M Petrifilm SALX Plate and streak onto the gel. Perform a single streak to obtain isolated colonies (Figure **2014.01**).

(*4*) Roll down the top film to close the 3M Petrifilm SALX Plate.

(*5*) Using a gloved hand (while practicing GLP to avoid cross-contamination and/or direct contact with the plate), gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area.

(*6*) Streak each enriched test portion onto a 3M Petrifilm SALX Plate and incubate at 41.5 ± 1°C for 24 ± 2 h in a horizontal position with the colored side up in stacks of no more than 20 plates.

Table 2014.01D. Interpretation for presumptive positive *Salmonella* species

Colony color			Colony metabolism		
Red	Dark red	Brown	Yellow zone	Gas bubble	Result
√			√		Presumptive +
√				√	Presumptive +
√			√	√	Presumptive +
	√		√		Presumptive +
	√			√	Presumptive +
	√		√	√	Presumptive +
		√	√		Presumptive +
		√		√	Presumptive +
		√	√	√	Presumptive +

G. Confirmation of 3M Petrifilm Salmonella Express Plates

(*1*) Using a permanent ultra-fine tip marker, circle at least five presumptive positive colonies (red to brown colonies with a yellow zone or associated gas bubble, or both) on the plate top film (*see* Table **2014.01D**).

(*2*) Lift the top film of the 3M Petrifilm SALX Plate and insert the 3M Petrifilm SALX Confirmation Disk by rolling it onto the gel to avoid entrapping air bubbles. Close the 3M Petrifilm SALX Plate. Using a gloved hand, gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area and ensure good contact between the gel and the 3M Petrifilm SALX Confirmation Disk.

(*3*) Incubate the 3M Petrifilm SALX System (plate and disk) at 41.5 ± 1°C for 4–5 h in a horizontal position, right side up, in stacks of no more than 20 plates.

(*4*) Observe circled colonies for color change. Red/brown to green blue, blue, dark blue, or black confirms the colony as *Salmonella* spp. No color change indicates the colony is negative. If presumptive positive *Salmonella* colonies are not present, then report the results as *Salmonella* not detected in the matrix.

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