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Evaluation of Commercial Conjugates for Fluorescent Antibody Detection of Salmonellae

BERENICE M. THOMASON AND G. ANN HEBERT
Center for Disease Control, Atlanta, Georgia 30333

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Fluorescent antibody (FA) reagents for *Salmonella* produced by Difco, Sylvana, and Clinical Sciences, Inc., were evaluated for physicochemical and performance characteristics. The Difco panvalent (A through O64) and the Difco polyvalent (A through S) were similar in physicochemical characteristics. They had less than 60% gamma globulin with 3% albumin and had fluorescein to protein (F/P) ratios of less than 10. The Sylvana conjugate had 81% gamma globulin with less than 1% albumin. Its F/P was 33.9. The Clinical Sciences reagent contained 75% unlabeled albumin as packaged in the Fluoro-kit. Analysis of the original conjugate showed 86.5% gamma globulin with only 0.5% albumin. The (F/P) was 32.8. The performance characteristics were determined by using a variety of *Enterobacteriaceae* and food and feed samples. All conjugates stained the homologous *Salmonella* strains. The majority of cross-reactions were limited primarily to the *Arizona*, *Citrobacter*, and *Escherichia coli* groups. The Difco panvalent was more reactive with heterologous organisms. It stained 89% of the *Arizona* compared with 42% stained by the Difco polyvalent (A through S) and 39% stained by the Sylvana and Clinical Sciences reagents. We found 90% agreement between FA and culture when the Difco polyvalent was used to examine food and feed samples and 94% agreement when the Clinical Sciences Fluoro-kit was used on another group of samples.

The fluorescent antibody (FA) technique is a rapid, sensitive method for detecting salmonellae in a variety of specimens. However, the effective use of this technique in diagnostic laboratories is totally dependent upon reliable commercial reagents.

Several years ago the cooperative efforts of several groups (7) resulted in a design for the preparation of a commercial salmonellae conjugate that would cover both the somatic and flagellar antigens for 31 *Salmonella* serotypes of O groups A through S. These represented the vast majority of serotypes isolated from outbreaks of food poisoning in the United States during the years 1965 to 1970. The results of this cooperative effort became the basis for the specifications compiled and distributed to manufacturers by the Center for Disease Control (CDC) (1), which also evaluates premarket reagents for the manufacturers. Experience has shown that analyzing conjugates, identifying the factors responsible for deficiences, and suggesting means of improvement results in the production of better diagnostic reagents.

In this paper we describe the results obtained when we evaluated Salmonella polyvalent conjugates produced by Difco, Sylvana Co., and Clinical Sciences, Inc., for both their physicochemical and performance characteristics.

MATERIALS AND METHODS

Fluorescein-labeled conjugates. Bacto FA Salmonella Panvalent was a premarket sample of a Difco reagent. It was a lyophilized anti-*Salmonella* conjugate prepared with selected strains of *Salmonella* and *Arizona* representing somatic (O) antigens A through O64 and all flagellar (H) antigens in the genus *Salmonella*.

Bacto FA Salmonella Poly was a lyophilized Difco conjugate for the O antigens of *Salmonella* O groups A through S and H factors a-z, z4, z6, z10, z13, z15, z23, z24, z27, z28, z29, z32, z35, z38, and z42.

Salmonella Polyvalent OH Globulin (Groups A through S) produced by Sylvana Company was a lyophilized conjugate prepared with motile organisms representing the somatic antigens of *Salmonella* O groups A through S.

Salmonella Fluoro-kit produced by Clinical Sciences, Inc. contained a lyophilized conjugate prepared with motile organisms of *Salmonella* O groups A through S.

Physicochemical analysis. We determined the total protein per milliliter, the amount of fluorescein isothiocyanate (FITC), and the fluorescein to protein (F/P) ratio of each conjugate. In addition, cellulose acetate strip electrophoresis (CASE) gave a fluorescence profile, which included the unreacted fluores-

cent material (UFM), and a protein profile with the relative percentages of each serum protein (6).

Protein concentrations were measured by the biuret method with a Beckman DB spectrophotometer. FITC was measured as protein-bound FITC by absorbance at λ max (near 495 nm) in 0.1 N NaOH and related to a pure fluorescein diacetate reference standard. The F/P ratio was calculated from the FITC and protein measurements and expressed as micrograms of protein-bound FITC per milligram of protein. The protein compositions were determined by CASE with the Beckman Microzone equipment and procedure using uncleared membranes. The preparation of uncleared membranes for densitometric analysis and the interpretation of the densitometer tracings have been described (4). The presence of unlabeled protein and UFM were determined by comparing the fluorescence and protein profiles on a CASE membrane in the following manner. After electrophoresis, but before Ponceau S staining, a membrane was examined under a Wood's light (366 nm), and the number and location of fluorescent bands were recorded. The protein profile was visible after the membrane was stained with Ponceau S. Thus a nonfluorescent protein band or a nonprotein fluorescent band were easily noted by comparing the profiles.

Performance testing: (i) Strains. We used 133 *Salmonella* serotypes representing O groups A through O64. In addition, the following non-*Salmonella* strains were tested: 36 *Arizona* representing O groups 1 through 34; 142 *Escherichia coli* representing O groups 1 through 145; 32 *Citrobacter freundii* representing O groups 1 through 31; 13 *Providence* representing O groups 1 through 29; 15 *Serratia* representing O groups 1 through 15; 35 *Shigella* representing O groups A through D; five strains each of *Proteus morganii*, *P. vulgaris*, *P. rettgeri*, and *P. mirabilis*; five strains of *Pseudomonas fluorescens*; two strains each of *Pseudomonas diminuta*, *P. maltophilia*, *P. aeruginosa*, *P. putida*, and *P. stutzeri*; and one strain each of *Pseudomonas alcaligenes* and *P. denitrificans*.

(ii) Specimens. Various food, feed, and environmental samples were examined by both FA and cultural procedures. Cultural methods were those recommended by Galton et al. (2).

(iii) Titration of conjugates. Twofold dilutions of the Difco and the Sylvana conjugates were used to stain smears of *Salmonella* serotypes representing O groups A through S. The staining titer was defined as the highest dilution which gave a 4+ fluorescence intensity of cells walls. The routine test dilution was one dilution below the titer. The Clinical Sciences reagent was tested at the dilution obtained by rehydrating as recommended by the manufacturer.

(iv) Preparation and staining of smears with fluorescent antibodies. Smears of pure cultures of *Salmonella* or other organisms were made either from saline suspensions or directly from nutrient broth cultures. Specimens were cultured first in either selenite broth or tetrathionate broth containing brilliant green dye. Smears on Teflon-coated slides were prepared by the method of Goldman (3). After they were dried, the slides were fixed for 3 min in a solution containing six parts of absolute ethanol, three parts of chloroform, and one part of 37% formaldehyde solution. The fixed slides were then rinsed with 95% ethanol and allowed to air dry.

Fixed smears were stained for 30 min at room temperature in a moist chamber. After the conjugate was drained off, the slides were rinsed briefly with agitation in a bath of phosphate-buffered saline at pH 7.6, put in a bath of fresh phosphate-buffered saline for 10 min, dipped in a bath of distilled water, and allowed to drain dry. A cover glass was mounted on each slide with buffered glycerol (pH 9), and the smears were examined for fluorescing organisms.

(v) Fluorescence equipment. Both a Leitz Ortholux microscope fitted with an oil immersion condenser and an Osram HBO-200 mercury vapor lamp and a Zeiss IF microscope with an oil immersion condenser and a 100-W iodine quartz lamp were used in this study. The primary filter for the Leitz microscope was a Schott BG 12 (3 mm), and the ocular filter was either an OG-1 or a Leitz blue-absorbing filter. The primary filter for the Zeiss microscope was a KP 500 interference filter, and the ocular filter was the Zeiss 50.

RESULTS

Physicochemical characteristics. The protein profiles obtained by CASE are shown in Fig. 1. Note the similarity of all profiles except the one for the packaged Clinical Sciences conjugate. The data from these profiles and other physicochemical analyses are presented in Table 1. The Difco panvalent and polyvalent conjugates were approximately 50 and 58% gamma globulin, respectively, with 3% albumin. The panvalent reagent had a higher protein concentration than the polyvalent, but its lower percentage of gamma globulin made the two reagents similar in gamma globulin concentration with 13 and 11 mg/ml. The Difco conjugates also had similar F/P ratios of 9.5 and 8.8, but the panvalent reagent contained an appreciable amount of UFM. The Sylvana conjugate was 81% gamma globulin with <1%

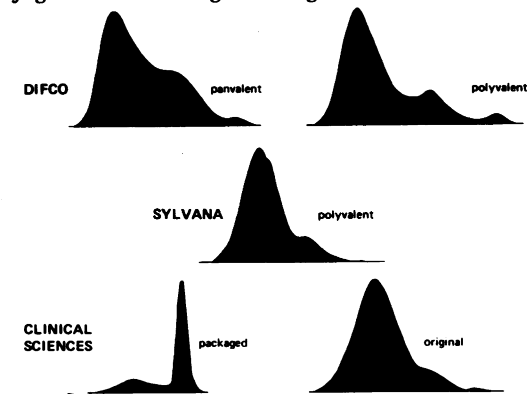

FIG. 1. *Protein electrophoretic profiles of Salmonella conjugates.*

TABLE 1. *Physicochemical characteristics of Salmonella conjugates*

Conjugate characteristics	Difco		Sylvana	Clinical Sciences	
	Panvalent	Polyvalent	Polyvalent	Packaged	Original[a]
CASE[b]					
Gamma globulin (%)	50	58	81.0	22[c]	86.5
Beta-alpha globulins (%)	47	39	18.4	2[c]	13.0
Albumin (%)	3	3	0.6	+[c]	0.5
Unlabeled protein (%)	0	0	0	75[c]	0
UFM[d]	++	−	±	−	−
Total protein (mg/ml)	26.0	19.5	19.8	nd[e]	17.5
Bound FITC[f] (μg/ml)	248.0	174.0	672.0	nd	574.0
F/P[g] ratio (μg/mg)	9.5	8.8	33.9	30[h]	32.8
Gamma globulin (mg/ml)	13	11	16	nd	15

[a] Conjugate prior to addition of albumin stabilizer and packaging.
[b] Cellulose acetate strip electrophoresis.
[c] Approximate value.
[d] Unreacted fluorescent material.
[e] Not done, too diluted for analysis.
[f] Fluorescein isothiocyanate.
[g] Fluorescein to protein.
[h] Approximate value based on electrophoretic migration.

albumin. Its gamma globulin concentration was 16 mg/ml, its F/P ratio was 33.9, and it contained a trace of UFM. The Clinical Sciences packaged conjugate was approximately 75% unlabeled albumin with an estimated 22% gamma globulin, 2% beta-alpha globulins, and a trace of labeled albumin. The fluorescein-labeled fraction of the protein was approximately 90% gamma globulin, and its anodic position after electrophoresis indicated an approximate F/P ratio of 30 (6). No further analysis was attempted with this conjugate, and Clinical Sciences very kindly supplied us with some of the original conjugate without the albumin stabilizer. This Clinical Sciences reagent was 86.5% gamma globulin with only 0.5% albumin. Its gamma globulin concentration was 15 mg/ml, and its F/P ratio was 32.8.

Performance characteristics: (i) Salmonella. For complete testing of the somatic antibodies of the conjugates, it was necessary to select one or more specific serotypes to represent each *Salmonella* O group. These are shown in Table 2. The working dilutions were determined to be 1:2 for the Difco panvalent conjugate and 1:8 for both the Difco and the Sylvana polyvalent conjugates. The working dilution of the Clinical Sciences reagent was predetermined by the manufacturer and was ready for use when rehydrated as recommended. The four conjugates stained all serotypes tested of *Salmonella* O groups A through S with a 4+ fluorescence intensity. The working dilutions of the Difco and Sylvana conjugates were tested against *Salmonella* serotypes representing O

TABLE 2. *Salmonella serotypes used for determining diagnostic dilutions of commercial Salmonella conjugates*

Serotype	O group
Salmonella paratyphi A	A
S. typhimurium	B
S. schwarzengrund	B
S. thompson	C_1
S. virginia	C_2
S. newport	C_2
S. kentucky	C_3
S. javiana	D_1
S. fresno	D_2
S. anatum	E_1
S. newington	E_2
S. illinois	E_3
S. senftenberg	E_4
S. rubislaw	F
S. poona	G
S. cubana	G
S. carrau	H
S. florida	H
S. gaminara	I
S. carmel	J
S. michigan	J
S. cerro	K
S. minnesota	L
S. aderike	M
S. dakar	M
S. urbana	N
S. alachua	O
S. lansing	P
S. inverness	P
S. champaign	Q
S. duval	R
S. waycross	S

groups T through O64. These results are shown in Table 3. The performance characteristics reported were based on fluorescence intensity of bacterial cell walls. However, fluorescent bacteria with attached fluorescing flagellae are additional evidence of the presence of *Salmonella*. We, therefore, cultured all salmonellae in Trypticase soy-tryptose broth to enhance flagella production. No attempt was made to determine which H antigen factors were present, but we noted that all salmonellae exhibited fluorescent flagellae with each conjugate tested.

(ii) Arizona. Of the 36 strains of *Arizona* tested, 33 fluoresced 2+ or better with one or more of the conjugates. Difco's panvalent conjugate stained 32 (89%) of all strains tested. The Difco polyvalent conjugate stained 15 (42%), and both the Sylvana and the Clinical Sciences conjugates stained 14 (39%) of the *Arizona* strains. These results are shown in Table 4.

(iii) E. coli. Difco's panvalent and polyvalent conjugates and the Clinical Sciences reagent stained 20 of 142 (14%) of the *E. coli* strains tested at 2+ or better intensity. The Sylvana conjugate stained 15 (11%) of the strains. These results are shown in Table 5.

(iv) Citrobacter. Difco's panvalent reagent stained 9 (27%) of the 33 *Citrobacter* strains at a 2+ intensity. The Difco polyvalent conjugate stained 55 (15%), the Sylvana conjugate stained 6 (18%), and the Clinical Sciences reagent stained 4 (12%) of the strains on which they were tested. These results are shown in Table 6.

(v) Providence and Serratia. None of the 13 *Providence* or the 15 *Serratia* strains tested were stained at more than a 2+ intensity by any of the conjugates with the exception of *Serratia* O11 which was stained 2 to 3+ by the Difco panvalent conjugate.

(vi) Shigella. None of the 35 *Shigella* strains examined was stained at more than a 2+ fluorescence with any of the conjugates.

(vii) Proteus. One of the five strains of *P. mirabilis* was stained at a 2+ intensity with the Difco panvalent reagent, but was negative with the other conjugates. The other 19 strains of *P. morganii*, *P. vulgaris*, *P. rettgeri*, and *P. mirabilis* were negative with all of the conjugates.

(viii) Pseudomonas. None of the 17 *Pseudomonas* strains tested was stained at more than a 2+ intensity with any of the conjugates, except for one strain each of *P. maltophilia* and *P. putida* which stained at a 3+ intensity with the Difco panvalent reagent.

(ix) Specimens examined. Six environmental swab specimens obtained at a fishmeal plant were stained with the Difco panvalent conjugate. Of these, four were positive. Only one was confirmed by culture, and salmonellae were isolated from one of the FA negative samples. Of the six swab samples of raw fish that we examined, two were FA positive, and one of these was confirmed by culture.

A total of 204 samples of various foods and

TABLE 3. *Staining reactions of diagnostic dilutions of Salmonella polyvalent conjugates with Salmonella O groups T through O64*

Antigen	O group	Difco panvalent (1:2)	Difco polyvalent (1:8)	Sylvana (1:8)
Salmonella nairobi	T	4+	2-4+	4+[a]
S. berkeley	U	4+	4+[a]	4+[a]
S. niarembe	V	4+	±	–
S. deversoir	W	2-3+	4+[a]	–
S. bergen	X	4+	–	–
S. dahlem	Y	4+	4+[a]	–[b]
S. greenside	Z	4+	4+[a]	–[b]
S. treforest	O51	4+	4+[a]	4+[a]
S. utrecht	O52	4+	–	–
S. humber	O53	4+	4+[a]	4+[a]
S. uccle	O54	4+	2-4+	4+
S. tranoroa	O55	4+	–	–
S. artis	O56	4+	4+[a]	–[b]
S. locarno	O57	4+	4+[a]	–[b]
S. basel	O58	4+	3+[a]	4+[c]
S. betioky	O59	2-3+	±	–
S. luten	O60	4+	4+[a]	–
S. eilbeck	O61	4+	–	–
S. O64	O64	4+	–[b]	–

[a] Only an occasional bacterium fluoresced 4+.
[b] Most bacteria showed speckled cell wall fluorescence.
[c] Approximately 50% of bacteria fluoresced 4+.

TABLE 4. *Heterologous reactions of Salmonella polyvalent conjugate dilutions with Arizona serogroups*

O group[a]	Difco panvalent (1:2)	Difco polyvalent (1:8)	Sylvana (1:8)	Clinical Sciences
1,2	4+	–	–	–
1,3	4+	–[b]	–	–
1,4	4+	–	–	–
5	4+	–[b]	–	–
6	4+	±	–	–
7ab	3+	4+	4+	4+
7ac	4+	4+	4+	4+
8	4+	±	–	–
9ab	2+	±	–	–
9ac	4+	–	–	–
10ab	4+	4+	4+	4+
10ac	4+	4+	4+	3+
11	4+	–[b]	–[b]	–
12	2+	4+	4+	2-4+
13	3+	4+	4+	4+
14	4+	2-4+	–[b]	–
15	3-4+	3-4+	–[b]	–
16	4+	4+	4+	4+
17	4+	4+	4+	4+
18	4+	4+	4+	4+
20	4+	4+	4+	4+
22	4+	4+	4+	4+
23	4+	–[b]	–	–
24	4+	–	–	–
25	1-4+	4+[c]	4+[c]	–
26	4+	–[b]	–	–
27	4+	4+	4+	4+
28	1+	±	2+	1-2+
29	4+	3+[c]	–	–
31	1-4+	–[b]	–[b]	–
32	1-3+	2-4+	4+	4+
33	4+	–[b]	–	–
34	4+	±	–[b]	–

[a] Tested 36 strains of *Arizona* representing O groups 1 through 34. Only those strains showing 2+ or better fluorescence are listed.

[b] Most bacteria showed a speckled fluorescence of the cell wall.

[c] An occasional bacterium fluoresced 4+.

feeds were examined by using the Clinical Sciences Fluoro-kit. Of these, 151 samples were positive by FA, and salmonellae were isolated from 139 of the 151 samples. There were 12 samples positive by FA that were negative by culture. No samples were FA negative and culturally positive. For each type of specimen the percentage that was FA positive but culturally negative was calculated as the percentage agreement between FA and culture (Table 7). The percentage of FA positive samples that were negative by culture varied for each food type and ranged from 0 to 17%.

TABLE 5. *Heterologous reactions of Salmonella polyvalent conjugate dilutions with Escherichia coli serogroups*

O group[a]	Difco panvalent (1:2)	Difco polyvalent (1:8)	Sylvana (1:8)	Clinical Sciences
O3	±	2+	–	2+
O9	1+	2+	–	1-2+
O11	3+	4+	4+	4+
O17	2+	4+	3+	2+
O18ac	4+	2+	±	4+
O21	3-4+	4+	3+	4+
O23	3+	1+	1+	2+
O35	3+	–	–	–
O40	3+	–	–	–
O44	3+	3+	3-4+	4+
O46	2+	–	–	–
O62	2+	2+	1+	2+
O66	–	3+	2+	1-2+
O68	2+	3-4+	3+	4+
O70	–	3+	4+	4+
O73	1+	3+	4+	4+
O75	2+	4+	4+	4+
O77	2-3+	4+	3+	4+
O78	3+	–	–	–
O85	3+	4+	4+	4+
O100	1+	2+	–	–
O106	3-4+	4+	4+	4+
O111	4+	4+	4+	4+
O118	2+	–	–	–
O120	3+	–	3-4+	–
O127	3+	4+	4+	4+
O135	–	2+	1+	2+

[a] Tested 142 strains of *E. coli* representing O groups 1 through 145. Only those strains showing 2+ or better staining are listed above.

TABLE 6. *Heterologous reactions of Salmonella polyvalent conjugate dilutions with Citrobacter freundii serogroups*

O group[a]	Difco panvalent (1:2)	Difco polyvalent (1:8)	Sylvana (1:8)	Clinical Sciences
9ab	2+	–	–	–
10, 9b	2+	–	–	–
17	2+	–	±	–
18	–	–	2+	–
19	1-2+	–	±	–
20	–	2+	4+	2+
21ab	4+	4+	4+	4+
22	4+	4+	4+	4+
23	3-4+	4+	4+	3+
24	4+	–	–	–
28	±	2+	2+	–
29	4+	–	–	–

[a] Tested 33 *C. freundii* strains representing O groups 1 through 31. Only those strains showing 2+ intensity or better are listed.

Another group of 769 samples of various types was examined with the Difco polyvalent conjugate. An overall agreement of 90.0% was obtained between FA and culture results. The percent agreement for each food group ranged from a low of 65.1% on raw turkey samples to a high of 100.0% on dried egg powders. One sample of meat meal which was negative by FA yielded one colony of *Salmonella* on one of three brilliant green agar plates.

DISCUSSION

Analysis of the data in Table 1 revealed three distinctly different concepts of basic reagent composition. (i) The Difco conjugates were less than 60% gamma globulin with 3% albumin, contained approximately 12 mg of gamma globulin per ml, and had F/P ratios of less than 10. (ii) The Sylvana conjugate and the undiluted prepackaged Clinical Sciences reagent were above 80% gamma globulin with less than 1% albumin, contained approximately 15 to 16 mg of gamma globulin per ml, and had F/P ratios above 30. (iii) The Clinical Sciences packaged reagent was prediluted before lyophilizing, and 75% of its total protein was unlabeled albumin. However, each of the conjugates met all performance requirements.

A direct comparison of two of these conjugates, Difco and Sylvana polyvalent, is interesting. Their working dilution titers were each 1:8, but the Sylvana conjugate contained nearly 50% more gamma globulin per milliliter and had about four times the F/P ratio of the Difco conjugate. If these reagents had been prepared from the same batch of antiserum, the reagent with the highest F/P ratio should have given the highest titer, unless the lower F/P ratio was an optimal one for *Salmonella*. This seems unlikely in view of our studies on samples of polyvalent *Salmonella* globulin labeled at various F/P ratios. In these studies, titers increased with increasing F/P ratios up to approximately 25 to 30. This indicates that an F/P ratio of 25 to 30 is optimal for polyvalent *Salmonella* reagents. Perhaps higher F/P ratios on the Difco conjugates would have resulted in higher staining titers.

Both UFM and appreciable amounts of labeled albumin should be eliminated from conjugates. Both have been shown to contribute to nonspecific staining (5). Although there was no evidence during this study of interference by either the UFM or albumin present in the Difco reagents, use of the conjugates on tissue sections could result in background staining.

All of the conjugates tested stained representative strains of *Salmonella* O groups A through S at maximal (4+) fluorescence with their appropriate routine test dilution. However, the Difco panvalent reagent which covers *Salmonella* O groups A through O64 did not stain all of the higher O groups 4+ with the routine test dilution determined for O groups A through S. At a 1:2 dilution, *Salmonella deversoir* (O45) and *S. betioky* (O59) were stained at a 2 to 3+ intensity. If the titer decreased at all during storage, this reagent would be unsatisfactory. However, this was not tested in our laboratory.

The Difco and Sylvana polyvalent conjugates gave varying degrees of fluorescence with a number of *Salmonella* serotypes of O groups 42 through 64. This was expected because of the known existence of shared antigens among the various salmonellae.

When the three polyvalent reagents for *Salmonella* O groups A through S were tested with both homologous and heterologous antigens, little difference in activity was observed. The pattern of cross-reactions obtained was similar to that reported by Thomason and Wells (10) who prepared and tested a CDC polyvalent

TABLE 7. *Comparison of FA and culture results from food specimens tested with the Salmonella Fluoro-kit*

Foods	Total no. of samples examined	No. FA +	No. culture +	Agreement[a] (%)	FA +, culture − (%)
Meat meal	41	39	38	98	2
Chicken (swabs)	29	8	3	83	17
Pet food	42	39	35	91	9
Pork sausage	21	3	2	95	5
Nonfat dry milk	10	1	0	90	10
Egg powder	61	61	61	100	0
Totals	204	151	139	94	6

[a] No samples were FA − and culture +.

conjugate for only 28 *Salmonella* serogroups as compared with the 31 covered by these reagents. The major cross-reacting groups with both the commercial and the CDC conjugates were *Arizona*, *E. coli*, and *Citrobacter*.

The Difco panvalent conjugate was much more reactive with heterologous organisms. It stained 89% of the *Arizona* strains compared with 42% stained by the Difco polyvalent reagent and 39% stained by Sylvana and Clinical Sciences reagents. The Difco panvalent and polyvalent conjugates and the Clinical Sciences conjugate each stained 14% of the *E. coli* tested, whereas the Sylvana conjugate stained 11% of the strains tested. The Difco panvalent conjugate stained 27% of the *Citrobacter* strains tested, whereas the Difco polyvalent reagent stained 15%, the Sylvana stained 18%, and the Clinical Sciences reagent stained 12% of the strains tested. The Difco panvalent conjugate was more reactive with the other genera tested; it stained a *Serratia*, a *Proteus*, and two *Pseudomonas* cultures. This increased cross-reactivity may make it impractical to use the Difco panvalent conjugate in routine practice, although too few specimens have been examined to permit final conclusions as to its effectiveness.

The evaluation of the Clinical Sciences Fluoro-kit on food and feed samples yielded results similar to those reported by Insalata et al. (7). They reported 97.5% agreement between FA and culture when the Fluoro-kit was used to examine 120 samples, whereas we found a 94.1% agreement on 204 samples. We did not experience the false-positive FA results reported by Insalata and co-workers caused by the washing of salmonellae from one slide well to another. However, unless care is taken during fixation, organisms can be flushed from one well to another by the stream of fixative. We directed the tip of the squeeze bottle at the center of the slide, allowing the fixative to flow from the center into each row of wells, thereby minimizing the chance of wash-over.

Our results with the Difco polyvalent reagent (A through S) on 769 samples of food and feed were almost identical to those reported by Insalata et al. (8). They found 90.8% agreement between FA and culture results on 250 samples when they used a mixture of Difco O (A through S) and Difco H (A through S) conjugates which was later supplied as the Difco polyvalent (A through S) reagent. The latter reagent yielded a 90.0% agreement in our studies.

The difference in the percent agreement among the various food groups is interesting (Tables 7 and 8). In every case but one (Table 8), the dehydrated products gave better than 90.0% agreement between the FA and cultural results. This is probably due to reduction of competitive bacteria resulting from industrial processing. The one exception was the food supplement. This was a large batch of material that originally had a most probable number (MPN) of over 10 *Salmonella* per 100 g, but when examined by us had an MPN of 3.6 *Salmonella* per 100 g. We think that in these samples, FA positive results that could not be confirmed by culture were caused by failure of the culture procedure to reveal viable *Salmonella*. The FA technique gave more unconfirmed positive results on smears from raw meat and poultry than on other foodstuffs. These products are not usually cultured in a preenrichment broth before going into enrichments. Smears made directly from the selective enrichment broths frequently contain fat and

TABLE 8. *Comparison of FA and cultural results from food specimens tested with the Difco polyvalent (A through S) Salmonella conjugate*

Foods	Total no. of samples examined	No. FA +	No. culture +	Agreement[a] (%)	FA +, culture − (%)
Meat meal	128[a]	75	69	94	6
Turkey parts	89	53	22	65	35
Spices	152	37	32	97	3
Food supplement	96	68	56	88	12
Frosting mixes	56	18	16	96	4
Chicken livers	80	37	28	89	11
Pork sausage	28	9	4	82	18
Nonfat dry milk	84	32	28	95	5
Egg powder	56	32	32	100	0
Totals	769	361	287	90	10

[a] One sample was FA − and culture +.

tissue debris that make interpretation difficult. Often the salmonellae may be overgrown by other enteric organisms, so that they are not detectable by culture although they are still detectable by FA tests. Agreements between FA and culture are biased in favor of the culture procedure, so they should be interpreted carefully before assessing the value of FA procedures for the detection of salmonellae in any type of sample.

We found a post-selective enrichment culture most helpful in eliminating background material in specimens to be examined by FA. We inoculated 2 ml of Trypticase soy-tryptose broth, prepared by mixing equal parts of Trypticase soy broth (BBL) and tryptose broth (Difco), with 0.05 to 0.1 ml of the selective enrichment broth culture. The new culture was incubated for 2 h in a 37-C water bath before smears were made and stained with the FA conjugates. This procedure eliminated many false-positive FA results. Specimens, such as dried milk, which require pre-enrichment prior to inoculation into either selenite or tetrathionate broth, yielded FA smears with little or no background material. This type of specimen did not require the postenrichment procedure; however, for simplicity, all specimens can be post-enriched without detrimental effect. When time was not a factor, we also subcultured the primary selective enrichment broth to a second selective enrichment broth for an additional 24 h of incubation with equally good results. Another procedure that has proved useful in eliminating background material while allowing multiplication of salmonellae is the microcolony technique described previously (9). This technique is more tedious than inoculating a post-selective enrichment broth and may not prove to be practical when processing large numbers of samples.

In our laboratory, the three commercial polyvalent reagents for *Salmonella* O groups A through S performed satisfactorily and met the CDC specifications. The major difference in the three conjugates is that the Clinical Sciences reagent is available only as a component of the *Salmonella* Fluoro-kit, and it has a predetermined test dilution which is obtained by rehydrating the product as recommended by the manufacturer. The Difco and Sylvana conjugates are marketed as lyophilized undiluted reagents which require the user to determine their routine test dilution.

With the advent of satisfactory commercial reagents, and with appropriate FA equipment, a well-trained technologist should be able to use FA techniques to advantage for detecting salmonellae in a variety of products. The economics of screening samples for salmonellae by FA tests depend to a large extent upon the fraction of samples expected to be positive. Laboratories expecting large numbers of *Salmonella*-contaminated samples may not find FA procedures as helpful as those laboratories in which only a small proportion of samples are positive by FA tests and, therefore, require confirmation by culture. All FA positive results must be confirmed by culture methods because of antigenic similarities among the enteric organisms. FA negative results should eliminate the need for further culture work.

LITERATURE CITED

1. Center for Disease Control. 1973. Specifications and evaluation methods for laboratory, immunological, and microbiological reagents, vol. 1. Bacterial, fungal, and parasitic reagents. 4th ed. U.S. Dept. of Health, Education, and Welfare, Center for Disease Control, Atlanta, Ga.
2. Galton, M. M., G. K. Morris, and W. T. Martin. 1968. Salmonellae in foods and feeds. Review of isolation methods and recommended procedures. U.S. Dept. of Health, Education, and Welfare, Communicable Disease Center, Atlanta, Ga.
3. Goldman, M. 1968. Fluorescent antibody methods, p. 148-149. Academic Press Inc., New York.
4. Hebert, G. Ann, Patricia L. Pelham, and Bertie Pittman. 1973. Determination of the optimal ammonium sulfate concentration for the fractionation of rabbit, sheep, horse, and goat antisera. Appl. Microbiol. **25**:26-36.
5. Hebert, G. Ann, Bertie Pittman, and W. B. Cherry. 1967. Factors affecting the degree of nonspecific staining given by fluorescein isothiocyanate labeled globulins. J. Immunol. **98**:1204-1212.
6. Hebert, G. Ann, Bertie Pittman, Roger M. McKinney, and William B. Cherry. 1972. The preparation and physicochemical characterization of fluorescent antibody reagents. U.S. Dept. of Health, Education, and Welfare, Center for Disease Control, Atlanta, Ga.
7. Insalata, N. F., W. G. Dunlap, and C. W. Mahnke. 1973. Evaluation of the *Salmonella* Fluoro-Kit for fluorescent antibody staining. Appl. Microbiol. **25**:202-204.
8. Insalata, N. F., C. W. Mahnke, and W. G. Dunlap. 1972. Rapid, direct fluorescent antibody method for the detection of salmonellae in food and feeds. Appl. Microbiol. **24**:645-649.
9. Thomason, Berenice M. 1971. Rapid detection of *Salmonella* microcolonies by fluorescent antibody. Appl. Microbiol. **22**:1064-1069.
10. Thomason, Berenice M., and Joy G. Wells. 1971. Preparation and testing of polyvalent conjugates for fluorescent antibody detection of salmonellae. Appl. Microbiol. **22**:876-884.