Henry Ford Hospital Medical Journal

Volume 13 | Number 3 Article 11

9-1965

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Henry Ford Hosp. Med. Bull.
Vol. 13, September 1965

A COMPARISON OF THE IMMUNOFLUORESCENCE TECHNIQUE WITH CONVENTIONAL METHODS FOR THE IDENTIFICATION OF GROUP A BETA HEMOLYTIC STREPTOCOCCI

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This study was undertaken to determine the 'present-day' advantages and disadvantages of the fluorescent antibody (FA) technique[1,2,3] and to evaluate this method with the conventional procedures in the identification of Group A beta hemolytic streptococci isolated from clinical specimens. Our previous experiences, during the period from 1956-1960, had demonstrated that the materials and methods could not be readily applied to routine diagnostic bacteriological procedures, even though they had been proven to be useful experimental tools. However, since the streptococcal fluorescein conjugates and equipment have been improved during the past few years, it was decided that the fluorescent antibody test for beta streptococci should be re-evaluated.

The advantages of the FA technique are rapidity and sensitivity. The two major disadvantages of this type of staining procedure are as follows: (a) the problems inherent in any serological system, namely, the very real possibility that the globulin or serum conjugate is non-specific, (b) the financial expense and time required to obtain the equipment and to train the technologist. Therefore, the decision to use this procedure must be based on the particular requirements of each clinical bacteriological laboratory.

The authors have compared the efficacy of several Group A streptococcal fluorescein conjugates, using both pure stock cultures and fresh isolates from clinical specimens. The organisms were, also, examined by such conventional methods as: (i) the Lancefield streptococcal precipitin test,[4] using either the Autoclave,[5] Hydrochloric acid,[6] or Lytase[7,8] extraction procedures, (ii) Maxted's Bacitracin disc test.[10]

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Materials and Methods

Study No. 1 — The fluorescent study was divided into two series of experiments. The first series which were undertaken consisted of the fluorescent staining of approximately 100 previously isolated and identified stock cultures of beta hemolytic streptococci selected at random. These organisms were isolated from a variety of clinical specimens. The streptococci were grown on sheep blood agar (SBA and/or in Todd-Hewitt Broth (THB). This will be referred to as *Study No. 1.*

Study No. 2 — The second phase of the project involved the processing by the fluorescent procedure of 550 throat cultures which were suspected to contain beta hemolytic streptococci. This will be referred to as *Study No. 2.* Some fluorescent antibody (FA) tests were performed on isolates from such clinical specimens as spinal fluid, sputa, throat swabs, urines, wounds, etc.

The second study consisting of five hundred and fifty throat swabs used in *Study No. 2* were processed in three groups as follows:

Group I — 79 throat swab specimens (No. 1 to No. 79) consisted of a comparison 3 hour Todd-Hewitt cultures with 18 hour SBA plate cultures.

(a) The sediment from the 3 hour THB was processed by the FA procedure.

(b) Colonies from the 18 hour plate were tested as follows:

(i) FA staining.
(ii) Lancefield precipitin testing.
(iii) Bacitracin disk testing.

Group II — 253 throat swab specimens were used to compare the efficacy of 18 hour THB cultures with 18 hour SBA platings. These were compared on the basis of the technique discussed for Group I above.

Group III — 218 throat swab specimens were tested according to the method outlined in Group I, part (b) above.

The three basic procedures used in *Studies No. 1 and 2* were as follows:

(a) bacitracin or Taxos A*
(b) the Lancefield precipitin test[4,6]

(i) Autoclave procedure[5]
(ii) Hydrochloric Acid Extraction[6]
(iii) Enzyme method[7]

(c) fluorescent procedures using conjugates of goat and/or rabbit serum globulins.

(i) Company A or
(ii) Company B.

The pure cultures and specimens were almost always inoculated onto 5-7 per cent sheep's blood-trypticase-soy agar in both *Studies No. 1 and 2.* Todd-Hewitt broth was also widely used throughout the experiments for growing both pure culture isolates as well as for initial isolation from throat swabs.

*Taxos A = bacitracin discs supplied by Baltimore Biological Laboratory (BBL), Baltimore, Maryland. These were used according to the BBL instructions.

IMMUNOFLUORESCENCE

The purpose of the first study using known positive beta streptococci was to determine whether the FA procedure might be superior to the conventional methods now used to identify Group A streptococci. The precipitin test was used as the basis for judging whether or not the streptococcus belonged to the Lancefield Group A.

The primary purpose of *Study No. 2* was to evaluate whether or not the Todd-Hewitt broth culture, either the 3 hour or 18 hour cultures, could possibly replace the SBA plate culture using the FA procedure for the identification of Group A beta streptococci.

The fluorescent antibody test is performed by placing a loop of saline emulsified culture on a clean microscope slide. After air drying and heat or ethanol fixation, the conjugate is applied to the smear. It was found that a 1:2 dilution of the commercial conjugates most frequently gave the best results. The literature indicates that as high as a 1:80 dilution of the 'stock conjugate' may give good results. The flooded smear is incubated in a moist chamber at 37°C. for 30 to 45 minutes, either with or without gentle shaking. The preparation is next washed for 5 minutes each in successive baths of buffered saline and distilled or tap water. When dry it is mounted in a drop of buffered glycerin, Ph 8.2. The slide is then examined by fluorescence microscopy. The OSRAM HBO 200 mercury vapor lamp was used in conjunction with the BG 12 exciter filter and the number 50 Zeiss barrier filter. The planapochromatic oil immersion objective lens (100X) was employed. The positive fluorescence was graded according to the following subjective schema: $-$ = negative, $\pm$ = questionable (autofluorescence), 1+ to 4+ readings were indicative of positive fluorescence.

Both negative and positive FA smears were included in this study. Strains of *streptococcus* group C and *staphylococcus aureus* were routinely used as the negative FA controls. One to two positive Group A controls were performed with each experiment.

In an attempt to gain some experience with the reliability of commercial sera, three sets of streptococcal antisera from three different sources were used. Three extraction procedures were also used on at least 21 organisms in order to determine what differences, if any, would be encountered. Group D organisms and Group E antisera were not as extensively used as other strains of organisms and antisera.

Twenty-one strains were serologically tested by Dr. M. Moody of the Communicable Disease Centre (CDC), Atlanta, Georgia. The authors are very grateful for the very kind assistance which Dr. Moody gave during this study.

Each beta hemolytic streptococci isolated was, also, tested for susceptibility or resistance to such commonly used antibacterial agents as Penicillin-G, Erythromycin and Tetracycline.

RESULTS

Study No. 1: The 100 streptococci isolated in this study were separated initially on the basis of the Lancefield serological procedures into "Group A" or "Not Group A" as shown in Table 1. It is evident from the data that more than one-half (57 per cent) of the streptococcal isolates were Group A. The table also shows the breakdown of the streptococcal groups according to the source of the specimen. It can be readily seen that the majority of Group A streptococci were isolated from the ear, nose, throat and wound specimens. Whereas, the majority of the non-Group A streptococci were obtained from sputa and wound specimens.

The data in Table II gives the Lancefield groupings of the 43 non-Group A streptococci using the precipitin test. The streptococcal Group B occurred more frequetnly (42 per cent) than the other groups. It should be pointed out that little

consideration was given to urine cultures isolates and thus the reason for the lack of Group D streptococci (enterococci) in this study.

Table 3 shows the degree of correlation using the three methods. The data shows relatively close agreement (96 per cent) between the FA and precipitin methods with a wider variation (9-14 per cent) using the bacitracin procedure.

Tables 4 and 5 show that all the beta streptococci tested regardless of group, were sensitive to Penicillin-G and only 1 per cent was resistant to Erythromycin. However, a larger number were resistant to tetracycline. Eighteen per cent of all the beta streptococci showed resistance to this antibiotic.

Study No. 2, Group I: The data in Table VI shows that there were 14 beta streptococci isolated and typed by the Lancefield procedure. The FA technique for Group A streptococci identified 7 or 100 per cent of the Group A streptococci present on the SBA plate and 6 of the 7 or 86 per cent of the Group A strains using THB. The bacitracin test was positive for 9 strains and thus would have been responsible for 2 Group A false positive if the precipitin and/or the FA test had not been performed. All three procedures were responsible for questionable or doubtful reactions (see Table VI).

Study No. 2, Group II: It was assumed that longer incubation times for THB would not only yield a larger percentage of positive but that more growth would assist the bacteriologist in identifying the Group A streptococci using FA techniques. The data in Table VII shows the results of the 18 hour THB and SBA cultures. Both the FA procedure using 18 hour SBA isolates and the bacitracin procedure identified 100 per cent of the Group A streptococci whereas only 19 of the 26 (73 per cent) Group A streptococci were detected by the THB-FA method. The 18-hour THB procedure also showed considerably more doubtful results than the 18 hour SBA and bacitracin techniques (see Table VII).

Study No. 2, Group III: Since the data using 3 and 18 hour THB cultures was not nearly as reliable in the detection of Group A streptococci as the SBA platings, the decision was made to perform FA Group A staining only on SBA cultures. Two hundred and eighteen throat swabs yield 30 beta streptococcal strains (see Table VIII). Twenty-three (77 per cent) belonged to Group A and 7 (23 per cent) were non-Group A streptococci. The FA was only slightly more effective in identifying Group A strains than the bacitracin test as demonstrated in Table VIII.

Study No. 2, Susceptibility Tests: The data in Table IX summarizes the susceptibility of the beta streptococci to Penicillin G (Pen G), Erythromycin (Erythro) and

Tetracycline (Tetra). The designations — (a), (b) and (c) refer to Groups I, II and III respectively of Study No. 2. It can be seen that 100 per cent of the Group A streptococci were susceptible to Penicillin G and Erythromycin whereas 20 per cent of these strains were Tetracycline resistant. It so happened in this study that one of 25 non-Group A strains was Penicillin resistant and Erythromycin susceptible (see Table IX).

It can be seen that the susceptibility patterns of the beta streptococci of Study No. 1 and 2 were comparable. In both studies all the strains showed high susceptibility to Penicillin G and Erythromycin and they exhibited greater resistance to Tetracycline (see Tables IV, V and IX).

Extensive serological procedures were undertaken in order to evaluate the degree of specificity of streptococcal extracts and commercial group antisera. Not only were the extracts prepared by three commonly used procedures but three sources of antisera were also used (see Table X). In addition, twenty-one strains were tested by Dr. Max Moody of the Communicable Disease Centre, Atlanta, Georgia.

The data shown in Table X and XI demonstrates the large number of cross reactions which were obtained using the autoclave extract and two sources of commercial antisera (i.e. A,[1] B[2]). The HC1 extracts of the 21 organisms showed extremely close correlation with all three batches of antisera. A significant number of cross over reactions were obtained with the autoclave and enzyme extracts (see Table X and XI).

A summary of the members of both the Group A and non-Group A streptococci is shown in Table XII and XIII. Examination of the results in these tables will show that members of Group A predominated in our control study No. 1 (see Table XII). Strains of Group B, C, D, G, etc. were also included to determine the degree of cross-reactions with Group A conjugates. The data in Table XIII shows that the incidence of Group A streptococci was sixty-nine per cent of the total streptococci isolated from throat swabs in study No. 2, not nearly as high as reported by some authors[3] in the literature. The results in Table XIV summarizes the data obtained from both studies No. 1 and 2. This information supports the thesis that streptococcal Groups A, B, C, G and the non-typable are the most prevalent isolates in clinical specimens with the possible exception of Group D which occurs with relative frequency in urine but was examined only briefly in this study.

The percentage of false reactions with bacitracin and FA are shown in Table XV. The preliminary screening with both techniques demonstrated from 2-14 per cent false results (see Table XV).

Table I

Beta Streptococci Isolates Grouped According to A or Not A By Study No. 1

Group	1		2		3		4		5		
Specimens	Ear, Nose and Throat		Sputa		Wounds		Genitourinary		Spinal Fluids		
	No.	%	No.	%	No.	%	No.	%	No.	%	Total
Beta streptococci	31	31	19	19	43	43	6	6	1	1	100%
Group A	26	84	4	21	25	58	1	17	1	100	57%
Not Group A	5	14	15	79	18	42	5	83	0	0	43%

Table II

Grouping of Those Streptococci Not Group A By Study No. 1

Specimens	Ear, Nose and Throat	Sputa	Wounds	Genitourinary	Spinal Fluids	Total	
	No.	No.	No.	No.	No.	No.	%
Group B	1	8	8	1	0	18	42
Group C	1	2	4	0	0	7	16
Group D	0	0	0	0	0	0	0
Group E	0	0	0	0	0	0	0
Group F	0	1	1	1	0	3	7
Group G	1	1	3	0	0	5	12
Non-typable	0	2	2	2	0	6	14
Not done	2	1	0	1	0	4	9
						43	100

Table III

Comparison of the Three Identification Procedures for Study No. 1

Procedures	57 Group A Streptococci						43 Non-Group A Streptococci					
	Positive		Negative		Questionable		Positive		Negative		Questionable	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Fluorescent Antibody Technique	56	98	0	0	1	2	0	0	42	98	1	2
Bacitracin or Taxos-A disc	52	91	0	0	5	9	0	0	37	86	6	14
Precipitin Test	57	100	0	0	0	0	43	100	0	0	0	0

Table IV

Antibiotic Susceptibility Pattern of Group A Streptococci for Study No. 1

Specimens	Total	Penicillin-G		Erythromycin		Tetracycline	
		Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
Ear, Nose and Throat	26	26	0	26	0	23	3
Sputa	3	3	0	3	0	1	2
Wounds	26	26	0	26	0	25	1
Genitourinary	1	1	0	1	0	1	—
Spinal Fluids	1	1	0	1	0	—	1
Total	57	57	0	57	0	50	7
Per Cent	100	100	0	100	0	88	12

Table V

Antibiotic Susceptibility Pattern of Non-Group A Streptococci of Study No. 1

Specimens	Total	Penicillin-G		Erythromycin		Tetracycline	
		Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
Ear, Nose and Throat	5	5	0	5	0	5	0
Sputa	16	16	0	16	0	11	5
Wounds	17	17	0	16	1	12	5
Genitourinary	5	5	0	5	0	4	1
Spinal Fluids	0	0	0	0	0	0	0
Total	43	43	0	42	1	32	11
Per Cent	100	100	0	98	2	74	26

Table VI

Comparison of the 3-hour Todd-Hewitt Broth and 18-hour SBA Throat Cultures (79) Using the FA and Bacitracin Tests in Study No. 2

No. of beta streptococci = 14		Todd-Hewitt Broth	18-hour SBA Plate	
		FA	Bacitracin	FA
No. of Group A	= 7	6 = +* (43%)	9 = +	7 = +
No. of Not Group A	= 7	7 = −** (50%)	4 = −	5 = −
		1 = ?***(7%)	1 = ?	2 = ?

* + = positive.
** − = negative.
*** ? = questionable.

Table VII

Comparison of the 18-hour Todd-Hewitt Broth Cultures Using the FA and Bacitracin Tests on 253 Throat Swabs in Study No. 2

No. of beta streptococci = 37		Todd-Hewitt Broth	18-hour SBA Plate	
		FA	Bacitracin	FA
No. of Group A	= 26	19 = +* (52%)	26 = +	26 = +
No. of Not Group A	= 11	10 = −** (27%)	10 = −	10 = −
		5 = ?*** (13%)	1 = ?	0 = ?
		3 = No****(8%)	0 = No	1 = No

* + = positive.
** − = negative.
*** ? = questionable.
****No = not done.

Table VIII

Beta Streptococcal Identification of 218 SBA Cultures of Throat Swabs Using the FA and Bacitracin Procedures

No. of cultures containing beta streptococci	= 30
No. of Group A beta streptococci	= 23
No. of Non-Group A beta streptococci	= 7
No. showing positive FA	= 23
No. showing positive Bacitracin	= 22
No. showin questionable FA	= 0
No. showing questionable Bacitracin	= 1

Table IX

Susceptibility Patterns of Beta Streptococci Isolated in Study No. 2

		Penicillin-G		Erythromycin		Tetracycline	
		Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
Group A (a)*	7	7	0	7	0	6	1
(b)*	26	26	0	26	0	19	7
(c)*	23	23	0	23	0	20	3
Totals	56	56	0	56	0	45	11
Per Cent	100	100	0	100	0	80	20
Not Group A (a)	7	7	0	7	0	5	2
(b)	11	10	1	11	0	8	3
(c)	7	7	0	7	0	6	1
Totals	25	24	1	25	0	19	6
Per Cent	100	96	4	100	0	76	24
SUMMARY:							
Group A	56	56	0	56	0	45	11
Not Group A	25	25	1	25	0	19	6
Totals	81	80	1	81	0	64	17
Per Cent	100	99	1	100	0	79	21

* (a), (b) and (c) represent the three groups of experiments in Study No. 2 — See Section on Materials and Methods.

Table X

STREPTOCOCCAL TYPING

Evaluation of Three Extraction Methods and Three Different Sources of Lancefield Streptococcal Grouping Antisera Using 21 Strains of Beta Streptococci

Strain Number	Communicable Disease Center (CDC)*	Autoclaved Extract Method of Rantz and Randall			HC1 Extract of Lancefield			Enzyme		
		A[1]	B[2]	C[3]	A	B	C	A	B	C
115	A	ABCDFG	ADC	A	A	A	A	AB	AD	A
172	C	CG	CD	C	C	C	C	ABCD	—	C
30	A	AB	AD	A	A	A	A	ABD	D	A
59	B	B	BD	B	B	B	B	AB	B	B
133	A	ABD	AD	A	A	A	A	AB	D	A
31	A	AD	AD	A	A	A	A	ABD	D	A
137	B	ABCDF	BD	B	B	B	B	ABD	BD	B
357	C	C	CD	C	C	C	C	B	D	C
297	A	A	AD	A	A	A	A	BD	—	A
281	C	ABCDFG	CD	C	C	C	C	D	D	C
91	A	AB	AD	A	A	A	A	BDC	D	G
100	G	AG	D	G	G	—	G	AB	D	G
201	C	ABCDF	C	C	C	BC	C	ABCD	C	C
262	A	AB	AD	A	A	A	A	BD	C	A
526	A	ABD	AD	A	A	A	A	ABD	D	A
65	A	AB	AD	A	A	A	A	A	AD	A
8	G	BCDF	C	G	G	G	G	DG	—	G
87	A	ABD	AD	A	B	A	A	ABD	AD	A
9	B	B	C	B	B	B	B	AB	BD	B
20	G	BDF	—	G	G	G	G	ABDG	D	G
18	A	ABDF	F	A	A	A	A	AB	D	A

*Communicable Disease Center — These were the results obtained by Dr. Max Moody, of the CDC.

A^1,B^2,C^3 represent the three sources of streptococcal typing sera,which were prepared by three different methods.

Table XI

Summary of the Results Obtained by Three Extraction Methods and Three Different Sources of Streptococcal Grouping Antisera.

Streptococcal Group	Communicable Disease Center (CDC)	NUMBER OF STRAINS BY								
		Autoclaved Extract Method of Rantz and Randall			Hot HC1 Extract of Lancefield			Lytase Extract Method of Maxted		
		A	B	C	A	B	C	A	B	C
A	11	11(10)*	10(11)	11(0)	9(1)	11(0)	11(0)	8(10)	3(10)	11(0)
B	3	3(1)	2(3)	3(0)	3(0)	3(0)	3(0)	3(3)	3(2)	3(0)
C	4	4(3)	4(3)	4(0)	4(0)	4(1)	4(1)	2(4)	1(3)	4(0)
D, E and F	0									
G	3	1(3)	0(2)	3(0)	3(0)	2(0)	3(0)	2(3)	0(2)	3(0)
Other	0	0	1	0	0	1	0	0	0	3

Antigen and serum controls negative.

*( ) = Number of strains showing cross-reactions or typing in groups other than that classified by CDC procedure.

Table XII

Summary of Streptococcal Groupings for Strains Examined in Study No. 1

Lancefield Group	Specimen Group Number*					Total	% of Total Beta Strep. Isolates	% of Beta Strep. Not Group A
	1	2	3	4	5			
No. of beta strep.	31	19	43	6	1	100	100	43
Beta strep., Group A	26	4	25	1	1	57	57	0
Beta strep., Not Group A	5	15	18	5	0	43	43	0
Group B	1	8	8	1	0	18	18	42
Group C	1	2	4	0	0	7	7	16
Group D	0	0	0	0	0	0	0	0
Group E	0	0	0	0	0	0	0	0
Group F	0	1	1	1	0	3	3	7
Group G	1	1	3	0	0	5	5	12
Non-Typable	0	2	2	2	0	6	6	14

*Specimen Group Numbers were as follows:
(1) Ear, Nose and Throat
(2) Sputa
(3) Wounds
(4) Genitourinary
(5) Spinal Fluids.

Table XIII

Summary of the Beta Streptococcal Groupings in Study No. 2

Categories	Group I	Group II	Group III	Total	% of Total Throat Swabs (550)	% of Total Beta Strep. Isolates (81)	% of Total Beta Strep. Not Group A (25)
	Specimen Numbers						
	1 -79	80-332	333-550				
No. of specimens	79	253	218	550			
No. of beta strep.	14	37	30	81	15		
Beta strep., Group A	7	26	23	56	10	69	
a) by THB and BA	6	26	0				
b) by BA only	1	0	0				
Beta strep., Not Group A	7	11	7	25	5	31	
Group B	4	1	1	6		7	24
Group C	2*	7**	2	11		14	44
Group D	0	0	0	0		0	0
Group E	0	0	0	0		0	0
Group F	0	0	1	1		1	4
Group G	0	2	2	4		5	16
Non-Typable	1	0	1	2		3	8
Not Done	0	1	0	1		1	4

*Both were Taxos-A positive, but FA negative.

**1 beta strep. was Taxos-A positive, but FA negative.

Table XIV

Summary of Study No. 1 and No. 2

		Study No. 1	Study No. 2	Total	% of Total Beta Strep. Isolates (181)
Total No. of beta strep.		100	81	181	100
Total No. of beta strep.	Group A	57	56	113	62
Total No. of beta strep.	Not Group A	43	25	68	38
Total No. of beta strep.	Group B	18	6	24	13
	Group C	7	11	18	10
	Group D	0	0	0	0
	Group E	0	0	0	0
	Group F	3	1	4	2
	Group G	5	4	9	5
	Non-Typable	6	2	8	5
	Not Done	4	1	5	3

Table XV

Comparison of the Preliminary and Follow-Up Results Using Both Bacitracin and FA
In Studies No. 1 and No. 2

	BACITRACIN			FLUORESCENT ANTIBODY								
				SBA			3 hr. T-HB			18 hr. T-HB		
		False Results			False Results			False Results			False Results	
	No.	Prelim. Report	With Repeat	No.	Prelim. Repeat	With Report	No.	Prelim. Report	With Repeat	No.	Prelim. Report	With Repeat
Study No. 1	100	7	1	50	0	0	50	2	0	0	0	0
Study No. 2	81	5	3	81	2	0	14	1	0	37	5	0
Total No.	181	12	4	131	2	0	64	3	0	37	5	0
Percentage		7	2		2	0		5	0		14	0

Discussion

Some authors have indicated that 95 per cent of the Beta Hemolytic Streptococci isolated from throat cultures are Group A, but our experience does not support this high percentage (see Tables I, VI, VII and VIII). In fact, our data shows that about 70 per cent of the hemolytic streptococci were Group A streptococci. This is a strong argument in favor of perfoming throat cultures before or at the initiation of antibiotic therapy. Another obvious advantage in performing the culture is to determine the predominating organism which may be a species other than the streptococcus. If it is the latter, a susceptibility test can be performed using the antibiotic(s) (Penicillin G, Erythromycin, Tetracycline) intended for the patient.

The value of the immunofluorescence test for grouping the Group A beta streptococci has been debated by clinical bacteriologists for the past several years.[9] These investigators have criticized the possible usefulness of the FA technique for hospital laboratories for several reasons. They have stated that the methods and materials are presently rather complex for the routine laboratory. Some of their reasons are as follows:

(i) the commercial conjugates have shown considerable variation in specificity.

(ii) the equipment required for the FA technique is expensive and difficult to assemble and maintain.

(iii) unless the laboratory has a large number of FA specimens, the procedures are too time consuming.

Our experience with the FA streptococcal staining technique has led us to draw several conclusions. Firstly, the 3 hour Todd-Hewitt broth cultures, or for that matter the 18 hour THB cultures, did not always yield the beta hemolytic streptococcus upon FA staining of the sediment. The SBA plates in conjunction with FA staining detected a higher percentage of Group A streptococci than did the FA THB preparations. Therefore the recommendation is being made that if the FA — Todd-Hewitt Broth procedure is used, the SBA must be employed for more complete bacteriological evaluation of both Group A streptococci and other pathogens. The FA — THB technique, either the 3 hour or the 18 hour, should be considered a screening and/or supplementary procedure, since there is the strong possibility of overlooking positive specimens.

The 18 hour Todd-Hewitt Broth cultures are more logically used instead of the 3 hour THB because throat swabs are received throughout the day and can be processed by the FA technique by the following morning. Another reason for selecting a period longer than 2-4 hours is that the FA microscopic staining is made less tedious due to the presence of a higher concentration of organisms. Another

possibility is that the THB and SBA can be set up and the negative SBA specimens can be checked by FA staining of the THB.

The workload in much increased by FA streptococcal staining of either the THB or SBA cultures. Therefore the supervisor of the bacteriology laboratory and the clinicians must make the decision as to whether or not the FA examination for Group A streptococci is a desirable procedure within their particular hospital, State laboratory etc.

Another approach to this question of grouping the streptococci involves the more conventional precipitin test. Either the Group A precipitin or complete streptococcal grouping should be considered. The drawback for this suggestion is that another 24-48 hours is required. Furthermore, the bacteriologist should employ the HC1 extraction technique and have a satisfactory source of streptococcal grouping antisera which produce a minimum of cross-reactions.

One other possible alternative is the use of bacitracin (Taxos A) discs. If the beta hemolytic streptococcus produces a 12-15 mm. or larger zone, the tentative identification of a Group A streptococcus can be made. However, one should bear in mind that this is purely a screening procedure with a prediction accuracy level of 85-90 per cent.

Summary and Conclusion

A relatively large percentage (31 per cent) of beta hemolytic streptococci isolated from throat swab specimens did *not* belong to the Lancefield Group A.

Three and 18-hour Todd-Hewitt Broth Cultures did not yield as many Group A streptococci as the sheep's blood agar plates, using the FA Group A conjugates. Therefore, the sheep's blood agar plate should also be used in conjunction with the FA broth procedure, if the latter is used for the screening of Group A streptococci.

The serological studies indicate that the streptococcal extracts, which are to be used for Lancefield grouping procedures, should be prepared preferably by the HC1 extraction procedure in order to avoid major cross-reactions between the various groups.

The Bacitracin disc test, which is used for the presumptive screening of Group A streptococci, may be in eror 15-20 per cent of the time.

Almost all beta hemolytic streptococci tested in this project, especially the Group A strains, were very susceptible to Penicillin-G and Erythromycin, but 15-30 per cent were resistant to Tetracycline. Therefore, the clinician must be aware of the relatively high incidence of Tetracycline resistant beta streptococci, especially the Group A strains, which may be present in his patients.

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