Salmonella Isolated in Florida During 1943 with the Combined Enrichment Method of Kauffmann

MILDRED M. GALTON AND MUN S. QUAN

Bacteriologists, Bureau of Laboratories, Florida State Board of Health, Jacksonville, Fla.

NUMEROUS enrichment methods for fecal bacteriology have been described in the literature, and we shall not attempt to enumerate them. It is well known that a suitable selective enrichment is essential for the isolation of enteric pathogens from feces.

A tetrathionate broth for enrichment of typhoid and paratyphoid bacilli was first used successfully in 1923 by Mueller,[1] who showed that it inhibited coliform organisms and allowed typhoid and paratyphoid bacilli to grow profusely. Kauffmann, 1930,[2] described the combined enrichment method for *Salmonella,* which consisted of the original tetrathionate broth of Mueller plus 5 per cent bile and brilliant green 1:100,000 used in combination with Kauffmann's modification of the brilliant green-phenol red agar of Kristensen, Lester, and Jurgens.[3] In 1935, Kauffmann,[4] reported the results of 10,182 feces examinations made by this method in which an increase in *Salmonella* isolations of 700 per cent over previous findings was obtained. In typhoid isolations an increase of 30–40 per cent was noted, and in dysentery isolations an increase of 10–20 per cent. As a result of his experiences he recommends for the examination of pathogenic intestinal bacteria, in addition to the direct streak method, the routine use of the combined enrichment method.

Extensive investigations with this method have been reported by many European workers,[5] by Hormaeche and Peluffo [6] in Montevideo, by Varela [7] in Mexico, and by others with equally favorable results. It was highly recommended to us by Dr. P. R. Edwards, Kentucky Agricultural Experiment Station in Lexington, Ky., who has also confirmed its efficacy. Since it has not, to our knowledge, been used routinely in public health laboratories in this country, the following results are reported to confirm its usefulness.

From March 15, 1943, to January 1, 1944, 8,093 feces specimens have been examined in the central laboratory of the Florida State Board of Health at Jacksonville by the combined enrichment method of Kauffmann, and by direct streaking. A large percentage of these specimens were received from normal food handlers, the remainder were from suspected cases and contacts of enteric infections.

PROCEDURE

Specimens were received in 1 oz. screw-capped bottles containing 10 to 15 ml. of glycerine-saline preservative. A portion of the specimen was streaked directly onto a Wilson-Blair bismuth sulfite agar (WB) plate and an SS agar (Difco) plate. About ½ ml. was placed in tetrathionate-brilliant green

bile enrichment broth. After 16–18 hours' incubation at 37° C. a large loopful of the tetrathionate broth was streaked to a brilliant green agar (BG) plate and also to an SS agar plate as the BG is not favorable for the growth of *Shigella*. Typical or suspicious colonies were picked to Kligler's iron agar (Difco) with 1 per cent sucrose added, and the procedure for identification already described by Galton and A. L. Quan [8] was followed.

The tetrathionate broth and brilliant green agar were prepared according to the formulae of Kauffmann [4] as follows:

Tetrathionate broth—Enrichment medium

A. 90 ml. sterile infusion broth—pH 7.4
5 gm. sterile $CaCo_3$ (precipitated chalk)
10 ml. sterile 50% solution $Na_2S_2O_3$
Sterilize in autoclave
Add 2 ml. unheated solution I + KI
(20 gm. I + 25 gm. KI + 100 ml. H_2O)

B. To 100 ml. of tetrathionate broth add 1 ml. brilliant green solution (1 to 1,000)

C. Sterile beef bile 5%
(5% of a 10% solution of dehydrated oxgall, Difco, was used as fresh bile was difficult to obtain)

The $CaCO_3$ is evenly suspended by continued shaking of the mixtures.

The medium is dispensed into sterile tubes with a sterile pipette in 7–8 ml. amounts. It will keep for some time and is not further heated. We usually prepare 2 or 3 liters at one time.

Brilliant Green Agar (Kristensen, Lester, and Jurgens, modified by Kauffmann)

	Per cent	
Meat extract	0.5	
Peptone (Difco)	1.0	
NaCl	0.5	
Lactose	1.0	
Sucrose	1.0	
Phenol red solution	4.0	(40 ml. N/10 NaOH + 460 ml. H_2O + 1 gm. phenol red)
Brilliant green solution (0.5%)	2.5 ml. per liter	
Agar	2 %	

Sterilize in autoclave. Reaction should be pH 7.2 − 7.4.

We have found it satisfactory to pour a sufficient number of brilliant green agar plates for 2 or 3 days if they are kept in the refrigerator until used.

RESULTS

The number and per cent of *Salmonella*, typhoid and dysentery isolations obtained by the combined enrichment method and by direct streaking are shown in Table 1.

For several years, prior to the time we adopted the combined enrichment method, Selenite F enrichment, together with direct streaking to SS or DC and WB plates, was used routinely in our laboratory. The results with this method during 1942 and until March 15, 1943, are shown in Table 2 for comparison. Due to the decided decrease in the percentage of positive typhoid cultures with the combined enrichment method, we have given the results obtained with Selenite F in 1941 in Table 3, to show the much greater decrease that occurred in positive typhoid cultures before the change in methods was made. We may also add that the percentage of typhoid isolations in our blood-clot cultures has decreased from 2.07 per cent in 1941 to 0.007 per cent in 1943. The majority of our positive typhoid feces cultures come at present from repeated check specimens on the 18 to 20 known carriers in the state. In spite of this fact, Selenite F was superior to the combined method for the enrichment of *Eberthella typhosa*. We believe better results could be obtained if the tetrathionate broth were also inoculated onto WB agar, as this is, apparently, the most favorable plating medium for the typhoid bacillus.

It may be seen from the results of these examinations that our percentage of positive dysentery isolations has remained almost constant during the 3 year period. We have not, as yet, found the tetrathionate-brilliant green bile broth particularly conducive to the

TABLE 1

Positive Results from the Examination of 8,093 Feces Specimens by the Direct Streak Method and Combined Enrichment Method of Kauffmann

(1943)

	Direct Streak		Tetrathionate Broth			
Isolations	*SS*	*WB*	*SS*	*BG*	*Total Positive*	*Per cent Positive*
Salmonella	17	42	74	85	107	1.32
Typhoid	36	76	42	8	79	0.97
Dysentery	77	1	10	1	84	1.03

TABLE 2

Positive Results from the Examination of 3,888 Feces Specimens by the Direct Streak Method and Selenite F Enrichment

(1942–1943)

	Direct Streak		Selenite F		
Isolations	*SS or DC*	*WB*	*DC*	*Total Positive*	*Per cent Positive*
Salmonella	9	11	17	22	0.5
Typhoid	49	87	91	110	2.82
Dysentery	50	8	12	54	1.38

TABLE 3

Positive Results from the Examination of 2,096 Feces Specimens by the Direct Streak Method and Selenite F Enrichment

(1941)

	Direct Streak		Selenite F		
Isolations	*SS or DC*	*WB*	*DC*	*Total Positive*	*Per cent Positive*
Salmonella	5	4	12	14	0.6
Typhoid	46	78	123	141	6.7
Dysentery	40	0	15	40	1.8

growth of *Shigella*. Kauffmann, however, recommends inoculating the tetrathionate broth to plates after 5 and 16 hours' incubation for best results with *Shigella*. In our work we have been able to streak only after 16 hours' incubation, which probably accounts for our failure.

The results in these tables clearly demonstrate that with the combined method (Table 1), our *Salmonella* isolations have more than doubled those obtained through Selenite F enrichment. It is also significant that 20 cultures were isolated from the brilliant green agar alone. From our experience this method is far superior to Selenite F and direct streak methods for the isolation of *Salmonella*. Although the tetrathionate broth is rather time consuming to prepare, it is well worth the effort. Coliforms are inhibited to such an extent that frequently no growth at all appears on the BG and SS agar plates in negative cultures. *Salmonella* grow readily on BG agar. The colonies are pink or red (when appearing in pure culture), circular, convex and transparent, with even borders. *Pseudomonas aeruginosa* also develops well on this medium, but the colonies can be recognized usually by their flatness, irregular borders and purplish red color. *Alcaligenes* produces red colonies which are raised and slightly opaque. The lactose and sucrose fermenting organisms which grow on BG agar produce opaque, raised colonies, yellowish green in color. We have observed comparatively few BG agar plates on which

pink colonies were found to be slow lactose or sucrose fermenters instead of *Salmonella,* thus the picking of colonies is reduced to a minimum.

Since our results with the combined method were so favorable we began using it in our Miami branch laboratory, late last year. Reports from W. H. Miller help to confirm our opinion of its value. In 2½ months 324 feces specimens were examined and 10 *Salmonella* isolated. During the first 9½ months of 1943, 472 specimens were examined with the Selenite F enrichment broth and no *Salmonella* were found.

TYPES

During the 9½ months that we have used the combined method, 107 strains of 27 types of *Salmonella* have been isolated from 89 persons, both normal carriers and cases, as shown in Table 4.

It is notable that more cultures of *S. meleagridis* were isolated than of any other type. Strangely enough, 12 of the 14 cultures of this species were isolated from food handlers in the same community over a period of 6 weeks. It was found that the persons harboring this unusual type were not ill and that they were employed in the same canning establishment. An investigation by the county health officer has failed to reveal the source of infection. Of the other two cultures, one was obtained from a 5 year old child with enteritis, and one from a food handler. These persons lived in different sections of the state.

One new *Salmonella* type was found, which has been described by Edwards.[9]

TABLE 4

Types Isolated from Feces of 89 Patients

Types of Salmonella	*Group*	*Number Isolated*	*Case*	*Normal*		*History Unknown*
				Non-F.H. *	*F.H.*	
bredeney	A-B	4	..	..	3	1
paratyphi B	"	1	..	..	1	..
paratyphi var. Java	"	3	1 [b]	2 [a]	..	..
derby	"	5	1	..	3	1
san diego	"	2	1	..	1	..
typhimurium	"	5	2	..	1	1
typhimurium var. copenhagen	"	2	2 [c]	..	..	..
bareilly	C	3	..	1	2	..
hartford	"	1	1	..	..	..
litchfield	"	2	..	..	2	..
montevideo	"	8	2	1	3	..
newport	"	9	3	..	3	..
oranienburg	"	11	4 [d]	1	3	1
oregon	"	4	..	..	3	..
sendai	D	4	2	..	1	..
anatum	E	10	2	1	6	1
give	"	5	2	..	2	..
meleagridis	"	14	1	..	12 [e]	..
newington	"	4	1 [f]	..	1	..
senftenberg	"	1	..	..	1	..
carrau	Further groups	1	..	..	1	..
coli	" "	1	..	1	..	..
florida	" "	2	..	..	2	..
gaminara	" "	1	..	..	..	1
inverness	" "	1	..	..	1	..
madelia	" "	2	..	..	2	..
rubislaw	" "	1	..	..	..	1

* F.H.=food handler

[a] One culture isolated from feces of a normal spider monkey.

[b] *S. coli* was isolated from this patient 2 months later. Subsequent specimens failed to reveal either type.

[c] Isolated from feces of two sick pigeons.

[d] *E. typhosa* also isolated from the same specimen in one case which proved fatal.

[e] Flexner dysentery bacilli isolated from same specimen of one food handler.

[f] Sonne dysentery bacilli isolated from patient 1 month later. Neither organism was found in subsequent specimens.

He has given it the name of *S. inverness*.

The occurrence of more than one type in the same person was noted once. *S. anatum* and *S. bredeney* were isolated from an apparently normal Negress, a food handler, 27 years old. Mixed infections were encountered twice. *Salmonella oranienburg* and *Eberthella typhosa* were isolated simultaneously from a fatal infection. *S. meleagridis* and *Shigella dysenteriae* (Flexner) were obtained from the same feces specimen of a normal food handler, whose history failed to reveal previous enteric disturbance.

Approximately two-thirds of the *Salmonella* isolations were obtained from normal persons, 54 being food handlers and 6 not food handlers. A detailed questionnaire was sent to the physician in charge of each person from whom specimens were obtained, when each culture was isolated. Data have been received regarding 46 of the 60 normal carriers. The information so obtained has not revealed a single instance in which an intestinal disturbance was noted prior to isolating a pathogenic organism. This indicates that the occurrence of *Salmonella* in the the stools of apparently healthy persons is not uncommon in this region.

CONCLUSIONS

In our hands, the combined enrichment method of Kauffmann has proved to be excellent for the isolation of *Salmonella* from routine feces specimens. We attribute our 164 per cent increase in positive *Salmonella* finding to the efficacy of this medium.

It did not prove as favorable for the isolation of typhoid and *Shigella*. However, with the inclusion of the following measures in our present method, more satisfactory results could, no doubt, be obtained: (1) Inoculation of tetrathionate broth after 16 hours' incubation to a WB agar plate. (2) Inoculation of tetrathionate broth after 5 hours' incubation to an SS agar plate.

We have continued to find in Florida, a wide variety of *Salmonella* types, including all of the antigenic groups. It is possible that this occurrence is due in part to the large numbers of transient persons continually moving into and out of the state from many points of the globe.

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