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MICROBIOLOGICAL QUALITY OF FISH FOR SALE IN GUATEMALA CITY

Presentado por el ICAITI a

INTERNATIONAL CENTER FOR MARINE RESOURCE DEVELOPMENT

UNIVERSITY OF RHODE ISLAND

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1. Introduction

A contract between the Central American Research Institute for Industry (ICAITI) in Guatemala City and The International Center for Marine Resource Development from the University of Rhode Island whose purpose was to study the sanitary conditions and fish quality at the retail level in Guatemala City was signed on February 15, 1977.

The parameters chosen were microbiological quality and sanitation indexes of handled fish. They include aerobic plate count at 25° C, faecal coliforms (MPN) and a Staphylococcus aureus estimate. The three parameters were investigated in every sample taken whether it be fish, fish surfaces, surfaces of tables and floors, ice water or drippings. Both coliforms and staphylococci are not part of the natural flora of raw seafood and their presence is considered to be contamination from human sources.

The work covered by the contract was divided in three parts: the first, a trial test in collaboration with the Centro de Investigación en Tecnología de Alimentos (CITA) from Costa Rica consisting in the shipment of swab samples between ICAITI and CITA and analysis of those samples to evaluate the effect of transport on such samples when shipped via air freight in an ice-chest. The purpose was to discover if in the future such a procedure could be followed with samples taken in places where no local

laboratory can do microbiological analysis.

The second part of the survey studied the conditions under which fish are sold retail at two public markets. Those sites generally lack sophisticated equipment like refrigerators, freezers, electric saws, stainless steel tables and so forth. Installations are usually reduced to a large wood or cement table, another small table where fish are cleaned and cut, and generally a wooden chest lined with galvanized tin to keep fish with ice; if there is no such ice chest, cane baskets lined with plastic are usually used with "pacaya" leaves as insulation. The study was carried out during a 6 day period in order to follow the sanitary conditions of the market during the week. The fish were studied while kept at the market under those conditions usually found in that particular site.

The third part of the study dealt with fish subjected to processing such as filleting, freezing, drying and salting. The frozen fish products are mainly processed by commercial fisheries and distributed at retail level to supermarkets where they are kept frozen in display freezer.

Four supermarkets and one popular market were visited, the later because in the particular season when this study was carried out (April) there was a high production

and consumption of dried-salted fish, practically nonexistent during other times of the year. Most of the dried fish are prepared at artisan level. To complement the laboratory data, several questions where asked at each site, markets or supermarkets, involving topics like source of fish, means and conditions of transport, rejections and criteria for doing so, storage of fish, use of ice, sanitation practices, wholesale and retail prices.

2. Materials and methods

The procedures for taking samples and analyzing them were those recommended by the International Committee on Microbiological Specifications for Foods (Thatcher & Clark, 1968). A photocopy has been included in Section 9.

Samples of processed fish were bought in supermarkets and popular markets and transported in the original packaging (when provided with such) to the laboratory. Also, samples of freezer surfaces were taken using the swab method; this involved a sterile swab in its own tube (Falcon Plastics), a sterile metal frame to expose 15 cm^2 (2.325 square inches) and sterile peptone solution (0.1%) to wet the swab. Once the the surface was sampled the swab was kept in its own tube with about 5 ml of peptone solution.

During the study of the popular market conditions, sampling was done every day for a 6 day period. In this case, only fresh fish were studied and they were not transported to the laboratory but remained at the market with the rest of seafood items on display. Each day a sample of the skin surface and of internal cavity were taken according to the procedure described above. In addition, the surfaces of the table for cutting and filleting fish and the floor were sampled, and samples of ice, water and fish-ice dripping were taken.

Aerobic plate count

The APC was carried out following the method No. 1 (Pour Plate Method) of Thatcher and Clark, 1968, with a modification in the incubation temperature. Since not only normal flora, largely psycrophilics, but also mesophilic contaminants were of interest, an intermediate temperature of incubation of 25°C was used. Plates were to be kept for at least three days but in that time spreaders and giant colonies had developed so a 48 hr period was used instead.

Faecal coliforms

Determinations of the most probable number of faecal coliforms were performed

as sanitation index. Procedure followed was method No. 1 from Thatcher & Clark, 1968 with no further modifications.

Staphylococcus aureus count

Method No. 2 of Thatcher and Clark, 1968 was followed with no further modifications. Staphylococcus count was performed as an index of mishandling.

3. Description of the places visited

The results from the questionaire provided by ICMRD are presented in Tables 1-A and 1-B as well as a copy of the questionnaire. Brief descriptions of the markets and supermarkets follow. Graphs 1 and 2 show the temperatures and relative humidities present in the markets at the time the samples were taken, at 10 am each morning.

Colón Market

This is a popular market located in one of the older quarters of the city; the large, high ceiling building is divided into two main sections: one where vegetables and staples are sold and the other, of interest to us, housing the tiny "comedores" or eateries and butcher shops. Like other markets, the place is crowded with vending posts.

There is no specific area where fish vendors are located. There are only three stands where fish are sold; two of them are located with the kitchens and butcher stalls, though they do not occupy stalls but are placed in the hallways. The other one is found among the vegetable stands.

The stand selected for doing the study is owned by woman that personally takes care of it with the help of a daughter. The place allows only a narrow passage for shoppers and it is difficult to stay in front of the stand to purchase any seafood. Problems were encountered for this reason while collecting the samples. Although there are windows, natural light is scarce because of walls separating stalls, and the smoke from the kitchens that over the years has deposited soot on the walls and window panes. No artificial light is available in the stand.

The area was infested with flies that continuously are standing on the seafood, tables and scale. No control measures are taken to solve the problem. Since the stand does not occupy a designed stall, there is no water available; it has to be collected in cans from a central faucet.

To scale and fillet the fish, the people in charge use a large knife and a brush made from wood and nails, both instruments are rarely washed, nor desinfected. The table is made of wood and the surface is full of cuts from the use of the knife.

The ice is bought in big chunks and is broken by the people there, but the pieces are still too large and there is poor distribution of ice among the fish. When fish are stored overnight, a lot of plantain leaves and plastic are used to cover the fish in a large wood ice-chest lined with galvanized metal.

La Terminal Market

This market was designed to function as a wholesale distribution center, receiving products from the countryside and then distributing them to retail markets in different quarters of the city. However, at present it also functions as a retail market and it is the largest market in the country.

There is an specific area for selling seafood and the stalls are built for that purpose. Each stand has cement benches covered with glazed tiles and tap water is available in every stall. There is a greater variety of seafood items on display, not only from Guatemala but also from El Salvador. Even though the installations are adequate, people selling there do not use them correctly. In many cases they cover the benches with plastic thus preventing the ice drippings from going down the drain. As in the Colón Market, flies are also abundant and the problem of light is even more severe, because the place has a low ceiling and the windows are covered with cardboard since the panes are probably broken.

The stand chosen for the study is owned by a young couple who personally man it. Even though there are tap water facilities, the salesman do not use the washstand but have placed a huge washbowl where they dip things to wash them. The water in the bowl was observed to be turbid. To prepare the seafood for sale, a wood table is used where the fish is scaled and filleted, using a large knife and a brush made from wood and nails. The table and instruments were not cleaned or desinfected during the time the samples were collected.

Ice is used in large chunks and placed among the seafood; no pieces of ice were observed with the fresh fish. Also, the temperature of the fresh fish was 9°C. Upon entering the market the preparation of ice to be sold was observed. Large rectangular pieces are cut into smaller pieces to be sold to the fish stands. The ice was being sawed on a wood platform outside the market on the streets, exposed to dust.

At the stand seafood was stored overnight in cane baskets lined with plastic and pacaya (a type of palm) leaves. It was the only case observed where seafood was placed between layers of ice.

La Placita Market

This place is located in a very centric quarter and used to be a larger market but a fire destroyed part of it. The reason to visit it was to sample dried-salted fish that is consumed in the days preceeding and during the Holy Week. This market occupies a tall building with a high ceiling. Windows are located on the walls under the ceiling providing good illumination. The stand where fish were sampled is not located in an specific area for such items; there were many different items on sale in neighboring stands. Fish was piled on wood tables against a wood counter, without any kind of packaging. Since they are dry-salted, no ice or refrigeration is used. There is no running water and cleaning is seldom carried out; when done wiping with a damp cloth is used.

Description of the supermarkets

The supermarkets that were sampled were located in different places in the city, but in general, they were a similar regarding sanitary aspects. They all had an open freezer where fish were placed for sale. The supermarkets and samples are listed in Table 2, as well as price per kilogram of sample.

At two supermarkets, Norte and La Torre, fish were not distributed orderly in the freezer and some opened bags were found. In these same two supermarkets there was no a fish-man or vendor who could give us information on the fish for sale. In many cases the bags of fish were unidentified and contents were of unknown species. Also there were some unpackaged fish for sale, mixed with the packaged fish.

It was found that the same printed plastic bag was used for packing different kinds of fish, thus the content could not be confirmed particularly because the product is filleted.

4. Results and discussion

Unfortunately, the first part of the experimental program consisting in analysis of samples to be sent to ICAITI's laboratories by CIDA has not been carried out due to the fact at the time of writting the report the samples had not been received. Upon finishing that portion of the project, the results will be sent to Rhode Island.

Parts two and three are discussed in the following pages. The results for each individual sample can be found in the laboratory reports in Section 10. It can be noted that part three has been discussed first due to its relative simplicity.

During the part three, that of sampling the processed fish the aim was to analyze the microbiological condition of the products and the places. All samples are sold inside plastic bags so there is no real contact between the freezer walls and the fish. In only one place, Supermarket Paiz Montúfar, unpackaged frozen fish is displayed on ice, not for preservation but for ornamental reasons, and the advantage of doing this is the maintainance of the fish surface wet and shiny.

The microbial loads of the products, measured by the aerobic plate count (APC) at 25°C are shown on Table 3. The major part of samples consists of filleted and frozen fish;therefore according to the American Public Health Association (APHA, 1976) recent interpretation of results, figures higher than 10^6 per gram or cm^2 are considered indicative of incipient spoilage. As it can be seen in the Table 3, samples from two supermarkets, Norte and La Torre have products with APC results in the order of 10^7 per gram.

In the case of La Placita where only dry-salted fish was sold, the situation is similar; counts in excess of $10^6/g$ are suggestive of spoilage. When it comes to faecal coliforms and Staphylococcus aureus, their presence is considered a consequence

of human handling. If faecal coliforms are in excess of 10/g for frozen fish and 40/g for salted dried fish it is supposed that products have been subjected to mishandling, (APHA, 1976). As for S. aureus, they should not be in excess of 10^2/g; higher counts are also indicative of gross mishandling. As can be noted in Table 3 there are samples where both indicators, faecal coliforms and S. aureus are in excess of the limits, indicating mishandling.

Phase two is a more complicated situation. In this case two popular markets were studied for 6 days to obtain a picture of the sanitary conditions that exist daily and so that the effects on the quality of fresh chilled fish sold there would also be known. In each market, three species of fish were chosen among the most popular with the public and sampled with the swab method for skin surface and cavity surface. During several days they remained there to be sampled every day.

Results indicate that in the case of Colón Market, there is gross contamination of the table, floor, and ice. The liquid drippings from the piled fish are heavily contaminated (Tables 4, 5, and 6). Ice and the liquid drippings are the most important factors, both are in contact with fish and are the source for cross contamination. One of the major problems is the tendency of people selling fish to cover tables and benches with plastic thus allowing accumulation of the liquid. At the end of three days, how-

ever, APC of fish samples were $10^6/cm^2$ or less, even inside cavities. General appearance of the fish was also good; no signs of spoilage were evident.

At the other market, La Terminal, the location sampled showed a similar situation. The most heavily contaminated samples were ice and liquid drippings (Tables 7, 8 and 9). Fish sampled stayed for four days and were spoiled at the end of the trial. The exact day of their arrival to the market was not clearly defined and they could have been in ice longer than assumed. In this market the failure to provide a good draining of the liquid is even more negligent than in the other market because these people have a cement bench covered with glazed tiles with inclination and drainage construction; but the bench was covered with plastic.

5. References

- APHA, 1976. Compendium of Methods for the Microbiological Examination of Foods. M.L. Speck, editor, APHA Inc. Washington, D.C., 701 p.
- Thatcher, F.S., and D.S. Clark, 1968. Microorganisms in Foods. International Committee on Microbiological Specifications for Foods. University of Toronto Press. Toronto, Canada, 234 p.

6. Tables

TABLE 1-A

QUESTIONAIRE RESULTS FOR PROCESSED FISH SAMPLES

PLACE SURVEYED	Paiz-Montúfar Supermarket	La Placita Market	Residencial Supermarket	Norte Supermarket	La Torre Supermarket
PRODUCTS SOLD	Frozen fish	Solid , dried fish	Frozen fish	Frozen fish	Frozen fish
How are the fish delivered to the store?	Wooden boxes with ice in insulated truck	Dried fish in nets in ordinary trucks	Plastic bags in refrigerated truck	Plastic bags in small vans	Personnel questioned was not sure
How are fish displayed?	Open freezer whole evicescerated fish, fillets in plastic bags	On a wooden table, rough surface, uncovered	Open freezer	Open freezer	Open freezer
Are the fish washed?	NO	NO	NO	NO	NO
Rejection criteriu	i) firmness, ii) color iii) odor	Rejection uncommon	Odor, gill color	Gill color	Personnel questioned was not sure
Storage	Frozen fish is kept onvernight in closed freezer	Covered with burlap bag and tied with rope	In the same freezer	In the same freezer	In the same freezer

TABLE 1-A

(Continuation)

PLACE SURVEYED	Paiz-Montúfar Supermarket	La Placita Market	Residencial Supermarket	Norte Supermarket	La Torre Supermarket
PRODUCTS SOLD	Frozen fish	Solid, dried fish	Frozen fish	Frozen fish	Frozen fish
Ice	Used to wet surface, preservation relies on freezer	Not used (dried fish)	Not used	Not used	Not used
Relation Ice : Fish	Not important	Not applicable	Not applicable	Not applicable	Not applicable
Relation fish volume : chamber size in storage	They use closed cabinets and one room with refrig. equipment	Not applicable	Half full	More than half full	Half full
How are the fish arranged in the storage box	Piled according to kinds	Piled at random	Piled according to kinds	Piled according to kinds	Piled at random
Maximum storage time before selling	4 days	5 days	One week	One week	One week

TABLE 1-A

(Continuation)

PLACE SURVEYED	Paiz-Montúfar Supermarket	La Placita Market	Residencial Supermarket	Norte Supermarket	La Torre Supermarket
PRODUCTS SOLD	Frozen fish	Solid, dried fish	Frozen fish	Frozen fish	Frozen fish
Description of retail site	Freezing and chilling facilities, place seems adequate with sufficient light. No desinfection is applied, only cleaning with detergents	The place has no metal tables, only of wood. There are no refrigeration facilities. Light comes from windows but seems adequate. No desinfection. Cleaning is done rarely and poorly	All fish is inside the display freezer which seems to be clean but not desinfected. Light is adequate	---	---
Other observations	Personnel in charge has experience in handling fish and differentiate between genera of fish (kinds). Fish sold is mainly filleted either by commercial companies or at the supermarket. Whole fish is sold frozen	People in charge do not know well how to differentiate between kinds. In the season when the survey was done only dried fish was on sale	Almost all fish is processed by commercial fish companies. Once in a while frozen fish is filleted at the supermarket.	Almost all fish is processed by commercial fish companies. Once in a while frozen fish is filleted at the supermarket.	Filleted by commercial fish companies and some at the supermarket Several bags of fish were unicenti fied and contents were of unknown species.

TABLE 1-B

QUESTIONAIRE RESULTS FOR MARKETS SELLING FRESH FISH

PLACE SURVEYED	Colón	Terminal
How are the fish delivered to the market?	Wooden boxes with ice and sawdust; fish are bought at Terminal Market	Wooden boxes with ice and cane baskets with ice
How are the fish displayed?	On a wood bench, with small amount of ice. The bench is covered by plastic	On a cement bench covered with glazed tile with small amount of ice. However although the bench has drain, plastic is placed over it.
Washing of fish	All fish are washed with ice-water (melted ice)	Only when ice is dirty
Rejections	Occasionally, on basis of : texture, gill odor, cavity color	Occasionally, on basis of : texture, slime
Ice for storage	YES	YES
Relation Ice : Fish	They buy 75 lb of ice per each 50 lb of fish but it is not properly distributed with the fish	About 1 : 1 by volume. Ice is properly placed in layers over and under fish

TABLE 1-B

(Continuation)

PLACE SURVEYED	Colón	Terminal
is the storage chamber adequate in size?	Large enough for the amount stored	---
How is the fish placed in the chamber?	Inside plastic bags and pacaya leaves for insulation	Sandwiched between layers of ice
Storage time	Maximum 2 days	Maximum 3 days
Retail side conditions	Wood bench plastic covered without drain. No tap water at the site. Water is brought in from a faucet in small cans. No place to wash hands, fish or instruments. A large knife and nail brush to scale fish but seldom washed or desinfected. The place is dark with not enough room. Table for cutting and scaling fish is not clean and is made of wood. Weighing scale is also not clean. Flies are abundant.	They have running water (chlorinated) but use a large tub where they wash instruments, knife and nail brush. Table where seafood is cut and scaled is not clean and is made of wood. Flies are abundant
Observations	People in charge have years of experience and differentiate among different kinds of fish. Almost all merchandise is fresh fish.	Sale of various kinds of seafood besides fresh fish, turtle eggs, sea water and fresh water, shrimp, crabs and molluscs. Prices among the week of survey were fluctuating because of a nation wide strike by meat producers and distributors

TABLE 1-C

PROYECTO PESCA ARTESANAL

CONVENIO URI - CITA

CUESTIONARIO

TERMINAL

I) PESCADOR:

1. Nombre
2. Fecha
3. Familia (N° miembros)
4. Zona de captura
5. Hora de pesca
6. Lugar en que vive
7. Tipo de arte
7a. Especie
8. Lava usted la caja en que almacena el pescado SI NO
9. Cada cuánto?
10. Temperatura del agua
11. Humedad ambiental
12. Profundidad del pescado en la caja
13. Peso estimado de la captura
14. Peso real de la captura

TABLE 1-C

(Continuation)

		SI	NO
15.	Usa hielo		
16.	Cantidad de hielo		
17.	Capas de pescado por capa de hielo		
18.	Tiempo de eviscerado después de la captura		
19.	Estimado de vísceras rotas en la cavidad abdominal		
20.	Peso promedio del pescado		
21.	Forma de lavado del pescado		
22.	Cantidad de especies en la captura		
23.	Especies predominantes		
24.	Tiempo que tarda en regresar a puerto		
25.	Si el pescador no entrega diario el pescado en el puesto de compra, pasar a N° 39		
26.	Forma de almacenamiento de la captura		
	en el bote		
	en nevera		
	en congelador		
	en cajas		
	en bolsas plásticas		
	otros.		

TABLE 1-C

(Continuation)

26a.	Cubierto o descubierto		
27.	Con hielo	SI	NO
28.	¿Qué cantidad?		
29.	Temperatura del medio de almacenamiento		
30.	Humedad del medio de almacenamiento		
31.	¿Consumen parte de este pescado?	SI	NO
32.	¿Con qué frecuencia?		
33.	¿Lava el recipiente de almacenamiento?	SI	NO
34.	¿Cada cuánto?		
35.	Tiempo que tarda el pescado almacenado en este sitio		
36.	¿Se suministra hielo durante éste tiempo?	SI	NO
37.	Profundidad del pescado en el recipiente de almacenamiento		
38.	Peso total en el recipiente		

NOTA: en observaciones se especificarán otros detalles.

39. Tiempo transcurrido en llegar al puesto de compra desde el momento de la captura

40. Manejo del pescado del bote al puesto:

Cajas
Canastas plásticas
Canastas de madera
Bolsas plásticas
Otros

TABLE 1-C

(Continuation)

41. Colocación del pescado en el puesto antes de vender

    Piso
    Mesa
    Tabla
    Balanza
    Nevera

42. Lavado del pescado SI NO

43. Rechazo de pescado SI NO

44. Indice de calidad para el rechazo

45. Forma de almacenamiento

46. Con hielo SI NO

47. Distribución hielo-pescado

48. Tamaño de cámara con respecto a la cantidad de pescado

49. Posición del pescado en la cámara

50. Tiempo de almacenamiento

51. Forma de transporte al mercado

52. Con hielo SI NO

53. Tiempo de transporte

54. Condiciones de manejo antes de entrega al puesto de venta consumidor

55. Condiciones del puesto de venta

TABLE 1-C

(Continuation)

56. Tiempo de permanencia en el puesto antes de ser vendido

57. Diagrama de flujo

58. Observaciones generales

TABLE 2

SAMPLE CODE FOR SUPERMARKET SAMPLES AND PRICES

IN US $ PER KILOGRAM

PLACE	CODE	IDENTIFICATION	US$/Kilogram
Paiz-Montúfar Supermarket	PA	Curvina fillets	3.19
	PB	Roballo slices	3.30
	PC	Dried roballo	2.84
	PD	Whole frozen curvina	1.54
	PE	Ice	
La Placita Market	MPA	Dried roballo	3.30
	MPB	Dried juilín	1.76
	MPC	Dried codfish	2.75
	MPD	Table surface	
	MPE	Counter surface	
Residencial Supermarket	RA	Curvina fillet	3.30
	RB	Whole frozen salmonete	0.55
	RC	Pristis perotetti (frozen)	2.86
	RD	Front wall of freezer	
	RE	Back wall of freezer	
Norte Supermarket	NA	Whole salmonete	0.46
	NB	Curvina fillet	3.52
	NC	Pristis perotetti fillets	2.33
	ND	Side wall of freezer	
	NE	Back wall of freezer	
La Torre Supermarket	LTA	Pristis perotetti fillets	2.49
	LTB	Curvina fillets	2.42
	LTC	Curvina fillets	2.42
	LTD	Curvina fillets	2.42
	LTE	Unidentified fish fillets	1.36

TABLE 3

MICROBIAL COUNTS OF SUPERMARKET SAMPLES

SAMPLE	Aerobic Plate Count at 25° C Colonies/gram	Faecal Coliforms MPN / gram	Staphylococcus aureus Coagulase positive
PA	9.8×10^5	23	0
PB	2.7×10^5	23	0
PC	1.5×10^4	0	0
PD	3.7×10^6	7.3	10^3
PE	1.6×10^5/ml	0/ml	10/ml
MPA	2.3×10^6	3.6	0
MPB	2.4×10^6	0	0
MPC	1.2×10^7	0	10
MPD	$5.0 \times 10^4/cm^2$	0	$6.6/cm^2$
MPE	$1.6 \times 10^2/cm^2$	0	0
RA	6.3×10^6	7.3	10^2
RB	2.6×10^4	0	10^2
RC	6.9×10^6	460	10^3
RD	$8 \times 10^6/cm^2$	0	0
RE	$1.3 \times 10^1/cm^2$	0	0
NA	1.3×10^7	0	0
NB	3.4×10^5	7.3	10^2
NC	5.3×10^7	460	10^2
ND	$0 / cm^2$	0	0
NE	$0 / cm^2$	0	0
LTA	5.3×10^7	0	10^1
LTB	1.4×10^7	150	10^2
LTC	1.4×10^7	150	0
LTD	6.6×10^5	23	10^2
LTE	3.5×10^6	23	0

TABLE 4

AEROBIC PLATE COUNT AT 25°C PER cm^2 OR PER ml*

(Colón Market)

	Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
A	Juilin skin surface	3.8×10^4	7.3×10^5	6.4×10^5			
B	Juilin cavity surface	1.6×10^4	1×10^6	1.9×10^6			
C	Palometa skin surface	4.5×10^5	2.6×10^6	1×10^6			
D	Palometa cavity surface	4.6×10^4	8×10^5	7.3×10^5			
E	Pepemechin skin surface	1.9×10^5	1.3×10^6	8.6×10^5			
F	Pepemechin cavity surface	8.6×10^4	8×10^5	1.8×10^6			
G	Floor surface	1.8×10^6	2.4×10^6	1.5×10^6	3.2×10^6	3.8×10^6	3.5×10^6
H	Table surface	1.4×10^6	1.6×10^6	2.8×10^6	1.9×10^6	3.9×10^6	6.6×10^5
I	Water*	$< 10^3$	8×10^4	$< 3.0 \times 10^1$	7.0×10^1	7.0×10^5	1.9×10^4
J	Ice*	2.7×10^6	3.4×10^6	1.3×10^7	4.7×10^6	4.3×10^6	1.9×10^7
K	Fish-ice drippings*	3.6×10^7	1.1×10^8	7×10^7	9.3×10^7	2.5×10^8	5×10^7

TABLE 5

FAECAL COLIFORMS COUNT AS MPN PER cm^2 OR PER ml*
(Colón Market)

	Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
A	Juilin skin surface	3	15	0			
B	Juilin cavity surface	10	160	60			
C	Palometa skin surface	1.5	30	0			
D	Palometa cavity surface	3	0	0			
E	Pepemechin skin surface	1	15	150			
F	Pepemechin cavity surface	.73	4.8	150			
G	Floor surface	73	310	60	60	2.4	24
H	Table surface	31	73	0	24	6.0	0
I	Water*	0	930	0	0	390	0
J	Ice*	1 100	150	360	0	430	730
K	Fish-ice drippings*	> 2 400	2 400	4 300	9 300	> 24 000	4 300

TABLE 6

STAPHYLOCOCCUS AUREUS CELL COUNT PER cm^2 OR PER ml*
(Colón Market)

	Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
A	Juilin skin surface	0	70	7			
B	Juilin cavity surface	0	0	0			
C	Palometa skin surface	0	0	0			
D	Palometa cavity surface	0	1	0			
E	Pepemechin skin surface	0	0	0			
F	Pepemechin cavity surface	0	1.	0			
G	Floor surface	0	70	0	0	70	700
H	Table surface	0	1	700	7	0	70
I	Water*	0	0	0	0	0	0
J	Ice*	0	10^3	10^2	10^2	0	10^2
K	Fish-ice drippings*	0	0	0	10^3	10^4	10^3

TABLE 7

AEROBIC PLATE COUNT AT 25°C PER cm^2 OR PER ml*
(La Terminal Market)

	Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
A	Roballo skin surface	2.4×10^6	5.2×10^6	1.13×10^7	1.2×10^7		
B	Roballo cavity surface	2.5×10^7	8.6×10^6	1.5×10^7	2.0×10^7		
C	Mojarra skin surface	1.6×10^6	5.6×10^6	3.5×10^6	1.9×10^7		
D	Guapote skin surface	1.2×10^6	6.6×10^6	1.3×10^7	1.6×10^7		
E	Floor surface	2.2×10^7	1.1×10^6	2.4×10^6	5.6×10^6	1.1×10^6	1.4×10^6
F	Table surface	2.4×10^7	2.4×10^6	5.5×10^6	1.4×10^7	8.0×10^6	1.0×10^6
G	Ice*	4.1×10^7	3.1×10^7	3.3×10^7	1.0×10^8	1.3×10^7	8.4×10^7
H	Water*	2.0×10^1	4.0×10^1	2.5×10^1	5	0	0
I	Fish-ice drippings*	2.0×10^7	4.0×10^8	4.3×10^8	3.9×10^8	1.6×10^9	2.0×10^9

TABLE 8

FAECAL COLIFORMS COUNT IN MPN PER cm^2 OR PER ml*

(La Terminal Market)

	Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
A	Roballo skin surface	31	160	31	62		
B	Roballo cavity surface	31	31	6.2	15.3		
C	Mojarra skin surface	73	31	14	160		
D	Guapote skin surface	>160	>160	73	62		
E	Floor surface	>160	31	31	160	6	4.6
F	Table surface	>160	8	>160	733	2.6	15.3
G	Ice*	> 2 400	240	1 100	930	230	2 400
H	Water*	0	0	0	0	0	0
I	Fish-ice drippings*	> 2 400	2 100	11 000	1 500	> 24 000	> 24 000

TABLE 9

STAPHYLOCOCCUS AUREUS CELLS COUNT PER SQUARE CENTIMETER OR PER ML*

(La Terminal Market)

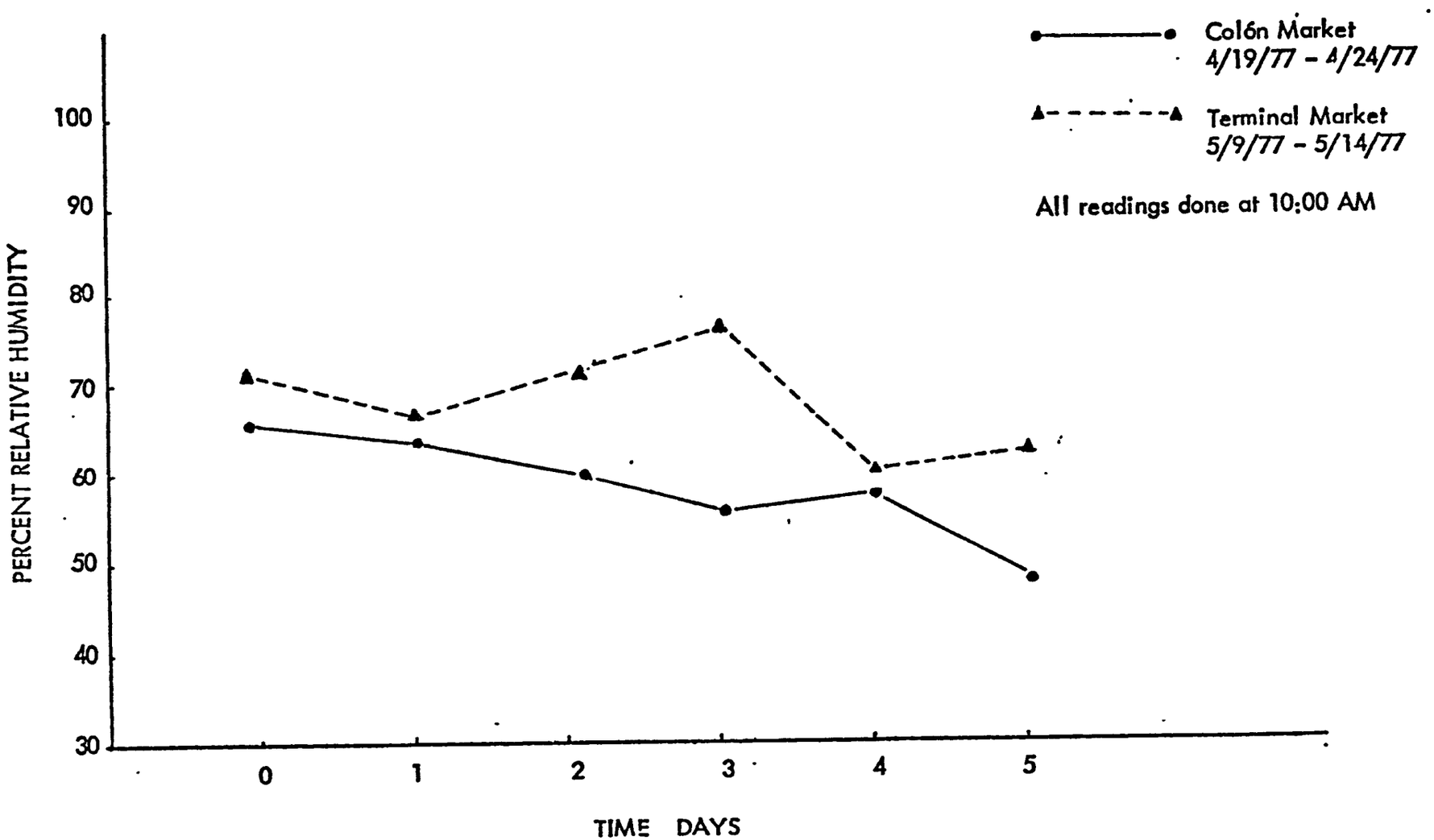
	Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
A	Roballo skin surface	0	0	0.6	0		
B	Roballo cavity surface	0	0	0	10^1		
C	Mojarra skin surface	0	0	0	0		
D	Guapote skin surface	-	0	6.6×10^1	0		
E	Floor surface	0	0	0	0	0	10^1
F	Table surface	0	0	0.6	0	0	10^3
G	Ice*	0	0	10^1	10^1	10^1	10^2
H	Water*	0	0	0	0	0	0
I	Fish-ice drippings*	0	0	10^2	10^4	0	10^2

7. Graphs

GRAPH 1

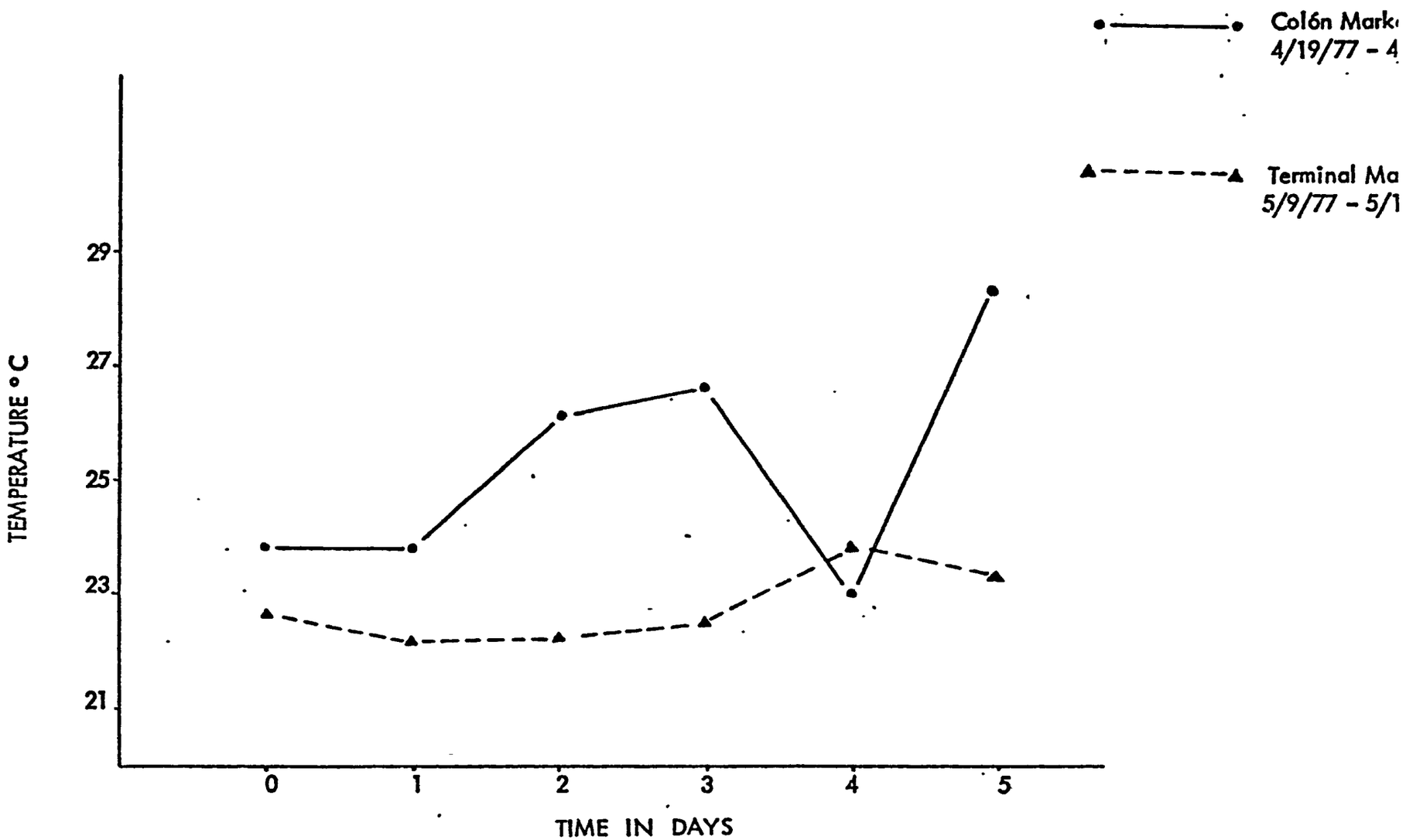
% OF RELATIVE HUMIDITY AT THE POPULAR MARKETS DURING

THE SIX DAY STUDY PERIOD


Colón Market
4/19/77 - 4/24/77
Terminal Market
5/9/77 - 5/14/77
All readings done at 10:00 AM
PERCENT RELATIVE HUMIDITY
100
90
80
70
60
50
40
30
0
1
2
3
4
5
TIME DAYS

GRAPH 2

TEMPERATURE AT MARKETS


Colón Mark
4/19/77 - 4
Terminal Ma
5/9/77 - 5/1
TEMPERATURE °C
29
27
25
23
21
0
1
2
3
4
5
TIME IN DAYS

8. Methods

countries and almost exclusively so in North America, where it has been specifically recommended for dairy products (APHA, 1960) and for frozen foods (APHA, 1966; AFDOUS, 1966); it is used in most public health and food control agencies in the United States and Canada. A modification of the method, used for some years in France and regions influenced by French technology, has been adopted by the International Dairy Federation (1958) for determination of aerobic mesophiles in liquid and dried milk. In the method, milk is added to Standard Methods Agar (Medium 77, Part III) and the poured plates are incubated at 30° ± 1° C for 3 days. The Drop Plate method is used by some laboratories in the United Kingdom. The Surface Plate method is used widely in Europe and may soon be officially recommended by the International Standards Organization for use with meat and meat products containing more than 3000 organisms per gram (Barraud *et al.*, 1967).

The standard Pour Plate procedure is so well established, and its accuracy and limitations so well understood, that the Committee recommends its retention, for purposes of arbitration. Nevertheless, one of the other methods may be chosen later, if warranted by comparison with the Pour Plate method under sufficiently diverse circumstances. The Committee will undertake international testing to compare the relative accuracy of different plating procedures.

Method 1[4] *(The Pour Plate or Standard Plate Count Method)*

I. Apparatus and materials

1 Requirements for Preparation and Dilution of the Food Homogenate, Method 1 or 2, above.

2 Petri dishes, glass (100 × 15 mm) or plastic (90 × 15 mm).

3 1-, 5-, and 10-ml bacteriological pipettes (milk dilution specifications; APHA, 1960).

4 Water bath or air incubator for tempering agar, 45° ± 1° C.

5 Incubator, 35° ± 1° C. (*Note* The temperature constancy and uniformity of air incubators are notoriously bad. To check constancy, continuous or frequent interior temperature readings should be made over a period of several hours by suitable thermocouples or thermometers. To check uniformity, similar readings over a period of several hours should be made at various positions in the incubator while it is filled with agar plates (see also APHA, 1960).)

4/The method follows that recommended for dairy products (APHA, 1960) and for frozen foods (Lewis and Angelotti, 1964; AFDOUS, 1966).

6 Colony counter (Quebec dark field model or equivalent recommended).

7 Tally register.

8 Standard Methods Agar (Plate Count Agar: Medium 77, Part III).

II. Procedure

1 Prepare food sample by one of the two procedures recommended in the section on Preparation and Dilution of the Food Homogenate (p. 60).

2 To duplicate sets of Petri dishes, pipette[5] 1 ml aliquots from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions, and a 0.1 ml aliquot from the 10^{-6} dilution to give 10^{-1} to 10^{-0} g of food per Petri dish. The above series is suggested in cases where the approximate range of bacterial numbers in the specimen is not known by experience. The level of dilution prepared and plated can be varied to fit the expected bacterial count.

3 Promptly pour into the Petri dishes 10–15 ml of Standard Methods Agar, melted and tempered to 45° ± 1° C.

4 Immediately mix aliquots with the agar medium by tilting and rotating the Petri dishes. A satisfactory sequence of steps is as follows: (*a*) tilt dish to and fro 5 times in one direction, (*b*) rotate it clockwise 5 times, (*c*) tilt it to and fro again 5 times in a direction at right angles to that used the first time, and (*d*) rotate it counter-clockwise 5 times.

5 As a sterility check, pour one or two plates with agar medium alone (control(s)).

6 After the agar has solidified, invert the Petri dishes and incubate them at 35° ± 1° C for 48 ± 3 hours.

7 Using the colony counter and tally register, count all colonies on plates containing 30–300 colonies.

8 Compute the number of mesophilic aerobes per gram of specimen.

Method 2[6] *(The Surface Plate Method)*

I. Apparatus and materials

1 The requirements for Method 1 above, I, items 1, 2, 4, 6, 7, and 8.

2 A drying cabinet or incubator for drying the surface of agar plates, preferably at 50° C (see step 9, p. 158, for note on drying plates).

5/Pipettes must not be used to deliver less than 10% of their volume.

6/This method is widely used, particularly in Europe, and as described, follows closely that being recommended by the International Standards Organization (Barraud *et al.*, 1967). The method is not recommended for samples in which the number of mesophilic organisms is likely to be lower than 3000 per g.

of gas after 24 or 48 hours of incubation is considered sufficient evidence of the presence of coliforms.

6 To determine the MPN proceed as follows. Determine the highest dilution in which all three tubes are positive for gas production and the next two higher dilutions. If this is not possible because none of the dilutions yielded three positive tubes or because further dilutions were not made beyond the one yielding three positive tubes, use the last three dilutions. Following the example described in Method 1 above, II, steps 6 and 9, and referring to Table 2, determine the MPN.

Method 3[13]

I. Apparatus and materials

1 The requirements for Method 2 above, I, items 1 through 3.

2 Brilliant-Green Lactose Bile Broth 2% (Medium 10, Part III), 10 ml volumes in 150 × 15 mm tubes containing inverted Durham fermentation tubes (75 × 10 mm).

3 Violet-Red Bile Agar (Medium 100, Part III) or Endo Agar (Medium 21, Part III), for plates.

II. Procedure

1 Prepare food samples by one of the two procedures recommended in the section on Preparation and Dilution of the Food Homogenate (p. 60). The techniques of dilution should be the same. Material remaining from dilution blanks employed in the determination of the plate count can be used.

2 Pipette 1 ml of each of the decimal dilutions of the food homogenate into each of three separate tubes of Brilliant-Green Lactose Bile Broth 2%.

3 Incubate tubes at 35–37° C for 24 and 48 hours.

4 After 24 hours, record tubes showing gas production.[14] Return tubes not displaying gas to incubator for an additional 24 hours.

5 After 48 hours, record tubes showing gas production.[14]

6 Select tubes for confirmation tests and MPN determination as described in Method 1 above, II, step 6.

7 Confirm that tubes of Brilliant-Green Bile Broth 2% selected in step

13/This method is used in numerous laboratories on the European continent.

14/If tests for faecal coliforms are to be made, select a number of gas-positive tubes and follow the procedure described in Method 2 of the section on Determination of Coliform Organisms of Faecal Origin below.

6 immediately above are positive for coliform organisms by streaking a loopful from each on the surfaces of a plate of either Violet-Red Bile Agar or Endo Agar. Incubate plates inverted at 35–37° C; examine Violet-Red Bile Agar plates after 24 hours and Endo Agar plates after 48 hours. The formation of dark red colonies with diameters greater than 0.5 mm on Violet-Red Bile Agar or the formation of red colonies surrounded by red haloes on Endo Agar confirms the presence of coliform organisms.

8 Record the number of tubes in each dilution that were confirmed as positive for coliform organisms.

9 Determine the MPN of coliforms as described in Method 1 above, II, steps 6 and 9.

DETERMINATION OF COLIFORM ORGANISMS OF FAECAL ORIGIN

As a rule, sufficient measurement of the sanitary quality of foods is accomplished by means of the coliform determination. In certain cases, however, it may be desirable to determine the probable origin of coliform bacteria isolated from food and to obtain an estimate of the proportion of the total coliform density representative of recent faecal contamination. The following procedures may be expected to differentiate between coliforms of faecal origin (intestines of warm-blooded animals) and coliforms from other sources, with reasonable accuracy.

Method 1[15]

I. Apparatus and materials

1 Inoculating needle, with a 3-mm-diameter loop, preferably of nichrome or platinum-iridium wire.

2 Agitated water bath with thermo-regulation capable of maintaining a temperature of 45.5° ± 0.2° C.

3 E.C. Broth (Medium 19, Part III), 10 ml volumes in 150 × 15 ml tubes containing inverted Durham fermentation tubes (75 × 10 mm).

II. Procedure

1 Select tubes of Lauryl Sulphate Tryptose Broth that are positive for gas production in Method 1 of the section on Enumeration of Coliforms above.

15/This method (modified Eijkman test; Eijkman, 1904) follows that recommended for water (APHA *et al.*, 1965) and foods (AFDOUS, 1966; Lewis and Angelotti, 1964).

2 Inoculate a loopful of broth from each of the selected cultures into a separate tube of E.C. Broth.

3 Incubate E.C. Broth tubes at 45.5° ± 0.2° C and read for gas production after 24 and 48 hours.

4 E.C. Broth tubes displaying gas production may be presumed to be positive for faecal coliform organisms.

Method 2[16]

I. Apparatus and materials

1 Inoculating needle with a 3-mm-diameter loop, preferably of nichrome or platinum-iridium wire.

2 Agitated water bath with thermo-regulation capable of maintaining a temperature of 44° ± 0.1° C.

3 5-ml bacteriological pipettes.

4 Brilliant-Green Lactose Bile Broth 2% (Medium 10, Part III), 10 ml volumes in 150 × 15 mm tubes containing inverted Durham fermentation tubes (75 × 10 mm).

5 Peptone Water (Medium 59, Part III), 10 ml volumes in 150 × 15 mm tubes.

6 Indole Reagent (Reagent 3, Part III).

II. Procedure

1 Select tubes of MacConkey Broth that are positive for gas production in Method 2 of the section on Enumeration of Coliforms above.

2 Inoculate a loopful of broth from each selected culture into a tube of Brilliant-Green Lactose Bile Broth 2% and a tube of Peptone Water.

3 Incubate tubes at 44° ± 0.1° C.

4 Read the Brilliant-Green Lactose Bile Broth 2% tubes for gas production after 24 and 48 hours of incubation.

5 After 24 hours of incubation, pipette aseptically a 5 ml portion of each Peptone Water tube to a separate test tube and add 0.2–0.3 ml of Indole Reagent. Shake the tubes and let them stand for 10 minutes. A dark red colour in the amyl alcohol surface layer constitutes a positive indole test; the original colour of the reagent represents a negative test. Cultures showing gas production in Brilliant-Green Lactose Bile Broth 2% and

16/This method is used widely in several European countries in conjunction with Method 2 of the section on Enumeration of Coliforms above. As described, it follows the procedure outlined by Mackenzie *et al.* (1948).

indole fermentation in Peptone Water may be presumed to be positive for faecal coliform organisms.

IDENTIFICATION TESTS FOR COLIFORM ORGANISMS: THE IMViC PATTERN[17]

In routine analyses of foods, further confirmation of the presence of *E coli* beyond that obtained from the tests described in Methods 1 and 2 o the section on Determination of Coliform Organisms of Faecal Origi above is not usually feasible because of the time and labour involved. I special instances, however, where the extra effort is merited, the differen tiation of the coliform group into species and varieties can be carried ou on the basis of the results of four tests (indole, methyl red, Voges-Pros kauer, and sodium citrate) referred to collectively as the "IMViC tests. A grouping of reaction combinations is presented in Table 3.

TABLE 3*

Differentiation of coliforms

(All of these organisms are capable of producing acid and gas from lactose in 48 hours at 37 °C)

	Gas in lactose bile-salt medium at 44–45.5° C	Indole test	Methyl red test	Voges-Proskauer test	Growth in citrate
Escherichia coli					
Type I (typical)	+	+	+	−	−
Type II	−	−	+	−	−
Intermediates					
Type I	−	−	+	−†	+
Type II	−	+	+	−†	+
Enterobacter‡ *aerogenes*					
Type I	−	−	−	+	+
Type II	−	+	−	+	+
Enterobacter cloacae	−	−	−	+	+
Irregular					
Type I	−	+	+	−	−
Type II	+	−	+	−	−
Type VI	+	−	−	+	+
Irregular, other types		Reactions variable			

*This table is similar to the one given in Ministry of Health ... (1956).

†Weak positive reactions are occasionally found.

‡The Judicial Commission of the International Committee on Nomenclature Bacteria of IAMS has officially substituted the term *Enterobacter* as the generic desig tion for the genus previously known as *Aerobacter* (Enterbacteriaceae Subcommit 1953).

17/The procedure for the IMViC tests follows closely those described in *Stand Methods for the Examination of Water and Wastewater* (APHA *et al.*, 1955).

5 A drying cabinet or incubator for drying the surfaces of agar plates.

6 Glass spreaders (hockey-stick-shaped glass rods, fire-polished). Approximate measurements: 3.5 mm diameter, 20 cm long, bent at a 90° angle 3 cm from one end.

7 Baird-Parker Agar (Medium 1, Part III), for plates.

II. Procedure

1 Prepare food samples by one of the two methods described in the section on Preparation and Dilution of the Food Homogenate (p. 60), using the same technique and extent of dilution.

2 Pour plates of Baird-Parker Agar (15 ml in each) and dry surfaces in drying cabinet or incubator (see item 9, p. 158, for note on drying plates).

3 Pipette 0.1 ml of homogenate and dilutions of homogenate onto the surfaces of separate plates and spread each portion with a sterile bent glass rod; in each case continue spreading until surface appears dry. Duplicate plates should be prepared for each dilution.

4 Incubate plates inverted at 37° ± 1° C for 24 and 48 hours.

5 After 24 hours of incubation, select plates possessing 30–300 separate colonies and count all colonies which are black and shiny with narrow white margins, and surrounded by clear zones extending into the opaque medium. These are colonies of *Staphylococcus aureus*.

6 Mark the position of these colonies and incubate plates for a further 24 hours.

7 Count all colonies with the above appearance that developed during the extended period of incubation and submit these, or a significant number of them (square root of count) if the count is high, to a coagulase test procedure (see section on Testing for Coagulase Production below, following Method 5).

8 With some batches of egg yolk, colonies of a few strains of *Staph. aureus* may be surrounded by an opaque zone at 24 hours, and a larger number of strains may show this appearance after 48 hours. Count these after 48 hours and submit them, or a suitable number of them (square root of the count) if there are many, to a coagulase test. This will distinguish these cultures (coagulase-positive) from *Staph. epidermidis* (coagulase-negative) which may give a similar appearance.

9 Total the colonies which produced clear zones after 24 hours of incubation, and those in steps 7 and 8 above which were coagulase-positive, and calculate from the dilutions used the total number of coagulase-positive staphylococci.

Note Over 90% of all coagulase-positive staphylococci show the characteristic black colony surrounded by a clear zone after incubation for 24–26 hours at 37° C; it is important to read results at this time and *not* at times shorter than 24 hours. A further 5–7% of coagulase-positive staphylococci show this characteristic clearing, often with an inner opaque zone, after 48 hours, at which time coagulase-negative staphylococci may also show this appearance; it is for this reason that the coagulase test should be made on suspect colonies appearing at 48 hours.

★ *Method 2*[26]

I. Apparatus and materials

1 Requirements for Method 1 above, items 1 through 5.

2 Inoculating needle with nichrome or platinum-iridium wire.

3 Vogel-Johnson Agar (Medium 102, Part III), for plates.

4 Trypticase Soy Broth with 10% Sodium Chloride (Medium 96, Part III), 5–10 ml volumes in tubes.

II. Procedure

1 Prepare food samples by one of the two procedures in the section on Preparation and Dilution of the Food Homogenate (p. 60), using the same technique and extent of dilution. In cases where food samples contain very high numbers of staphylococci, dilutions higher than 10^{-3} may be needed, since this method depends on at least one dilution giving negative results.

2 Inoculate single tubes of Trypticase Soy Broth (10% sodium chloride) with 1 ml aliquots of decimal dilutions of the food homogenate. The maximum dilution of sample must be sufficiently great to yield negative results for at least one dilution.

3 Incubate the tubes at 35–37° C for 48 hours.

4 Using a 3 mm loop, transfer 1 loopful from each inoculated tube to a separate plate of Vogel-Johnson Agar and streak in such a manner as to give isolated colonies.

5 Incubate plates at 35–37° C for 48 ± 2 hours.

6 Select at least one of each visibly different colony type, which has reduced tellurite, from all sample dilutions tested, and test these for

26/Recommended for enumeration of coagulase-positive staphylococci in foods by the U.S. Food and Drug Administration (Baer, 1966).

coagulase production (for procedure, see section on Testing for Coagulase Production below, following Method 5).

7 From highest dilution containing coagulase-positive staphylococci, estimate the number in the original specimen.

Method 3[27]

I. Apparatus and materials

1 Requirements for Method 1, above, items 1 through 6.

2 Egg-Yolk Azide Agar (Medium 20, Part III), for plates.

II. Procedure

1 Prepare food samples by one of the two methods described in the section on Preparation and Dilution of the Food Homogenate (p. 60), using same technique and extent of dilution.

2 Pour plates of Egg-Yolk Azide Agar and dry surfaces in drying cabinet or incubator (see item 9, p. 158, for note on drying plates).

3 Pipette 0.2 ml of homogenate and dilutions of homogenate onto the surfaces of separate plates and spread each portion with a sterile bent glass rod; in each case continue spreading until surface appears dry. Duplicate plates should be used for each dilution.

4 Incubate plates inverted at 37° ± 1° C for 24 and 48 hours. After 24 hours, clear areas (haloes) containing little or no granular precipitate surround colonies of staphylococci. The surface of the halo has an oily appearance due to Newton ring phenomena following the liberation of free fatty acid by a lipase esterase action of the staphylococci. After 48 hours the haloes are larger and contain a white granular precipitate (calcium salts of fatty acids). If the colony is yellow, owing to pigmentation, the whole picture resembles a fried egg.

5 Count all colonies having the above characteristics, on plates possessing between 30 and 300 colonies.

6 Test these colonies, or a significant number of them (square root of the count) if the count is high, for coagulase production (for procedure, see section on Testing for Coagulase Production below, following Method 5).

7 From the proportion which is coagulase-positive and the dilution, calculate the total count in the specimen and report as coagulase-positive staphylococci.

27/Used in a number of laboratories in Europe, particularly in the Scandinavian countries (Lundbeck and Tirunarayanan, 1966).

Notes The correlation between the lysis precipitate and coagulase production is currently being studied (H. Lundbeck, personal communication). Although most of the coagulase-positive staphylococci produce the lipase-esterase enzymes, a few coagulase-negative strains also demonstrate the lytic-precipitation phenomenon.

Sodium azide at the concentrations used does not inhibit staphylococci but prevents growth of all Gram-negative organisms except some strains of *Proteus* and *Pseudomonas*.

Method 4[28]

This method includes procedures for direct plating and enrichment culturing.

I. Apparatus and materials

1 Requirements for Method 1 above, items 1 through 6.

2 Milk Salt Agar (Medium 43, Part III), for plates.

3 Salt Egg-Yolk Agar (Medium 70, Part III), for plates.

4 Blood Agar (Medium 6, Formula 2, Part III), for plates.

5 Salt Broth 6.5% (Medium 67, Part III), 7–10 ml volumes in tubes.

6 Salt Broth 10% (Medium 68, Part III), 7–10 ml volumes in tubes.

7 Glucose Broth (Medium 27, Part III), 7–10 ml volumes in tubes.

8 Nutrient Agar (Medium 46, Part III), slants in tubes.

9 Materials for staining smears by Gram's method (Reagent 1, Part III).

II. Procedures

A. DIRECT PLATING

1 Prepare food samples by one of the two procedures described in the section on Preparation and Dilution of the Food Homogenate (p. 60), using the same technique and extent of dilution.

2 When estimating the total number of cells, pipette 0.1 ml of food homogenate and dilutions of homogenate onto the surface of separate, previously prepared and dried plates of Milk Salt Agar. Spread each portion with a sterile bent glass rod or inoculating needle over entire surface of plate, continuing the spreading action until surface appears dry.

28/This method is used throughout the Soviet Union (Meshalova and Mikhailova, 1964) for examination of foods for coagulase-positive staphylococci and in epidemiological studies.