The practical use of fluorescent antibody technic for bacteriologic diagnosis of beta hemolytic streptococci in a pediatric group practice is described. This use is based on modification and simplification for use in the group pediatric laboratory. Various aspects including costs are discussed.

THE USE OF THE FLUORESCENT ANTIBODY TECHNIC FOR IDENTIFICATION OF GROUP A STREPTOCOCCI IN PEDIATRIC PRACTICE

B. B. Breese, M.D.

THIS investigation was undertaken to determine if the use of the fluorescent antibody technic for the identification of group A streptococci had any practical application in the laboratory of a small pediatric group practice. We wished to know whether the method could replace the use of blood agar plates entirely, be used as an adjunct to them, or be entirely eschewed. The chief advantages claimed for it are two: (1) increased speed of identification of beta hemolytic streptococci (in from two to four hours); (2) ability to differentiate the group A organisms that are pathogenic for man from other groups that are not. Although the method has been widely used in state laboratories, to our knowledge this is the first analysis of its use in a private pediatric office.

The method depends on the fact that group A beta hemolytic rabbit antisera, when combined with a fluorescent dye and mixed with group A streptococci on a microscope slide, will attach to them and fluoresce brightly if exposed to a high intensity ultraviolet light in a dark field microscope.

The fluorescent antibody technic, which we finally evolved after almost a year of trial and error, turned out to be a cross between that recommended by the Staphylococcus-Streptococcus Unit of the Communicable Disease Center of the US Public Health Service, Atlanta, Ga.,[1,2] and that reported by Redys, et al., from the Connecticut State Department of Health[3] with some modifications of our own. The method for culturing on sheep blood agar plates was the one which we have used for many years.[4]

Methods

Specimens were obtained by swabbing of the infected area (usually the throat) with a cotton-tipped swab. This swab was rubbed across one-third of a blood plate for the blood agar method ("BA") and then placed in 1 ml of Todd Hewitt broth for the fluorescent antibody ("FA") method.

Blood Agar Plate Method (BA Method)

The blood agar plates were cross-streaked and incubated aerobically for from 15 to 18 hours at 37°. The plates were inspected for beta hemolysis and

then allowed to stand at room temperature for another 24 hours. At the end of this time, a final inspection was made and the number of colonies that showed beta hemolysis were classified as follows: Rare—less than 10 colonies; 1+—from 10 colonies to roughly 25 per cent of the visible colonies; 2+—from 25 per cent to 50 per cent; 3+—from 50 per cent to 75 per cent; 4+—more than 75 per cent.

Fluorescent Antibody Method (FA Method)

The tubes of Todd Hewitt broth were also incubated overnight with the swabs in them. The swabs were then removed and placed in order on a piece of paper toweling while the tubes were centrifuged. The supernatant was poured off and the swabs replaced in their respective tubes, their tips stirred in the sedimented material, and then touched on a marked area of a 1 x 3-inch glass microscope slide. These slides were marked off into ten squares with a diamond glass-cutting pencil and nail polish was painted over the marks, so that each square was separated from the others by an elevated rim of nail polish. After air drying, the smears were fixed with 95 per cent ethyl alcohol. They were then placed in petri dishes kept moist with wet filter paper and the conjugate (anti-group A streptococcus globulin) labeled with fluorescin isothiocyanate—(Baltimore Biologic Laboratory 09-005)[2] was added. After standing for 20 minutes, the slide was rapidly washed off with buffered salt solution, rinsed momentarily in distilled water, and gently blotted dry. A drop of glycerol-saline was placed in each square, and a cover slip large enough to cover all ten specimens was carefully placed over the entire slide. Care was taken that no mixing of the saline glycerol from one square to the next occurred.

These slides were then inspected with a Bausch and Lomb monocular Dyna Zoom laboratory microscope fitted with a cardioid condenser and illuminated with a mercury arc lamp which operated through the appropriate exciter and barrier filter (B & L "fluorescent Group One").

The number of fluorescing streptococci was read as "None: (0), "Few" (1+), "Moderate" (2+), and "Many" (3+). The brightness of fluoresence was described from the lowest, but definite, fluorescence (1+) to the brightest (4+). No controls with normal rabbit sera were used routinely.

Recognition of Group A Streptococci

The frequency with which group A organisms were found was investigated by two methods—the FA technic, described above, and the bacitracin disc method of Maxted.[5] The latter was used only on positive isolates from the blood agar plates, employing the Baltimore Biologic Laboratory Taxos A discs (0.02 unit Bacitracin).

Source of Bacteriologic Specimens and Method of Obtaining

The specimens for the initial studies on the method were obtained from patients seen in our pediatric office in the first six months of 1964, and are those on which this paper is based in the first four months of 1965. The majority were cultured from the throat but some were from nose, ear, skin, or other infected areas.

Of the later specimens, 201 were taken in triplicate. This was done by holding three swabs together and rubbing them across the tonsilar and posterior pharyngeal area while simultaneously rotating them, so that all would be equally exposed. One of these swabs was immediately streaked on blood agar, and then put into broth and processed with our routine cultures for that day. The other two swabs were each placed in pill envelopes, given duplicate code numbers, and held until about 20 such pairs were ac-

cumulated. Approximately once a week, one set of about 20 swabs was mailed to the Communicable Disease Center of the US Public Health Service in Atlanta, Ga. (CDC), where they were processed. In order to control the effect, if any, of sending the cultures through the mail, the other duplicate set was mailed back to our own office where they were processed in the usual manner. Thus, in each of these, one swab was immediately cultured in the routine manner (termed "our original culture"), one was dried and mailed to CDC (termed "CDC mailed culture"), and one was mailed back to our office (termed "our mailed culture"). Since we had shown previously that the viability of such cultures when plated on sheep blood agar was not interfered with by this drying process in the period of time involved,[6] we believed that the results obtained were comparable to freshly plated cultures using blood agar plates. After both laboratories (ours and CDC) had completed the work on cultures the code was broken and the results compared.

In addition to the bacteriologic procedures performed in our laboratory, an attempt was made to estimate the time and material costs involved in the two methods. In each of the patients whose cultures were included in the study, the author or one of his two associates, Dr. Frank Disney or Dr. William Talpey, made a tentative diagnosis of the probability of a streptococcal infection in one of three ways: "No," "Maybe," or "Yes," prior to the report of the bacteriologic results.

It was then possible to correlate the laboratory results with the physician's original clinical diagnosis (before he knew the results of the culture). In order to do this, however, both the laboratory results and the physician's clinical diagnosis had to be expressed in quantitative terms.

The quantitative means of expressing the laboratory results for both the FA and BA cultures have already been described. The clinical diagnoses were expressed quantitatively by using a ratio. This ratio was termed the "Clinical Estimate of the Probability of a Streptococcal Infection" (CEPSI).

It was equal in any group of patients to:

The number in the "Yes" category

The number in the "No"+ the number in the "Yes" category. (Those in the "Maybe" group were omitted since no clinical diagnosis was possible in them.)

For example, suppose that we had 50 cultures which were 4+ on the blood agar plates. In the 50 children from whom these cultures were obtained, the physicians tentatively diagnosed 5 in the "No" group, 10 in the "Maybe" group, and the remaining 35 in the "Yes" group. Clinical Estimate of the Probability of a Streptococcal Infection in these 50 cultures, which were 4+ on the blood agar plates, was then: $\text{CEPSI}=\frac{35}{40}=87.5$ per cent (the 10 in the "maybe" group were omitted from the calculations). This ratio gives us another parameter by which one may compare two culture results obtained by different methods. We believe that if the results in a group of patients who used one laboratory method (i.e., the result of culture on blood agar) agrees better with the physician's independent clinical diagnosis than the results in the same group of patients who used another method (i.e., the results of cultures that used the fluorescent antibody technic), that the first method is probably the better one for clinical use. In this study, we have used this Clinical Estimate of Probability of a Streptococcal Infection (CEPSI) as one of the means of evaluating the bacteriologic results. It should be emphasized that these CEPSI results are not considered as necessarily true or correct diagnostic appraisals. They are quantitative values

Table 1—Number of beta hemolytic streptococci found by fluorescent antibody technic in our mailed-in cultures and CDC mailed-in cultures

Our pediatric laboratory	Communicable Disease Center									
	0	%	Few	%	Moderate	%	Many	%	Total	
0	22	10.9	20	10.0	7	3.5	4	2.0	53	26.4
Few	2	1.0	11	5.5	3	1.5	1	0.5	17	8.4
Moderate	1	0.5	8	4.0	9	4.5	33	16.4	51	25.4
Many	3	1.5	7	3.5	9	4.5	61	30.3	80	39.8
Total	28	13.9	46	22.9	28	13.9	99	49.3	201	100.0

that give us some other base by which to judge the relative clinical significance of the two cultural technics that we are trying to compare: namely, the blood agar and the fluorescent antibody.

Data relevant to each patient and his cultures was coded and punched on I.B.M. cards. The results reported here were based on an analysis of these cards made at the Data Processing Center at the Strong Memorial Hospital.

Results

Comparison of Fluorescent Antibody Results in Triplicate Cultures

A comparison of our original cultures, our mailed-in cultures and CDC mailed-in cultures showed that the CDC mailed-in cultures had the largest number of fluorescent organisms and our original cultures the smallest number. Our mailed-in cultures were intermediate between the two. If we consider the finding of "0" and "Few" organisms as clinically insignificant and "Moderate" and "Many" as significant, the per cent of clinically significant positive cultures was: Original culture—52.7 per cent, our mailed-in culture—65.8 per cent, and CDC mailed-in cultures 63.2 per cent. These findings would suggest that (1) the process of drying and mailing cultures enhanced the percentage of recovery of streptococci (a result previously reported in the use of dry cotton swabs for the transport of cultures presumably due to selective death of common contaminates)[6]; (2) that our "modified" FA technic gave essentially the same results as did the standard method.

A more detailed analysis of the comparable mailed-in cultures by our modified method and the "standard" method of CDC is given in Table 1. This shows that in 164 of the 201 duplicate cultures (82 per cent) there was agreement, both finding positive or both finding negative cultures, and that in only 15 instances (7.5 per cent) did one find strongly positive cultures and the other negative ones. Although the CDC laboratory found 12.5 per cent more positive cultures than did our laboratory, the larger number of positive cultures was due to the CDC laboratory finding many more cultures in the "Few" (or insignificant) category than we had (22.9 per cent compared with 8.4 per cent).

When the "Clinical Estimate of the Probability of a Streptococcal Infection" (CEPSI) is used to measure the relative clinical significance of the results in our laboratory and CDC, the results are also comparable (Table 2). These two comparisons indicated that although we found somewhat fewer streptococci than the CDC did, our modified FA method compared well enough with the standard method to be usable.

Table 2—The clinical estimate of the probability of a streptococcal infection (CEPSI) in relation to number of fluorescent organisms seen. Our laboratory and CDC (initial visits only)

Number organisms	Number cases*		Total	Clinical strep diagnosis
	"No"	"Yes"		CEPSI %
Negative and few				
CDC	33	14	47	30
Our	31	14	45	31
Moderate				
CDC	5	15	20	75
Our	6	30	36	83
Many				
CDC	1	71	72	99
Our	2	54	56	96
Total				
CDC	39	100	139	28
Our	39	98	137	28

* 23 "Maybe" omitted.

Comparison of Modified FA Technic with Blood Agar Method of Culturing

There was good correlation between the results obtained by the blood agar and the FA technic in our laboratory (Table 3). In 920 of the 1,110 cultures (82.9 per cent), there was complete agreement (both significantly positive or both negative). In an additional 104 (9.4 per cent), there was partial agreement (one significantly positive, the other weakly positive or one negative and the other only weakly positive). In 86 cultures (7.8 per cent), there was complete disagreement in that one was strongly positive and the other negative. Neither method was found to show positive results more consistently than the other.

Using the Clinical Estimate of the Probability of a Streptococcal Infection (CEPSI) as a measure of the clinical significance of the two laboratory methods, there seems little to choose between the two. Both show that the more streptococci found in the cultures, the greater is the likelihood that an initial clinical diagnosis of streptococcal infection had been made (Table 4). These results indicate that from the standpoint of the correlation between the clinical impression and the laboratory result neither test seems superior to the other, although the larger number (six) of quantitative gradations on the blood agar plates may be somewhat more useful clinically than the four with the FA technic.

Combination of BA and FA Technic

Since the quantitative evaluation of the number of organisms by either test has great significance clinically, the question arises whether a combination of the two tests would be clinically more important than either test alone. With six quantitative measures of the number of colonies on the blood agar plates (0, Rare, 1+, 2+, 3+, 4+), and four of the fluorescent antibody (0, Few, Moderate, Many), 24 combinations of the two should be possible. However, so few cases fall into some of these com-

Table 3—The relation of cultures for beta hemolytic streptococci by the blood agar and fluorescent antibody technic (our laboratory)

	Number	%
Complete agreement		
Both significantly positive*	345	31.08
Both weakly positive†	14	1.26
Both negative	561	50.54
	920	82.88
Partial Agreement		
FA significantly positive, BA weakly positive	16	1.44
FA weakly positive, BA significantly positive	22	1.98
FA negative, BA weakly positive	21	1.89
FA weakly positive, BA negative	45	4.06
	104	9.37
Complete disagreement		
FA significantly positive, BA negative	35	3.15
FA negative, BA significantly positive	51	4.60
	86	7.75
Total	1,110	100.00

* Significantly positive — FA = Moderate or many, BA = 2+, 3+, 4+

† Weakly positive — FA = Few, BA = 1+

(6 cultures in which degree of positivity unknown omitted).

binations that statistical significance is lost. We have therefore divided both the BA and FA results into two large groups on the basis of the number of beta hemolytic streptococci found — "Occasional" and "Numerous." "Occasional" on the blood agar cultures is defined as "0," "Rare," and "1+" and "Numerous" as 2+, 3+, 4+. With the FA "Occasional" is defined as "Few"; "Numerous" as "Moderate" and "Many."

Using these definitions for the number of organisms found, four combinations are possible: (1) Both "Occasional"; (2) Both "Numerous"; (3) BA "Occasional"; FA "Numerous"; and (4) BA "Numerous"; FA "Occasional."

Study of these combinations (Table 5) indicates that, where there is agreement between the results and both show "Occasional" organisms, the Clinical Estimate of the Probability of a Streptococcal Infection (CEPSI) is very low (11.4 per cent). Conversely, where both results show "Numerous" organisms the CEPSI is very high (87.6 per cent). In the 121 cases where one is high and the other low, the results are intermediate. However, when the BA cultures are "Numerous" and the FA cultures are "Occasional," the CEPSI is higher (78.2) than when the FA are "Numerous" and the BA "Occasional" (42.4).

Table 4—Relation of number of streptococci found in cultures to clinical estimate of the probability of a streptococcal infection (CEPSI); comparison of BA and FA technics

Blood agar		Fluorescent antibody	
Number strep	CEPSI* %	Number strep	CEPSI %
0	13 (622)	0	16 (631)
Rare	19 (18)	Few	36 (81)
1+	32 (50)	Moderate	73 (158)
2+	58 (28)	Many	89 (240)
3+	84 (91)		—
4+	89 (296)	Total	43 (1,110)
	—		
Total	43 (1,105)		

* Number in parenthesis = number cultures.

Table 5—Clinical estimate of the probability of a streptococcal infection to combination of results of cultures on blood agar (BA) and by fluorescent microscopy

	No	Yes	Total	CEPSI
Both occasional	411	53	464	11.4
Both numerous	35	248	283	87.6
BA occasional,* FA numerous	19	14	33	42.4
BA numerous,† FA occasional	12	43	55	78.2
	477	358	335	42.9

* "Occasional" on BA=0, Rare, 1+; on FA="0" and "Few."
† "Numerous" on BA=2+, 3+, 4+; on FA="Moderate" and "Many."

This suggests that the BA results are more important than the FA results.

These results indicate that the number of streptococci found in the culture by either method is highly correlated with the physician's initial clinical impression of the probability of a streptococcal infection. The more organisms found, the more probable is a clinical diagnosis of streptococcal infection. They also indicate that the results using the blood agar technic are somewhat better correlated with the clinical impression than are the results using the FA technic. This is shown in those situations where the two culture methods have diverse results (one showing "Occasional" organisms, the other "Numerous"). Under these circumstances, the CEPSI is higher in the group of patients who had numerous organisms in the BA cultures and only occasional fluorescent organisms by the FA method.

It would appear, then, that if only one method were to be used in our laboratory, with the significance of the results the only consideration, we would choose the blood agar method for culturing beta hemolytic streptococci. However, there are three other important factors that must be considered in a pediatric office laboratory: (1) speed of obtaining the results; (2) the ability to differentiate at the time of initial bacteriologic diagnosis group A streptococci from other groups that are generally not pathogenic for man, and lastly (3), the cost of the procedure.

Speed of Obtaining Results

Using our modified FA technic, which involved overnight incubation, we sacrificed maximum speed to obtain results for simplification of the method. It thus took our laboratory workers longer to find the results of the FA cultures than results with blood agar plates. Although inspection of an average of 20 to 30 blood agar plates took 30 minutes at most, the processing of those same specimens, even by our abbreviated "Modified" FA method, took at least two hours after incubation. Consequently, we usually knew the blood agar results by 9:00 A.M. of the day after the patient was seen, but it was not until 12 noon that the FA results were available.

If it were sufficiently important to the health of the patient to know the result of a particular culture promptly, it is possible to get the result the same day the patient is seen by using the standard FA method and processing the culture separately. However, this situation was encountered very rarely.

Differentiation of Group A Streptococci from Other Groups

Another advantage claimed for the FA technic is that it differentiates group A organisms from other human nonpathogenic (non A) strains, and this can not be done when using blood agar plates alone. Actually, this is not particularly important in dealing with sick children because almost all strongly positive cultures on blood agar are group A. In this series, 98.5 per cent of 407 cultures found positive on the blood agar plates were shown to be group A by the bacitracin disc method. Although we found

Table 6—Cost analysis: Blood agar technic *vs.* fluorescent antibody for diagnosis of beta hemolytic streptococci

Item		Total or unit cost	Blood agar Cost per culture	Fluorescent antibody Cost per culture
Permanent equipment*				
Microscope		475.00	0.032	0.032
Fluorescent lamp		510.00		0.034
12 place centrifuge		160.00		0.011
Incubator		125.00	0.008	0.008
Miscellaneous	BA	100.00		
	FA	150.00	0.007	0.010
Consumable supplies				
Repairs and parts	BA	15.00	0.005	0.013
	FA	40.00		
Mercury arc		45.00		0.045
Media—blood agar		0.30	0.100	
Media—broth		0.15		0.150
Conjugate—group A	5 cc	15.00		0.075
Conjugate—control	5 cc	15.00		0.008
Cover slips, slides		3.00/100		0.030
Miscellaneous	BA	100.00	0.033	0.050
	FA	150.00		
Personnel				
technician	Per hr	2.50	0.050	0.250
Laboratory space	50 sq ft at			
elect. etc.	400/ft/yr=	200.00	0.066	0.066
Total			0.301	0.782

* Depreciated over 5 yr.

that 4.6 per cent of the cultures strongly positive on the blood agar plates were negative by the FA technic, we think that this discrepancy was due to technical errors for the most part and not because they were not group A.

Cost of the Determinations

Last, we wish to consider the costs involved with the FA technic compared with the blood agar method. The estimates are approximations, and we have excluded charges for the clerical work involved in recording the results, phoning patients, and so on, or the cost of training an office worker to use the FA technic; nor has any charge been made for physician supervision. Actually, time in training represented at least three weeks in the aggregate, and was performed by the senior investigator who subsequently acted in a supervisory capacity. Had these costs been added, even if spaced over three years, estimates would have been increased materially.

The costs are calculated on a basis of 3,000 cultures per year. Although we do about 8,000 cultures for beta hemolytic streptococci on blood agar in our office yearly, our special interest makes this number greater than it would obtain in most comparable offices. Using these figures, we found that the blood agar method costs approximately 30 cents per culture, and the fluorescent antibody method approximately 80 cents (Table 6). This means a difference of approximately 50 cents per culture between the

blood agar and FA methods, as done in the relatively simple manner used in our office. Had we adapted the more complex and therefore more costly technics conducted at research institutions such as the Communicable Disease Center, the costs would have been higher by both methods.

Discussion

In this investigation, the use of the fluorescent antibody method of diagnosis of streptococcal disease has been studied in a small pediatric office. The use of such an office for bacteriologic studies has one marked advantage over most large laboratories. It is possible in such a setting to correlate closely the laboratory and clinical results—a situation rarely possible in the larger laboratory with its superior technical facilities.

The studies have shown that by simplifying and modifying the standard method, the technic could be used on a high proportion of cultures for beta hemolytic streptococci in a relatively small medical pediatric group practice. Compared with the standard method used at the US Public Health Communicable Disease Center, our method seemed to be reasonably accurate, although a longer incubation time than the standard method was required.

Our studies indicated that the FA technic did not show appreciably more or less beta hemolytic streptococci than were found with the blood agar technic; nor were the findings with the FA technic any better correlated with the clinical diagnosis than were those with the blood agar technic.

The increased speed of diagnosis possible with the standard FA technic over the blood agar technic had little practical application in our pediatric office, and was therefore sacrificed in favor of simplification. In large laboratories, this increased speed counterbalanced the lags incidental to getting specimens to the laboratory and a report of the results back to the physician. In many cases, this reduces the usual three-day delay to two days. However, in pediatric offices that do their own bacteriology, as in our case, the usual delay between culturing the patient and finding the result of the culture is rarely over 24 hours. To try to decrease this time appreciably by any technic presents so many practical problems that it is rarely worth while. It would mean, for example, that if maximum speed were to be obtained that the cultures on each individual patient would have to be started when he was first seen and processed independently of all the rest. We therefore allowed our fluorescent antibody cultures to grow overnight rather than to try to determine the cultural results within a few hours. Consequently, since the blood agar technic is simpler, our culture results were available with that technic before they were read by the fluorescent antibody method. Therefore, from this standpoint the FA technic did not offer any advantage over the blood agar method of culturing.

Another advantage claimed for the FA technic is that it distinguishes group A beta hemolytic streptococci from other nonpathogenic groups that may be found in throat cultures. As mentioned earlier, in dealing with sick children almost all cultures strongly positive for beta hemolytic streptococci are group A, and thus from this standpoint the FA technic also did not offer any advantages over the blood agar technic.

Again, there is the matter of costs. Our figures show that the FA technic costs about 50 cents per culture more than the blood agar method of culturing. In our office, this would amount to about $4,000 annually, and we consider this an excessive figure. Accordingly, from almost every angle, we can see no practical advantage of the fluorescent antibody technic over the blood agar method in the bacteriologic diagnosis of beta hemolytic

streptococcal illness in pediatric practice. We do not believe that it should replace the blood agar method. Nonetheless, as an adjunct to the blood agar method, we believe that it may have value; indeed, there are at least four situations in clinical practice where the FA technic could be helpful.

The first is the rare situation where—for some clinical or social reason—an immediate answer is desired as to whether a child has a streptococcal infection. Under these circumstances, both the direct method of Rauch and Rantz[7] or the standard FA method could be utilized to give an answer the same day that the patient was seen. The second situation occurs when the blood agar plates show hemolysis but streptococci can not be identified, usually due to overgrowth of other bacteria. In this instance, another diagnostic method would be helpful. The third situation occurs when the child clinically has a streptococcal infection, and treatment has been initiated but the blood agar culture is negative. Since this may be a technical error, it would be helpful to have a second method of checking the culture result. The fourth situation occurs when it is desirable to find out whether a particular beta hemolytic streptococcus is a group A organism. Here the FA technic may be used profitably despite the fact that the bacitracin disc method is simpler. When used on a pure culture, the FA technic is more rapid and probably more accurate.

In the writer's opinion, none of the above situations is sufficient to warrant the use of both the blood agar *and* the FA method routinely. However, it is possible to check the former method by the latter, when indicated, without putting every swab in broth initially. This may be done by the simple expedient of saving every swab from patients clinically suspected of streptococcal disease for 24 hours in a dry state. Those that show a discrepancy between the clinical opinion and the result on the blood agar plate can then be rechecked by both the FA method and a second attempt on the blood agar plate.

Desirable as such a double check on our streptococcal results might be, we do not believe that this is sufficient to warrant the cost of the apparatus, supplies, and the time consumed in even a relatively large pediatric group doing its own bacteriology. However, if the FA technic is also used for other clinical purposes, such as the immediate diagnosis of influenza meningitis or pathogenic E. coli, it is possible that an investment of this magnitude might be worth while.

Summary and Conclusions

The practicality of the use of the fluorescent antibody technic for the bacteriologic diagnosis of beta hemolytic streptococci in a pediatric group practice has been studied. It was found that the method could be modified and simplified, so that it could be utilized in our pediatric laboratory.

When compared with the standard FA method, it was reasonably accurate. When compared with the use of blood agar plates, the method gave about the same quantitative and qualitative results bacteriologically. Moreover, compared with the physician's clinical diagnosis, the results on the blood agar plates are as good or better than those obtained with the fluorescent antibody technic. Since 98.5 per cent of cultures of beta hemolytic streptococci from sick children were group A, there is little advantage in determining the specific group of each organism in pediatric illnesses.

Cost estimates showed that in our laboratory, excluding training and supervising costs, the FA technic came to 78 cents per culture and the blood agar method to 30 cents.

It is concluded that the FA technic offered no advantage over the blood agar

method in routine cultures for beta hemolytic streptococci in our pediatric office. Under some circumstances, the use of the FA technic as an adjunct to the routine use of blood agar plates could be advantageous; from a practical standpoint, however, this would be warranted only if the FA apparatus were used for other purposes.

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Dr. Breese is Clinical Professor of Pediatrics, University of Rochester School of Medicine and Dentistry (2333 Elmwood Avenue), Rochester. N. Y. 14618.
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Harold G. Wolff, M.D. Lecture Award

The American Association for the Study of Headache announces the Fourth Annual Harold G. Wolff, M.D., Lecture Award. Physicians are invited to submit an original paper on headache, head pain, or on the nature of pain itself. The paper may deal with basic research, clinical studies, or both. All physicians, including those in training as fellows or residents, are eligible.

A prize of $1,000 will be awarded to the recipient of the lectureship, and he will be invited to present his paper at the annual meeting of the American Association for the Study of Headache at the New York Hilton Hotel, New York City, on July 12, 1969. Papers should be submitted in duplicate to: Donald J. Dalessio, M.D., 8878 Nottingham, La Jolla, Calif. 92037. The deadline date is March 15, 1969.