APPLIED MICROBIOLOGY, March, 1965 Vol. 13. No. 2
 Printed in U.S.A.

Comparative Evaluation of Five Selective and Differential Media for the Detection and Enumeration of Coagulase-Positive Staphylococci in Foods

F. D. CRISLEY, J. T. PEELER, AND R. ANGELOTTI

Milk and Food Research, Robert A. Taft Sanitary Engineering Center, U.S. Public Health Service, Cincinnati, Ohio

Received for publication 31 August, 1964

ABSTRACT

CRISLEY, F. D. (Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio), J. T. PEELER, AND R. ANGELOTTI. Comparative evaluation of five selective and differential media for the detection and enumeration of coagulase-positive staphylococci in foods. Appl. Microbiol. **13**:140–156. 1965.—Five selective media for the detection and enumeration of coagulase-positive staphylococci were evaluated for their efficiency in the recovery of 17 strains of coagulase-positive staphylococci from foods. They were Staphylococcus Medium 110 (SM-110), tellurite-glycine-agar (TGA), egg-tellurite-glycine-pyruvate-agar (ETGPA), tellurite-egg-agar (TEA), and tellurite-polymyxin-egg yolk-agar (TPEY). Statistical analysis by the rank correlation method of the efficiency with which these media recovered staphylococci from pure 24-hr Brain Heart Infusion cultures revealed the following efficiencies in descending order: (i) TPEY, (ii) ETGPA, (iii) TGA, (iv) TEA, (v) SM-110. Growth of 17 strains of coagulase-negative cocci on these media showed the following approximate descending order of inhibition to these organisms: (i) ETGPA, (ii) TEA, (iii) SM-110, (iv) TGA, (v) TPEY. The appearance of colonies of the various coagulase-negative strains on each medium was studied for the degree to which they could be confused with colonies of coagulase-positive strains. Nineteen food contaminants, including *Proteus vulgaris*, *Bacillus* sp., *Escherichia coli*, *Erwinia* sp., fecal streptococci, and others, were also studied for similarities in appearance to staphylococci and for ability to grow on the selective media. The influence of five sterile food homogenates (frozen chicken and tuna pies, custard, smoked ham, and raw whole egg) on recovery of 1,500 enterotoxigenic staphylococci (three strains) per milliliter was determined by statistical analysis. Three main effects (culture, media, and food) and three interactions (media with food, food with cultures, and media with culture) were found to be significant. Recovery on TPEY was influenced less by food than the other selective media and showed optimal recovery ability from sterile custard, eggs, and ham. TGA recovered well from sterile chicken pie and custard, SM-110 from sterile custard, and TEA from sterile ham. None of the media was outstanding in recovering staphylococci from tuna pie. The ability of the five selective media to recover 1,500 enterotoxigenic staphylococci (three strains) per ml from three sterile foods in the presence of 10 strains of contaminating bacteria added at the 0, 10^5, and 10^6 levels per milliliter was also studied and analyzed statistically. Only three factors were significant under these conditions—cultures, foods, and the interaction of media with the level of added contamination. Efficiency of recovery of TGA, SM-110, and ETGPA was found not to be dependent upon the level of contamination. Recovery on TPEY decreased with increases in the number of contaminants. TEA increased in efficiency at the 10^5 level, but decreased at the 10^6 level. When recovery on Trypticase Soy Agar was considered to be 100%, the average percentage of recovery by each of the selective media under all experimental conditions was determined.

Many media have been proposed for the detection and enumeration of pathogenic staphylococci from various habitats, based on characteristics more or less correlated with pathogenicity. Examination of foodstuffs for potentially enterotoxigenic staphylococci presents special problems that limit the usefulness of methods designed for clinical use. This has resulted in a continuing search for methods and media better suited for use with food.

Staphylococcus Medium 110 (SM-110) has been widely employed for this purpose. It was designed to take advantage of the ability of the organism to grow in relatively high salt concentrations and to enhance the properties of gelatin liquefaction (Stone reaction), mannitol fermentation, and chromogenesis, all of which at various times were thought to have been correlated with coagulase production and pathogenicity. Of these criteria, chromogenesis is currently the most widely used by the food worker for selection of staphylococci on SM-110. Frequently, coagulase-positive strains from food either do not develop the characteristic pigment, or do so only after extended incubation at room temperature or lower. This makes necessary the confirmation of greater numbers of isolates by the coagulase test and also delays the results, often beyond the time limit of usefulness.

The incorporation of tellurite into selective media such as the tellurite glycine agar (TGA) of Zebovitz, Evans, and Niven (1955), a modification of the medium of Ludlam (1949), has been very useful. The striking black appearance of staphylococci on tellurite media after about 24 hr eliminates the necessity of subjective judgments of pigmentation. Tellurite media are, however, widely considered to be inhibitory to many food-borne coagulase-positive staphylococci, and as such offer a distinct disadvantage in quantitative determinations of these bacteria. Moreover, *Proteus vulgaris* has been shown to grow out on tellurite glycine medium with the production of black colonies that closely resemble, and may be confused with, those of coagulase-positive staphylococci (Deneke and Blobel, 1962; Crisley, *unpublished data*).

Recently, Finegold and Sweeney (1961) described a polymyxin-containing medium useful in hospital work. This medium was not considered by Baird-Parker (1962*a*) to be entirely satisfactory for examination of foods.

Gillespie and Alder (1952) observed that most of the coagulase-positive strains they studied produced opacity when grown in media containing egg yolk. Subsequently, egg yolk was incorporated into media for examination of foods (Carter, 1960; Herman and Morelli, 1960). Clearing of egg yolk in a selective medium has also been employed as a basis for selection of coagulase-positive staphylococci from platings of food samples (Hopton, 1961).

Egg yolk has also been combined with tellurite in selective media on which both development of opacity (Innes, 1960) and egg yolk clearing (Baird-Parker, 1962*a*) have been used as criteria for identification of staphylococci in foods. We have employed a tellurite-polymyxin-egg yolk-agar (TPEY) for investigations of commercial foods, foods implicated in outbreaks of food poisoning (Crisley, *unpublished data*), and, more recently (Crisley, Angelotti, and Foter, 1964), of synthetic cream pie fillings and pies. On TPEY, both the development of opacity and clearing in egg yolk are employed to identify staphylococci.

The relative efficacy of diagnostic media for staphylococci in food has not been adequately reported, particularly in the case of the recently developed recipes, and from the standpoint of quantitative recovery, which is an important factor in examinations concerned with development and use of microbiological standards. An evaluation of the egg-tellurite-glycine-pyruvate-agar (ETGPA) of Baird-Parker (1962*a*) was compiled by him (Baird-Parker, 1962*b*) from the results of various laboratories. The results showed the method to be quite successful, but recovery results and other pertinent material were not included in the report.

The present work was done to provide useful data concerning the efficacy of five selective and differential media for determinations of *Staphylococcus aureus* in foods. The media were SM-110, TGA of Zebovitz et al. (1955), the tellurite-egg-agar (TEA) of Innes (1960), ETGPA of Baird-Parker (1962*a*), and TPEY of Crisley et al. (1964).

MATERIALS AND METHODS

Media. Trypticase Soy Agar (TSA; BBL) was employed as a nonselective recovery medium against which recoveries on the five selective media were compared. It was prepared according to the manufacturer's directions. The dehydrated versions of SM-110 (Difco) and TGA (Difco) were similarly made. TEA was made up according to the author's instructions (Innes, 1960). Potassium tellurite for both TGA and TEA was purchased from Difco. ETGPA was made up according to Baird-Parker (1962*a*, *personal communication*), by employing potassium tellurite (The British Drug Houses, Ltd., Laboratory Chemical Division, Poole, England), since he considered this brand of chemical to be essential. Glycine and sodium pyruvate for ETGPA were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Preparation of TPEY has been previously described (Crisley et al., 1964).

Selective media were prepared, poured into sterile petri dishes, allowed to dry for 24 hr at room temperature, and used immediately or stored for 1 week in a refrigerator before use, except for ETGPA, which was poured and used within 24 hr as recommended (Baird-Parker, 1962*a*).

Cultures. Sources of the 17 coagulase-positive staphylococci are listed in Table 1. The 17 coagulase-negative cocci employed were isolated from frozen ocean perch (1), frozen codfish (1), frozen haddock (1), turkey salad from a food-poisoning

episode (3), turkey meat from another outbreak (1), delicatessen potato salad (1), raw milk (2), frozen chicken pie (1), ham implicated in food poisoning (1), fomites (food tray, refrigerator shelf) at the scene of a food-poisoning outbreak (2), nasal swab from a food handler (1), and Colby cheese (2). Common food contaminants (Table 3) were all obtained from the collection of the Food Microbiology Section.

All cultures were maintained on a semisolid stock culture medium (Crisley et al., 1964) at room temperature. Before use, they were subcultured once in the same medium in a water bath for 24 hr at 35 C and seeded into tubes of Brain Heart Infusion (BHI Difco) broth. BHI cultures were incubated in the same way for 24 hr ± 30 min before use. At this time, sterile glass beads were added, and each tube was mixed on a Vortex Junior Mixer (Scientific Industries, Inc., Queen's Village, N.Y.) for 10 sec. A homogenous suspension of bacterial cells was obtained that was remarkably uniform in viable count from one experiment to another and could be diluted for mixed-culture studies with reasonable certainty of the numbers added.

Coagulase-positive staphylococci used in studies of recovery from sterilized foods in pure or mixed cultures were grown on TSA slopes in Pyrex bottles. Cell suspensions were prepared from these bottle cultures in phosphate-buffered dilution water (American Public Health Association, 1960), as previously described (Crisley et al., 1964).

Food homogenates. Single lots of commercial brands of frozen chicken and tuna pies were purchased at a local chain grocery store and were stored in a deep freeze at −20 C until needed. A large smoked ham was similarly obtained and stored. Custard filling was made up in the laboratory according to the recipe of Angelotti et al. (1959). Raw whole egg was made by sterilizing fresh shell eggs in $HgCl_2$ solution as for preparation of egg yolk for TPEY (Crisley et al., 1964), and aseptically removing and blending the egg for 20 sec in a Waring Blendor at low speed to minimize foaming. Frozen foods were thawed, cut up into small pieces, weighed into large-mouth, screw-cap refrigerator jars, and sterilized in an autoclave at 121 C for 15 min. Food homogenates, except for raw egg, which was used undiluted, were made in a ratio of 1 part food to 7 parts of buffer by adding 64 g of sterile food to 448 ml of standard buffered dilution water (American Public Health Association, 1960). The resulting 1:8 homogenates contained the greatest concentration of solid foods that could be pipetted with accuracy.

Recovery studies. Surface plating was employed by delivering 0.1 ml of dilutions of cultures or seeded food homogenates to the surface of triplicate plates of media and spreading the inocula over the surface with sterile glass spreader bars. Seeded plates were incubated at 35 C and counted after 24 and 48 hr on a Quebec colony counter, except for counts of mixed cultures in foods on SM-110, which were incubated for 48 hr at 35 C and additionally for 72 hr at room temperature to encourage optimal pigmentation.

Recovery on sterile and experimentally contaminated foods was studied on food homogenates inoculated with approximately 1,500 staphylococci per milliliter, so that 0.1 ml contained 150 organisms when delivered and counted on each plate of TSA control medium. It was felt that this level of inoculum provided approximately the smallest number of organisms that would respond to the effects studied in a manner consistent with statistical validity. In addition, the placement of unduly large quantities of homogenate on each plate surface was avoided.

In the studies of recovery of staphylococci from experimentally contaminated foods, approximately equal numbers of each of 10 common food contaminants were added to the staphylococcal inoculum at two levels: 10^5 and 10^6 bacteria per milliliter of homogenate. This means that each plate of selective medium received totals of 10^4 and 10^5 contaminants, together with approximately 150 staphylococci. The contaminants were four coagulase-negative cocci (one each isolated from Colby cheese, raw milk, frozen chicken pie, and ham from a food-poisoning outbreak); *P. vulgaris* 4669; *Bacillus* sp. No. 5; *B. subtilis* H.; *Alcaligenes faecalis*; *Escherichia coli*, M. C. 34; and *Streptococcus faecalis*. These species of contaminants were selected because of their demonstrated ability to grow on the media studied, or their common presence in food.

After incubation, the plates of selective media containing contaminants in mixed culture with staphylococci were counted for colonies presumed to be *S. aureus* by the criteria accepted for each medium. Confirmation on a limited scale was accomplished by fishing characteristic colonies to TSA slants from plates at both levels of contamination after all incubation periods, incubating the slants for 24 hr at 35 C, and testing for coagulase by the tube method, with the use of fresh frozen human plasma and a 4-hr incubation period at 37 C. Since the coagulase-negative cocci and *P. vulgaris* strains used in the mixed-culture study were those bacteria previously noted to resemble coagulase-positive staphylococci on the tellurite-containing media, it was felt that the method constituted a fairly stringent test.

RESULTS

Recovery of pure cultures of coagulase-positive staphylococci. Comparative growth of the 17 strains recovered from 24-hr BHI broth cultures is presented in Table 1. After 24 hr of incubation on TPEY, colonies were convex and about 1.0 to 1.5 mm in diameter, they were jet-black or dark-gray in color, and they exhibited one or more of three types of egg yolk reaction. One was a discrete zone of egg yolk precipitate around and beneath the colony. The second was a clear zone or halo often occurring together with a zone of egg yolk precipitation beneath the colony. A

TABLE 1. *Comparison of five selective media for the enumeration of coagulase-positive staphylococci, based on the recovery on Trypticase Soy Agar*

Culture	Source of culture	Plate count × 10^6/ml on TSA		Per cent recovery from 24-hr BHI broth cultures after 24- and 48-hr incubation									
				SM-110		TGA		TPEY		TEA		ETGPA	
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
196E	Stock	290	316	TSTC*	24	70	63	85	89	36	36	86	90
CHP-4-5	Frozen chicken pie	271	336	47	52	76	77	106	103	41	42	94	98
C-71	Colby cheese	895	945	15	15	54	52	89	75	44	41	69	67
C-33	Colby cheese	515	610	39	38	53	50	50	54	44	53	61	49
RM-1	Raw milk	410	480	33	56	41	68	76	75	18	32	73	86
P-1	Fresh water perch	555	625	TSTC	11	103	108	91	87	TSTC	56	122	117
SH-1	Frozen shrimp	313	354	49	49	89	89	99	99	62	51	134	125
S-6	Stock	390	460	14	31	45	55	76	86	TSTC	29	64	78
255FR	W. C. Frazier, University of Wisconsin	430	460	TSTC	52	72	100	114	121	37	37	81	102
DPS-4-43	Delicatessen potato salad	625	705	54	63	90	85	110	104	80	76	82	79
LAB-1	Cream-filled doughnut, food-poisoning episode	450	630	TSTC	42	78	82	104	112	56	48	114	115
OHD	Egg, food-poisoning episode	155	346	TSTC	16	40	47	73	100	TSTC	38	66	80
OTSH-1	Turkey salad, food-poisoning episode	314	369	TSTC	25	85	67	116	118	TSTC	61	73	81
OTMNY-1	Turkey meat, food-poisoning episode	717	750	8	74	TSTC	5	100	107	70	80	0.002	0.003
OBHH-2	Baked ham, food-poisoning episode	580	610	65	73	97	93	126	127	94	88	129	126
OBHH-9	Food handler, food-poisoning episode	815	870	TSTC	70	109	105	91	88	80	93	TSTC	94
OBHH-10	Steam table, site of food-poisoning episode	1,050	1,080	TSTC	51	TSTC	53	90	93	71	74	TSTC	66

* Too small to count.

third type of reaction was the absence of either a precipitation zone or halo, but visible precipitation beneath the colony. All strains showed these typical characteristics, except 196E, C-33, and P-1, in which the egg yolk zone was slightly more diffuse ("fuzzy") in appearance, but all other characteristics were typical. Egg yolk reactions were generally better after 48 than after 24 hr.

The zones of clearing of egg yolk on ETGPA were not as clear or definite in a number of strains as might have been expected from the report of Baird-Parker (1962*a*). This is presumably due to the tendency of the medium to remain more translucent in our hands than is desirable, though we attempted to follow closely the directions and precautions of Baird-Parker (1962*a*, *personal communication*). Frequently, an oily zone, somewhat similar to a clear zone, was discernible, and this, together with the colonial morphology and clearing, was accepted as the criterion for coagulase-positive staphylococci on ETGPA. Thirteen strains showed the slight clearing reaction in 24 to 48 hr. Clearing was accompanied by development of an egg yolk precipitate in five strains. According to Baird-Parker (1962*a*), the precipitation is characteristic of coagulase-negative *Staphylococcus saprophyticus* or occasional strains of *S. aureus* after incubation for 48 hr.

Furthermore, the variation in colony size of coagulase-positive strains after both 24 and 48 hr was troublesome on ETGPA and was much more marked than on the other media. Colony size generally ranged from pinpoint to about 1.0 or 1.5 mm in diameter. We feel that this variation may lead to lower counts in some foods when the extremely small colonies are overlooked, because they are so similar to coagulase-negative colonies.

On TEA, the appearance of coagulase-positive colonies was much closer to the published description (Innes, 1960) after 48 hr of incubation than after 24 hr. Colonies did not appear to take up sufficient tellurite for clear differentiation after only 24 hr. The clear or oily zones around colonies, described by Innes (1960), occurred in 11 strains, and clearing was accompanied by, or followed by, egg yolk precipitation in nine strains. Inhibition or atypical reactions at 24 hr were exhibited by four strains.

Appearance of colonies of coagulase-positive staphylococci on SM-110 and TGA conformed to the accepted criteria for these media.

Table 1 presents the number of colonies enumerated on the selective media, expressed as the percentage of the counts observed on TSA agar, which were considered to be 100% for purposes of this study.

The relative efficiency of the media studied was determined by a rank correlation method reported by Kendall (1955). The media were ranked in Table 1 by rows (horizontally) for both the 24- and 48-hr incubation periods; the rank of 1 was assigned to the medium exhibiting the highest recovery, and subsequent ranks were assigned in the descending order of recovery. Identical scores were resolved by averaging the ranks; i.e., two media that possess identical scores but should occupy ranks 4 and 5 would each be considered as ranking 4.5 (yielding a sequence of 1, 2, 3, 4.5, 4.5). The sum of ranks was compared for each of the five media vertically (down the column). A coefficient of concordance was then calculated and tested to determine whether significant concordance existed according to Kendall's (1955) method.

The null hypothesis that the five media did not grow staphylococci in any set order from the 17 cultures was proposed. The alternative hypothesis is that the media do grow staphylococci in a set order from the 17 cultures. It was decided to let the null hypothesis be rejected one time in 20 when it is true ($\alpha = 0.05$). Since N (number of media) was less than seven, the value of S (sum of squares of column rank totals) must be compared with a critical value in the tables for (number of cultures) and equals 17; N equals 5. The critical value for S is 421.0. For an S value larger than 421.0, the null hypothesis was rejected (the fact that the ranks were unordered).

By this method it was concluded for both the 24- and 48-hr data that the value of S exceeded 421.0 (i.e., $S_{24\text{ hr}} = 1{,}790.5$ and $S_{48\text{ hr}} = 1{,}804$); hence, the media were considered to be ordered. The best estimate of the ranks of the media in descending order of efficiency of recovery of coagulase-positive staphylococci was 1-TPEY, 2-ETGPA, 3-TGA, 4-TEA, and 5-SM-110.

Recovery of pure cultures of coagulase-negative cocci. Expressed as the number of strains recovered at various percentage levels of the TSA count, the relative recovery of coagulase-negative cocci is presented in Table 2. Better outgrowth occurred after 48 hr of incubation than after 24 hr. SM-110 seemed to be somewhat more inhibitory to coagulase-negative cocci than TGA, particularly after 24 hr, when the appearance of colonies on TGA was distinctly different from the usual appearance of coagulase-positive strains, though colonies of seven of our strains on TGA strongly resembled positive types after 48 hr. Our results again confirm the desirability of a 24-hr incubation period for TGA as recommended by Zebovitz et al. (1955) and other workers, and also that many coagulase-negative organisms grow out in 48 hr on SM-110, necessitating further incubation for pigment develop-

TABLE 2. *Recovery of 17 strains of coagulase-negative cocci on five selective media and Trypticase Soy Agar*

Medium	Time of incubation	No. of strains recovered at various percentage levels of the TSA count (100%)*				
		0.0 to 0.1%	0.11 to 10%	11 to 50%	51 to 100%	over 100%
	hr					
SM-110	24	14	0	0	3	0
	48	5	2	3	6	1
TGA	24	9	4	1	3	0
	48	4	5	4	3	1
TPEY	24	5	1	1	6	4
	48	0	0	2	9	6
TEA	24	11	0	1	3	2
	48	4	0	3	7	3
ETGPA	24	14	3	0	0	0
	48	11	3	1	2	0

* TSA counts per milliliter for the 17 strains ranged from 250×10^5 to 65×10^6.

ment and coagulase tests on both pigmented and unpigmented colonies.

Of the three egg yolk recipes tested, ETGPA (Baird-Parker, 1962*a*) was most suppressive to coagulase-negative cocci. None of the visible colonies growing out at 24 hr resembled coagulase-positive types. After 48 hr of incubation, however, four strains produced colonies similar to coagulase-positive strains, morphologically and in egg yolk-clearing ability, which Baird-Parker (1962*a*) considered to be a characteristic of positive types. One of these strains also showed precipitated egg yolk beneath the colony.

TEA (Innes, 1960) was less inhibitory to coagulase-negative cocci than was ETGPA. Two of our strains produced colonies that resembled coagulase-positive strains according to the criteria of Innes (1960). Our turkey-meat strain differed only in that the colony was slightly more brown than the gray or black color usually associated with coagulase-positive colonies.

Of the egg yolk media, TPEY was least inhibitory to coagulase-negative cocci. After 24 hr, coagulase-negative cultures produced colonies that were usually small (pinpoint to 0.5 mm in diameter), and white, gray, or black, with a light periphery. Usually there was no discrete zone of clearing or precipitation of egg yolk. In four of our strains, on crowded areas of the plate, there was occasionally a diffuse type of egg yolk precipitation quite different from the discrete zonal reaction noted with coagulase-positive colonies. Some of our strains, after 48 hr of incubation on TPEY, had developed larger dark colonies similar to coagulase-positive organisms, but had not developed an egg yolk zone, or had shown only the diffuse type of reaction. One strain, our turkey-meat isolate, developed a zone of clearing and egg yolk precipitate similar to those associated with coagulase-positive colonies. This strain also gave trouble on ETGPA and TEA.

Growth of common food contaminants. Growth of 19 bacteria that may be encountered in food is summarized in Table 3. Generally, none of the media was completely inhibitory to *A. faecalis*, members of the genus *Bacillus*, and *P. vulgaris*. Particular attention was paid to the appearance of *P. vulgaris* colonies on the media as a result of their resemblance to staphylococci on tellurite-containing media (Deneke and Blobel, 1962; Baird-Parker, 1962*b*; Crisley, *unpublished data*). Outgrowth of *P. vulgaris* on TPEY was not completely suppressed, but was markedly less than on TGA or ETGPA. Only a few of the colonies present were equal in size to staphylococcal colonies. They were flatter, more brown, and showed no egg yolk reaction. *P. vulgaris* showed a tendency to spreading film formation on ETGPA, which increased with the number of successive rapid transfers prior to plating. Although the organism grew out on TEA, there was considerable inhibition, and the colony morphology was characteristically different from staphylococci. In several other cultures, a coalescing film of growth was present on plates of the 10^{-1} dilution of broth culture, but there was no growth at higher dilutions.

The *Bacillus* cultures that we tested grew slightly better on TPEY and TEA than on ETGPA, TGA, and SM-110. *Bacillus* growth on TEA was especially troublesome, because it showed a tendency to spread more extensively than on TPEY. *Bacillus* sp. were easily differentiated from staphylococci.

Factorial analyses of (i) the effect of sterile foods and its interactions with media and cultures and (ii) the effect of the level of experimental contamination and its interactions on the recovery of coagulase-positive staphylococci. Essentially the same statistical models and assumptions were made to determine the relative effect of these aspects on recovery on the five media as related to overall recovery on TSA. The analysis of the effect of sterile food is based on data from Table 4 and the effect of experimental contamination on data from Table 5. Triplicate plates were counted for each of the 75 combinations of five foods, five media, and three cultures, and the results (225 observations in all) are presented in Table 4. In the analysis of the effect of experimental contamination (Table 5), triplicate plates were counted for all combinations of four factors (i.e., three cultures, five media, three

TABLE 3. *Comparative growth response of various bacterial food contaminants on Trypticase Soy Agar and five selective media after incubation at 35 C*

Species	No. of organisms per ml[a] recovered from 24-hr BHI broth cultures determined by plating in										
	TSA	SM-110		TGA		TPEY		TEA		ETGPA	
	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Alkaligenes faecalis	4.8×10^7	+	2.4×10^5	−	−	+	6.9×10^{6b}	+	4.7×10^5	−	−
Bacillus cereus	1.3×10^7	±	±	−	−	±	±	+	1.1×10^5	−	−
B. subtilis H	6.9×10^7	+	+	+	7.7×10^3	1×10^2	1.2×10^3	+	2.8×10^7	1.3×10^3	4.0×10^7
B. subtilis SEC	3.1×10^6	+	3.0×10^5	±	±	3.2×10^{5b}	3.5×10^5	+	2.8×10^5	±	+
Bacillus sp. No. 5	1.7×10^7	2.4×10^6	3.4×10^6	±	4.0×10^2	1.4×10^7	1.6×10^7	7.6×10^6	9.0×10^6	±	5×10^1
Erwinia carotovora	7.5×10^8	−	−	−	−	−	−	−	−	−	−
E. nimipressuralis	2.2×10^8	−	−	−	−	−	−	−	−	−	−
Escherichia coli G	6.8×10^8	−	−	−	−	−	−	−	−	−	−
E. coli MC34	1.6×10^9	−	−	−	−	−	−	−	−	−	−
Proteus morgani	2.7×10^9	−	−	−	−	+	5.7×10^{4b}	−	−	−	−
P. rettgeri	1.1×10^9	±	±	−	−	−	−	−	−	−	−
P. vulgaris ATCC 4669	1.2×10^9	+	3.4×10^{7b}	+	9.9×10^{7c}	+	9.5×10^{6b}	±	5.0×10^{5b}	2.0×10^{8c}	2.0×10^{8c}
P. vulgaris M	1.1×10^9	+	2.8×10^8	1.0×10^{8c}	1.3×10^{8c}	+	$3.8 \times 10^{7b,d}$	±	1.0×10^{5b}	1.3×10^{8c}	1.3×10^{8c}
Pseudomonas aeruginosa KR1	6.6×10^8	−	−	−	−	−	−	±	±	−	−
Pseudomonas sp	6.1×10^8	−	−	±	±	−	−	±	±	−	−
Salmonella typhi ATCC 6539	5.4×10^8	−	−	−	−	−	−	−	−	−	−
Serratia marcescens	2.7×10^9	±	+	−	−	±	+	±	+	±	±
Streptococcus faecalis	1.2×10^9	±	1.0×10^{9b}	−	−	−	−	−	−	−	−
S. faecium	5.5×10^8	±	+	−	−	−	−	−	−	−	−

[a] Symbols: − = no growth from surface plating of 0.1 ml of concentrated culture (10^{-1} dilution); ± = growth on plates containing 10^{-1} dilution but not higher dilutions (usually these plates showed a coalescing film of growth or colonies too numerous to count); + = slight growth on dilutions above 10^{-1} (colonies were usually barely visible or microscopic in size).

[b] Colonies that were very small and difficult to count.

[c] Significant numbers of black colonies that could be confused with those of coagulase-positive staphylococci.

[d] Contained a few black colonies on low dilutions that could be confused with coagulase-positive staphylococci.

TABLE 4. *Influence of five sterile foods on the recovery of Staphylococcus aureus 196E, LAB-1, and 71 on five selective media and Trypticase Soy Agar in a typical experiment*

Food homogenate	Plate counts of triplicate plates after 48 hr of incubation at 35 C																	
	SM-110			TGA			TPEY			TEA			ETGPA			TSA		
	196-E	LAB-1	71	196-E	LAB-1	71	196-E	LAB-1	71	196-E	LAB-1	71	196-E	LAB-1	71	196-E	LAB-1	71
Frozen chicken pie	198	129	112	164	168	157	138	158	103	129	96	94	105	145	97	163	157	157
	178	138	129	177	161	99	145	167	111	178	89	83	118	126	102	176	155	118
	146	137	125	176	139	164	173	165	129	143	94	96	123	136	113	165	165	178
Frozen tuna pie	159	169	132	204	114	119	169	118	125	162	87	90	151	139	115	178	133	106
	156	156	114	161	111	108	176	152	118	195	104	101	138	163	101	208	130	122
	174	150	109	186	139	103	198	131	126	158	95	141	162	144	103	177	121	105
Laboratory custard	184	164	110	172	144	115	196	145	98	125	106	76	104	95	96	198	132	139
	186	155	91	138	155	103	139	148	96	161	107	66	99	102	73	201	127	83
	179	131	84	160	159	83	220	153	128	136	116	73	101	102	72	161	140	86
Smoked ham	127	110	101	129	74	109	129	141	96	144	140	91	130	80	59	180	142	193
	163	81	131	137	107	108	126	147	88	150	150	92	133	101	54	105	209	74
	128	97	121	114	77	98	134	148	96	116	138	100	132	86	62	147	113	119
Concentrated whole raw egg	161	77	61	149	107	117	184	164	112	127	165	80	181	136	87	184	87	96
	119	100	88	156	91	119	163	133	94	150	130	74	184	103	79	173	102	92
	121	75	96	162	90	133	219	136	86	139	128	81	207	99	77	195	118	84

foods, and three levels of contamination by concomitant bacteria). The data used in Table 5 were averages of triplicate plate counts in a typical experiment (135 observations in all).

The factors were tested for average differences within the factors, such as the differences among the five media, among the three foods, etc., and the first-order interactions among the food, media, cultures, etc. The mathematical models for factorial experiments involving data from Tables 4 and 5 were as follows. Model for testing data in Table 4:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \tau_l + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \epsilon_{ijkl}$$
$$i = 1, \cdots, 5$$
$$j = 1, \cdots, 5$$
$$k = 1, \cdots, 3$$
$$l = 1, \cdots, 3$$

Model for testing data in Table 5:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \delta_l + (\alpha\delta)_{il} + (\beta\delta)_{jl} + (\gamma\delta)_{kl} + \epsilon_{ijk'}$$
$$i = 1, \cdots, 3$$
$$j = 1, \cdots, 5$$
$$k = 1, \cdots, 3$$
$$l = 1, \cdots, 3$$

where: Y_{ijkl} = the log plate count in the $ijkl$th cell; μ = overall log mean; α_i = the ith food effect; β_j = the jth media effect; γ_k = the kth culture effect; τ_l = the lth replicate effect; δ_l = the lth level of contamination effect; $(\alpha\beta)_{ij}$ = the ijth media × food interaction; $(\alpha\gamma)_{ik}$ = the ikth food × culture interaction; $(\alpha\delta)_{il}$ = the ilth food × level of contamination interaction; $(\beta\gamma)_{jk}$ = the jkth media × culture interaction; $(\beta\delta)_{jl}$ = the jlth media × level of contamination interaction; $(\gamma\delta)_{kl}$ = the klth culture × level of contamination interaction; ϵ_{ijkl} = the $ijkl$th error effect.

TABLE 4a. *Analysis of variance for factorial analysis of culture, media, and foods at 48 hr*

Source	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F-ratio
Replications..	0.00134	2	0.00067	0.15
Media.....	0.29291	4	0.07322	16.02*
Food.....	0.25289	4	0.06322	13.83*
Culture.....	1.30438	2	0.65219	142.71*
Media × food.....	0.38265	16	0.02391	5.23*
Media × culture.....	0.09662	8	0.01207	2.64*
Food × culture.....	0.11301	8	0.01412	3.09*
Error.....	0.82352	180	0.00457	
Total.....	3.26732	224		

* Significant at $\alpha = 0.05$.

TABLE 4b. *Media recovering significantly more Staphylococcus aureus for each food and culture**

Food homogenates					Cultures		
Frozen tuna pie	Frozen chicken pie	Custard	Raw egg	Ham	196E	LAB-1	C-71
None	TGA	TPEY TGA SM-110	TPEY	TPEY TEA	TPEY TGA SM-110	TPEY	TGA

* Where more than one medium is listed, they showed approximately equal trends that are significantly larger than the remaining media.

The assumptions chosen for the mathematical experiments were as follows. (i) The observations Y_{ijkl} are random variables distributed about true means that are fixed constants. (ii) The model contains additive effects. (iii) The Y_{ijkl} are independently distributed in a normal distribution. (iv) The random variables Y_{ijkl} have a common variance, σ^2. (v) The model used is a fixed-effects model (i.e., we limit our conclusions to the foods, cultures, media, and level of contamination actually present).

Auxiliary assumptions were that the plate counts made on the experimentally contaminated plates were all *S. aureus* colonies (i.e., colonies of other bacteria were not counted as *S. aureus*), and that SM-110 plates that were incubated for 5 days to allow for pigment development were assumed to behave like the other selective media at 48 hr of incubation. These assumptions were generally borne out by previous experiments, though confirmation by coagulase test was necessarily limited by the volume of work.

The plan of statistical analysis (Ostle, 1954) was to test the effects within the factors and between the factors of the first-order interactions to determine whether any of them were different from zero. All tests were performed on the assumption of rejection of the hypothesis that the main effects and the interaction effects are equal to 0 only once in 20 times when the hypothesis is true ($\alpha = 0.05$).

To assure that two of the assumptions in Table 4b were satisfied, logs were taken to make the count data approximately normally distributed, and Bartlett's tests (Snedecor, 1956) were performed to examine homogeneity of variances. The Bartlett's test confirmed statistically the experimental observations that the 24-hr counts were frequently incomplete, owing to the erratic outgrowth of staphylococci and variations in the diagnostic reactions. Hence, only the data collected after 48 hr of incubation at 35 C were analyzed.

Analyses of variance for factorial analysis of the main effects, first-order interactions, and the experimental error are presented in Tables 4a and 5a for the experiments on sterile food homogenates and the experimentally contaminated systems, respectively. Variation in techniques may include, among other things, a gain in efficiency, owing to experience in counting plates.

Other quantities in the tables include the sum of squares (SS), which are divided by the degrees of freedom (df) to get the mean square estimate (MS) for the main effects and the interactions. Ratios of mean squares to the mean square error are listed in the F-ratio column. If there is no effect due to the particular main factor or interaction being tested, the F-ratio will be near 1. As the F-ratio becomes larger, it can be said that there is more evidence that there is an effect due to the main effect or interaction in question. For example, if a factor such as the culture effect is found to be significant on the average, some strains tend to grow out better than others. To test which strains were significantly different from each other, the test suggested by Duncan (1955) would be used. If an interaction is present, some quantities of one factor significantly influence the effect of another.

Effect of sterile foods. Based on the data in Table 4, the analysis of variance presented in Table 4a indicates that three main effects and three interactions were significant. They were culture, media, and food; and interactions media × food, food × culture, and media × culture. By Duncan's (1955) test, the 196E strain was recovered in greater numbers than LAB-1, and the LAB-1 strain was recovered in larger numbers than the C-71 culture when logarithms of the plate counts were averaged over the whole experiment.

On TPEY, recovery was better than on TGA and SM-110; on TGA, it was better than on TEA and ETGPA. Moreover, recovery of staphylococci was better from tuna and chicken than from custard, eggs, and ham.

Figures 1, 2, and 3 present the trends in the interactions. In these figures, points close to the

TABLE 5. *Effect of experimental contamination on recovery of coagulase-positive staphylococci from sterile food homogenates*

Staphylococcus culture	Food homogenate	Avg counts* of sterile food homogenates containing only staphylococci					Avg counts* in sterile food homogenates containing staphylococci and 10^5 contaminants/ml					Avg counts* in sterile food homogenates containing staphylococci and 10^6 contaminants/ml				
		SM-110	TGA	TPEY	TEA	ETGPA	SM-110	TGA	TPEY	TEA	ETGPA	SM-110	TGA	TPEY	TEA	ETGPA
196E	Custard	183	157	185	141	101	170	166	92	174	148	147	132	135	163	155
	Chicken pie	174	172	152	150	115	167	209	161	210	158	178	178	108	182	177
	Raw whole egg	134	156	189	139	191	132	151	147	147	111	117	152	126	147	102
LAB-1	Custard	150	153	149	110	100	126	151	154	144	123	180	117	117	129	144
	Chicken pie	135	156	163	93	136	142	139	139	137	139	137	137	120	94	123
	Raw whole egg	84	96	144	141	113	108	109	135	149	76	98	104	111	131	66
C-71	Custard	95	100	107	72	80	96	107	134	110	106	100	103	135	128	119
	Chicken pie	122	140	114	91	104	77	108	183	134	149	137	153	129	133	112
	Raw whole egg	82	123	97	78	81	109	123	104	125	94	67	129	79	103	84

* Average of triplicate 48-hr plate counts of *S. aureus* 196E, LAB-1, and C-71 recovered from three sterile and experimentally contaminated foods on five selective media in a typical experiment.

TABLE 5a. *Analysis of variance for factorial analysis of culture, media, foods, and level of contamination at 48 hr*

Source	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F-ratio
Media	0.07963	4	0.01240	2.37
Level of contamination	0.02273	2	0.01136	2.17
Food	0.16361	2	0.08180	15.64*
Culture	0.47692	2	0.23846	45.59*
Media × level of contamination	0.09393	8	0.01174	2.24*
Media × food	0.07177	8	0.00897	1.71
Media × culture	0.06233	8	0.00779	1.48
Level of contamination × food	0.03689	4	0.00922	1.76
Level of contamination × culture	0.04147	4	0.01036	1.98
Food × culture	0.03797	4	0.00949	1.81
Error	0.46105	88	0.00523	
Total	1.54830	134		

* Significant at $\alpha = 0.05$.

ordinate $r = 0$ show little or no interaction with the quantities of the factors given on the abscissa, as compared with points lying to either side of $r = 0$. Points above "0" indicate a positive interaction as measured by an increase in numbers of staphylococci recovered. Points below "0" indicate negative interaction in terms of a decrease in staphylococcal recovery.

For example, in Fig. 1, TPEY was influenced less by food than were the other selective media, and SM-110 was greatly influenced by raw eggs in which the count was reduced. Marked positive interactions occurred between TGA and chicken pie; SM-110 and custard; TEA and ham; and between ETGPA and raw eggs and tuna pie. Negative interactions took place between TGA and ham; SM-110 and eggs; TEA and chicken; and between ETGPA and custard and ham.

Food-culture interactions (Fig. 2) were less important than media-food interactions. Positive interactions occurred between 196E and raw egg and between LAB-1 and custard. Negative results were found between C-71 and custard and 196-E and chicken.

Culture-media interactions (Fig. 3) had even less effect than food-culture interactions. Positive values were found between LAB-1 and TPEY and between C-71 and TGA. Negative values were shown between LAB-1 and TGA.

The question of which medium recovered the most staphylococci when employed with a particular food or culture was answered by employing the statistical method of Scheffé (1953), which allows determination of the significance of certain comparisons after the data have been examined. Table 4b indicates the media that recover significantly more organisms for each food and culture at the $\alpha = 0.05$ level. On this basis, TPEY can be recommended for

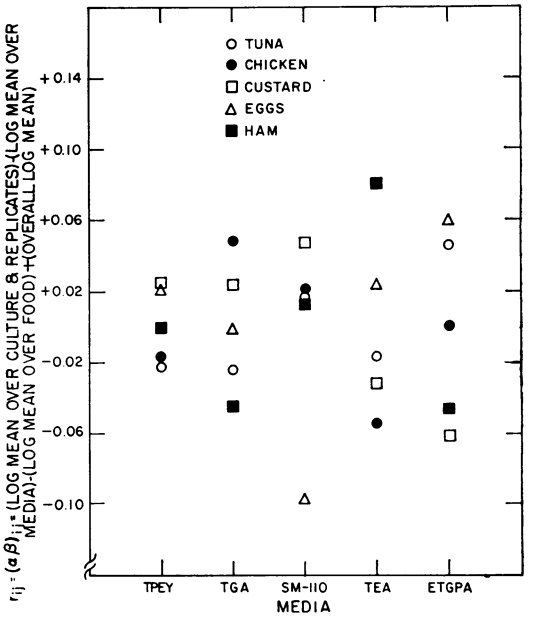

FIG. 1. *Interaction effects between sterile food homogenates and staphylococcal selective media after 48 hr of incubation at 35 C, based on logarithms of individual plate counts.*

optimal recovery from custard, eggs, and ham; TGA from chicken pie and custard; SM-110 from custard; and TEA from ham. None of the media was outstanding in recovering staphylococci from tuna pie.

Three media (TPEY, TGA, and SM-110) were outstanding in recovering 196E. TPEY also recovered more organisms of the LAB-1 strain, and TGA was best in recovering C-71.

When recovery on TSA was considered to be 100%, the calculated average per cent recoveries

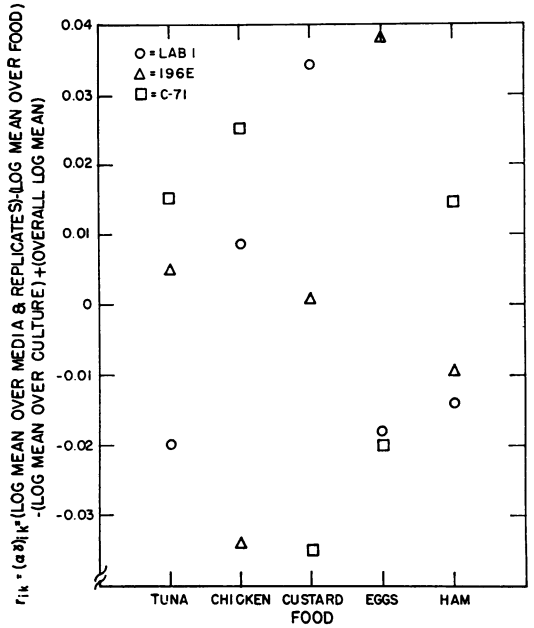

FIG. 2. *Interaction effects between sterile food homogenates and test cultures after 48 hr of incubation at 35 C, based on logarithms of individual plate counts.*

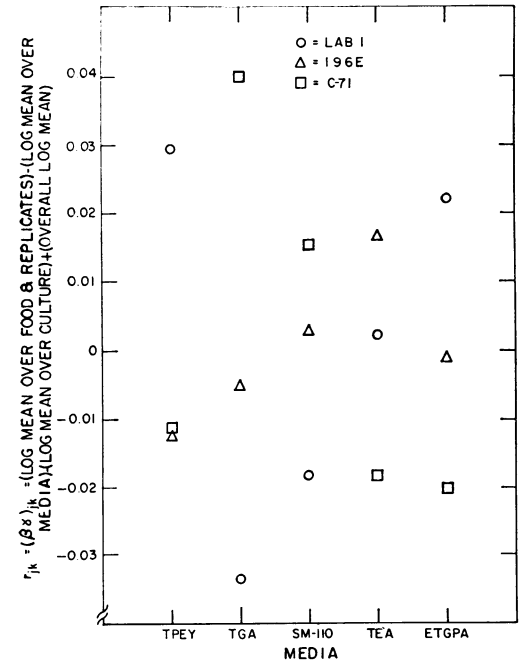

FIG. 3. *Interaction effects between selective staphylococcal media and test cultures after 48 hr of incubation at 35 C, based on logarithms of individual plate counts.*

of staphylococcal media under all conditions in the experiment on sterile foods were: TPEY, 99.3%; TGA, 93.0%; SM-110, 91.5%; TEA, 83.0%; and ETGPA, 80.3%.

Effect of experimental contamination. Factorial analysis of main effects and interactions was carried out on data presented in Table 5 for the effect of three levels of experimental contamination (0, 10^5, and 10^6 contaminants). The estimate of absolute recovery on each medium as compared with recovery on TSA from sterile food homogenates under identical conditions is included in the overall calculations.

The analysis of variance (Table 5a) shows that only two factors and one interaction reached the level of significance. They were cultures and foods and the interaction media × level of contamination. Duncan's (1955) test was again used to show differences between quantities within factors.

Significantly (α = 0.05 level), more colonies were found on platings of 196E and of LAB-1 and more on platings of LAB-1 than of C-71. The culture factor did not interact significantly at the first-order level with the other factors; that is, better growth of one strain when compared with another seemed to be independent of the other factors.

Platings of chicken pie revealed significantly more colonies than platings of custard, which in turn showed more colonies than platings of eggs.

Averaged over the experiment, the three levels of added experimental contamination (0, 10^5, and 10^6 bacteria per milliliter) showed no significant difference among media. Only one of the six first-order interactions (media × level of contamination) was found to be significant at the α = 0.05 level.

Figure 4 shows the effect of the interaction between media and contamination level. The ratio in Fig. 4 should be "0" if the efficiency of the media is not dependent on the level of contamination. The media TGA, SM-110, and ETGPA are nearly "0" for all three levels, but TPEY decreases in recovery with an increase in the number of contaminants, and TEA peaks at a level of 100,000 added contaminants per milliliter. TPEY and TEA seem to be responsible for the largest part of the interaction of media × contamination level.

When recovery on TSA was considered to be 100%, the calculated average per cent recoveries of staphylococci in the presence of experimental contamination under all conditions was: TGA, 97.2%; TPEY, 94.4%; TEA, 93.0%; SM-110, 90.1%; and ETGPA, 83.8%.

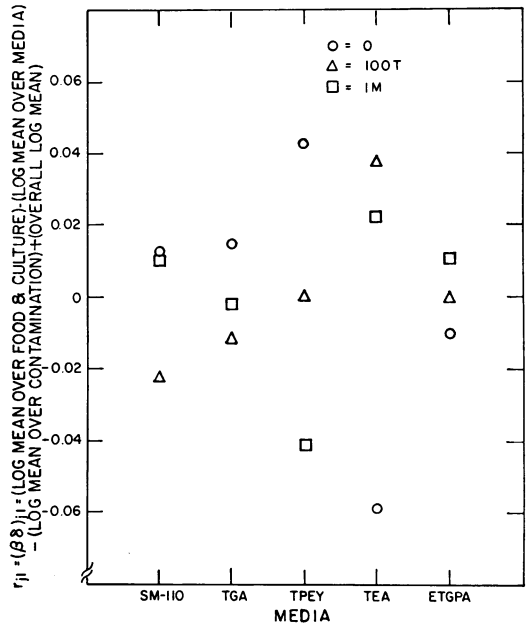

FIG. 4. *Interaction effects between selective staphylococcal media and the level of experimental contamination after 48 hr of incubation at 35 C, based on logarithms of the means of triplicate plate counts.*

DISCUSSION

We feel that the three egg yolk media generally possess advantages for differentiation of coagulase-positive staphylococci over media without egg, in spite of conflicting opinions concerning the value of the egg reaction as a diagnostic tool. Gillespie and Alder (1952) found 80% of coagulase-positive staphylococci from human sources outside the hospital environment producing opacity in egg yolk media, whereas all of their 60 coagulase-negative organisms were egg yolk-negative. Similar results were found by Alder, Gillespie, and Herdan (1952). Based on clearing and precipitation of egg yolk, only 76 egg yolk-positive strains of 186 coagulase-positive *S. aureus* strains were isolated from umbilical stumps of newborn infants by Graber et al. (1958). The same source yielded 14 egg yolk-positive strains of 24 coagulase-negative strains. Nevertheless, Graber et al. (1958) considered the egg yolk reaction diagnostically helpful, because 95 strains of *S. albus*, *S. citreus*, and *S. aurantiacus* were egg yolk-negative on plates, and their pigmentation is not always sufficiently distinct from *S. aureus* on other media. Silverman et al. (1961), working with isolates from shrimp, also felt that the egg reactions increased their ability to find staphylococci in their samples, since coagulase-positive staphylococci were not isolated from samples that did not yield egg yolk-reacting colonies. Carantonis and Spink (1963) found that only 11 of 505 opacity-producing strains (60% from lesions and the rest from nasal swabs of health carriers), considered by them to be "pathogenic," were coagulase-negative. These authors also found 98 of 101 "pathogenic" egg yolk-negative strains to be coagulase-positive. However, 100 additional "presumptively nonpathogenic" egg yolk-negative strains were also found to be coagulase-negative. The results of Carantonis and Spink (1963) indicate a heavy preponderance of coagulase-positive cultures among isolates from clinical sources showing a positive egg yolk reaction. In the study of various foods, the egg yolk reactions (either clearing or opacity) were found to be superior to other criteria in differentiating staphylococci by Hopton (1961), Baird-Parker (1962*a*, *b*), Carter (1960), Innes (1960), and Jay (1963).

On the other hand, in their studies of isolates from hospital patients, Mandel and Warholak (1962) found 362 of 382 (95%) coagulase-positive strains and 73 of 142 (51%) coagulase-negative strains to produce clearing and opacity on egg yolk media. Although they felt that the frequency of positive egg yolk reactions in coagulase-negative strains (51%) nullified the differential value of the egg yolk reaction, Mandel and Warholak (1962) noted that most of their coagulase-negative, egg yolk-positive cultures were from the nose, throat, and abscesses; and the majority of coagulase-negative, egg yolk-negative strains were from urine cultures. This apparent source specificity of the egg yolk reaction should be further investigated. If the relationship were found to be a firm one, it might prove to be valuable in determining whether foods have been significantly contaminated in handling, since important sources of staphylococci in food are the respiratory tract and lesions of food handlers.

The presence of coagulase or egg yolk reactivity may not always be a reliable indicator of enterotoxigenicity. Silverman et al. (1961) called attention to variations in both of these properties within strains or substrains of staphylococci. Coagulase-negative strains derived from positive cultures have been noted. The present availability of serological methods for enterotoxin detection (Casman, McCoy, and Brandly, 1963; Hall, Angelotti, and Lewis, 1963) greatly reduces the need for animal tests. More accurate and comprehensive work directed at re-examining the relationship between enterotoxin production and coagulase (and other attributes of staphylococci) than was heretofore possible should be carried out.

The results clearly show that recovery of

staphylococci from pure laboratory cultures is only one of several factors that must be considered in selecting a medium for analysis of food. For example, though TPEY was clearly superior in this respect to the other media tested and also in recovery from sterile foods, it recovered more coagulase-negative cocci and lost some of its recovery efficiency in experimentally contaminated foods. We feel that the chief value of the present work is to shed light on some of the factors that should be considered in the choice of a selective medium for the analysis of foods for staphylococci. In addition, several recommendations can be made.

TPEY was designed by us for optimal recovery as a result of many experiments on a few test strains of staphylococci. Its relatively low level of inhibitory effect on coagulase-negative cocci is probably directly related to its superior recoverability of coagulase-positive strains. ETGPA recovered from pure cultures nearly as well as TPEY, but without allowing the coagulase-negative strains to grow. TGA was less inhibitory to coagulase-positive staphylococci than was SM-110. This may not be true of staphylococci on primary isolations from food, because many workers have expressed contrary opinions.

The studies on pure cultures indicate, to some extent, the accuracy of diagnosis that can be expected on the five media. The great variation in size of colonies on ETGPA in our hands must be considered a disadvantage to be numbered among others already reported (Baird-Parker, 1962*a*, *b*). Of the egg yolk recipes, TPEY showed fewer atypical results than the other egg media, though one of the coagulase-negative strains showed strong egg yolk reactions usually identified with coagulase-positive types. This is not unexpected, in view of the reports in the literature. On the egg yolk media, generally, a 48-hr incubation period yielded better results than were obtained at 24 hr with our laboratory test strains. We have, however, noticed that on actual platings of food samples the egg yolk reaction is often more pronounced after 24 hr than in our test cultures. Although we count and select colonies on food plates on the basis of a discrete egg yolk zone, consideration must be given to the production of diffuse egg yolk reaction zones by some coagulase-positive cultures on TPEY, since three of our strains showed tendencies to produce diffused egg zones. Generally, our experience with foods shows that where such zones appear in the colonies in crowded areas of the plate, they are indicative of coagulase-negative cocci. When they appear in well-isolated colonies, we make a practice of coagulase testing a few to make certain of their reaction.

With a few exceptions, approximately the same species of miscellaneous contaminants tended to grow out on all of the selective media. This may simply reflect the possession by the media of common mechanisms for selection of staphylococci from food. The two strains of *P. vulgaris* studied were generally less troublesome on TPEY than on ETGPA or TGA. We feel that only minimal experience with TPEY would be necessary to distinguish easily these *Proteus* strains from staphylococci. Even more troublesome than its morphology on ETGPA was the spreading tendency of *P. vulgaris*, which might result in overgrowth of staphylococci on Baird-Parker's medium.

Baird-Parker (1962*b*) pointed out that *P. vulgaris* also grows out on the polymyxin agar of Finegold and Sweeney (1961) and the egg yolk azide agar of Hopton (1961), as well as on TGA and ETGPA. He attempted to overcome this deficiency in ETGPA by the addition of phenylalanine and detection of phenylpyruvic acid produced by *P. vulgaris* on this substrate.

The appearance of coalescing films of growth with several other common food contaminants at the 10^{-1} dilution, but absence of any growth at higher dilutions, is suggestive of the presence of metabolic products in high enough concentration to overcome the suppressive effects of selective media. A similar situation is possible in platings of heavily contaminated spoiled foods.

Growth of *Bacillus* sp. on the selective media is important, because members of this genus have been shown to be inhibitory to staphylococci (Graves and Frazier, 1963). Production of antibiotics by members of the *Bacillus* group is well known. TEA particularly suffered from *Bacillus* contamination, but TPEY was also considerably affected.

Analysis of the effect of sterile food confirms statistically the frequent observation that cultural differences among strains are of primary importance. They were the most important single factor in our experiments. Differences among media and foods were second and third, respectively, in rank of importance.

Paralleling its performance in recovering pure cultures from BHI broth, TPEY recovered best from sterile foods and appeared to be markedly better than the other two egg yolk media. ETGPA, in contrast to its ability to recover pure cultures, did not recover staphylococci from food as well as the other selective media, a good example of the discrepancy that may occur between recovery of pure cultures and recovery from food. Higher overall recovery on all media

from tuna and chicken pies than from custard, eggs, and ham may be an indication of the presence of an inhibitory substance in the latter three foods or the presence of a "clumping" factor, such as has been reported to occur in fresh raw milk (Smith, 1957). This point, however, requires further testing.

Our analysis of the main effects and significant interactions in the sterile food experiments may also help to explain some of the presently conflicting data and opinions regarding the efficacy of various selective media for food work. Media showing positive values (above 0 in the figures) would be most valuable in quality control work or other situations requiring relatively sensitive staphylococcal determinations. These media are listed in Table 4b, which is based on the test of Scheffé (1953). For example, TGA would appear to be best for analysis of chicken pie and TPEY for raw egg. For overall general use, TPEY might well be the medium of choice, since it appears to recover well with three of the five foods. On the other hand, if custard samples are presently being run on SM-110, no benefit will accrue from a change to TPEY, especially if laboratory personnel are experienced in the use of the older medium.

To a large extent, it is possible to make such recommendations from our experiments, because under our conditions culture × food and culture × media interactions were less significant than those of media × food. In other systems, it is quite possible that different interactions would assume greater significance. Hence, more studies of a variety of foods and media might tend to broaden and solidify recommendations for standardized methods that could profitably be utilized for better quality control of foods. Whether such studies are of sufficient value to be undertaken at all is dependent on the scientific and economic importance of the results. Development of meaningful microbiological standards in foods, for example, would seem to require such studies.

The effect of experimental contamination tended to alter the relative plating efficiency of the media noted in the experiments on sterile foods. As main effects, cultures and foods were not significantly changed from results in sterile foods. The interaction between media and level of contamination illustrated in Fig. 4 clearly shows that recovery on two media, TPEY and TEA, was affected. The reason for the apparent increase in counting efficiency on TEA in the presence of approximately 100,000 added contaminants is not clear and requires further testing. A very definite "experience factor," which may be partly responsible, was, however, noted in our study—that is, counting efficiency tended to increase on all media in proportion to our experience with them. Nevertheless, in the presence of comparatively large numbers of experimental contaminants, TPEY, the medium that we found least inhibitory to coagulase-positive staphylococci, was reduced in efficiency, and this must be considered if this medium is to be used in quality control and other work involving large numbers of similar contaminants. TEA also suffered from the same deficiency, apparently because of the presence of *Bacillus* organisms, which seemed to spread extensively on this medium.

Because no significant culture × media or food × media interactions were found to exist in the face of experimental contamination, the cultures and foods were essentially constant effects for the five media—that is, the five media recovered essentially the same numbers of the three cultures relative to each other in the contamination experiment. If present results were also true for the large number of cultures encountered in routine food work, then a specific medium-culture effect does not have to be considered. While it may be convenient for the routine worker to accept the absence of a medium × culture effect (because it presently cannot be predicted for all staphylococcal strains in existence), it is necessary for the researcher concerned with evaluation of media employing a fixed group of strains, to consider this effect. The same relationship may also be true of other contaminated foods, even though a food-media interaction may be present in sterile foods, as we have already shown.

Our results indicate that TPEY is generally superior in recovering staphylococci from pure cultures and foods, though other media may be approximately as useful for specific purposes. Its efficiency in the presence of contaminants may be expected to vary directly with the number of contaminants. To a great extent, the loss in efficiency of recovery would also necessarily depend on the type of contaminants present in the food, a variable that cannot be tested experimentally beforehand. Furthermore, our observations suggest that the worker's experience with a selective medium cannot be overlooked in judging the efficacy of such media when the many variables are considered.

Our experience with TPEY thus far indicates that the egg yolk-tellurite reactions as employed in this medium are highly satisfactory for analysis of a variety of foods, despite the small percentage of unusual coagulase-negative types that may give a characteristic egg yolk reaction. Of 91 isolates (from market foods and foods involved in food-poisoning outbreaks) considered pre-

sumptively coagulase-positive on the basis of their morphology and egg yolk reactions, 84, or 92%, were confirmed by the tube test. In one instance, we isolated and identified by the egg yolk reaction two staphylococcal colonies on a plate covered with an almost solid film of growth of other food contaminants. To date we have not noticed an adverse effect on staphylococcal recovery from market and outbreak foods of heavy contamination by concomitant bacteria, including *Bacillus* species. This may be partially due to what appears to be more pronounced egg yolk reactivity by food isolates on primary isolation as well as experience with our medium. For these reasons, its superiority in recovering staphylococci, and the fact that recovery on TPEY appears to be less influenced by food constituents in sterile foods than the other media tested, we feel that the extra work and care necessary in preparing TPEY should generally yield better results in the examination of a wide variety of foods for staphylococci. A more definite recommendation must await the accumulation of further data and experience by us and others. Our experience also indicates that poured plates of TPEY can be stored in a refrigerator for approximately 1 week before any change in selectivity is noted. After this time, the medium seemed to become less inhibitory to our coagulase-negative staphylococcal strains.

ACKNOWLEDGMENTS

We sincerely appreciate the excellent technical assistance of Nancy Batterson and Gayle Mulberry.

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