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Control of Nonspecific Staining in the Fluorescent Antibody Technique for the Detection of Salmonellae in Foods

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A fluorescent antibody conjugate, prepared from the IgG (immunoglobulin G) fraction of *Salmonella* polyvalent flagellar antiserum, gave better specific staining intensities and significantly lower nonspecific staining than did conjugates prepared from globulin fractions of ammonium sulfate-fractionated *Salmonella* polyvalent antisera. IgG was purified by affinity chromatography against protein A, a normal cell wall component of *Staphylococcus aureus*. Affinity chromatography yielded high-purity IgG in a one-step purification procedure. The conjugate prepared from affinity-purified IgG was compared with commercially available fluorescent antibody conjugates for the detection of salmonellae in retail samplings of meats and poultry and gave better correlations with the cultural method than did the commercial conjugates.

The direct fluorescent antibody (FA) technique for the detection of salmonellae in foods was accorded Official First Action status by the Association of Official Analytical Chemists in 1975 (2). The FA technique has several advantages over the conventional cultural methods. Isolation of pure cultures is not necessary for this technique, and results can be obtained within 50 to 55 h of sampling. However, the technique needs further refinement before it finds wide applications as a screening method for the detection of salmonellae in foods and feeds. There is great need for conjugated antisera of high specificity and high titers (14).

Nonspecific staining (NSS) is a major problem associated with the FA technique. The complex phenomenon of NSS depends upon several factors, including the quality of the antiserum, the quality of the fluorescent dye, the method of conjugation of the dye to the protein, the presence of unreacted fluorescent material in the conjugate, the dye protein ratio of the conjugate, and the titer of the conjugate (22). Although several of the above factors have been investigated and optimal conditions for reducing NSS have been established, very little attention has been given to the quality of the antiserum and the effects of different components of the antiserum on NSS.

The treatment of FA preparations with chelated azo dyes has been reported to reduce NSS without affecting the specific staining intensities (19). Erichrome black T was used to reduce NSS in clinical specimens infected with salmonellae and stained with an antisalmonella conjugate (28). Flazo-orange has been recommended as a counterstain for reducing NSS in the examination of rendered animal by-products (11) and swine tissues (20) for salmonellae. However, flazo-orange reduced the intensity of specific staining of *Salmonella typhimurium* with an antisalmonella conjugate (28).

Fractionation with ammonium sulfate is the currently accepted procedure for purifying the antiserum before conjugation with fluorescein isothiocyanate (FITC). Ammonium sulfate fractionation of rabbit antiserum yields a product containing approximately 64% gamma globulins, 35% other globulins, and 1% albumin (23). Some research workers have further purified the antiserum and isolated the immunoglobulin G (IgG) fraction by applying techniques such as diethylaminoethyl (DEAE)-cellulose chromatography (13), chromatography on DEAE-Sephadex columns (9), and zonal centrifugation (39). In each instance, the investigators reported a significant reduction in nonspecific fluorescence without any change in specific staining intensity. However, these purification procedures were cumbersome and time consuming and were not adaptable to routine preparation of conjugates.

In this investigation, affinity chromatography was used for the rapid, single-step purification of IgG from *Salmonella* polyvalent antiserum. The specific and nonspecific staining characteristics of FITC-labeled IgG and labeled globulin fraction of antisera were compared for use in

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examining raw meats and poultry samples for salmonellae.

MATERIALS AND METHODS

Cultures. *Staphylococcus aureus* ATCC 12598 was obtained from Earl Edwards, Naval Health Research Center, San Diego, Calif. Serotypes of salmonellae were obtained from B. M. Thomason, Center for Disease Control, Atlanta, Ga. Cultures of nonmotile salmonellae, *Arizona hinshawii* (*Salmonella arizonae*), *Citrobacter freundii, Escherichia coli, Proteus vulgaris*, and *Pseudomonas fluorescens* were obtained from the culture collections of the Southeast Poultry Research Laboratory and the Department of Food Science, University of Georgia. Stock cultures of salmonellae were maintained on a semisolid stab medium (37) at room temperature. Stock cultures of other bacteria were maintained on nutrient agar slants at 4°C. Before the stock cultures were used, they were activated by at least two passages through tryptic soy broth incubated overnight at 37°C.

Chemicals. Deoxyribonuclease and Sepharose (4B-200) were obtained from Sigma Chemical Co., St. Louis, Mo.; lysostaphin from Schwartz/Mann, Orangeburg, N.Y.; cyanogen bromide from J. T. Baker Chemical Co., Phillipsburg, N.J.; bovine IgG from Miles Laboratories, Elkhart, Ind.; *Salmonella* polyvalent H antiserum from the Center for Disease Control, Atlanta, Ga.; and *Salmonella* polyvalent O antiserum from Difco Laboroatories, Detroit, Mich.

FITC-conjugated globulin fractions of *Salmonella* antisera. The following FITC-conjugated globulin fractions of antisera were used in the comparative investigations using the FA technique: (i) FA *Salmonella* poly (Difco, lot no. 636734); (ii) FITC-labeled immune rabbit globulin-*Salmonella* polyvalent OH groups A through S (Sylvana, Millburn, N.J., lot no. 091170-AB); and (iii) FITC-conjugated *Salmonella* polyvalent H antiserum (E. M. Ellis, National Animal Disease Center [NADC], Ames, Iowa.) The Difco antiserum was received late and could not be included in the initial phase of the study.

Meat and poultry samples. Samples of raw meats and poultry were purchased from retail outlets in the Athens, Ga., area in 1-lb (450-g) lots, iced, and transported to the laboratory. Upon receipt of the samples in the laboratory, 25-g portions of the samples were placed immediately in enrichment broths.

Crude protein A preparation from *S. aureus*. *S. aureus* was cultured in tryptic soy broth containing 0.5% yeast extract in 1-liter Erlenmeyer flasks with four oblique indentations at the bottom. The flasks were incubated for 18 h at 37°C in a Gyrotary water bath shaker (New Brunswick Scientific Co., New Brunswick, N.J.). The cells were harvested by centrifugation and subjected to digestion by lysostaphin in the presence of deoxyribonuclease according to the procedure of Sjoquist et al. (36). After the digest was neutralized with 5 M NaOH, it was clarified by passing through a 0.2-μm, 47-mm-diameter membrane filter (Millipore Corp., Bedford, Mass.). The clarified crude digest containing protein A was applied to a column of Sepharose-IgG for the purification of protein A.

Preparation of Sepharose-IgG column. Sepharose was activated with cyanogen bromide, and bovine IgG was coupled to the activated Sepharose according to the procedure of March et al. (31). After the ligand was coupled to the gel, the gel was filtered and resuspended in 1.5 liters of 0.05 M 2-aminoethanol–0.2 M $NaHCO_3$, pH 9.0, and stirred overnight to inactivate the free active sites. The suspension was again filtered, and the cake was washed with 1 liter of a solution containing 0.1 M NaH_2PO_4 and 4 M urea, pH 6.0, and finally with 1 liter of a solution containing 0.1 M NaH_2PO_4 and 0.5 M NaCl, pH 7.0. The cake was suspended in the final buffer, and the slurry was poured into a column (1.2 by 100 cm). The column was washed with a solution containing 0.1 M NaH_2PO_4 and 0.5 M NaCl, pH 7.0, until the absorbance of the eluant at 280 nm was less than 0.01.

Purification of protein A. The clarified lysostaphin digest of *S. aureus* was applied to the Sepharose-IgG column. The column was washed with 0.1 M NaH_2PO_4–0.5 M NaCl, pH 7.0, until the absorbance of the eluant was less than 0.01. Protein A was eluted from the column with 0.1 M glycine-hydrochloride buffer, pH 3.5 (24). Fractions of eluant showing an absorbance of more than 0.8 were pooled. The pooled protein A extract was dialyzed against three changes of 0.1 M phosphate-buffered saline (PBS), pH 7.0.

Preparation of Sepharose-protein A column. The protein A solution was dialyzed at 4°C against 0.2 M $NaHCO_3$, pH 9.0. Protein A was coupled to Sepharose by the same procedure as that described for the Sepharose-IgG column. Ten milliliters of Sepharose and 4 mg of protein A per ml were used for the conjugation.

Isolation of IgG from *Salmonella* polyvalent antisera. *Salmonella* polyvalent H antiserum was dialyzed against several changes of borate-saline buffer, pH 7.0, to remove its glycerin content. *Salmonella* polyvalent O antiserum was rehydrated according to the instructions of the manufacturer. Each antiserum was separated into an IgG fraction and an eluant fraction by passing through the Sepharose-protein A column, washing the column with PBS (pH 7.0) to remove all the non-IgG components, and eluting the IgG fraction with glycine-hydrochloride buffer. The two fractions of each antiserum were dialyzed at 4°C against PBS (pH 7.0), and their protein content was determined. The protein content of each fraction was adjusted to 10 mg/ml by concentrating the solution in polyethylene glycol, molecular weight 20,000 (Sigma Chemical Co., St. Louis, Mo.). The IgG fraction of the antiserum obtained by passage through the Sepharose-protein A column will henceforth be referred to as immunopurified IgG, and the non-IgG fraction will be referred to as the eluant fraction.

Labeling with FITC. The eluant and the immunopurified IgG fractions of the two antisera were labeled separately with FITC by the dialysis labeling procedure (7). The labeled conjugate was centrifuged at 16,000 × *g* to remove fine particles. Merthiolate at a concentration of 1:10,000 was added as a preservative. The labeled conjugate was stored at 4°C until use.

Analytical methods. Protein was estimated by the biuret procedure (16), using a Beckman DU spectrophotometer and cells of 10-mm light path. The purity of protein A was checked by disc electrophoresis (5).

The identity of protein A was established by a double-immunodiffusion test (33) using Ionagar no. 2 (Consolidated Laboratories, Chicago, Ill.) in barbital buffer, pH 8.5, against human, bovine, and goat IgG. Immunoelectrophoresis of the whole antisera and immunopurified fractions was conducted by the method of Graber (17), using Ionagar no. 2 in barbital buffer, pH 8.5. The electrophoresis was conducted in a Gelman electrophoresis chamber (Gelman Instruments Co., Ann Arbor, Mich.). A relatively high voltage (5 V/cm) was applied for 1 h to minimize diffusion effects. The FITC content of conjugates was determined by the procedure of McKinney et al. (30), with fluorescein diacetate as the reference standard.

Determination of FA titers of conjugates. The FA titers of conjugates were determined by preparing 1:1, 1:2, 1:4, 1:8, 1:16, and 1:32 dilutions of the conjugates in PBS and using the dilutions to stain representative strains from different serogroups of salmonellae. The staining titer of a conjugate was defined as the highest dilution that gave maximum fluorescence intensity of the cell walls and clear-cut cell outline with all serotypes. The working dilution was one dilution below the titer.

Evaluation of staining characteristics of the FA conjugates. Twenty-five grams of dried egg white (The C. F. Sauer Co., Richmond, Va.), previously examined and found to be free from salmonellae, was rehydrated with 25 ml of a 0.3% yeast extract solution in a sterile 500-ml Erlenmeyer flask. Each flask was inoculated with known concentrations (about 10^5 organisms) of pure cultures of motile salmonellae (seven serotypes), nonmotile salmonellae (four serotypes), and non-salmonellae. Selenite cystine broth (225 ml) was added to each sample, and the samples were incubated at 37°C for 18 h. The samples were prepared for FA examination; recommended methods were followed (2).

Fluorescence microscopy. The smears were examined under a Leitz Dialux epifluorescence microscope fitted with a 75-W xenon lamp, a double KP 490 interference filter, a K 515 barrier filter, and a TK 510 dichroic beam splitter. The smears were scanned and rated for specific staining by the Association of Official Analytical Chemists procedure (2). Each smear was also evaluated for nonspecific fluorescence subjectively on an arbitrary scale of 0 to 4 as follows: 0 = negligible or complete lack of nonspecific fluorescence; 1 = subdued NSS scattered at different locations, particularly on the periphery of the smear; 2 = uniform but dull NSS throughout the smear; 3 = uniform, less intense NSS throughout the smear, with intensely fluorescing localized areas; 4 = excessive, high-intensity NSS; fluorescing cells extremely difficult to observe.

Examination of meat and poultry samples for salmonellae. Samples of raw meat and poultry were examined for salmonellae by the cultural method (40) and by the direct FA technique (2), using the various FA conjugates.

RESULTS

The yield of cells of *S. aureus* from 2 liters of tryptic soy-yeast extract broth was 34 g (wet weight). Sjoquist et al. (36) obtained 250 g of wet bacterial cells from the fermentation of *S. aureus* in 10 liters of CCY broth (3). However, in their studies, the organism was grown in stirred fermentors with a continuous supply of sterile air to the organisms; this procedure may have contributed to the higher yields. Affinity-purified protein A, when subjected to disc electrophoresis, revealed a single protein band (Fig. 1). In immunodiffusion tests, the isolated material reacted with human, bovine, and goat IgG to form distinct precipitin bands. The precipitin bands with IgG from the three species formed a continuous band that indicated complete identity of reaction (Fig. 2).

When the Sepharose-protein A column was used to separate IgG from the other components of *Salmonella* polyvalent antisera, the column could be made essentially free of all substances except bound IgG within 1 h after the sample was applied. IgG was eluted as a single peak

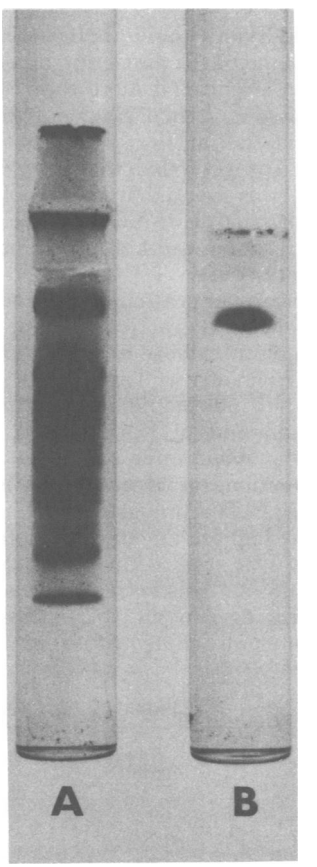

FIG. 1. *Polyacrylamide gel electrophoresis pattern of (A) crude protein A preparation and (B) purified protein A preparation.*

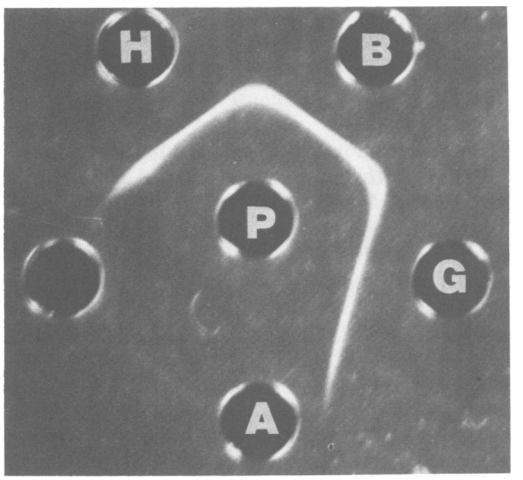

FIG. 2. *Immunodiffusion reaction of protein A against IgG from various species. P, Protein A; H, human IgG; B, bovine IgG; G, goat IgG; A, bovine serum albumin.*

with glycine-hydrochloride buffer (Fig. 3). The original volume of the antiserum applied to the column was 10 ml, and more than 90% of the IgG was eluted in 18 ml of glycine-hydrochloride buffer. Thus, the purification did not lead to a high dilution of IgG. When the purity of affinity-purified IgG was checked by immunoelectrophoresis against goat anti-rabbit whole serum, a single precipitation arc indicated that the IgG was essentially pure.

The physicochemical characteristics of fluorescein-labeled IgG fractions, eluant fractions, and ammonium sulfate-fractionated globulin fractions are shown in Table 1. Although the fractions were labeled under identical conditions, the amount of FITC bound to eluant fractions was about three times that bound to the IgG fractions of H and O antisera. The commercial FITC-conjugated OH antiserum showed the highest amount of protein-bound FITC. However, this conjugate also had the maximum protein concentration. In a comparison of fluorescein protein (F/P) ratios of different conjugates, all conjugates except H IgG were observed to have F/P ratios ranging from 22.8 to 32.2, whereas the H IgG had an F/P ratio of 10.2. The FA titer of the H IgG fraction was, however, greater than that of all other conjugates except the OH conjugate.

The six conjugates were evaluated for their specific and NSS characteristics on smears prepared from broth cultures of egg white samples inoculated with motile salmonellae, nonmotile salmonellae, and other organisms. Egg white was selected as the menstruum because it has been implicated in NSS (18). H IgG gave the highest staining intensities with all seven serotypes of motile salmonellae (Table 2). Specific staining of motile salmonellae varied from 1+ to 3+ for the H eluant fraction. The H eluant fraction did not stain *Salmonella typhi*. Similar results were obtained for O IgG and O eluant conjugates. However, the distinctions were not as sharply defined as those for H fractions. The ammonium sulfate-fractionated H conjugate gave 4+ staining for two serotypes and 3+ staining for four serotypes. *S. typhi* was stained to an intensity of 2+. The ammonium sulfate-fractionated OH conjugate stained all seven serotypes at an intensity of 4+. The mean specific staining intensities for all the seven serotypes with six conju-

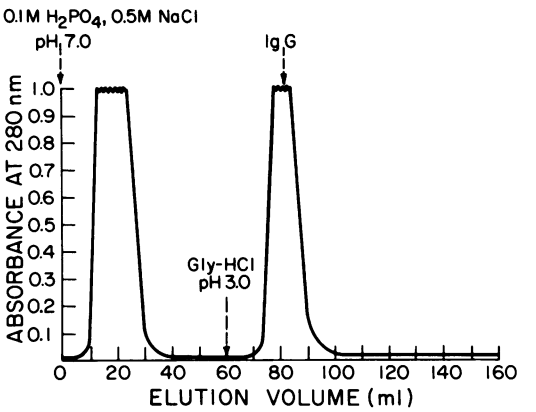

FIG. 3. *Elution profile of IgG from Sepharose-protein A column. Column dimensions, 1.0 by 10 cm; bound protein A, 4 mg/ml; flow rate, 48 ml/h.*

TABLE 1. *Physicochemical characteristics of FA conjugates*

Conjugate	Protein (mg/ml)	Protein-bound FITC (μg/ml)	F/P ratio	FA titer	Working titer
H eluant	4.2	106.7	25.1	2	1
H IgG	3.0	30.8	10.2	8	4
O eluant	4.2	137.8	32.4	4	2
O IgG	1.5	34.2	22.8	4	2
H (NADC)	0.75	24.9	33.2	2	1
OH (Sylvana)	20.0	535.0	26.8	16	8

gates indicated that the H IgG and OH conjugates were superior to O IgG, H, H eluant, and O eluant conjugates. Specific intensities were very low with conjugates of the H and O eluants.

An examination of the NSS values for the six conjugates revealed that H IgG and O IgG conjugates gave significantly lower values than the other four conjugates. The NSS observed with conjugates of OH and H eluants was intense.

The performance of the conjugates in the FA staining of nonmotile salmonellae can be ascertained from the data presented in Table 3. The two strains of *S. gallinarum* are genotypically nonmotile, whereas *S. europe* 8CL and *S. typhimurium* P-10 are nonmotile variants of normally motile strains. The H IgG conjugate stained all four nonmotile strains. The specific staining intensity values for the six conjugates were similar to those observed for motile salmonellae. The O IgG, H, and OH conjugates gave acceptable specific staining intensities. The specific staining intensity of O IgG conjugate was only 2+ for the two strains of *S. gallinarum*. The O eluant and H eluant conjugates gave negligible to low specific staining with the nonmotile salmonellae. A comparison of NSS values again established the superiority of conjugates of H IgG and O IgG over the conjugates of H and OH.

The reactions of various FA conjugates with non-salmonellae are shown in Table 4. Once again, H IgG was superior to the other conjugates in terms of specific staining intensities and cross-reactions; H IgG did not react with any of the non-salmonellae tested except arizonae.

Cross-reactions occurred between *Citrobacter freundii* and O IgG, H, and OH conjugates. The OH conjugate also stained one strain of *Escherichia coli*. The H and O IgG conjugates stained the *E. coli* strain weakly. In terms of NSS, H IgG again was superior to all the other conjugates. The NSS was maximal with the OH conjugate. Interestingly, NSS was moderate to high when smears were made from uninoculated selenite cystine broth containing egg white and stained with H and O eluants and with H and OH conjugates.

From the results obtained, the inference was that the H IgG fraction was superior to the O IgG fraction with respect to specific staining characteristics and to NSS. The H IgG conjugate was, therefore, used in a later study to compare its efficiency in detecting salmonellae

TABLE 2. *Comparison of the staining intensities of FA conjugates: motile salmonellae*

Serotype	SS[a] (and NSS)					
	H eluant	H IgG	O eluant	O IgG	H (NADC)	OH (Sylvana)
S. typhimurium	1+ (3)	4+ (1)	2+ (3)	4+ (1)	4+ (3)	4+ (3)
S. newport	1+ (3)	4+ (1)	1+ (4)	4+ (1)	3+ (3)	4+ (3)
S. anatum	2+ (3)	4+ (0)	3+ (2)	4+ (0)	3+ (2)	4+ (3)
S. senftenberg	2+ (3)	4+ (0)	3+ (2)	2+ (1)	3+ (3)	4+ (3)
S. infantis	2+ (2)	4+ (2)	2+ (3)	2+ (1)	4+ (2)	4+ (3)
S. typhi 2V	3+ (3)	4+ (0)	0 (2)	3+ (1)	3+ (2)	4+ (3)
S. derby	0 (3)	4+ (1)	0 (3)	4+ (1)	3+ (3)	4+ (2)
Mean SS	1.6+	4+	2.7+	3.4+	3.3+	4+
Mean NSS	2.9	0.7	1.6	0.9	2.6	2.9

[a] SS, Specific staining intensity rated subjectively on a scale of 0 to 4+; NSS, nonspecific staining intensity rated subjectively on a scale of 0 to 4.

TABLE 3. *Comparison of staining intensities of FA conjugates: nonmotile salmonellae*

Serotype	SS[a] (and NSS)					
	H eluant	H IgG	O eluant	O IgG	H (NADC)	OH (Sylvana)
S gallinarum (A)	0 (2)	4+ (1)	0 (3)	2+ (1)	3+ (2)	3+ (3)
S. gallinarum (B)	0 (3)	3+ (1)	0 (3)	2+ (1)	2+ (3)	3+ (3)
S. europe 8CL	0 (1)	4+ (1)	2+ (3)	3+ (1)	4+ (2)	4+ (4)
S. typhimurium P-10	1+ (2)	4+ (0)	1+ (2)	3+ (1)	3+ (3)	4+ (3)
Mean SS	0.25+	3.75+	0.75+	2.5+	3+	3.5+
Mean NSS	2.00	0.75	2.75	1.00	2.50	3.25

[a] See Table 2.

with commercial FA conjugates, using the FA technique. The samples of raw meat and poultry were concurrently subjected to analysis by cultural methods. The other FA conjugates included in the study were FA *Salmonella* poly OH (Difco), *Salmonella* polyvalent OH (Sylvana), and FA *Salmonella* poly H (NADC).

The performances of the various FA conjugates in Table 5 were compared in the FA procedure with results obtained by the cultural technique. In terms of overall agreement with the cultural method, H IgG conjugate was superior to the other conjugates. It also gave the fewest number of positives that could not be culturally confirmed.

The evaluation of NSS characteristics of the various conjugates for beef, poultry, and pork samples are presented in Table 6. Results from an analysis of variance of the NSS values obtained for the same sample with different conjugates showed that the NSS values for the four conjugates were significantly different from each other. For each sample category, H IgG conjugate had the lowest NSS value. Differences be-

TABLE 4. *Comparison of staining intensities of FA conjugates: non-salmonellae*

Organism	SS[a] (and NSS)					
	H eluant	H IgG	O eluant	O IgG	H (NADC)	OH (Sylvana)
Arizona hinshawii (1)	0 (0)	3+ (1)	2+ (3)	3+ (1)	2+ (3)	2+ (4)
Arizona hinshawii (2)	2+ (3)	4+ (1)	2+ (2)	4+ (1)	2+ (3)	3+ (4)
Citrobacter freundii	0 (0)	0 (0)	0 (2)	1+ (1)	2+ (2)	1+ (3)
Escherichia coli	0 (2)	0 (0)	0 (3)	1+ (1)	1+ (2)	3+ (3)
Proteus vulgaris	0 (2)	0 (0)	0 (3)	0 (0)	0 (2)	0 (3)
Pseudomonas fluorescens	0 (1)	0 (0)	0 (2)	0 (1)	0 (1)	0 (3)
Controls						
Selenite cystine broth	0 (0)	0 (0)	0 (1)	0 (1)	0 (1)	0 (1)
Selenite cystine broth and dried egg white	0 (2)	0 (0)	0 (2)	0 (0)	0 (2)	0 (3)
Mean NSS	1.25	0.25	2.25	0.62	2.00	3.00

[a] See Table 2.

TABLE 5. *Comparison of the specific staining of FA conjugates with the cultural method*

Conjugate	Samples analyzed (no.)	Agreement with the cultural method (%)	Samples positive by trial method but negative culturally (no.)	Samples negative by trial method but positive culturally (no.)
H IgG	142	93.0	7 (4.9)[a]	3 (2.1)
NADC	99	87.9	9 (9.1)	3 (3.0)
Difco	119	85.7	15 (12.6)	2 (1.7)
Sylvana	142	86.6	16 (11.3)	3 (2.1)

[a] Numbers in parentheses are percentages.

TABLE 6. *Comparison of nonspecific staining intensities of FA conjugates with meat and poultry samples*

Sample	Mean NSS[a] value				Least significant difference (0.01)	F value
	H IgG	Difco	NADC	Sylvana		
Beef	0.78	2.38[b]	2.87	3.50	0.51	81.73
	(36)	(29)	(24)	(36)		
Poultry	0.58	1.91	2.10	3.19	0.62	46.28
	(41)	(34)	(28)	(41)		
Pork	0.34	2.19	2.36	3.27	0.41	137.32
	(65)	(56)	(47)	(65)		
All samples	0.52	2.15	2.41	3.30	0.29	240.97
	(142)	(119)	(99)	(142)		

[a] Rated subjectively on a scale of 0 to 4.

[b] Underlined mean values are not significant from one another at 99% confidence level. Figures in parentheses under NSS values indicate the number of samples analyzed.

tween the NADC conjugate and the Difco conjugate were not significant at the 0.01% level of significance. Sylvana conjugate had the highest NSS values in each sample category and in the overall evaluation of all samples.

DISCUSSION

NSS caused by conjugated serum proteins is a problem of serious concern in the application of the FA technique for detecting pathogens in foods. NSS is due mainly to the electrostatic forces between the microscopic preparation and the serum proteins. At pH 7.0, the serum proteins have a net negative charge that is further increased by conjugation (32). Hence, reduction of the proportion of dye to the protein in the conjugate has a favorable effect on NSS. Further, the ammonium sulfate-fractionated globulin fractions of antisera are heterogeneous with respect to net charge. If we are to reduce NSS to the minimum, the conjugate must consist only of the specific antibody with a low uniform degree of molecular labeling.

The method presented here for the purification of IgG from *Salmonella* antiserum differs from the other reported methods in that it is an extremely simple, one-step procedure that yields a product of high purity. In contrast to the separation of molecules on the basis of their physicochemical characteristics using methods such as DEAE-cellulose chromatography, gel filtration, and zonal centrifugation, specific biological interactions such as enzyme-inhibitor reaction or antigen-antibody reaction are used in affinity chromatography to provide extremely effective and rapid separations.

The ligand chosen for the separation of IgG from antisera was protein A. Protein A is the normal cell wall component of most strains of *Staphylococcus aureus* (27). It consists of a single polypeptide chain of molecular weight 42,000 containing several regions of internal homology (4). The most interesting property of protein A is its ability to interact and form precipitates with IgG from various species. This reaction is similar to antigen-antibody reactions and has been investigated extensively. One molecule of protein A combines with two molecules of IgG (24). The F_c fragment of IgG is involved in the reaction. The specific interaction of protein A with the F_c fragment of IgG was used in this investigation to isolate IgG from *Salmonella* polyvalent antisera.

An examination of the physicochemical characteristics of the FITC-labeled conjugates (Table 1) reveals certain interesting facts. Although the different serum fractions were labeled under identical conditions of protein/FITC ratio, time of reaction, and temperature, their F/P ratios were considerably different. Because FITC reacts with the ϵ-amino groups of lysyl and arginyl residues of the protein molecule, we can infer that IgG contains fewer such reaction sites than do other components of the antiserum. NSS also increases directly in proportion with F/P ratio. Increases in F/P ratio up to about six produced higher specific staining titers, but F/P ratios above six did not give higher specific titers (34). NSS also increases directly in proportion to the FITC content of a conjugate (21). Results in Table 1 show that the FITC contents of H IgG, O IgG, and H conjugates were significantly lower than those of H eluant, O eluant, and OH conjugates. The conjugates of OH and H IgG also had higher FA titers than conjugates of O IgG, O eluant, H eluant, and H. Because NSS can be reduced significantly by dilution of a conjugate (21), H IgG could be presumed to have lower NSS characteristics. These deductions from the physicochemical data have been borne out by the experiments with egg white and meat and poultry samples.

H IgG and H conjugates stained the cell wall of nonmotile and motile salmonellae. The staining of the cell surface of motile salmonellae by flagellar antisera has been reported previously (26, 35). The cell wall was the site of staining in an investigation of the site of fluorescence on the cell surface with flagellar antisera (13).

Flagellar antisera to salmonellae are prepared by intravenous immunization of rabbits with highly motile, formalinized cultures (10, 29). The flagellar antiserum thus produced contains considerable (1:640 to 1:2,560) titers of O agglutinins (12). The production of potent salmonella flagellar antisera with low or negligible O antibodies requires special techniques such as shearing of the flagellae from the bacterial cells and isolating them by ion-exchange chromatography (1) or by immunosorption (12).

Goepfert and Hicks (13) attempted to explain the presence of somatic antibodies in flagellar antiserum by suggesting that formalinization does not reduce the antigenicity of cells to the extent that heating or acetone drying does. They hypothesized that formalinized cells stimulate production of H as well as O antibodies. Further, they hypothesized that "blocking" or "incomplete" antibodies may be present in H sera. These antibodies react with a specific antigen, but do not allow agglutination to occur (8). Incomplete antibodies are usually present in higher concentrations than are normal antibodies in a given serum. Such antibodies participate in the FA reaction, but do not cause agglutination of cells. The phenomenon of incomplete antibodies appears to offer a feasible explanation for the staining of cell surface that is observed with flagellar antibodies.

The use of a conjugate prepared from flagellar antiserum for the detection of salmonellae in foods has been criticized on the basis that it does not adequately cover *Salmonella* O groups either qualitatively or quantitatively (6). However, several research workers have obtained satisfactory results with FITC-conjugated *Salmonella* polyvalent H antiserum (15, 18, 20, 36). Cross-reactions did not occur when H antibodies were used in the indirect FA technique (18). A conjugate prepared from *Salmonella* polyvalent H antiserum gave a higher degree of agreement (95.7%) with the cultural technique than did two commercially purchased FA conjugates prepared from globulin fractions of OH antiserum (25). The H conjugate recorded fewer false positives and detected salmonellae in all of the culturally positive samples.

In terms of both specific staining intensities and NSS characteristics, the use of immunopurified H IgG is clearly superior to ammonium sulfate-fractionated globulin fractions of antisera. Although other workers had arrived at similar conclusions previously, the method presented here for the isolation of IgG from an antiserum is an extremely sensitive, rapid procedure. This method can easily be adopted for the routine preparation of conjugates for the FA procedure. The immunopurification is the only step required for the isolation of IgG from the antiserum.

On the basis of results presented here, immunopurified IgG should be used for preparing FA conjugates for use in detecting salmonellae in foods. The use of immunopurified IgG conjugates in semiautomated systems, such as the one evaluated by Thomason et al. (38), will prove to be particularly beneficial. With the semiautomatic system, a fluorometric reader rather than a fluorescence microscope is used to evaluate specific staining intensities. With such a system, in which the smears are not examined for the morphological characteristics of fluorescing cells but are evaluated just for the fluorescing intensities on the smear, it is imperative that NSS be reduced to a minimum.

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