CAPÍTULO V

CONCLUSIONES

Según el análisis estadístico realizado a los encuestados se concluye lo siguiente:

- Las personas encuestadas fueron 63, siendo 28 mujeres que representan el 44 % y 35 hombres que corresponden al 56 %, habiendo un mayor número de hombres.

- De la muestra tomada, 23 personas prefirieron el Pollo A, es decir el 36.5 %, y 40 el pollo B, 63.5 %, observándose claramente la aceptación del pollo B.

- Tanto los estudiantes universitarios como los empleados prefirieron el pollo B en un 65 %, las amas de casa en un 100 % y los profesionales lo prefirieron en un 25 %, teniendo así, mayor aceptación el pollo B.

- De las personas que degustaron por primera vez pollo ahumado, el 100 % prefirió el pollo B y de las que ya habían probado, el 55 % prefirió el pollo B.

- De las que prefirieron el pollo B, el 79 % lo hicieron por el sabor y de los que eligieron el pollo A el 57% lo hicieron por su sabor, por lo que ésta fue la característica sensorial predominante en la preferencia. Los porcentajes anteriores, tanto del pollo A como del pollo B discrepan mayormente, por lo que se puede concluir que el humo líquido contribuye a mejorarlo.

- Tanto el pollo A como el pollo B tuvieron el mismo porcentaje (13%) en cuanto a textura, es así que el humo líquido no influye en la textura del pollo.

- El pollo ahumado B tiene un puntaje de aceptación mayor en cuanto al olor con respecto al pollo A.

- Los porcentajes relacionados al aspecto que tenía el pollo A y el pollo B fueron iguales por lo que el humo líquido no altera el aspecto del pollo.

- Los porcentajes relacionados al color que tenía el pollo A y el pollo B fueron iguales por lo que el humo líquido no altera el color del pollo.

- El pollo A agradó parcialmente en cuanto a sabor y color, mientras que el pollo B, el agrado máximo se logró en el sabor y un agrado parcial en el color y aspecto, concluyéndose que el sabor influyó para elegir al pollo B.

- El análisis microbiológico del pollo crudo (materia prima) revela presencia de coliformes totales, aerobios totales, psicrófilos, pero sin embargo, cumple con los requisitos microbiológicos, esto puede deberse a contaminaciones cruzadas o a malas operaciones realizadas durante el proceso.

- El pollo crudo no tiene presencia de E. coli, Salmonella ni S. aureus, por lo que no respresenta un peligro para la salud de los consumidores.

- Según el análisis microbiológico de los pollos ahumados, tanto el A como el B, tienen ausencia de Coliformes totales, aerobios totales, E. coli, Salmonella, S. aureus, por lo que el humo líquido no altera las propiedades microbiológicas del pollo.

De acuerdo a los resultados se puede concluir que el humo líquido ***no altera las propiedades microbiológicas*** pero sí mejora la característica organoléptica ***del sabor y olor*** y es un producto que tuvo una buena aceptación por los encuestados.

RECOMENDACIONES

- Los pollos no deben ser grasosos porque influye en el sabor a ahumado y no hay una transferencia del humo hacia los tejidos internos.

- Es preferible utilizar pollos que tenga pesos inferiores a 4 libras para lograr una buena cocción.

- Los pollos ahumados deben ser colocados en fundas de polipropileno para evitar la contaminación microbiológica.

- Utilizar el humo líquido en la preparación de otros productos como embutidos, pescados.

- Un nuevo estudio revelará el tiempo máximo de consumo de los pollos ahumados.

- Realizar un estudio de la estabilidad natural del producto, pollo ahumado, para determinar la vida útil del mismo con fines comerciales.

- Realizar estudios cromatográficos para determinar la composición del humo líquido y a su vez determinar los valores mínimos de Hidrocarburos Policíclicos Aromáticos (HPA).

- Se recomienda la utilización de este método alternativo de humo líquido en la producción de pollo ahumado ya que permite reducir los costos de producción debido a la disminución en el gasto de energía eléctrica.

ANEXOS

ANEXO # 1

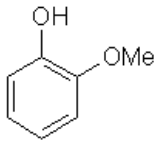
COMPUESTOS ENCONTRADOS EN EL HUMO

Peak Identifications

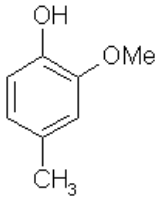
Back to (Top) (RT 0 - 30 min) (RT 31 - 67 min) (RT 67 - 101 min)

1. Methyl acetate
2. Formic acid
3. Unknown
4. Acetic acid
5. Ethyl formate
6. Acetoin
7. Propanal
8. Furfural
9. 2-Butanone
10. Acetol acetate
11. 2-Acetylfuran
12. gamma-Butyrolactone
13. 2(5H)-Furanone
14. 3-Methylsuccinic anhydride
15. 3-Methylenesuccinic anhydride
16. 3-Methylcyclopentenone
17. 3-Methyl-2(5H)-furanone
18. Phenol
19. Methylcyclopentenolone
20. 3,5-Dimethylcyclopentenolone
21. o-Cresol
22. Guaiacol
23. Maltol
24. Ethylcyclopentenolone
25. 4-Methylguaiacol
26. gamma-Hexalactone
27. Pyrocatechol
28. 5-Hydroxymethylfurfural
29. 4-Ethylguaiacol
30. Artifact
31. Syringol
32. Eugenol
33. 4-Propylguaiacol
34. Vanillin
35. cis-Isoeugenol
36. 4-Methylsyringol
37. trans-Isoeugenol
38. Acetovanillone
39. 4-Ethylsyringol
40. 4-(2-Propio)-vanillone (part peak)
41. 4-(1-Propio)-vanillone
42. 4-(2-Propenyl)-syringol
43. Z-4-(1-Propenyl)-syringol
44. Syringaldehyde
45. E-4-(1-Propenyl)-syringol
46. 4-Acetosyringone
47. 4-(2-Propio)-syringone
48. 4-(1-Propio)-syringone
49. Palmitic acid
50. Stearic acid

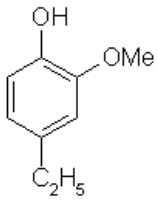
GUAIACOL RELATED



GUAIACOL



4-METHYL



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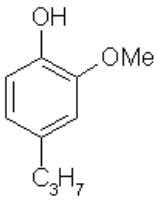

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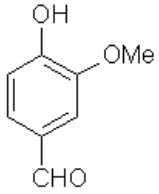
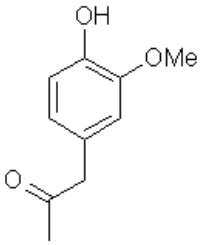
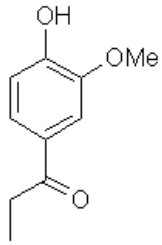


VANILLIN



4-(2-PROPIO)-
VANILLONE



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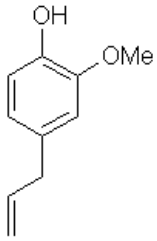
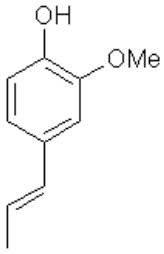
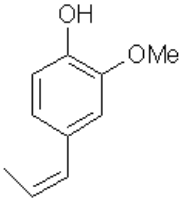


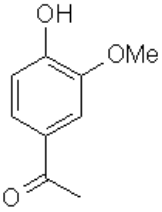
EUGENOL



E-ISOEUGENOL

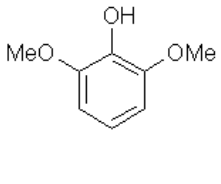


Z-ISOEUGENOL

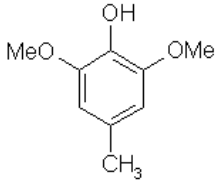


ACETO-
VANILLONE

SYRINGOL RELATED



SYRINGOL



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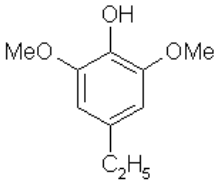

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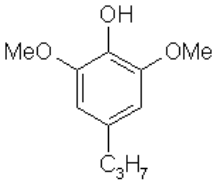

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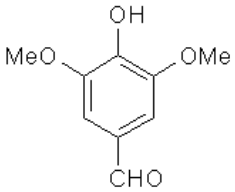
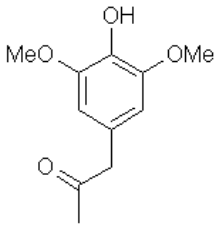
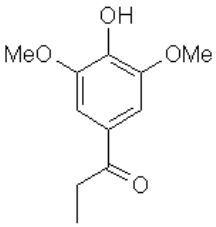


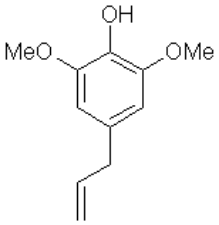
SYRINGALDEHYDE



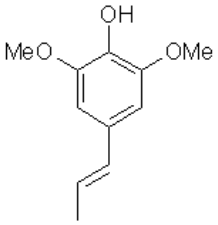
4-(2-PROPIO)-SYRINGONE)



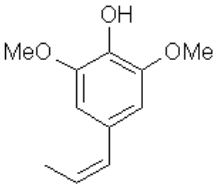
4-(1-PROPIO)-SYRINGONE)



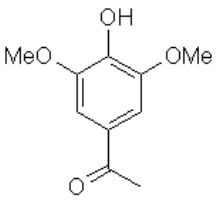
4-(2-PROPENYL)-SYRINGOL



E-4-(1-PROPENYL)-SYRINGOL



Z-4-(1-PROPENYL)-SYRINGOL



ACETOSYRINGONE

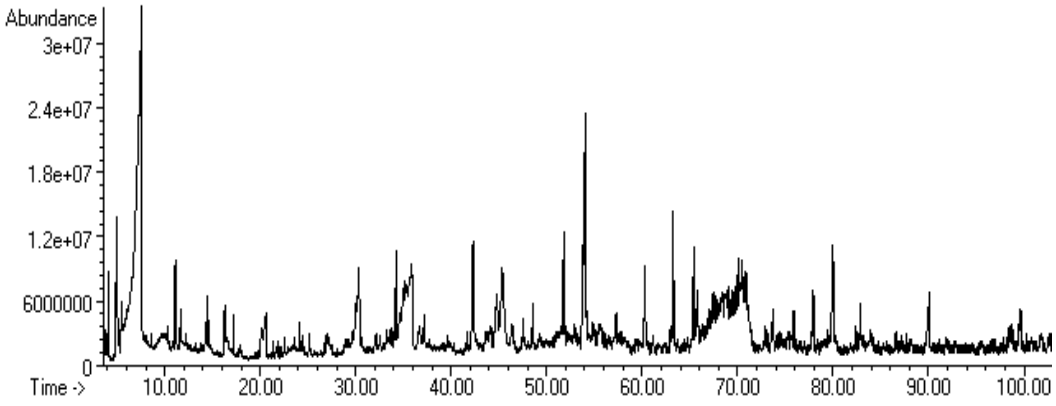
Back to In The News ** **Back to HomePage**

Email:
leffingwell@mindspring.com

GC/MS of Liquid "Hardwood Smoke" Extract (E.D. Alford and J.C. Leffingwell, 1998)

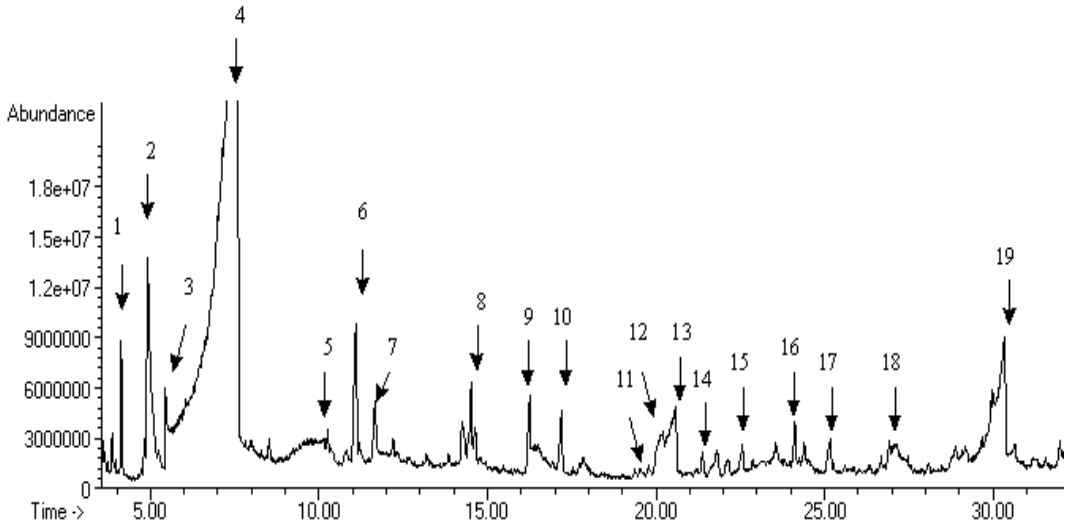
Analysis was conducted on a HP 5890 GC with a 5970 MSD on a 60 meter DB-5 column (0.32 I.D. and 0.25 micron film) programed from 60 degrees C. to 250 degrees C. with a beginning hold of 2 minutes at 60° and final hold of 30 minutes at 250°.

Click Here for Peak Identifications



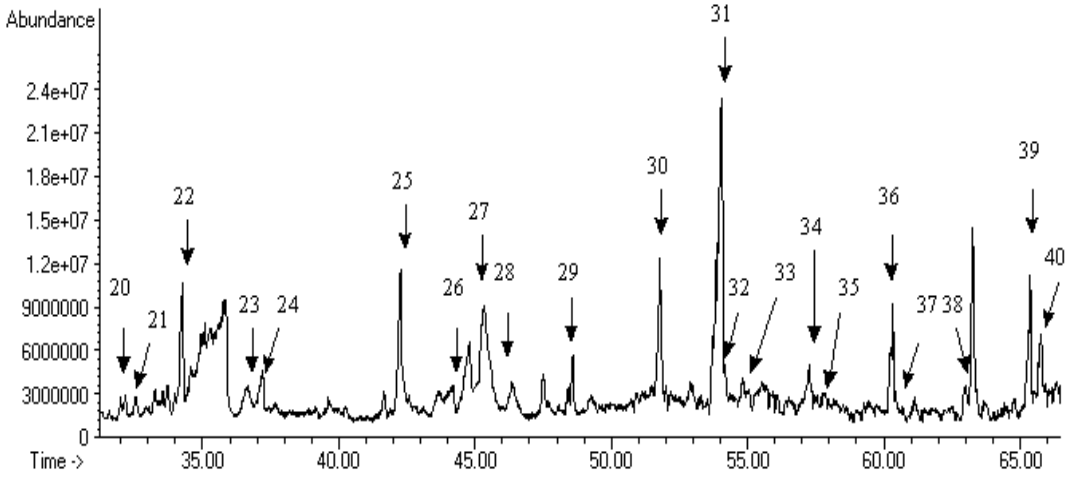
GC/MS of Liquid "Hardwood Smoke" Extract - RT of 0 to 30 minutes

Click Here for Peak Identifications



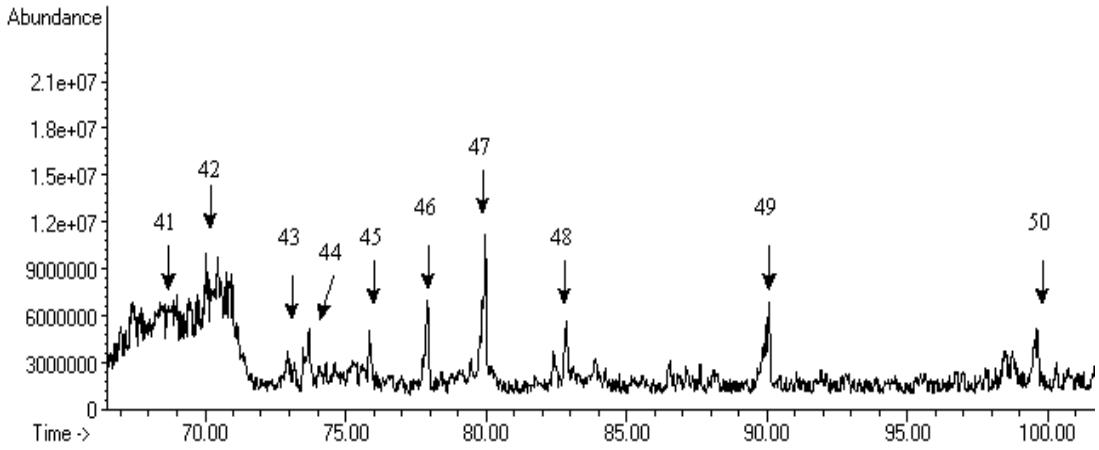
GC/MS of Liquid "Hardwood Smoke" Extract - RT of 31 to 67 minutes

Click Here for Peak Identifications



GC/MS of Liquid "Hardwood Smoke" Extract - RT of 67 to 101 minutes

Click Here for Peak Identifications



ANEXO # 2

CUESTIONARIO PARA MEDIR LA PREFERENCIA
DEL POLLO AHUMADO POR HUMO LIQUIDO

NOMBRE _____ (Opcional)

EDAD _____ **SEXO** **F** ☐ **M** ☐

OCUPACION (Opcional)

UNIVERSITARIO ☐ AMA DE CASA ☐ OTRO ☐

PROFESIONAL ☐ EMPLEADO ☐

1- Es la primera vez que come pollo ahumado?

SI ☐ NO ☐

2- Si la respuesta a la primera pregunta es NO, con qué frecuencia come el pollo ahumado?

Menos de una vez por semana ☐ Una vez por semana ☐ Dos o más veces por semana ☐

3- Qué tipo de pollo ahumado le gustó?

A ☐ B ☐

4- Por que le gusto el pollo elegido? (Una sola alternativa)

Su olor ☐ Su aspecto ☐

Su sabor ☐ Su textura ☐

Su color ☐ OTRO _____

5- A Ud. NO le gustó el pollo ahumado A por (Una sola alternativa)

Su olor ☐ Su aspecto ☐

Su sabor ☐ Su textura ☐

Su color ☐ OTRO _____

6 A Ud. NO le gustó el pollo ahumado B por (Una sola alternativa)

Su olor ☐ Su aspecto ☐

Su sabor ☐ Su textura ☐

Su color ☐ OTRO _____

7- Califique del 1 al 5 siendo 1 la calificación de menor aceptación del pollo ahumado A, y 5 la calificación de mayor agrado

	1	2	3	4	5
Su olor	☐	☐	☐	☐	☐
Su sabor	☐	☐	☐	☐	☐
Su color	☐	☐	☐	☐	☐
Su aspecto	☐	☐	☐	☐	☐
Su textura	☐	☐	☐	☐	☐

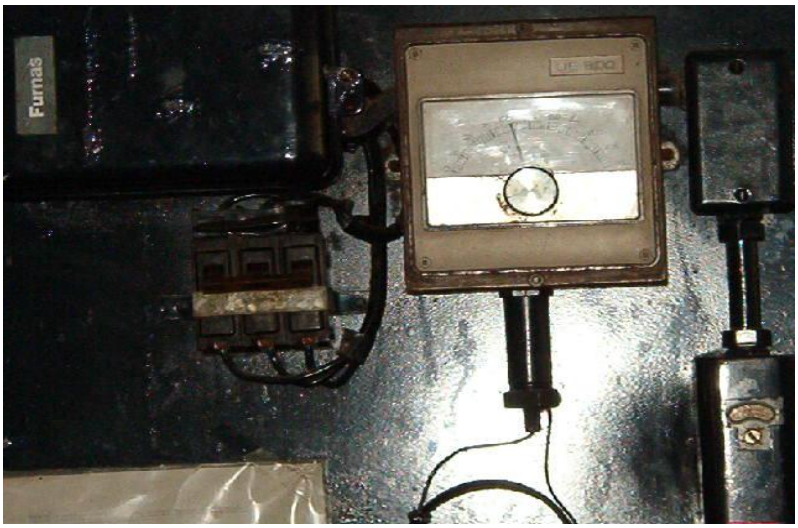
8- Califique del 1 al 5 siendo 1 la calificación de menor aceptación del pollo ahumado B, y 5 la calificación de mayor agrado

	1	2	3	4	5
Su olor	☐	☐	☐	☐	☐
Su sabor	☐	☐	☐	☐	☐
Su color	☐	☐	☐	☐	☐
Su aspecto	☐	☐	☐	☐	☐
Su textura	☐	☐	☐	☐	☐

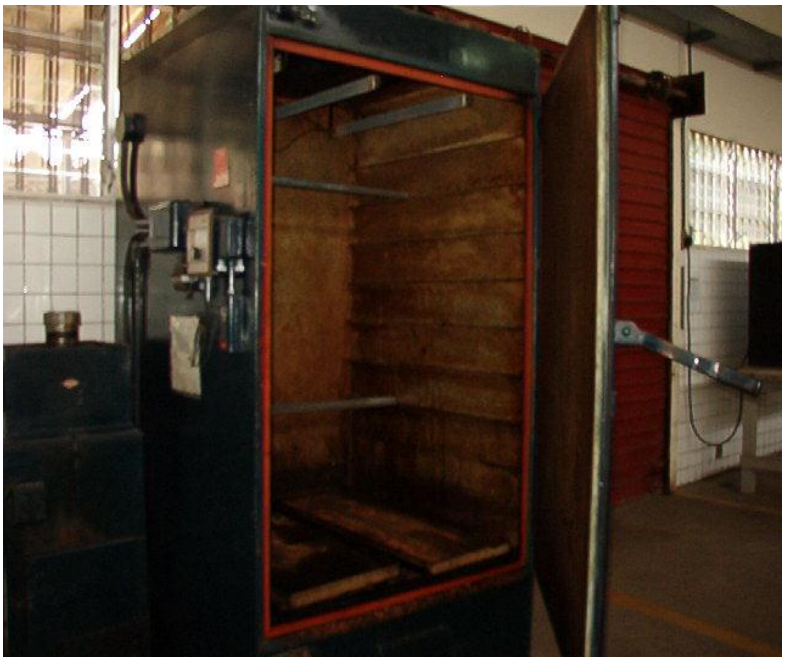
ANEXO # 3

AHUMADOR TRADICIONAL

TORRE AHUMADORA TRADICIONAL DEL PROGRAMA DE TECNOLOGÍA DE ALIMENTOS DE LA ESPOL



PANEL DE CONTROL DE TEMPERATURA



PARTE INTERIOR DE LA TORRE AHUMADORA

GENERADOR DE HUMO QUE CONTIENE ASERRÍN DE MADERA

ANEXO # 4

BASE DE DATOS DE ENCUESTA

ANEXO # 5

MÉTODOS UTILIZADOS EN LOS ANÁLISIS MICROBIOLÓGICOS

Subchapter 2
CHILLED, FROZEN, PRECOOKED, OR PREPARED FOODS, AND NUT MEATS

17.2.01

AOAC Official Method 966.23
Microbiological Methods
First Action 1966
Final Action 1989

(For the determination of aerobic plate count, most probable number of coliform bacteria, and *Escherichia coli* and *Staphylococcus* in products such as frozen cooked meat, poultry, and vegetable products; cooked and/or breaded seafood; bakery products; salads; tree nut meats; and ingredients of food laboratory samples collected during sanitation inspections of food-producing establishments, unless specific directions are given for that product.)

A. Media and Reagents

Ingredients and reagents used to prepare the following media may be products of any manufacturer if comparative tests show that satisfactory results are obtained. Use pure carbohydrates suitable for biological use, ACS reagent grade inorganic chemicals, and dyes certified by the Biological Stain Commission for use in media.

For convenience, dehydrated media of any brand equivalent to formulation may be used. Test each lot of medium for sterility and growth-promoting qualities of suitable organisms (e.g., inoculate media containing lactose with coliform bacteria, *Staphylococcus* media with *Staphylococcus*, etc.).

Determine pH before autoclaving with pH meter standardized against standard buffers, **964.24** (*see* A.1.04). Adjust pH, when necessary, by adding 1M NaOH or 1M HCl so that stated final pH results after autoclaving.

Use sterile glass or plastic, 100 × 15 mm, Petri dishes.

(**a**) *Plate count agar.*—*See* **940.36A(g)** (*see* 17.1.02).

(**b**) *Lauryl sulfate tryptose broth.*—Dissolve 20.0 g Trypticase or tryptose (pancreatic digest of casein), 5.0 g NaCl, 5.0 g lactose, 2.75 g K_2HPO_4, 2.75 g KH_2PO_4, and 0.1 g sodium lauryl sulfate in 1 L H_2O with gentle heat, if necessary. Dispense 10 mL portions into 20 × 150 mm test tubes containing inverted 10 × 75 mm fermentation tubes. Autoclave 15 min at 121°C. Final pH, 6.8 ± 0.1.

(**c**) *Brilliant green lactose bile (BGLB) broth.*—Dissolve 10.0 g peptone and 10.0 g lactose in ca 500 mL H_2O. Add solution (pH 7.0–7.5) of 20 g dehydrated oxgall or oxbile in 200 mL H_2O. Dilute to 975 mL and adjust pH to 7.4. Add 13.3 mL 0.1% solution of brilliant green, and dilute to 1 L with H_2O. Filter through cotton and dispense 10 mL portions into 20 × 150 mm test tubes containing inverted 10 × 75 mm fermentation tubes. Autoclave 15 min at 121°C. Final pH, 7.2 ± 0.1.

(**d**) *Eosin methylene blue agar (Levine).*—*See* **940.36A(d)** (*see* 17.1.02).

(**e**) *Baird-Parker medium (egg tellurite glycine pyruvate agar, ETGPA).*—(*1*) *Basal medium.*—Suspend 10.0 g tryptone, 5.0 g beef extract, 1.0 g yeast extract, 10 g sodium pyruvate, 12.0 g glycine, 5.0 g $LiCl·6H_2O$, and 20.0 g agar in 950 mL H_2O. Heat to bp with frequent agitation to dissolve ingredients completely. Dispense 95 mL portions into screw-capped bottles. Autoclave 15 min at 121°C. Final pH, 7.0 ± 0.2 at 25°C. Store ≤1 month at 4 ± 1°C.

(*2*) *Enrichment.*—Bacto EY tellurite enrichment (Difco Laboratories or equivalent) or prepare as follows: Soak fresh eggs ca 1 min in dilution of saturated $HgCl_2$ solution (1 + 1000, w/v). Aseptically crack eggs and separate yolks from whites. Blend yolk and physiological saline solution, **940.36B(c)** (*see* 17.1.02), (3 + 7, v/v) in high-speed blender ca 5 s. To 50 mL egg yolk emulsion, add 10 mL filter-sterilized 1% potassium tellurite solution (w/v). Mix and store at 4 ± 1°C.

(*3*) *Complete medium.*—Add 5 mL warmed enrichment to 95 mL molten basal medium cooled to 45–50°C. Mix well, avoiding bubbles, and pour 15–18 mL into sterile 100 × 15 mm Petri dishes. Store plates at room temperature (≤25°C) for ≤5 days before use. Medium should be densely opaque; do not use nonopaque plates. Dry plates before use by one of the following methods: (*a*) in convection oven or incubator 30 min at 50°C with lids removed and agar surface downward; (*b*) in forced-draft oven or incubator 2 h at 50°C with lids on and agar surface upward; (*c*) in incubator 4 h at 35°C with lids on and agar surface upward; or (*d*) on laboratory bench 16–18 h at room temperature with lids on and agar surface upward.

(*4*) *Interpretation.*—Colonies of *S. aureus* are typically circular, smooth, convex, moist, 2–3 mm in diameter on uncrowded plates, gray-black to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone (precipitate) and frequently with outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle. Occasional nonlipolytic strains may be encountered which have same appearance, except that surrounding opaque and clear zones are absent. Colonies isolated from frozen or desiccated foods which have been stored for extended periods are frequently less black than typical colonies and may have rough appearance and dry texture.

(**f**) *Trypticase (tryptic) soy broth with 10% sodium chloride.*—Add 95 g NaCl to 1 L of solution of 17.0 g Trypticase or tryptose (pancreatic digest of casein), 3.0 g Phytone (papaic digest of soya meal), 5.0 g NaCl, 2.5 g K_2HPO_4, and 2.5 g glucose. Heat gently if necessary. Dispense into 16–20 mm diameter tubes to depth of 5–8 cm. Autoclave 15 min at 121°C. Final pH, 7.3 ± 0.2.

(**g**) *EC broth.*—Dissolve 20.0 g Trypticase or tryptose (pancreatic digest of casein), 1.5 g Bacto bile salt No. 3 or bile salt mixture, 5.0 g lactose, 4.0 g K_2HPO_4, 1.5 g KH_2PO_4, and 5.0 g NaCl in 1 L H_2O. Dispense 8 mL into 16 × 150 mm test tubes containing inverted 10 × 75 mm fermentation tube. Autoclave 15 min at 121°C. Final pH, 6.9 ± 0.1.

(**h**) *Brain-heart infusion.*—*See* **967.25A(r)** (*see* 17.9.01). Dispense into bottles or tubes for storage and autoclave 15 min at 121°C.

(**i**) *Desiccated coagulase plasma (rabbit) with EDTA.*—Reconstitute according to manufacturer's directions. If not available, reconstitute desiccated coagulase plasma (rabbit) and add Na_2H_2EDTA to final concentration of 0.1% in reconstituted plasma.

(**j**) *Tryptophane broth.*—*See* **940.36A(h)** (*see* 17.1.02) but dispense in 10 mL portions.

(**k**) *Buffered glucose broth (MR-VP medium).*—*See* **940.36A(b)** (*see* 17.1.02).

(**l**) *Koser's citrate broth.*—*See* **940.36A(e)** (*see* 17.1.02).

(**m**) *Butterfield's buffered phosphate diluent.*—(*1*) *Stock solution.*—Dissolve 34.0 g KH_2PO_4 in 500 mL H_2O, adjust to pH 7.2 with ca 175 mL 1M NaOH, and dilute to 1 L. Store in refrigerator. (*2*) *Diluent.*—Dilute 1.25 mL stock solution to 1 L with H_2O. Prepare dilution blanks with this solution, dispensing enough to allow for losses during autoclaving. Autoclave 15 min at 121°C.

B. Preparation of Test Sample

(Prepare all decimal dilutions with 90 mL sterile diluent plus 10 mL previous dilution unless otherwise specified. Shake all dilu-

tions 25 times in 30 cm arc. Pipets must accurately deliver required volume. Do not use to deliver <10% of their total volume. For example, to deliver 1 mL, do not use pipet >10 mL; to deliver 0.1 mL, do not use pipet >1 mL.)

(**a**) *Frozen and/or prepared foods.*—Use balance with capacity of ≥2 kg and sensitivity of 0.1 g to aseptically weigh 50 g unthawed (if frozen) test sample into sterile high-speed blender jar. Add 450 mL diluent, (**m**)(*2*), and blend 2 min. (If necessary to temper frozen test sample to remove 50 g portion, hold ≤18 h at 2–5°C.) Not >15 min should elapse from time test sample is blended until all dilutions are in appropriate media.

If entire test sample consists of <50 g, weigh portion equivalent to ½ test sample and add volume of sterile diluent required to make 1:10 dilution. Total volume in blender jar must completely cover blades.

(**b**) *Tree nut meat halves and larger pieces.*—Aseptically weigh 50 g test sample into sterile jar. Add 50 mL diluent, (**m**)(*2*), and shake vigorously (50 times through 30 cm arc) to obtain 10^0 dilution. Let stand 3–5 min and shake just before making serial dilutions and inoculations.

(**c**) *Nut meal.*—Aseptically weigh 10 g test sample into sterile jar. Add 90 mL diluent, (**m**)(*2*), and shake vigorously (50 times through 30 cm arc) to obtain 10^{-1} dilution. Let stand 3–5 min and shake to resuspend just before making serial dilutions and inoculations.

C. Aerobic Plate Count

Seed duplicate Petri dishes in dilutions of 1:10, 1:100, 1:1000, etc., using plate count agar, (**a**). Ordinarily, 1:100 through 1:10 000 are satisfactory. Place 1 mL appropriate dilution in each plate, and add molten agar (cooled to 42–45°C) within 15 min from time of original dilution. Incubate 48 ± 2 h at 35°C and count duplicate plates in suitable range (30–300 colonies). If plates do not contain 30–300 colonies, record dilution counted and note number of colonies found. Average counts obtained and report as aerobic plate count/g.

17.2.02

AOAC Official Method 966.24 Coliform Group and *Escherichia coli* in Tree Nut Meats

Microbiological Method
First Action 1966
Final Action 1971

Seed 3-tube most probable number (MPN) series into lauryl sulfate tryptose broth, (**b**), using 1 mL inocula of 1:10, 1:100, and 1:1000 dilutions, with triplicate tubes at each dilution. (For nut meats [halves and larger pieces], begin MPN determination with 10^0 dilution; for nut meal, begin with 10^{-1} dilution.) Incubate 48 ± 2 h at 35°C for gas formation as evidenced by displacement of liquid in insert tube or by vigorous effervescence when tubes are shaken *gently*. Examine tubes for gas formation at 24 and 48 h intervals. Transfer, using 3 mm loop, from gassing tubes to BGLB, (**c**; omit this transfer for tree nuts), and EC broth, (**g**), at time gas formation is noted.

Incubate BGLB broth 48 ± 2 h at 35°C. Using MPN Table **966.24A**, compute MPN on basis of number of tubes of BGLB broth producing gas by end of incubation period. Report as MPN of coliform bacteria/g.

Incubate EC broth 48 ± 2 h at 45.5 ± 0.05°C in covered H_2O bath. Submerge broth tubes in bath so that H_2O level is above highest level of medium. Examine tubes for gas formation at 24 and 48 h intervals. Streak gas-positive tubes on Levine's eosin methylene blue agar plates, (**d**), and incubate plates 24 ± 2 h at 35°C.

Pick 2 or more well-isolated typical colonies from Levine's eosin methylene blue agar plates and transfer to agar slants prepared from agar medium, (**a**). Incubate 18–24 h at 35°C. If typical colonies are not present, pick 2 or more colonies most likely to be *E. coli*. Pick ≥2 from every plate.

Table 966.24A Most probable numbers (MPN) per 1 g test portion, using 3 tubes with each of 0.1, 0.01, and 0.001 g portions

Positive tubes				Positive tubes				Positive tubes				Positive tubes			
0.1	0.01	0.001	MPN	0.1	0.01	0.001	MPN	0.1	0.01	0.001	MPN	0.1	0.01	0.001	MPN
0	0	0	<3	1	0	0	3.6	2	0	0	9.1	3	0	0	23
0	0	1	3	1	0	1	7.2	2	0	1	14	3	0	1	39
0	0	2[a]	6	1	0	2	11	2	0	2	20	3	0	2	64
0	0	3[a]	9	1	0	3[a]	15	2	0	3[a]	26	3	0	3[a]	95
0	1	0	3	1	1	0	7.3	2	1	0	15	3	1	0	43
0	1	1	6.1	1	1	1	11	2	1	1	20	3	1	1	75
0	1	2[a]	9.2	1	1	2[a]	15	2	1	2	27	3	1	2	120
0	1	3[a]	12	1	1	3[a]	19	2	1	3[a]	34	3	1	3	160
0	2	0	6.2	1	2	0	11	2	2	0	21	3	2	0	93
0	2	1[a]	9.3	1	2	1	15	2	2	1	28	3	2	1	150
0	2	2[a]	12	1	2	2[a]	20	2	2	2	35	3	2	2	210
0	2	3[a]	16	1	2	3[a]	24	2	2	3[a]	42	3	2	3	290
0	3	0	9.4	1	3	0	16	2	3	0	29	3	3	0	240
0	3	1[a]	13	1	3	1[a]	20	2	3	1	36	3	3	1	460
0	3	2[a]	16	1	3	2[a]	24	2	3	2[a]	44	3	3	2	1100
0	3	3[a]	19	1	3	3[a]	29	2	3	3[a]	53	3	3	3	>1100

[a] Such highly improbable results suggest that factors were present that interfered with recovery or identification at the lower dilutions. Therefore, the indicated MPN value could be much lower than the true concentration.

Table 966.24B Classification of biochemical types[a]

Indole	MR	VP	Citrate	Type
+	+	–	–	Typical *E. coli*
–	+	–	–	Atypical *E. coli*
+	+	–	+	Typical intermediate
–	+	–	+	Atypical intermediate
–	–	+	+	Typical *Enterobacter aerogenes*
+	–	+	+	Atypical *Enterobacter aerogenes*

[a] Other groupings may appear; in such cases, cultures are usually mixed. Restreak to determine their purity. Compute MPN of *E. coli*/g, considering Gram-negative, nonspore-forming rods producing gas in lactose and producing + + – – or – + – – IMViC patterns as *E. coli*.

Transfer growth from plate count agar slants into following broths for identification by biochemical tests:

(**a**) *Tryptophane broth.*— Incubate broth, (j), 24 ± 2 h at 35°C and test for indole by adding 0.2–0.3 mL Kovacs reagent, **967.25B(a)** (*see* 17.9.01), to 24 h culture. Test is positive if upper layer turns red.

(**b**) *MR-VP medium.*—Incubate medium, (**k**), 48 ± 2 h at 35°C. Aseptically transfer 1 mL culture to 13 × 100 mm test tube to test for acetylmethylcarbinol. Add 0.6 mL 5% alcoholic α-naphthol solution (w/v), 0.2 mL KOH solution (4 + 10), and few crystals of creatine. Shake and let stand 2 h. Test is positive if eosin pink develops. Alternatively, *see* **967.27D(c)**(*1*) (*see* 17.9.03).

Incubate remainder of MR-VP medium for additional 48 h and test for methyl red reaction by adding 5 drops methyl red solution to culture. Test is positive if culture turns red; negative, if yellow. (Prepare methyl red solution by dissolving 0.1 g methyl red in 300 mL 90% alcohol and diluting to 500 mL with H_2O.)

(**c**) *Koser citrate broth, **966.23A(l)**.*—Incubate 96 h at 35°C and record growth as + or –.

(**d**) *Lauryl sulfate tryptose broth, **966.23A(b)**.*—Incubate 48 ± 2 h at 35°C. Examine tubes for gas formation.

(**e**) *Gram stain.*—Perform Gram stain on 18 h agar slant (*Standard Methods for the Examination of Water and Wastewater*, 18th Ed., 1992, American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC, USA). Coliform organisms will stain red (negative); Gram-positive organisms will stain blue-black.

(**f**) *Classification.*—Classify biochemical types as in Table **966.24B**.

References: *JAOAC* **49,** 270, 276(1966); **51,** 865, 867(1968); **58,** 1154(1975).

Revised: March 1999

17.2.03

AOAC Official Method 977.27
Bacteria in Foods and Cosmetics
Spiral Plate Method
First Action 1977
Final Action 1981

A. Principle

Bacterial suspension from prepared test portion of food or cosmetic is deposited continuously on surface of rotating agar plate. Resultant track on surface is in form of Archimedes spiral. Volume is decreased while dispensing stylus moves from center to edge so that exponential relationship exists between volume deposited and radius of agar. On incubation, colonies develop along lines where liquid was deposited. Counting grid is calibrated for test portion volume associated with different areas of agar. Number of colonies per known area is counted and calculated to bacterial concentration.

B. Apparatus

Spiral plating machine.—For use with 150 × 15 mm (100 × 15 mm may be used) Petri dishes and adjusted to deliver total volume of 0.035 mL/plate. Platform carrying plate is rotated at ca 50 rpm and is connected mechanically to lead screw driving hollow syringe dispenser. Backflow syringe, 2-way valve, and vacuum trap control loading and dispensing of test portion, disposal of residual test portion, and rinsing of system. Liquid is dispensed from backflow syringe through thin wall Teflon tubing through stylus to surface of agar plate.

C. Plates

Pour 40–45 mL portions plate count agar, **940.36A(g)** (*see* 17.1.02), into 150 × 15 mm (100 × 15 mm may be used) Petri dishes; let harden and dry to smooth, even surface.

D. Calibration of Spiral Counters

To determine volume associated with different parts of counting grid, prepare 11 bacterial suspensions by diluting 1:1 from 10^6 to 10^3 cells/mL (use nonspreaders). Plate all dilutions in duplicate by both **966.23C** (*see* 17.2.01) and spiral plater, using same medium and incubator. Count spiral plates as in **G** and divide by average count/mL by **966.23C** (*see* 17.2.01) to calculate volume of counted grid area.

$$\text{mL in Counted area} = \frac{\text{number spiral colonies on area}}{\text{count} \,/\, \text{mL by } \textbf{966.23C} \text{ (}\textit{see}\text{ 17.2.01)}}$$

E. Preparation of Test Portion

Weigh 50 g test portion into sterile blender jar, add 450 mL dilution H_2O, **940.36A(a)** (*see* 17.1.02), and blend 2 min. If necessary, let settle few min before removing portion of supernate for spiral plating. (Presence of particles may clog tubing.)

Liquids may be used directly or after diluting 1 + 9 with dilution H_2O.

F. Operation

Check stylus tip angle by letting vacuum hold microscope cover slip against face of stylus tip at 1 mm above platform. Cover slip should be parallel to rotating platform in all directions. Adjust angle if necesary. Check stylus at start position.

Clean stylus tip before use and between plating each test solution by rinsing 1 s with commercial 5.25% NaOCl solution and then 1 s with sterile H_2O. Identify 3 disposable polyethylene cups and fill with commercial 5.25% NaOCl solution, sterile H_2O, and test solution. Turn vacuum filling valve to "on" and move test sample holder into position under stylus tip. Lower stylus into NaOCl solution and lift out twice. Repeat with H_2O. Lower stylus into test solution. Draw solution through stylus until continuous column of liquid is present in tube above vacuum filling valve. With tip of stylus still below surface of test solution, close vacuum valve. Raise stylus and move test sample holder out of way.

Identify lid of agar plate and remove lid. Place dish on turntable and lower stylus until tip rests freely on agar surface. Start apparatus and let rotate until stylus is lifted and apparatus stops automatically. Remove dish and replace cover. Incubate 48 ± 3 h at 35 ± 1°C.

Subchapter 5
STAPHYLOCOCCUS

17.5.01

AOAC Official Method 987.09
Staphylococcus aureus in Foods
Most Probable Number Method for Isolation and Enumeration
First Action 1987
Final Action 1991

(Applicable to detection and enumeration of small numbers of *S. aureus* in food ingredients and food expected to contain large population of competing species.)

A. Apparatus

(**a**) *Pipets.*—1.0 mL with 0.1 mL graduations; 5.0 and 10.0 mL with 0.5 and 1.0 mL graduations.

(**b**) *Blender.*—Waring, or equivalent 2-speed model, with high-speed operation at 16 000–18 000 rpm, and 1 L glass or metal blender jars with covers. One jar is required for each analytical unit.

(**c**) *Mixer.*—Vortex Genie, or equivalent.

(**d**) *Water bath.*—Maintained at 35–37°C.

(**e**) *Incubator.*—Maintained at 35–37°C.

B. Media and Reagents

(**a**) *Trypticase (tryptic) soy broth with 10% sodium chloride and 1% sodium pyruvate.*—Add 95 g NaCl to 1 L solution of 17.0 g Trypticase or tryptose (pancreatic digest of casein), 3.0 g Phytone (papaic digest of soya meal), 5.0 g NaCl, 2.5 g K_2HPO_4, 2.5 g dextrose (dehydrated Trypticase or tryptic soy broth is satisfactory), and 10 g sodium pyruvate. Adjust to pH 7.3. Heat gently if necessary. Dispense 10 mL into 16 × 150 mm tubes. Autoclave 15 min at 121°C. Final pH should be 7.3 ± 0.2. Store ≤1 month at 4 ± 1°C.

(**b**) *Physiological salt solution.*—Dissolve 8.5 g NaCl in 1 L H_2O. Autoclave 15 min at 121°C and cool to room temperature.

(**c**) *Baird-Parker medium (egg tellurite glycine pyruvate agar, ETGPA).*—(*1*) *Basal medium.*—Suspend 10.0 g tryptone, 5.0 g beef extract, 1.0 g yeast extract, 10.0 g sodium pyruvate, 12.0 g glycine, 5.0 g $LiCl·H_2O$, and 20.0 g agar in 950 mL H_2O. Heat to bp with frequent agitation to dissolve ingredients completely. Dispense 95 mL portions into screw-cap bottles. Autoclave 15 min at 121°C. Final pH should be 7.0 ± 0.2 at 25°C. Store ≤ 1 month at 4 ± 1°C.

(*2*) *Enrichment.*—Bacto EY tellurite enrichment (Difco Laboratories or equivalent) or prepare as follows: Soak fresh eggs ca 1 min in dilution of saturated $HgCl_2$ solution (1 + 1000). Aseptically crack eggs and separate yolks from whites. Blend yolk and physiological saline solution, (**b**), (3 + 7, v/v) in high-speed blender ca 5 s. To 50 mL egg yolk emulsion, add 10 mL filter-sterilized 1% potassium tellurite solution (w/v). Mix and store at 4 ± 1°C.

(*3*) *Complete medium.*—Add 5 mL warmed enrichment to 95 mL molten basal medium cooled to 45–50°C. Mix well, avoiding bubbles, and pour 15–18 mL into sterile 100 × 15 mm Petri dishes. Store plates at room temperature (25°C) for ≤ 5 days before use. Medium should be densely opaque; do not use nonopaque plates. Dry plates before use by one of following methods: (*a*) in convection oven or incubator 30 min at 50°C with lids removed and agar surface downward; (*b*) in forced-draft oven or incubator 2 h at 50°C with lids on agar surface upward; (*c*) in incubator 4 h at 35°C with lids on agar surface upward; or (*d*) on laboratory bench 16–18 h at room temperature with lids on and agar surface upward.

(**d**) *Brain-heart infusion (BHI) broth.*—Dissolve infusion from 200 g calf brain and from 250 g beef heart, 10.0 g proteose peptone or Gelysate, 5.0 g NaCl, 2.5 g $Na_2HPO_4·12H_2O$, and 2.0 g glucose in 1 L H_2O, heating gently if necessary. Dispense 5 mL portions into 16 × 150 mm test tubes and autoclave 15 min at 121°C. Final pH should be 7.4 ± 0.2.

(**e**) *Desiccated coagulase plasma (rabbit) with EDTA.*—Reconstitute according to manufacturer's directions. If not available, reconstitute desiccated coagulase plasma (rabbit) and add Na_2H_2EDTA to final concentration of 0.1% in reconstituted plasma.

(**f**) *Butterfield's buffered phosphate diluent.*—(*1*) *Stock solution.*—Dissolve 34.0 g KH_2PO_4 in 500 mL H_2O, adjust to pH 7.2 with ca 175 mL 1M NaOH, and dilute to 1 L. Store in refrigerator. (*2*) *Diluent.*—Dilute 1.25 mL stock solution to 1 L with H_2O. Prepare dilution blanks with this solution, dispensing enough to allow for losses during autoclaving. Autoclave 15 min at 121°C.

C. Preparation of Food Homogenate

Aseptically weigh 50 g unthawed food test sample into sterile blender jar. Add 450 mL phosphate-buffered dilution H_2O and homogenize 2 min at high speed (16 000–18 000 rpm). Use this 1:10 dilution to prepare serial dilutions from 10^{-2} to 10^{-6} by transferring 10 mL of 1:10 dilution to 90 mL dilution blank, mixing well with vigorous shaking, and continuing until 10^{-6} is reached.

D. Most Probable Number Technique

Inoculate 3 tubes of Trypticase soy broth with 10% NaCl and 1% sodium pyruvate, **B**(**a**), at each test dilution with 1 mL aliquots of decimal dilutions of sample. Maximum dilution of test portion must be high enough to yield negative end point. Incubate 48 h at 35°C.

Using 3 mm loop, transfer 1 loopful from each growth-positive tube to dried Baird-Parker medium plates, **B**(**c**)(*3*). Vortex-mix tubes before streaking if growth is visible only on bottom or sides of tubes. Streak so as to obtain isolated colonies. Incubate 48 h at 35–37°C.

E. Interpretation

Colonies of *S. aureus* are typically circular, smooth, convex, moist, 2–3 mm in diameter on uncrowded plates, gray-black to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone (precipitate), and frequently with outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle. Occasional nonlipolytic strains may be encountered which have similar appearance, except that surrounding opaque and clear zones are absent. Colonies isolated from frozen or desiccated foods which have been stored for extended periods are frequently less black than typical colonies and may have rough appearance and dry texture.

F. Confirmation Technique

For each plate showing growth, pick ≥ 1 colony suspected to be *S. aureus*. With sterile needle, transfer colonies to tubes containing 0.2 mL BHI broth, **B**(**d**), and to agar slants containing any suitable maintenance medium, e.g., Trypticase soy agar, standard plate count agar, etc. Incubate BHI culture suspensions and slants 18–24 h at 35°C. Retain slant cultures at room temperature for ancillary, or repeat test in case coagulase test results are questionable.

To BHI cultures, add 0.5 mL reconstituted coagulase plasma with EDTA, **B**(**e**), and mix thoroughly. Incubate at 35–37°C and examine periodically over 6 h interval for clot formation. Any degree of clot formation is considered positive reaction. Small or poorly organized clots may be observed by gently tipping tube so that liquid portion of reaction mixture approaches lip of tube; clots will protrude above

liquid surface. Coagulase-positive cultures are considered to be *S. aureus*. Test positive and negative controls simultaneously with cultures of unknown coagulase reactivity. Recheck doubtful coaglulase test results on BHI cultures which have been incubated at 35–37°C for >18 but ≤48 h.

Report most probable number (MPN) of *S. aureus*/g from table of MPN values, Table **966.24** (*see* 17.2.02).

Reference: *JAOAC* **70**, 35(1987).

17.5.02

AOAC Official Method 975.55
Staphylococcus aureus in Foods
Surface Plating Method for Isolation and Enumeration

First Action 1975
Final Action 1976

[Applicable for general purpose use in testing foods expected to contain ≥ 10 cells of *S. aureus*/g. For small numbers, *see* **987.09** (*see* 17.5.01).]

A. Apparatus

Sterile, bent glass streaking rods.—Hockey stick or hoe-shape, with fire-polished ends, 3–4 mm diameter, 15–20 cm long, with angled spreading surface 45–55 mm long.

B. Determination

At each dilution plated, aseptically transfer 1 mL test sample suspension, **987.09C** (*see* 17.5.01), to triplicate plates of Baird-Parker medium, **987.09B(e)(3)** (*see* 17.5.01), and equitably distribute the 1 mL inoculum over the triplicate plates (e.g., 0.4 mL–0.3 mL–0.3 mL). Spread inoculum over surface of agar using sterile, bent glass streaking rods. Avoid extreme edges of plate. Retain plates in upright position until inoculum is absorbed by medium (ca 10 min on properly dried plates). If inoculum is not readily absorbed, plates may be placed in incubator in upright position ca 1 h before inverting. Invert plates and incubate 45–48 h at 35–37°C. Select plates containing 20–200 colonies, unless only plates at lower dilutions (>200 colonies) have colonies with typical appearance of *S. aureus*, **987.09E** (*see* 17.5.01). If several types of colonies are observed which appear to be *S. aureus*, count number of colonies of each type and record counts separately. When plates at lowest dilution plated contain <20 colonies, these may be used. If plates containing >200 colonies have colonies with typical appearance of *S. aureus* and typical colonies do not appear at higher dilutions, use these plates for enumeration of *S. aureus*, but do not count nontypical colonies. Select one colony of each type counted and test for coagulase production, **987.09F** (*see* 17.5.01). Add number of colonies on triplicate plates represented by colonies giving positive coagulase test and multiply by test sample dilution factor. Report this number as number of *S. aureus*/g of food tested.

Reference: *JAOAC* **58**, 1154(1975).

17.5.03

AOAC Official Method 976.31
Staphylococcal Enterotoxin in Foods
Microslide Gel Double Diffusion Test

First Action 1976
Final Action 1977

(Detects 0.1–0.01 μg enterotoxin/mL and is applicable to detection of enterotoxin in culture fluids and concentrated food extracts.)

A. Principle

Precipitation line occurs when serological type of enterotoxin diffuses through gel and reacts with its specific antibody. Coalescence with reference precipitation line which results from serological reactivity of enterotoxin serotype and specific antibody confirms identity.

B. Apparatus

(a) *Debubblers.*—Fine glass rods. Prepare by pulling glass tubing very fine, as in making capillary pipets. Break into ca 6 cm lengths and seal ends in flame.

(b) *Electrical tape.*—Insulating tape, 0.25 × 19.1 mm (Temflex 1700, 3M Co., Electrical Products Division, Bldg 225-4N-05, 3M Center, St. Paul, MN 55144-1000, USA, or equivalent).

(c) *Microscope slides.*—Plain glass, precleaned, 7.62 × 2.54 cm (3 × 1 in.), 0.96–1.06 mm thick.

(d) *Pasteur pipets.*—Prepare by drawing out ca 7 mm od glass tubing or use disposable 30 or 40 μL pipets.

(e) *Petri dishes.*—20 × 150 mm and 15 × 100 mm.

(f) *Plastic templates.*—*See* Figure **976.31A**. (Available from Toxin Technology, 845 E. Johnson St, Madison, WI 53703, USA.)

(g) *Silicone lubricant.*—High vacuum grease (Dow Corning Corp., 2200 West Salzburg Rd, Midland, MI 48686, USA, or equivalent).

(h) *Staining jars.*—Coplin or Wheaton jars.

(i) *Sterile bent glass spreaders.*—Bend glass rods like hockey sticks and fire polish.

(j) *Water-saturated synthetic sponge strips.*—Approximately 1.5 × 1.5 × 6.5 cm H_2O-saturated absorbent cotton is also satisfactory.

C. Media and Reagents

(a) *Agar solution for coating slides.*—0.2%. Add 2 g bacteriological grade agar to 1 L boiling H_2O and heat until agar dissolves. Pour 20–30 mL portions agar into 180 mL (6 oz) prescription bottles or equivalent containers and store at room temperature. Remelt when needed for coating slides.

(b) *Brain-heart infusion (BHI) agar.*—0.7% (w/v). Adjust BHI broth to pH 5.3; add bacteriological grade agar to prepare 0.7% concentration and dissolve by boiling gently. Distribute in 25 mL portions into 25 × 200 mm test tubes, and autoclave 10 min at 121°C. Immediately before use, aseptically empty tubes of sterile medium into 15 × 100 mm Petri dishes.

(c) *Enterotoxin antisera.*—Dilute lyophilized sera with normal physiological saline according to specific instructions of supplier. Store liquid stocks (highly concentrated) and working dilutions of antisera at 4°C; for long term storage, freeze-drying or freezing is recommended.

(d) *Enterotoxin references.*—Rehydrate lyophilized enterotoxin preparations, (c), according to specific instructions of supplier.

(e) *Gel diffusion agar.*—Add 1.2% purified agar (Noble special agar, BD Biosciences, 7 Loveton Circle, Sparks, MD 21152, USA

(w) *Brilliant green (BG) water.*—Prepare sterile H_2O as **966.24(m)** (*see* 17.2.02), add 2 mL 1% aqueous brilliant green dye, **966.24(n)** (*see* 17.2.02), per L sterile H_2O, and mix well.

(x) *Rappaport-Vassiliadis medium.*—Use for raw, highly contaminated foods and animal feeds. (*Note*: Medium must be made from its individual ingredients because commercial formulations may not be effective.) Prepare following solutions: (*1*) *Solution A.*—Dissolve 5.0 g tryptone, 8.0 g NaCl, and 1.6 g KH_2PO_4 in 1 L H_2O. Make fresh on the day that the complete medium is made. Heat to ca 70°C to completely dissolve medium. (*2*) *Solution B.*—Dissolve 400 g $MgCl_2 \cdot 6H_2O$ in 1 L H_2O. Solution B can be stored at room temperature in a dark bottle up to 1 year. (*3*) *Solution C.*—Dissolve 0.4 g malachite green oxalate in 100 mL H_2O. Analytically pure malachite green oxalate (Merck) is recommended because other brands may not be equally effective. Solution C can be stored at room temperature in the dark up to 6 months. To prepare complete Rappaport-Vassiliadis medium, combine 1000 mL Solution A, 100 mL Solution B, and 10 mL Solution C. Dispense medium into 16 × 150 mm tubes and autoclave 15 min at 115°C.

B. Diagnostic Reagents

(a) *Kovacs reagent for indole test.*—Dissolve 5 g *p*-dimethyl-aminobenzaldehyde in 75 mL amyl alcohol and slowly add 25 mL HCl.

(b) *Voges-Proskauer (VP) test reagents.*—(*1*) *α-naphthol solution.*—5%. Dissolve 5.0 g α-naphthol in 100 mL absolute alcohol. (*2*) *Potassium hydroxide solution.*—40%. Dissolve 40 g KOH in H_2O and dilute to 100 mL.

(c) *Sodium hydroxide solution.*—1M. Dissolve 42.11 g 95% reagent NaOH in sterile H_2O and dilute to 1 L.

(d) *Hydrochloric acid solution.*—1M. Dilute 89 mL to HCl 1 L with sterile H_2O.

(e) *Methyl red indicator.*—Dissolve 0.10 g methyl red in 300 mL alcohol and dilute to 500 mL with H_2O.

(f) *Sterile physiological saline solution.*—*See* **940.36B(c)** (*see* 17.1.02).

(g) *Formalinized physiological saline solution.*—Add 6 mL HCHO solution (36–38%) to 1 L sterile saline solution, (**f**), mix, and store in tightly stoppered containers.

(h) *Salmonella polyvalent somatic (O) antiserum*.*—("Serological Identification of the *Salmonella* Serotypes," No. 1229, Difco Laboratories, November 1977, or equivalent.) Contains agglutinins for at least the following somatic (O) antigens 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 19, 22, 23, 24, 25, 34, and Vi. They are agglutinins for somatic (O) groups: A, B, C_1, C_2, D, E_1, E_2, E_3, E_4, F, G_1, G_2, H, I, and Vi.

(i) *Salmonella individual somatic (O) antisera*.*—[*See* reference in (**h**).] For at least each of the somatic (O) groups listed in (**h**).

(j) *Salmonella polyvalent flagellar (H) antiserum Poly a–z*.*—[*See* reference in (**h**).] Contains agglutinins for at least the following flagellar (H) antigens: a, b, c, d, e, f, g, h, i, k, l, m, n, p, q, r, s, t, u, v, w, x, y, z, z_4, z_6, z_{10}, z_{13}, z_{15}, z_{23}, z_{24}, z_{28}, z_{29}, z_{32}, 1, 2, 5, 6, 7.

(k) *Salmonella "Spicer-Edwards" flagellar (H) antisera*.*—[*See* reference in (**h**).] Consists of 7 pooled or polyvalent antisera which react as in Table **967.25**.

(l) *pH Test paper.*—Minimum range 6.0–7.6, with maximum gradations of 0.4 pH unit per color change.

Table 967.25 Spicer-Edwards *Salmonella* H antisera and H antigens with which each antiserum reacts

	Spicer-Edwards *Salmonella* H antisera			
H Antigens	1	2	3	4
a	+	+	+	–
b	+	+	–	+
c	+	+	–	–
d	+	–	+	+
e	+	–	+	–
G Complex[a]	+	–	–	+
l	+	–	–	–
k	–	+	+	+
r	–	+	–	+
y	–	+	–	–
z	–	–	+	+
z_4 Complex[b]	–	–	+	–
z_{10}	–	–	–	+
z_{29}	–	+	+	–
H Antigens	*Salmonella* H Antisera			
enx, enz_{15}	EN complex			
lv, lw, lz_{13}, lz_{28}	L complex			
1, 2; 1, 5; 1, 6; 1, 7	1 complex			

[a] The G complex component of Spicer-Edwards *Salmonella* H antisera 1 and 4 reacts with antigens f, g, m, p, q, s, t, and u.

[b] The z_4 complex component reacts with z_4, z_{23}, z_{24}, and z_{32} (from Difco Laboratories).

(m) *Sterile distilled water.*—Dispense 1 L H_2O into 2 L wide-mouth flask or wide-mouth jar; plug or cap loosely. Autoclave 20 min at 121°C.

(n) *Brilliant green dye solution.*—1%. Dissolve 1 g in sterile H_2O and dilute to 100 mL. (Because some batches of dye are unusually toxic, test all batches of dye before use and use only those producing satisfactory results when tested with known positive and negative test organisms.)

(o) *Bromocresol purple solution.*—0.2%. Dissolve 0.2 g in sterile H_2O and dilute to 100 mL.

References: *JAOAC* **50,** 753(1967); **51,** 870(1968); **52,** 455(1969); **56,** 1027(1973); **59,** 731(1976); **62,** 499(1979); **64,** 893(1981); **64,** 899(1981); **65,** 356(1982). *J. AOAC Int.* **79,** 1307(1996).

Revised: March 1999

* Conform to specifications issued by Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.

17.9.02

AOAC Official Method 967.26 *Salmonella* in Processed Foods

Detection
First Action 1967
Final Action 1974

A. Preparation of Test Portion

(a) *Dried whole egg, dried egg yolk, and dried egg white.*—Aseptically open laboratory sample container and aseptically weigh 25 g test portion into sterile, empty, wide-mouth, screw-cap pt (500 mL) jar. Add ca 15 mL sterile lactose broth, **967.25A(a)** (*see* 17.9.01). Stir with sterile glass rod, sterile spoon, or sterile tongue depressor to

smooth suspension. Add 3 additional portions lactose broth (10, 10, and 190 mL) for total of 225 mL. Stir after each addition until test portion is suspended without lumps. Cap jar securely and let stand at room temperature 60 min. Mix well by shaking, and determine pH with test paper, **967.25B(l)** (*see* 17.9.01). Adjust pH, if necessary, to 6.8 ± 0.2 with sterile 1M NaOH or HCl, **967.25B(c)** or **(d)** (*see* 17.9.01), capping jar securely and mixing well before determining final pH. Loosen jar cap ca ¼ turn and incubate 24 ± 2 h at 35°C.

(b) *Dry whole milk.*—Aseptically weigh 25 g test portion into sterile, wide-mouth screw-cap 500 mL (1 pt) jar. Add 225 mL sterile H_2O and mix well. Cap jar securely and let stand 60 min at room temperature. Mix well by swirling and determine pH with test paper, **967.25B(l)** (*see* 17.9.01). Adjust pH, if necessary, to 6.8 ± 0.2 with sterile 1M NaOH or HCl, **967.25B(c)** or **(d)** (*see* 17.9.01). Add 0.45 mL 1% aqueous brilliant green dye solution, **967.25B**(n) (*see* 17.9.01), and mix well. Loosen jar cap ca ¼ turn and incubate 24 ± 2 h at 35°C.

(c) *Dried active yeast.*—Aseptically weigh 25 g test portion into sterile, empty, wide-mouth, screw-cap pt (500 mL) jar. Add 225 mL sterile Trypticase (tryptic) soy broth, **967.25A(t)** (*see* 17.9.01), and let yeast form smooth suspension. Cap securely and let stand 60 min at room temperature. Determine pH with test paper, **967.25B(l)** (*see* 17.9.01). Adjust pH, if necessary, to 6.8 ± 0.2 with sterile 1M NaOH or HCl, **967.25B(c)** or **(d)**(*see* 17.9.01), capping jar securely and mixing well before determining final pH. (If pH is adjusted before yeast is evenly suspended, final pH will be less than desired.) Incubate 24 ± 2 h at 35°C, with jar cap loosened ¼ turn.

(d) *Onion powder and garlic powder.*—Aseptically weigh 25 g test portion into sterile, wide-mouth, screw-cap 500 mL (1 pt) jar. Test portion is pre-enriched in Trypticase (tryptic) soy broth, **967.25A**(t) (*see* 17.9.01), with added K_2SO_3(5 g/L) for final 0.5% K_2SO_3 concentration. Autoclave 225 mL portions in 500 mL flasks for 15 min at 121°C. Aseptically determine volume and adjust, if necessary, to 225 mL. Add 225 mL sterile Trypticase (trypic) soy broth, **967.25A(t)** (*see* 17.9.01), with K_2SO_3, to test portion and mix thoroughly, using sterile glass rod or spoon. Let stand 60 min and determine pH with test paper, **967.25B(l)** (*see* 17.9.01). Adjust pH, if necessary, to 6.8 ± 0.2 with sterile 1M NaOH or 1M HCl, **967.25B(c)** or **(d)** (*see* 17.9.01). Incubate 24 ± 2 h at 35°C, with jar cap loosened ¼ turn.

(e) *Milk chocolate and casein.*—Aseptically weigh 25 g test portion into sterile blender jar. Add 225 mL sterile reconstituted NFDM, **967.25A(v)** (*see* 17.9.01), to chocolate samples, and add 225 mL lactose broth, **967.25A(a)** (*see* 17.9.01), to casein samples. Blend each test portion/broth mixture 2 min at high speed and decant blended homogenate into sterile 500 mL jar. Cap jar securely and let stand 60 min at room temperature. Mix well by shaking, and determine pH with test paper, **967.25B(l)** (*see* 17.9.01). Adjust pH, if necessary, to 6.8 ± 0.2 with sterile 1M NaOH or HCl, **967.25B(c)** or **(d)** (*see* 17.9.01), capping jar securely and mixing well before determining final pH. To chocolate-reconstituted NFDM test samples, add 0.45 mL 1% aqueous brilliant green dye, **967.25B(n)** (*see* 17.9.01), and mix well. Loosen jar caps ¼ turn and incubate jar 24 ± 2 h at 35°C.

(f) *Instant nonfat dry milk (NFDM).*—Aseptically open laboratory sample container and aseptically weigh 25 g test portion into sterile beaker (250 mL) or other appropriate container. Cover with sterile foil cover or sterile cap to prevent contamination. Using sterile glass or paper (made with tape to withstand autoclaving) funnel, pour 25 g analytical unit gently and slowly over surface of 225 mL brilliant green H_2O, **967.25A(w)** (*see* 17.9.01), contained in sterile 500 mL Erlenmeyer or other appropriate container. Let container with test portion–pre-enrichment broth stand undisturbed 60 ± 5 min. Incubate loosely capped container, without mixing or pH adjustment, for 24 ± 2 h at 35°C.

B. Isolation

(a) *Growth in selective broth.*—Gently shake incubated test portion mixture, **A**, and transfer 1 mL to 10 mL selenite cystine broth, **967.25A(b)**(*1*) or (*2*) (*see* 17.9.01), and additional 1 mL to 10 mL tetrathionate broth, **967.25A(c)** (*see* 17.9.01). Incubate 24 ± 2 h at 35°C. (For dried active yeast, substitute lauryl sulfate tryptose broth, **967.25A(u)** (*see* 17.9.01), for selenite cystine broth, **967.25A(b)**(*1*) or (*2*) (*see* 17.9.01). Vortex-mix, and streak 3 mm loopful of incubated selenite cystine broth on selective media plates of XLD, **967.25A(d)** (*see* 17.9.01), HE, **967.25A(e)** (*see* 17.9.01), and BS, **967.25A(f)** (*see* 17.9.01). Repeat with 3 mm loopful of incubated tetrathionate broth. Incubate plates 24 ± 2 h at 35°C.

(b) *Appearance of typical Salmonella colonies.*—(*1*) *On XLD.*—Pink colonies with or without black centers. Many *Salmonella* may have large, glossy black centers or may appear as almost completely black colonies. Atypically, a few *Salmonella* cultures produce yellow colonies with or without black centers. (*2*) *On HE.*—Blue-green to blue colonies with or without black centers. Many *Salmonella* colonies may have large glossy black centers or may appear as almost completely black colonies. (*3*) *On BS.*—Brown, gray, or black, sometimes with metallic sheen. Surrounding medium is usually brown at first, turning black with increasing incubation time. Some strains produce green colonies with little or no darkening of surrounding medium. Examine XLD and HE plates for typical or suspicious *Salmonella* colonies after 24 ± 2 h incubation at 35°C. BS plates should be examined for typical or suspicious *Salmonella* colonies after 24 ± 2 h and 48 ± 2 h incubation at 35°C.

C. Treatment of Typical or Suspicious Colonies

(a) *Inoculation of triple sugar iron (TSI) agar and lysine iron agar (LIA).*—Pick with needle 2 or more typical or suspicious colonies, if present, from each XLD, HE, and BS plates having growth. Inoculate TSI slant, **967.25A(g)** (*see* 17.9.01), with portion of each colony by streaking slant and stabbing butt. After inoculating TSI with needle, do not obtain more inoculum from colony and do not heat needle, but inoculate LIA, **967.25A(m)**(*1*) (*see* 17.9.01), as in **967.27C(a)** (*see* 17.9.03). Store picked selective plates at 5–8°C or at room temperature (ca 26°C).

(b) *Presumptive reactions.*—Incubate TSI and LIA slants at 35°C for 24 ± 2 h. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive H_2S production. *Salmonella* cultures typically have alkaline (red) slant and acid (yellow) butt, with or without H_2S (blackening of agar) in TSI. In LIA, *Salmonella* cultures typically have alkaline (purple) reaction in butt. Consider only a distinct yellow coloration in butt of tube as an acidic (negative) reaction. Do not eliminate cultures that produce discoloration in butt solely on this basis. Most *Salmonella* cultures produce H_2S in LIA. Retain all presumptive positive *Salmonella* cultures on TSI (alkaline slant and acid butt) agar for biochemical and serological tests whether or not corresponding LIA reaction is positive (alkaline butt) or negative (acid butt). Do not exclude a TSI culture that appears to be non-*Salmo-*

nella if the reaction in LIA is typical (alkaline butt) for *Salmonella*. Treat these cultures as presumptive positive and submit them to further examination. LIA is useful in detection of *S. arizonae* and atypical *Salmonella* strains that utilize lactose and/or sucrose. Discard only apparent non-*Salmonella* TSI cultures (acid slant and acid butt) if corresponding LIA reactions are not typical (acid butt) for *Salmonella*. Test retained presumptive positive TSI cultures as directed in **C(c)** to determine if they are *Salmonella* spp., **967.27D(e)**(*1*) (*see* 17.9.03), or *S. arizonae* organisms, **967.27D(e)**(*2*) (*see* 17.9.03). If TSI slants fail to give typical *Salmonella* reactions, pick additional suspicious colonies from selective medium plate not giving presumptive positive culture and inoculate TSI and LIA slants as in **(a)**.

(**c**) *Selection for identification.*—Apply biochemical and serological identification tests to 3 presumptive positive TSI cultures picked from selective agar plates streaked from selenite cystine broth and to 3 presumptive positive TSI cultures picked from selective agar plates streaked from tetrathionate broth.

If 3 presumptive positive TSI cultures are not isolated from one set of selective agar plates, test other presumptive positive TSI cultures, if isolated, by biochemical and serological tests. A minimum of 6 TSI cultures are examined for each 25 g test portion tested.

References: *JAOAC* **50,** 753(1967); **51,** 870(1968); **52,**455(1969); **56,** 1027(1973); **59,** 731(1976); **61,** 401(1978); **62,** 499(1979); **64,** 893(1981); **64,** 899(1981); **65,** 356(1982);**67,** 807(1984); **69,** 277(1986).

Revised: March 1997

17.9.03

AOAC Official Method 967.27 *Salmonella* in Foods

Identification
First Action 1967
Final Action 1968

A. Cultures

Pure cultures on TSI are required for inoculation of biochemical test media.

(**a**) *Pure cultures.*—Proceed to **B**.

(**b**) *Mixed cultures.*—Streak any culture that appears to be mixed on MAC, **967.25A(q)** (*see* 17.9.01), XLD, **967.25A(d)** (*see* 17.9.01), or HE, **967.25A(e)** (*see* 17.9.01). Incubate 24 ± 2 h at 35°C.

(**c**) *Appearance of Salmonella colonies*—(*1*) *On MAC.*—Typical colonies appear transparent and colorless, sometimes with dark centers. *Salmonella* will clear areas of precipitated bile caused by other organisms sometimes present in medium. (*2*) *On XLD.*—*See* **967.26B(b)**(*1*) (*see* 17.9.02). (*3*) *On HE.*—*See* **967.26B(b)**(*2*) (*see* 17.9.02).

Pick with needle 2 typical or suspicious colonies and inoculate TSI slants by streaking the slant and stabbing the butt as in **967.26C(a)** (*see* 17.9.02). Retest purified cultures as in **967.26C(b)** (*see* 17.9.02), and proceed with identification.

As alternative to conventional tube system for *Salmonella,* any one of the AOAC-approved commercial biochemical kits can be used for presumptive generic identification of foodborne *Salmonella*. *See* **978.24** (*see* 17.9.04), **989.12** (*see* 17.9.05), and **991.13** (*see* 17.9.06).

B. Subcultures

(**a**) *Urease test.*—Subculture small amount of growth from presumptive positive TSI agar culture to urea broth, **967.25A(k)**(*1*) (*see* 17.9.01), and incubate 24 ± 2 h at 35°C or inoculate rapid urea broth, **967.25A(k)**(*2*) (*see* 17.9.01) with two 3 mm loopfuls of growth from each presumptive-positive TSI slant culture, and incubate 2 h in H_2O bath at 37 ± 0.5°C. Discard all cultures that give positive test (purple-red color). *Salmonella* spp. are urease negative (no change in orange color of medium).

(**b**) *Serological flagellar (H) screening test.*—To reduce number of presumptive positive TSI agar cultures carried through identification tests, perform serological flagellar (H) screening test by transferring one 3 mm loopful of each urease-negative TSI agar culture to either: (*1*) Brain–heart infusion broth, **967.25A(r)** (*see* 17.9.01), (for test on same day) and incubate at 35°C until visible growth occurs (ca 4–6 h); or (*2*) Trypticase soy–tryptose broth, **967.25A(s)** (*see* 17.9.01), (for test on following day) and incubate 24 ± 2 h at 35°C.

To 5 mL of each of the 6 broth cultures, add ca 2.5 mL formalinized physiological saline solution, **967.25B(g)** (*see* 17.9.01). Select 2 formalinized broth cultures and test with *Salmonella* flagellar (H) antisera, **967.25B(j)** or **(k)** (*see* 17.9.01), as in **967.28C** or **D** (*see* 17.9.07).

If selected formalinized broth cultures are positive, perform additional tests on these cultures, beginning with **C**, except step **C(d)** may be omitted.

If both formalinized broth cultures are negative, perform serological test on the 4 additional broth cultures [**B(b)**(*1*) or (*2*)] to obtain, if possible, 2 positive cultures for additional testing, **C**.

If all urease-negative TSI cultures from test portion are *Salmonella* serological flagellar (H) test negative, then perform additional tests, beginning with **C**, on these cultures.

C. Testing Urease-Negative Cultures

Using needle, transfer portion of presumptive positive TSI culture to LIA medium and small amount of growth from the TSI culture to each of other media:

(**a**) *Lysine iron agar, 967.25A(m)(1) (see 17.9.01).*—Stab butt twice and then streak slant. Replace tube cap loosely and incubate 24 ± 2 h at 35°C. Most *Salmonella* spp. give purple color of alkaline

Table 967.27A Characteristics of *Salmonella*

Test or substrate	Results[a]
Urease, **967.27B(a)**	– (Orange-red)
Lysine decarboxylase, **967.27C(a)**	+ (Alkaline; purple throughout medium)
Phenol red dulcitol broth, **967.27C(b)**	+ (Yellow and/or gas)[b]
KCN broth, **967.27C(c)**(*1*)	– (No growth)
Malonate broth, **967.27C(c)**(*2*)	– (Unchanged green)[c]
Indole test, **967.27C(c)**(*3*)	– (No red color)
Polyvalent flagellar test, **967.27B(b)** and **C(d)**	+ (Visible agglutination)
Polyvalent somatic test, **967.27C(f)**	+ (Visible agglutination)

[a] + = ≥90% positive in 1–2 days; – = ≥90% negative in 1–2 days.

[b] Majority of *S. arizonae* cultures are negative.

[c] Majority of *S. arizonae* cultures are positive.

ANEXO # 6

RESULTADOS DE LOS ANÁLISIS MICROBIOLÓGICOS DE LOS POLLOS

INSTITUTO DE TECNOLOGÍAS

Programa de Tecnología en Alimentos

Laboratorios PROTAL

R 4.1

INFORME : 0175-81M.06.03

DATOS DEL SOLICITANTE DE LOS ANÁLISIS	
NOMBRE:	Q.F. EMMA MORENO
DIRECCIÓN:	GERANIOS 2 MZ 3002 VILLA 4
TELÉFONO:	2891540

IDENTIFICACIÓN DE LA MUESTRA / ETIQUETA		
NOMBRE: POLLO CRUDO		
MARCA COMERCIAL: SIN MARCA		
TIPO DE ALIMENTO: Carnes y derivados	**CODIGO MUESTRA:**	0175-81M.06.03
TIPO DE ENVASE INMEDIATO: Funda de polipropileno	**LOTE :**	S/L
	FECHA DE ELABORACION:	S/F
	FECHA DE EXPIRACION:	S/F
	VIDA UTIL:	S/T
	CONTENIDO :	3.8 libras

ANÁLISIS ORGANOLÉPTICO

ENSAYOS REALIZADOS	RESULTADOS	REQUISITOS
COLOR	Normal	Normal
OLOR	Normal	Normal
SABOR	Normal	Normal
TEXTURA	Normal	Normal

ANÁLISIS FÍSICOS-QUÍMICOS

ENSAYOS REALIZADOS	UNIDAD	RESULTADOS	REQUISITOS	METODOS / REF.
No requeridos	-----	-----	-----	------

ANÁLISIS MICROBIOLÓGICOS

ENSAYOS REALIZADOS	UNIDAD	RESULTADOS	REQUISITOS	METODOS / REF.
COLIFORMES TOTALES	NMP/ g	7.3×10^2	----	AOAC 17 th 966.24
AEROBIOS TOTALES	UFC/ g	1.6×10^2	10^6	AOAC 17 th 966.23
AEROBIOS PSICROFILOS	UFC/ g	8.0 x 10	----	ICMSF
AEROBIOS PSICROFILOS	UFC/ g	20	-----	ICMSF
E. COLI	AUS/PRES	AUSENCIA	AUSENCIA	AOAC 17 th 966.24
SALMONELLA	AUS/PRES	AUSENCIA	AUSENCIA	AOAC 17 th 967.26
S. AUREUS	NMP/ g	0	AUSENCIA	AOAC 1 th7 987.09

Los resultados emitidos corresponden exclusivamente a la muestra proporcionada por el cliente

OBSERVACIONES : La muestra analizada **SI** cumple con los requisitos Microbiológicos del Ayto de Bilbao

Guayaquil, Julio 2 del 2003

MAE. Gloria Bajaña de Pacheco
Gerente Técnico y de Calidad

Laboratorios PROTAL acreditados ante el MNAC
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INSTITUTO DE TECNOLOGÍAS

Programa de Tecnología en Alimentos

Laboratorios PROTAL

R 4.1

INFORME : 0175-82M.06.03

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TELÉFONO:	2891540

IDENTIFICACIÓN DE LA MUESTRA / ETIQUETA		
NOMBRE: POLLO AHUMADO		
MARCA COMERCIAL: SIN MARCA		
TIPO DE ALIMENTO: Carnes y derivados	**CODIGO MUESTRA:**	0175-82M.06.03
TIPO DE ENVASE INMEDIATO: Funda de polipropileno	**LOTE :**	S/L
	FECHA DE ELABORACION:	S/F
	FECHA DE EXPIRACION:	S/F
	VIDA UTIL:	S/T
	CONTENIDO :	3.5 libras

ANÁLISIS ORGANOLÉPTICO		
ENSAYOS REALIZADOS	**RESULTADOS**	**REQUISITOS**
COLOR	Normal	Normal
OLOR	Normal	Normal
SABOR	Normal	Normal
TEXTURA	Normal	Normal

ANÁLISIS FÍSICOS-QUÍMICOS				
ENSAYOS REALIZADOS	**UNIDAD**	**RESULTADOS**	**REQUISITOS**	**METODOS / REF.**
No requeridos	----	----	-----	-----

ANÁLISIS MICROBIOLÓGICO				
ENSAYOS REALIZADOS	**UNIDAD**	**RESULTADOS**	**REQUISITOS**	**METODOS / REF.**
COLIFORMES TOTALES	NMP/ g	0	----	AOAC 17 th 966.24
AEROBIOS TOTALES	UFC/ g	0	10^5	AOAC 17 th 966.23
E. COLI	AUS/PRES	AUSENCIA	AUSENCIA	AOAC 17 th 966.24
SALMONELLA	AUS/PRES	AUSENCIA	AUSENCIA	AOAC 17 th 967.26
S. AUREUS	NMP/ g	O	AUSENCIA	AOAC 17 th 987.09

Los resultados emitidos corresponden exclusivamente a la muestra proporcionada por el cliente

OBSERVACIONES : La muestra analizada **SI** cumple con los requisitos Microbiológicos del Ayto de Bilbao

Guayaquil, Julio 2 del 2003

MAE. Gloria Bajaña de Pacheco
Gerente Técnico y de Calidad

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INSTITUTO DE TECNOLOGÍAS
Programa de Tecnología en Alimentos
Laboratorios PROTAL

R 4.1

INFORME : 0212-6M.09.03

DATOS DEL SOLICITANTE DE LOS ANÁLISIS	
NOMBRE:	Q.F. EMMA MORENO
DIRECCIÓN:	GERANIOS 2 MZ 3002 VILLA 4
TELÉFONO:	2891540

IDENTIFICACIÓN DE LA MUESTRA / ETIQUETA		
NOMBRE:	POLLO AHUMADO TRADICIONAL	
MARCA COMERCIAL:	SIN MARCA	
TIPO DE ALIMENTO: Carnes y derivados		**CODIGO MUESTRA:** 0212-6M.09.03
TIPO DE ENVASE INMEDIATO: Funda de polipropileno		**LOTE :** S/L **FECHA DE ELABORACION:** S/F **FECHA DE EXPIRACION:** S/F **VIDA UTIL:** S/T **CONTENIDO :** 3.3 libras

ANÁLISIS ORGANOLÉPTICO		
ENSAYOS REALIZADOS	**RESULTADOS**	**REQUISITOS**
COLOR	Normal	Normal
OLOR	Normal	Normal
SABOR	Normal	Normal
TEXTURA	Normal	Normal

ANÁLISIS FÍSICOS-QUÍMICOS				
ENSAYOS REALIZADOS	**UNIDAD**	**RESULTADOS**	**REQUISITOS**	**METODOS / REF.**
No requeridos	---	---	---	---
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ANÁLISIS MICROBIOLÓGICOS				
ENSAYOS REALIZADOS	**UNIDAD**	**RESULTADOS**	**REQUISITOS**	**METODOS / REF.**
COLIFORMES TOTALES	NMP/ g	0	---	AOAC 17 th 966.24
AEROBIOS TOTALES	UFC/ g	0	10^5	AOAC 17 th 966.23
E. COLI	AUS/PRES	AUSENCIA	AUSENCIA	AOAC 17 th 966.24
SALMONELLA	AUS/PRES	AUSENCIA	AUSENCIA	AOAC 17 th 967.26
S. AUREUS	NMP/ g	O	AUSENCIA	AOAC 17 th 987.09

Los resultados emitidos corresponden exclusivamente a la muestra proporcionada por el cliente

OBSERVACIONES : La muestra analizada **SI** cumple con los requisitos Microbiológicos del Ayto de Bilbao

Guayaquil, Julio 02 del 2003

MAE. Gloria Bajaña de Pacheco
Gerente Técnico y de Calidad

Laboratorios PROTAL acreditados ante el MNAC
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ANEXO # 7

FICHA TÉCNICA DEL HUMO LÍQUIDO UTILIZADO

FOREST FLAVORS

Forest Flavors Product Specification
Product Code FF-BS

FF-BS is an aqueous solution of Natural Smoke Flavors manufactured from hickory and other select hardwoods. This product contains Polysorbate 80 for solubility.

COMPOSITION

Acids	9.5-10.5% (volume percent)
Staining Index	65-80
Carbonyls	15-20 (g/100ml)
Phenols	12-20 (mg/ml)
Specific Gravity	1.078-1.088 (25°C)
Density average	9.04 (lbs./gal)
pH Level	2.0- 3.0

APPEARANCE
Clear, brown liquid with wood smoke aroma.

APPROVALS
Considered GRAS by the FDA and USDA. Kosher and Pareve certified. Approved for use by Agriculture Canada and compliant with EEC Regulations.

STORAGE
One year shelf life when stored under cool conditions (45-70°F)

PACKAGING
Available in 5, 55, 320 gallon containers and bulk

SUGGESTED USAGE
This product is designed to provide a smoke color and flavor to food products. Forest Flavors can assist you in determining appropriate application methods.

MISCELLANEOUS
A tar precipitate may form upon standing as a natural progression.

Date effective 5/7/99
Supersedes 1/99

Post Office Box 444, Glasgow, Kentucky 42142 USA • Telephone/Fax: 270-651-6075 • 888-996-9962 • E-mail: forest@glasgow-ky.com

Forest Flavors International
P.O. Box 444
Glasgow, Ky 42141
Telephone: 270-651-6075
Fax: 270-651-5657

CERTIFICATE OF ANALYSIS

Date:	**08/08/02**
Product:	**FF-BS**
Customer:	**Distribuidora Descalzi SA**
Batch Number:	**070302BS1**
Staining Index:	**61.8**
Acidity:	**10.66**
Specific Gravity:	**1.078**
pH:	**2.14**
Phenols:	**17.3**

One year shelf life when stored under cool conditions 45°-75°F

Test conducted by: Stephen Glover

Sam Crace
Sam Crace, Quality Assurance

ANEXO # 8

POLLO AHUMADO

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