Fluorescent Antibody Techniques for *Salmonella* and Other Enteric Pathogens

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OUR OBJECTIVE is to clarify the present status of immunofluorescence tests for the detection of *Salmonella*, *Shigella*, and enteropathogenic *Escherichia coli* in clinical specimens and foodstuffs. Among enteric bacteria, these genera are of primary importance as incitants of human infections.

Salmonellae

The detection of salmonellae by fluorescent antibody (FA) procedures is not as easy as some workers have suggested. Two major potential applications are the screening of fecal specimens and commercial foods and feeds. Infections due to salmonellae are a major public health problem because of the frequency with which these organisms occur in milk, meat, seafoods, eggs, and poultry. The last four of these products are not always decontaminated before being transported into the home or into commercial food preparation areas. Thus, infections may occur in human beings following consumption of rare meat, undercooked eggs, shellfish, or foods contaminated by contact with meat, poultry, or seafood containing salmonellae. Pasteurized liquid milk is not a source of infection unless it becomes contaminated with salmonellae after pasteurization. If salmonellae are present in liquid milk, however, these organisms may survive the drying process. Liquid, frozen, and dried eggs may contain viable salmonellae posing a threat to the consumer if the eggs or other foods to which they are added are not heated sufficiently to destroy the organisms. Contaminated water supplies may occasionally be the cause of outbreaks of salmonellosis, an excellent example being the 1965 Riverside, Calif., epidemic of *Salmonella typhimurium* (*1*).

Early work by Thomason and associates (*2*) directed toward the development of FA procedures for detecting salmonellae in feces was discouraging. Cross-reactions with normal intestinal flora were numerous when either monovalent or polyvalent conjugates were used for staining. These results were based on the staining of smears prepared directly from suspensions of feces in buffered-glycerol-saline preservative or from suspensions of stool specimens in physiological saline. Poor results were due to several factors: (*a*) enteric bacteria which were serologically related to salmonellae, (*b*) the presence of "normal antibodies" against enteric bacteria in the serum of the rabbits providing the conjugates, and (*c*) the use of conjugates of lower titer and poorer quality than those which can be prepared today.

The serologic specificity of the Vi antigen suggested the possibility of using the FA procedure to rapidly screen fecal specimens from known or suspected typhoid carriers. The work of Thomason and McWhorter (*3*) proved that the FA procedure was at least equal in sensitivity and specificity to the cultural examination for detecting typhoid bacteria in the fecal specimens of 129 registered chronic carriers. The

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specificity of the reagents that these workers used is well illustrated in table 1.

Ninety-one of the stool specimens from the 129 carriers were positive by culture; 90 were positive by the FA test. Six of the carriers who were positive by culture were negative by the FA procedure, and five of the carriers positive by the FA procedure were negative by culture. Both the O, Vi (*Salmonella typhi*) conjugate and the sorbed Vi (*Citrobacter freundii*) conjugate were used—the O, Vi reagent for detecting Vi negative cells. With these conjugates, Thomason and McWhorter observed no fluorescent organisms in fecal specimens from 64 normal persons. The slight differences between the two conjugates in results obtained on the carriers' specimens were not considered significant. Chronic carriers apparently excrete predominantly Vi positive cells. If true, this is a fortunate circumstance. It permits use of the highly specific sorbed Vi conjugate to detect typhoid bacteria without risk of obtaining false positive tests from other salmonellae of group D or from enteric bacteria possessing somatic antigens 9, 12, or related moieties.

In the work just reported a single FA examination was made with each of the two conjugates. Culturally, however, eight opportunities existed to isolate *S. typhi* since each specimen was streaked on one plate of MacConkey's agar, one plate of *Salmonella-Shigella* agar, and two plates of bismuth-sulfite agar, both before and after enrichment in selenite broth. The cultural results represent the totals from all media. Thus, the FA test may ultimately prove to have far greater sensitivity than conventional cultural isolation procedures for *S. typhi*. The specificity of the O, Vi and the Vi conjugates used in the study by Thomason and McWhorter is undoubtedly a function of their high titer (1:80), which reduces cross-staining of serologically related bacteria and almost eliminates nonspecific staining of tissue cells and debris.

Because of the success achieved with the FA test on carriers, detection of the typhoid bacillus in the stools of patients in the acute or convalescent stage was not expected to be difficult. This view, however, has been shown to be naive. In collaboration with Dr. Merrill Snyder at the University of Maryland, we have demonstrated that it is not unusual to obtain FA negative results on fecal specimens from experimentally infected human volunteers who yield a large number of typhoid bacillus colonies upon culture. The fecal typhoid bacteria resisted staining with the sorbed Vi conjugate and stained poorly with the O, Vi reagent. Yet, following growth on isolation media (or enrichment in tetrathionate or selenite broth), the bacteria fluoresced brilliantly. These results emphasize the necessity of enriching the bacteria in appropriate selective media before staining them with FA reagents.

Table 1. Reactions of representative strains of *Salmonella* antigenically related to *Salmonella typhi*

Salmonella strains tested	Antigenic schema of strains	Fluorescence with labeled globulins	
		O, Vi (1:80)	Vi (1:80)
S. typhimurium	1, 4, 5, 12	1+ to 2+	Negative.
S. paratyphi B	1, 4, 5, 12	2+	Do.
S. saintpaul	1, 4, 5, 12	2+	Do.
S. derby	1, 4, 5, 12	1+ to 2+	Do.
S. javiana	1, 9, 12	2+	Do.
S. pullorum	9, 12	2+	Do.
S. dublin	1, 9, 12	2+	Do.
S. gallinarum	1, 9, 12	2+	Do.
S. enteritidis	1, 9, 12	2+	Do.
S. typhi 0901	9, 12	2+	Do.
S. typhi Me 1325.	9, 12, Vi	4+	4+
S. typhi 2V	9, 12, Vi	4+	4+
S. paratyphi C	6, 7, Vi	1+ to 2+[1]	1+ to 2+[1]

[1] Approximately 1 percent of the cells were stained. Results of the slide test for Vi agglutination were doubtful.

SOURCE: reference 3.

These observations suggest several possibilities, namely, (*a*) the inhibition of staining by blocking antibody, (*b*) the removal of the Vi antigen or failure to synthesize it in organisms within the intestinal tract, and (*c*) the possible blocking of immunofluorescent staining by slime-wall formation. The ease with which most salmonellae form slime walls on phosphate agar (*4*) suggests that if slime-wall polysaccharide formed in vitro is found to inhibit FA staining of salmonellae, the possibility of slime-wall formation in the intestinal tract should be investigated.

These experiences have led to the hypothesis that the inability of the patient's defense mechanisms or of his intestinal flora to prevent the

synthesis of Vi antigen in the typhoid bacillus may produce the chronic carrier state.

An outbreak of typhoid fever at Stanford University provided an opportunity for Bissett and associates (*5*) to compare the FA detection of *S. typhi* with conventional isolation procedures on specimens from patients with acute disease. Examinations were performed directly on fecal specimens in the manner reported by Thomason and McWhorter (*3*) and also with selenite-F enrichment cultures. The enrichment cultures proved more sensitive, yielding positive FA results from 40 of the 41 patients positive by culture. There were nine positive and two questionable FA results on specimens that were negative by culture. *Citrobacter* organisms which fluoresced with the Vi conjugate were isolated from three of the specimens. Only one specimen was negative by the Vi FA test and positive by culture—a specimen from a patient undergoing treatment. It is unfortunate that the *Citrobacter* which was isolated was not studied further to see if it was *C. freundii* 029, Vi or another *Citrobacter* for which the rabbits had "normal" antibodies.

At present, sorbed Vi conjugate for detecting typhoid bacteria can be recommended for use in monitoring known carriers, in searching for new carriers, or for use on specimens from persons having acute cases of typhoid fever. It may be used on selenite or tetrathionate enrichment cultures but not directly on smears of fecal suspensions.

Demissie (*6*) successfully used high-titered (1:500) conjugates prepared with Roschka-type antigens to detect *Salmonella tubigen* in tetrathionate enrichment cultures of fecal specimens obtained during an outbreak. He also identified colonies from plates by FA staining of smears. The staining of smears prepared directly from saline suspensions of feces was unsatisfactory because of the small number of organisms present and their poor staining characteristics.

Similarly, Stulberg and associates (*7*) prepared individual and pooled OH conjugates. A mixture of *Salmonella typhimurium* and *Salmonella paratyphi* B conjugates was used to stain formalin-killed cells in suspensions. These cells had been collected by centrifugation of dextrose broth cultures of feces which had been incubated overnight. Stulberg and associates used this method with considerable success in monitoring the progress of an extensive outbreak of *S. typhimurium* infection in infants in a nursery. The reliability of the diagnosis depended upon the specificity conferred by the FA identification of flagellar antigens. For reasons not apparent, specificity was confined to specimens from infants; specimens from adults frequently contained organisms giving good somatic (O) staining in the absence of stained flagella. Cultures of such specimens were always negative.

Recently, national attention has been focused on salmonellosis, partly because of the decline of other communicable diseases and partly because of rapid increases in the consumption of foods processed from animal products which may be contaminated with salmonellae. Federal laws require that the ingredients of these foodstuffs must be free of *Salmonella* before they are released into interstate commerce. Conventional bacteriological examination for this genus requires a minimum of 36 to 48 hours. Thus, manufacturers are highly motivated to devise more rapid methods for screening their products for these organisms. These stimuli have resulted in a reexamination of the use of FA tests for this purpose.

Although foodstuffs present some special obstacles to the use of FA tests, they usually do not contain the large number and species of cross-reacting bacteria found in feces. All foods must be inoculated into selective enrichment media before they are examined; some require pre-enrichment in a noninhibitory medium followed by selective enrichment. These steps serve to hydrate dried food products and to permit multiplication of the salmonellae along with concurrent synthesis of fresh surface antigen so that good fluorescence is achieved.

Several research groups, including ours, are attempting to develop techniques to use in screening raw materials and finished food products for salmonellae (*8–17*). Success may provide reagents also suitable for use on fecal specimens. Some workers have used the indirect FA procedure to avoid labeling multiple antibody solutions (*8–10*, *13*). The disadvantages of the longer staining period and the increased cross-staining, however, have outweighed the

advantages of using a single labeled reagent. For example, Silliker and associates (*13*) found that their commercial goat-antirabbit conjugate required careful titration because, at low dilution, it stained a variety of enteric organisms.

Most current work is being done by direct FA staining, using antigen mixtures for antibody production (*6*, *7*, *12*, *15*). Both formalin-killed and Roschka- (heat,- acetone-, and alcohol-treated) type antigens are being used. Conjugates prepared from polyvalent OH serums appear promising. Serums prepared with boiled antigens are unsatisfactory (*6*), and their use may explain the low titers and poor results that we and some other earlier workers have reported.

In short, FA tests for salmonellae are needed in the clincial laboratory to screen fecal specimens for the presence of all common serotypes of the genus. Further improvements undoubtedly will be made, but at present reliable FA tests have been developed for detecting the typhoid organism in specimens from chronic carriers and persons with acute disease. In at least two instances, considerable success has been achieved in detecting the specific serotypes that have been shown, by isolation, to be incitants of outbreaks. Progress is also being made in developing polyvalent conjugates for screening foodstuffs (*16*, *17*). All of the data are not in, but apparently the requirements for success are (*a*) use of high-titered serums made with unheated antigens, (*b*) inoculation of specimens into suitable pre-enrichment and selective enrichment media, and (*c*) use of OH conjugates to enhance the specificity of the staining reagents. The mechanics of a screening procedure for detecting salmonellae by FA tests is shown in the flow chart.

Commercial FA reagents for salmonellae may not be a good investment because we do not yet know what types of reagents or what ancillary methods will prove most advantageous.

Shigellae

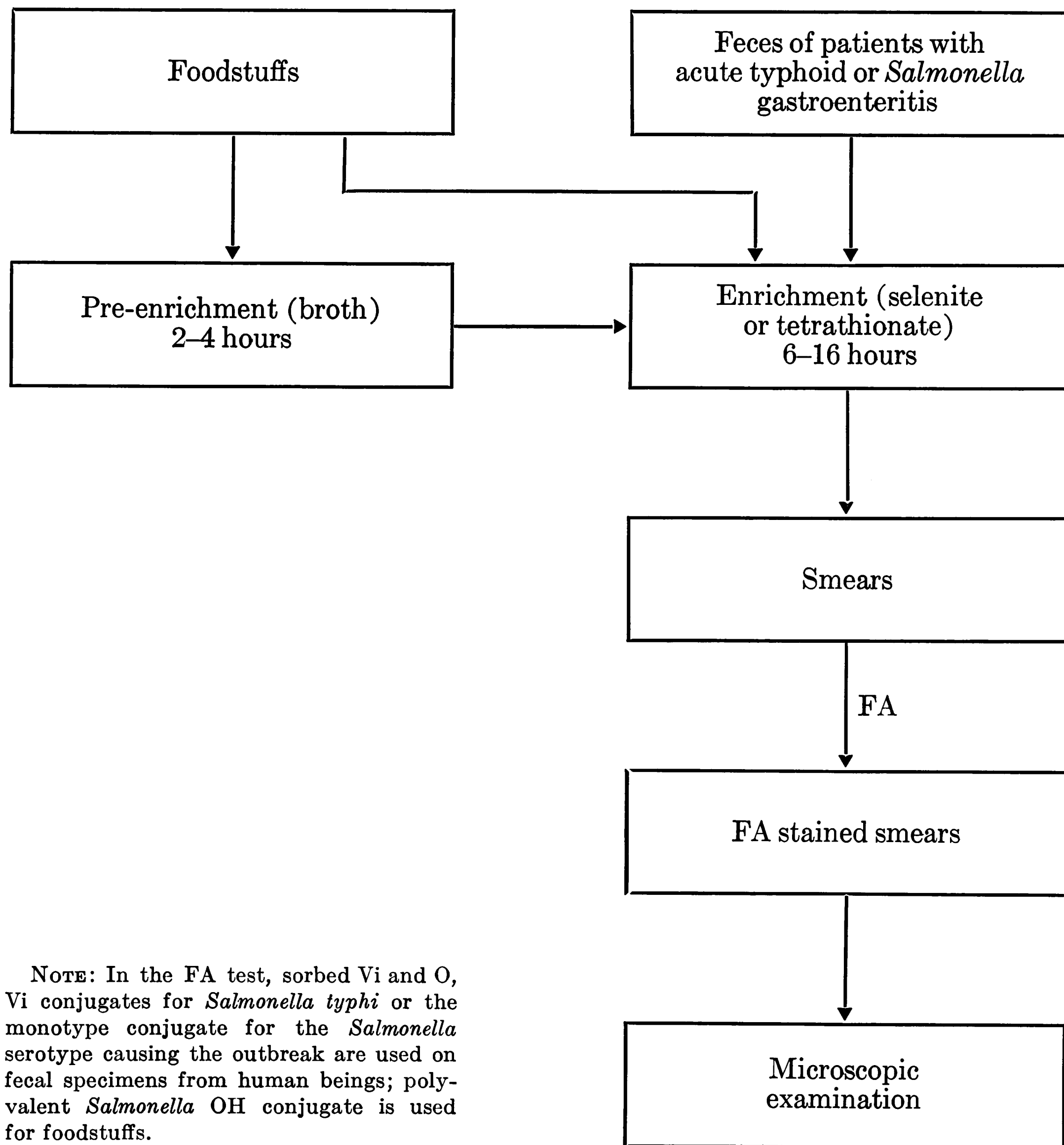
Several difficulties arise in devising a procedure for fluorescent antibody staining of shigellae in fecal specimens. Preparing sorbed conjugates for each *Shigella* serotype is not practical, although the work of LaBrec and associates (*18*) indicated that highly specific reagents could be obtained this way. These workers also reported that the indirect method was not satisfactory because the available sheep-antirabbit globulin contained normal antibody against various strains of shigellae. The requirement of the public health or hospital laboratory for FA reagents for shigellae can be met by preparing conjugates for the four serogroups A, B, C, and D. Such reagents should be usable at fairly high titer, require little, if any, sorption, and be highly specific for shigellae.

The detection of *Shigella dysenteriae* (A) and *Shigella boydii* (C) serotypes presents profound but not necessarily insoluble difficulties related to specificity (*19*). *S. dysenteriae* 2 and *Escherichia coli* 0112:B11 are serologically identical, as are *S. dysenteriae* 3 and *E. coli* 0124:B17 (see subsequent section on enteropathogenic *E. coli*). In addition, conjugates for *S. dysenteriae* 3 may be expected to cause strong fluorescence of some strains of types 21 and 22 of *Klebsiella pneumoniae*. The Group A and C shigellae, however, are of minor importance, each accounting for less than 1 percent of the total isolates of shigellae in the United States each year (*20*). Little evaluation of the FA reagents of these organisms has been done.

The detection of *Shigella flexneri* by FA tests also presents special problems. Because of the multiplicity of antigens within the group, cross-reactions with other enterobacteriaceae can be expected. Furthermore, the importance of culturing fecal specimens immediately after collection in order to obtain the greatest number of *S. flexneri* isolations possible is apparent in all the studies we have conducted (*19*, *21*). There is no satisfactory selective enrichment medium for isolating shigellae, and apparently the *S. flexneri* organisms do not compete well in viability and cultivability with intestinal flora. Transport media commonly used also are inadequate for maintaining the viability of these organisms. Further studies are needed in both these areas.

FA staining for the detection of *S. flexneri* is extremely sensitive and can be expected to give an excess of FA positive results which cannot be confirmed by conventional isolation methods. This fact may reflect the inadequacies of the cultural procedure and the cross-staining of other enteric organisms. In the most recent

Flow chart for fluorescent antibody (FA) detection of salmonellae in foodstuffs and feces



NOTE: In the FA test, sorbed Vi and O, Vi conjugates for *Salmonella typhi* or the monotype conjugate for the *Salmonella* serotype causing the outbreak are used on fecal specimens from human beings; polyvalent *Salmonella* OH conjugate is used for foodstuffs.

study in our laboratory (*21*), 76.7 percent of the specimens positive for *S. flexneri* by FA also were culturally positive. This proportion compares favorably with the 53.3 percent and the 39.3 percent which could be confirmed by culture in previous studies (*19*). Although the specificity of the FA tests for *S. flexneri* may be improved, results should be interpreted with caution. Use of commercial reagents should be restricted to evaluating the technique in comparison with isolation methods.

Shigella sonnei is one species of shigellae for which a definitive statement about FA tests can be made. *S. sonnei* may be detected with a high degree of reliability by applying an appropriate conjugate to smears prepared from fecal suspensions. As Taylor and Heimer (*22*) have shown, this test is of great value in England, where *S. sonnei* causes approximately 98 percent of all bacillary dysentery. Its value is somewhat less in the United States, although *S. sonnei* serotypes are accounting for an increasing pro-

portion (54 percent) of all cases of bacillary dysentery (*20*). Taylor and Heimer (*22*) compared results from FA and cultural methods and found them to agree in 95.6 percent of the 388 specimens cultured; of the remaining specimens, 1.8 percent were FA positive but culturally negative; and 2.6 percent were positive by culture only. These authors reported to the physicians by telephone all positive FA tests on specimens from patients with acute diarrhea. The report could usually be made within an hour, but they emphasized that it should be regarded as provisional and subject to confirmation by culture.

In four studies at the National Communicable Disease Center, we have evaluated the detection of *S. sonnei* by FA staining (*19*, *21*). The correspondence of the total positive and negative direct FA results with the cultural data in these four studies agreed very closely with the 95.6 percent reported by Taylor and Heimer (*22*), as the following table shows:

Author	*Percent of agreement between FA and cultural results*	*Percent of cultural recoveries from FA positive specimens* [1]
Taylor and Heimer, 1964 (*22*)	95.6	96.1
NCDC studies		
Thomason, Cowart, and Cherry, 1965 (*19*)	98.3	70.8
	94.6	45.4
	96.0	80.0
Thomason, Nahmias, and Mathews, 1967, (*21*)	97.6	84.2

[1] Positive cultures÷FA positives×100.

As the table shows, cultural recoveries of *S. sonnei* from FA positive specimens in the four studies were 70.8, 45.4, 80.0, and 84.2 percent. The comparable figure that Taylor and Heimer reported is 96.1 percent. Low recovery—45.4 percent—from the 1965 NCDC study was expected because 25 of the 37 patients were receiving antimicrobial treatment at the time the specimens were collected.

Thus, adequate data are available to insure confidence in the FA test as a detector of *S. sonnei*. A word of caution, however, about reagents is in order. A conjugate made from serum prepared with form 1 antigen must be used. Form 1 is the in vivo antigen, and organisms containing it stain more brilliantly with the FA reagent than those containing form 2 antigen. Cells in form 2 were not highly fluorescent even when treated with the undiluted homologous conjugate. The change from form 1 to form 2 antigen occurs rapidly in vitro.

If an outbreak of bacillary dysentery is due to *S. sonnei*, FA screening of specimens is a highly effective method of defining the scope of the disease and of selecting specimens for culture. When commercial reagents become available, the FA test for *S. sonnei* will be ready for use in public health and clinical laboratories. Before being used, these reagents should be titered against both of the antigenic forms of *S. sonnei*.

In contrast to enteropathogenic *E. coli*, the number of stained shigellae organisms seen in smears from patients with acute dysentery is low. As with *S. typhi*, negative FA smears are occasionally found on shigellae specimens whose isolation plates yield numerous colonies. These inconsistencies are unexplained.

The best method of handling fecal specimens before preparing smears for FA testing is not known. Taylor and Heimer (*22*) obtained excellent results with smears prepared from suspensions of feces in phosphate-buffered saline (pH 8.0). In our laboratory, smears of suspensions in physiological saline and smears prepared from rectal swabs transported in a soft medium gave the best results. The transport medium consisted of 0.25 percent of agar in distilled water, dispensed into 13 by 100 mm. tubes in 1.0 ml. amounts and autoclaved. Smears of fecal specimens enriched in broth yielded many more FA positive results than we obtained by any other method. A large percentage of these positive results, however, could not be confirmed by culture, and we have to assume that they represented false positive fluorescence. Hornung found that staining the fecal bacteria in suspension before the smears were prepared was not successful (*23*). We do not recommend this procedure because clumps of agglutinated organisms form that make the results difficult to interpret.

Enteropathogenic *Escherichia coli*

The use of immunofluorescence in detecting enteropathogenic *Escherichia coli* (EEC) is well established, and its effectiveness has been

affirmed in numerous studies (*24*). It is well adapted for diagnosis in hospitals or institutions and for the surveillance of infant diarrhea caused by *E. coli.* The procedure can be used to screen infants before admission to hospital nurseries, to rapidly determine (in 1 to 2 hours) the appearance of diarrhea induced by *E. coli*, to monitor the infant population at risk of possible colonization with EEC before symptoms appear, to follow the patient's excretion of EEC during the convalescent period, and to detect the presence of nonculturable EEC excreted during drug therapy.

These are things the FA procedure will do for the clinician; there are also things it will not do. First, the staining of EEC by FA reagents prepared from OB grouping serums does not result in definitive identification of *E. coli.* Such identification can be achieved only by determining the O, B, and H antigens after they have been titrated with appropriate antiserums in a laboratory specializing in such work. Thus, FA staining is not a substitute for complete serologic identification. In most hospital laboratories, however, the serologic study of EEC consists of performing slide agglutination tests on typical colonies obtained from isolation plates. Ample evidence shows that FA staining with OB serums gives results which are at least as reliable as those obtained with slide agglutination (*25*):

E. coli *identified*	*Positive reactions*	
	Agglutination	*Fluorescent antibody*
Enteropathogenic	118	118
Nonenteropathogenic	24	4
Total	142	122

The four cultures which gave positive reactions in the FA test also gave positive agglutination reactions. They consisted of (*a*) the *Citrobacter* species, related to *E. coli* 0127; (*b*) and (*c*) *E. coli* 071, K antigen related to B 15; and (*d*) *E. coli*, O antigen undetermined but no known relationship to EEC.

Only certain serotypes of some OB groups are judged, based on epidemiologic data, to be incitants of infant diarrhea. These serotypes cannot be distinguished by FA staining from the other serotypes within the OB group, since typing depends upon precise analysis for all antigens. However, in many evaluations over a number of years, in which all FA positive isolates of EEC have been typed, the great majority of cultures encountered belonged to serotypes known to be epidemiologically significant in human infections. This typing was done in Dr. W. H. Ewing's laboratory in the Enteric Bacteriology Unit at the National Communicable Disease Center.

Second, the FA test does not provide a culture of *E. coli* for detailed serologic study or for the important determination of sensitivity to antibiotics.

Third, the FA test is not a shortcut to cheap diagnosis by untrained laboratory workers. Proficiency in using fluorescence equipment and experience in interpreting stained smears are essential for reliable results.

The sensitivity of the FA tests in detecting EEC in fecal smears has been evaluated in several ways. The necessity for this evaluation arose from the fact that most workers found that fluorescent staining yielded at least 30 percent more positive results than conventional plating and isolation techniques. This result should not surprise anyone, since nonviable cells which are serologically intact and organisms of which the growth is inhibited by drugs, biological antagonists, or other agents retain their specific fluorescence. Nevertheless, one is more comfortable with a satisfactory explanation of the discrepancy between FA and cultural results.

The proponents of the FA test are no more required to prove that the excess of FA positive results over those obtained by culture represents true positives than the culture advocates are required to prove that the excess does not represent false negative cultural results. The time has come to re-examine the thoughtless acceptance of cultural results as the standard against which all detection procedures are judged. Enumerating all cultivable organisms of a given species is a worthy objective, but the result should not be equated with the actual viable content of the species in the specimen. If one wishes to demonstrate the actual presence of organisms in a specimen or the number of organisms irrespective of viability, the FA test frequently furnishes an estimate which is at least as accurate as that obtained by culture. The

only direct method of proving this estimate consists of repeatedly culturing the original FA positive, culturally negative specimen. When Moody and associates (*26*) did this, they recovered group A streptococci from 47 percent of the specimens from throat swabs which had been negative by culture. Upon re-examination, Cherry and associates (*25*) recovered EEC from an additional 12.5 percent of the specimens which had been culturally negative but FA positive when examined several weeks earlier.

Shaughnessy and associates (*27*) called attention to certain internal evidence in their data which supported the accuracy of the FA results. For example, of 12 FA positive, culture negative results, six were from patients from whom the epidemic strain of *E. coli* had been isolated by culture in other specimens that were taken either before or after the negative culture.

Freid and Lepper (*28*) and Freid and associates (*29*) showed that the FA test, in comparison with cultural tests for EEC, is from 10 to 100 times more sensitive. They concluded that the "body of unconfirmed FA-positive results obtained by screening populations during epidemic and endemic periods is largely due to the presence of EEC in numbers too small to be cultured." These authors derived their data from two sources: (*a*) cultural and FA examination of feces seeded with known numbers of EEC and (*b*) comparison of FA-positive tests with culturally positive tests of specimens from patients with and without diarrhea.

Chadwick and Abbott (*30*) and Chadwick (*31*), using the microcolony method of preparing smears for FA examination, reached similar conclusions. They showed that their method of staining coverslip impressions of the growth on plates that had been incubated for 3½ hours was sufficiently sensitive to allow detection of as few as 10 organisms in the original inoculum. This method was approximately 100 times as sensitive as direct smear examination by FA. Detection was essentially independent of the ratio of the blackground flora to that of EEC. Conversely, the investigators concluded that the recovery of EEC by culture was unlikely unless these bacteria constituted as much as 10 percent of the cultivable flora.

Hospital and institutional workers should be aware of observations by Boris and co-workers (*32*) that the EEC nasopharyngeal carrier rate was high in asymptomatic persons who had been closely associated during epidemic periods with infants who had diarrhea. Persons so colonized may transmit EEC to susceptible infants. In such carriers, the nasal rate of carriage may be three times or more the rate demonstrable by fecal examination.

In the United States at present, nine serogroups of *E. coli* can be implicated epidemiologically as the etiologic agents of infant diarrhea. These are:

026:B6
055:B5
0111:B4
0127:B8
086:B7
0119:B14
0125:B15
0126:B16
0128:B12

Carefully prepared reagents for these serogroups are remarkably specific when used on fecal smears. The key to their specificity and reliability for diagnostic use is the B antigen of *E. coli.* Conjugates prepared from OB serums have high staining titers and give brilliant peripheral staining. Conjugates from O serums have low titers and tend to produce a duller, uniform staining. As previously mentioned, when conjugates are used at appropriate working dilutions, few cross-reactions with fecal flora are encountered. An interesting enterococcus-like organism, which has not been isolated, is common in human fecal specimens and fluoresces brightly with most EEC conjugates (*33*). Its appearance is confusing to the novice, but with a little experience the organism can easily be differentiated, morphologically, from EEC. Usually, such organisms are also stained by several or all of the conjugates—a fact which aids in making the correct interpretation.

Thomason and associates (*33*) and Davis and Ewing (*34*) have pointed out some of the potential opportunities for cross-reactions with other *Enterobacteriaceae.* The laboratory worker should keep these possibilities in mind when

applying FA staining procedures. Practical experience, however, has proved that it is unusual to encounter, in fecal smears, non-EEC bacteria that morphologically and serologically resemble EEC and that stain with the specific conjugates. In our laboratory, examination of 4,652 fecal specimens in addition to those documented in the table on page 893 yielded 492 cultures which were FA positive for EEC. All except 26 (5.3 percent) of these specimens were confirmed as EEC. Six cultures were too rough to type, 10 were *E. coli* known to be related to EEC, six were *E. coli* not known to be related to EEC, five were *E. coli* of undetermined O groups, three were *Citrobacter*, and one was a *Proteus*. These cross-reacting organisms were positive by slide agglutination and FA tests.

In addition, 26 *E. coli* were isolated which were positive for one of the nine serogroups of EEC by slide agglutination tests but negative by FA examination. These cultures belonged to serogroups of *E. coli* other than the nine pathogenic ones. Thus, FA and slide agglutination tests showed the same degree of nonspecificity.

Conjugates for *E. coli* 0112:B11 and 0124:B17 should be excluded from pooled FA conjugates used for screening fecal smears. The reasons are that (*a*) these serogroups occur only rarely in the United States; (*b*) the OB antigen of *Shigella dysenteriae* 2 is identical to 0112:B11, and *S. dysenteriae* 3 is identical to 0124:B17; (*c*) 0124:B17 produces strong fluorescence of some strains of types 21 and 22 of *Klebsiella pneumoniae*, two types apparently very common in feces.

For several years we have attempted, with little success, to persuade manufacturers not to include 0112:B11 and 0124:B17 in pools of *E. coli* FA conjugates. The difficulty with the 0124:B17 conjugate is well illustrated in a study by Batshon (*35*). About 15 percent of the pregnant women in his study were found to be excreting EEC, as determined by FA staining, whereas only 2.4 percent were positive by culture. More than one-half, 33 of 62, of the total positive FA reactions for EEC were attributed to the 0124:B17 conjugate. However, not one culture of this serogroup was isolated. Thus, these reactions almost certainly represented false positive results and led to an estimate of the EEC excretion in the pregnant women which was at least twice the actual one. Freid and Lepper (*28*) were able to culture 0124:B17 (not definitely identified) from only three of 44 specimens which were FA positive, although the occurrence of FA positive specimens was correlated significantly with diarrheal patients. However, the variability of the FA results in repeated tests on the same patient led Freid and Lepper to exclude the 0124:B17 group from further consideration.

Generally, FA tests should be restricted to the examination of specimens from persons in the age group predominantly affected by EEC (birth to 2 years). Some workers have reported increased difficulty with cross-staining when the

Table 2. Fluorescent antibody and cultural study of fecal specimens of control groups for enteropathogenic *Escherichia coli*

Type and status of patients	Number of specimens examined	Number of specimens positive		Serotype of EEC isolated
		FA	Culture	
Hospitalized children, no diarrhea	32	[1] 4	3	0126:B16:NM; 055:B5:H7; and 0128ac:B12:H12.
Hospitalized adults, with diarrhea	25	0	0	0
Normal adults	25	0	0	0

[1] One specimen was positive for 0126 by fluorescent staining, but the organisms could not be isolated.

Source: reference 33.

reagents are applied to fecal smears from older persons. In our somewhat limited experience, however, such difficulties have not arisen (table 2).

The effectiveness of FA tests in detecting EEC is well documented, and the tests are practical. They can be applied advantageously to the rapid presumptive diagnosis of infant diarrhea and to the surveillance of EEC dissemination within hospitals or other institutions housing children in the age range from birth to 2 years. FA tests are valuable for screening, since their sensitivity makes cultural examination of specimens which are FA negative unnecessary.

A 14-minute, 16-mm., color-sound film entitled "Fluorescent Antibody Detection of Enteropathogenic *Escherichia coli*" was produced by the National Medical Audiovisual Center in 1967. It may be obtained on short-term loan, free of charge, from the National Medical Audiovisual Center (Annex), Atlanta, Ga. 30324—Sta. K or purchased from the Du-Art Film Laboratories, Inc., 245 West 55th Street, New York, N.Y. 10019.

This film, prepared for bacteriologists, medical technologists, general practitioners, pediatricians, and medical students, is designed to stimulate an awareness of the dangers of infant diarrhea and to teach the use of the FA technique in the rapid detection of EEC.

The availability of satisfactory reagents is vital to the routine use of FA methods. Fortunately, suitable reagents are available from some commercial companies. Much needs to be done, however, to standardize their production and to insure consistent performance. All commercial reagents should be titrated and checked for specificity in the user's laboratory. Qualified laboratories may obtain stock cultures, conjugates for control purposes, and guidance from the National Communicable Disease Center.

When effective reagents have been developed and the importance of enteropathogenic *E. coli* has been shown to the medical profession, progress will be made toward reducing a disease which in a 1961 outbreak in Chicago had an age-specific attack rate as high as 3,700 per 100,000 (*36*). After this outbreak, Dr. Warren Wheeler, editor of the *American Journal of Diseases of Children* and a pediatrician of note, wrote a hard-hitting editorial pointing out that our failure to recognize and cope with institutional diarrhea caused by EEC is a national disgrace (*37*). He has reiterated these sentiments in the introductory and concluding remarks to the film.

Summary

Industrial and public health laboratories are seeking to develop a reliable FA test for the detection of salmonellae in foods, feeds, and raw materials. Results indicate that conjugates prepared from OH serums may be useful in screening selective enrichment media for salmonellae. More information is needed, however, before specific recommendations can be made. However, a reliable FA test has been developed for detecting *Salmonella typhi* in fecal specimens obtained from chronic carriers and from persons with acute typhoid fever.

Several groups have evaluated conjugates for *Shigella flexneri* and *Shigella sonnei*. One difficulty in the use of *Shigella* conjugates is the inability to isolate *S. flexneri* from many of the FA positive specimens. Whether this difficulty is due to false positive FA reactions or to the failure of isolation procedures is not clear. The *S. sonnei* reagent has proved both sensitive and specific.

FA tests for enteropathogenic *Escherichia coli* are well adapted to the diagnosis and surveillance of infant diarrhea. The tests have proved to be 10 to 100 times more sensitive than cultural procedures. FA examination should be restricted to specimens from children up to 2 years of age.

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Tearsheet Requests

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New Medicaid Regulations

Secretary of Health, Education, and Welfare Robert H. Finch has issued a regulation limiting fees paid to physicians, dentists, and other individual providers of medical services under Medicaid.

The HEW regulation will limit payments to providers participating in State Medicaid programs to those received in January 1969, unless payments are below the 75th percentile of customary charges. It does not cover payments for prescription drugs, nursing home services, hospital care, or other services.

States whose payment structures provided payments below the 75th percentile of customary charges on January 1, 1969, may request permission from the Secretary to raise payments to that level. States whose payment structures provided fees above the 75th percentile of customary charges must adjust their payments so that they do not exceed reasonable charges as determined under title XVIII–B of the Social Security Act (Medicare).

The action became effective July 1, 1969, and remains in effect until July 1, 1970.

After July 1, 1970, States may request permission to increase fees paid to physicians and dentists only if two conditions are met: (*a*) the average percentage increase requested above the 75th percentile of customary charges on January 1, 1969, may not exceed the percentage increase in the all-services component of the Consumer Price Index (adjusted to exclude the medical component) or in an alternate index designated by the Secretary of Health, Education, and Welfare, and (*b*) evidence must be clear that the providers and the States have cooperatively established effective utilization review and quality control systems.

Regardless of which payment level was in effect in fiscal year 1970, in a given State, the 75th percentile of customary charges will provide the floor above which allowable Consumer Price Index increases will be measured.

The regulation requires States to revise their State Medicaid plans to include descriptions and details of their payment structures. A State that wishes to revise its payment structure for practitioners' services or to change the payments authorized under it may not do so until the proposed changes have been approved by the Secretary or his representative.

States that begin their Medicaid programs after July 1, 1969, must arrange their payment structures so that fees do not exceed the 75th percentile of customary charges.

The regulation implements the Secretary's budgetary decision to set Federal standards for vendor payments to physicians, dentists, and other medical practitioners to control escalating Federal and State expenditures for the program.

The Secretary appointed an Advisory Committee on Payments to Individual Practitioners under title XIX, chaired by Dr. James Haughton, first deputy administrator of the New York City Health Services Administration, to consider alternatives that would curb rising costs of payments to individual practitioners. Emphasis was to be placed on the control of future escalation.