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PROCEEDINGS

IN PURSUIT OF THE STREPTOCOCCUS

Newer Techniques for Their Recovery and Identification, and Clinical Implications

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THE PURSUIT of the streptococcus ends in the laboratory. Except in the hands of an expert, laboratory identification of beta-hemolytic streptococci frequently has failed. Laboratory failure certainly has not fostered the use of throat culture by pediatricians. Introduction of a new augmented culture medium has reduced the experience required for reliable recognition of beta hemolysis. A distinctive fluorescent-antibody technique minimizes error by affording a rapid means of specific identification of streptococcal group and type. A recently developed method of transport of material for culture permits the physician at a distance from the laboratory to utilize throat culture as a reliable diagnostic aid. Additional investigations are in progress, but a report is made at this time in order to describe these three improved laboratory aids in the recovery and identification of streptococci.

MATERIALS

Augmented Medium

The new augmented medium[1] is designed for use by the pediatrician or the microbiologist. In its preparation, the investigators added maltose, nucleic acid and neomycin to the classic beef-heart infusion, blood-agar base.[2] The antibiotic in the medium inhibits growth of micrococci and influenza bacilli and reduces the number of Neisseria.[3] Designated concentrations of nucleic acid and maltose enhance beta hemolysis which becomes sharply defined.[4]

In a clinical trial, 66 strains of Group A and 30 strains of Groups C and G beta-hemolytic streptococci were recovered from 755 consecutive throat cultures on the augmented medium and on classic media.

In a field trial pediatricians experienced no difficulty in recognizing beta hemolysis in cultures from the throat incubated 24-48 hours on the augmented medium and held to the light for reading.[2]

Isolation and grouping of the organisms was

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not done. Demand for this culture medium has increased and it is available from Palo Alto.

Fluorescein-Antibody

The rapid fluorescent-antibody technique of Coons and Kaplan[5] is appropriate for grouping and typing streptococci, in case it is not deemed advisable to institute therapy until an attempt has been made to identify the causative organism in streptococcal disease. The procedure is serologic; concentrated antisera containing specific, fluorescein-tagged antibodies are applied to throat smears. Any streptococci present act as specific antigens around which the homologous tagged antibody is precipitated and fixed. The unreacted or indifferent fluorescent proteins are removed by washing. The slide is examined microscopically under intense ultraviolet light.[6] The presence of streptococci is indicated by a yellow-green glow from deposited antibody. As equipment and specific tagged antisera become available, this half-hour grouping and typing technique will become more accessible.[7]

Inert Transport Materials

The foregoing techniques are most applicable when the laboratory is nearby. In an effort to bring the laboratory to the pediatrician, investigation was made of transport media for subsequent isolation of beta-hemolytic streptococci.[8] The fact that streptococci survive drying was accepted as established.[9-11] A search was made for an inert medium on which streptococci could be dried and recovered after transport. Two such media were found: Dacron-tipped swabs in a tube with a desiccant (DSD); and filter paper strips (FPS).[8]

PROCEDURES IN CLINICAL TRIALS

In a study previously described,[12] sequence throat swabbing was employed in the inoculation of a control culture and preparation of a paired Filter Paper Strip or a Dacron-tipped swab with desiccant for delayed culture on each of 1,519 children. The children were from closed population groups: those attending the clinics of four hospitals, three non-pay and one insurance; one pediatric office; and five private offices and two clinics forwarding specimens to a county health department (all co-operating in this study of transport materials for recovery of beta-hemolytic streptococci).

In the present study, three groups were continued in the investigation. In two of these, the insurance hospital and private pediatric office, 394 paired cultures were obtained. Personnel had changed both in the hospital and the laboratory and replacements were newly introduced to the techniques required. The county health department furnished 661 paired cultures.

Simultaneous swabbing was employed; two, Dacron-tipped* swabs being held in one hand and rotated during passage over and around the pharynx of each child. The first swab was placed in a glass tube with silica-gel desiccant contained in a screw cap which is replaced on the tube for transport. The second swab was rolled and streaked firmly over the surface of a Filter Paper Strip in a kit† as shown in Figure 1. The kit was left open 3-5 minutes until the filter paper strip appeared dry, then enveloped and mailed. Meanwhile the second swab was streaked onto a 10% defibrinated sheep-blood agar control plate; the culture was incubated overnight at 37°C and read. Controls at the health department were streaked from the first swab which was transported in a glassine bag, as the technique requiring a glass tube with desiccant was not investigated in the county laboratory.

After 2-10 days, each Filter Paper Strip was plated and each desiccated Dacron-tipped swab was streaked onto the sheep-blood agar which was incubated at 37°C and read visually for beta hemolysis at 24 hours. Beta-hemolytic streptococci were isolated from all cases tabulated as positive and were grouped and typed by courtesy of the Communicable Disease Center (CDC), Public Health Service, under the direction of Dr. Elaine Updyke. The number of beta-hemolytic colonies on each positive plate also was estimated. For this estimate on filter paper strip cultures, the strip was transferred to a second blood plate after a 6-hour primary incubation; both primary and replica plates then were incubated overnight, a colony count recorded and isolates picked for identification.

* Dupont Dacron® batting for preparation of Dacron-tipped applicators was obtained from Pacific Felt Co., 710 York Street, San Francisco, California. Dacron-tipped swabs are available from Econ Microbiological Laboratory, 2716 Humboldt Avenue So., Minneapolis 8, Minnesota.

† Available from Diagnostic Associates, Walnut Creek, California, and Econ Microbiological Laboratory, Minneapolis, Minnesota.

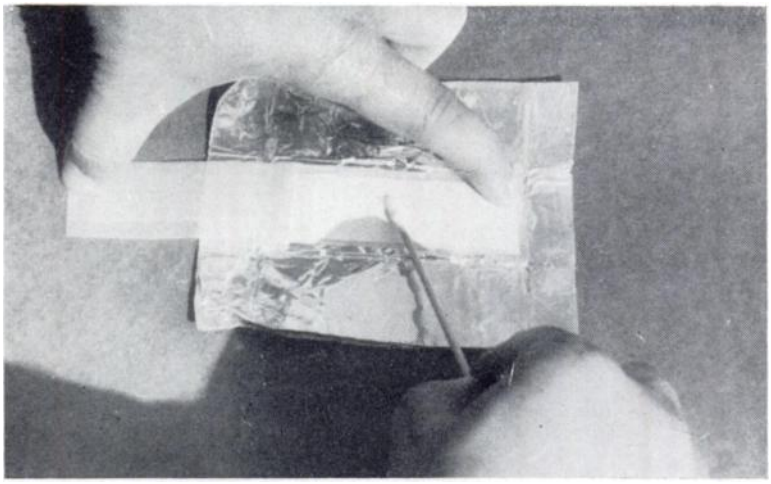

FIG. 1. Streaking a Filter Paper Strip in an open kit.

RESULTS AND DISCUSSION

Filter Paper Strip

The Filter Paper Strip technique was shown to be appropriate for reference laboratory study and clinical use in the transport and recovery of beta-hemolytic streptococci. In the earlier study, 216 (21%) of the Filter Paper Strip cultures were positive, compared to 17% of paired controls, using the techniques described for recovery and identification of beta-hemolytic streptococci from 1,014 children.[12] The number of positive paired cultures is shown by colony count (zero, few, over 25 per plate) in the upper part of Figure 2. Loss in transport on Filter Paper Strips paired with positive controls is more than compensated by recovery of beta-hemolytic streptococci on Filter Paper Strip cultures paired with negative controls and is not dependent on colony count. In the study presented here a similar loss and gain clearly is depicted in Table I.

At the left of Table I, the findings on control and Filter Paper Strip cultures are shown according to the number and location of patients. Of the beta-hemolytic streptococci, 168 (90%) of the 186 positives were obtained by the Filter Paper Strip technique and 106 (57%) on control cultures. In this series of paired cultures from 1,017 children, 16% were positive after Filter Paper Strip transport and 11% were positive control cultures. The 5% difference is in conformity with that found in the earlier study (4%).

The Filter Paper Strip in a kit currently is in use in the health department laboratories of 3 counties and 1 city and in 10 private laboratories. These laboratories serve a combined population of approximately a million persons in California. Pediatricians and microbiologists alike have reported the Filter Paper Strip technique highly satisfactory for throat cultures for beta-hemolytic streptococci. The kits can be stored conveniently and shipped with ease by letter mail.

Dacron-tipped Swab with Desiccant

The Dacron-tipped swab technique also is ready for reference laboratory study on the isolation of beta-hemolytic streptococci. In Figure 2 it can be noted that losses on Dacron-tipped swabs paired with positive controls are not quite balanced by gains from positive Dacron-tipped swabs paired with negative controls. The Dacron-tipped swab gave 241 confirmed isolates, or 16% positive cultures, from 1,519 children. Controls yielded 18% positives, a numerically greater but statistically equivalent finding.[12,13] In the present study the numerical findings are reversed, 48 of 60 positives being recovered from Filter Paper Strip cultures and 44 of 60 from controls in a series

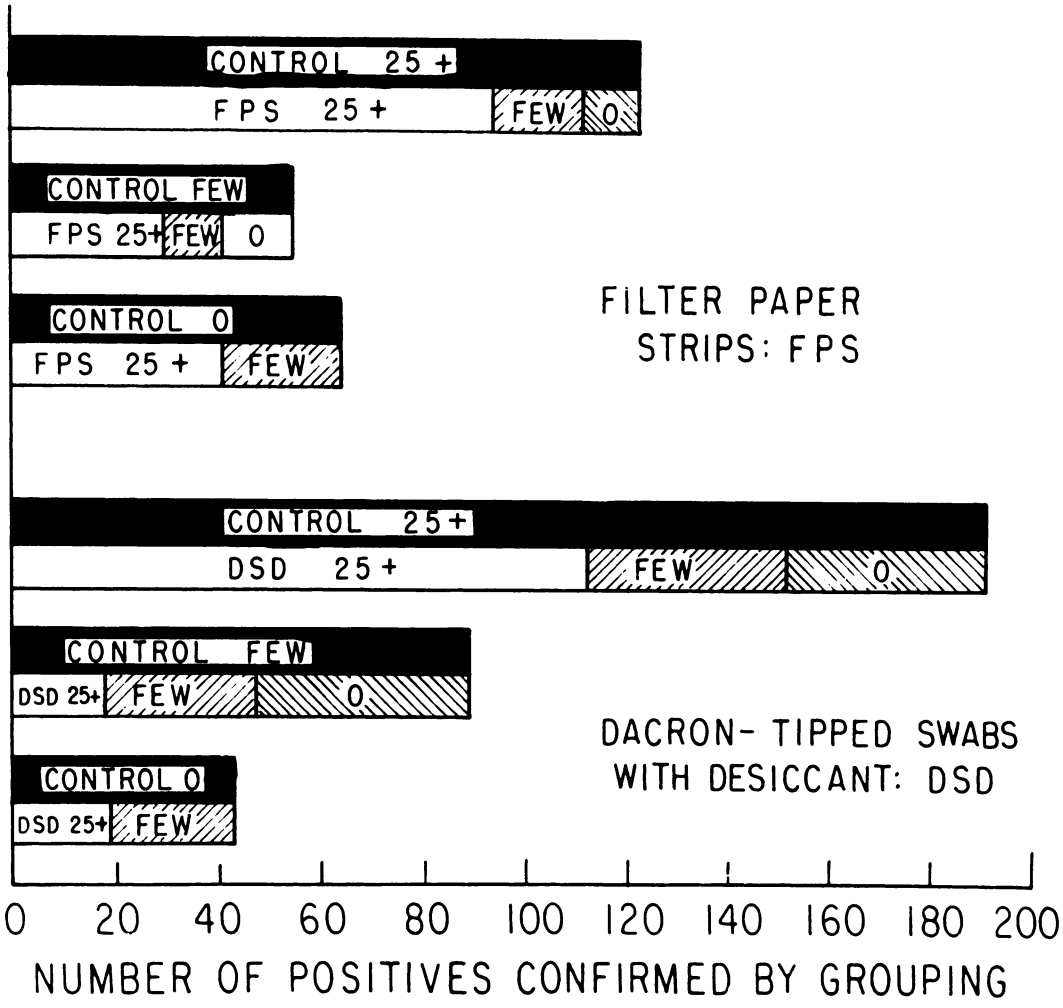

FIG. 2. Recovery of beta-hemolytic streptococci from paired cultures by colony count: 25+, few, 0.

of paired cultures from 552 children (Table I). Statistically these findings are not different.

The Dacron-tipped swab permits direct streaking and is preferred by some pediatricians and microbiologists because of the one-unit operation without transfer. The 8-inch glass tube with desiccant is too bulky for convenient mailing but is readily transported by the patient, a messenger, or for a house call.

In two hospitals, Dacron-tipped throat swabs without desiccant are transported to the laboratory in small glassine bags. This transport technique is reported as affording recovery of anticipated percentages of beta-hemolytic streptococci and of other less resistant organisms. The cotton-tipped applicator is no longer used as a throat swab for culture techniques in these hospitals.

Streptococcal Group and Type

Group A isolates constituted 82% of the 193 different positive cultures obtained (Table I). The remaining beta-hemolytic streptococci were classified as Group B, C or G; 3.6%, 6.6% and 7.8% respectively. Of the different Group A cultures: 19.7% were type 12; 6.9% were type 4; 5.2% were type 28; 2.1% were type 22; and 44% were untypable. The other types, 1, 2, 5, 6, 11, 13 and 33 each constituted 0.5-1% of the total. These percentages are equivalent to those previously reported.[12]

TABLE I

RECOVERY OF BETA-HEMOLYTIC STREPTOCOCCI BY POSITIVE (+) AND NEGATIVE (−) CULTURES AND BY LOCATION AND NUMBER OF PATIENTS

Cultures		*Number of Patients*				*Cultures*		*Number of Patients*		
Control	*FPS**	*Total*	*Pediatric Clinic*	*Pediatric Office*	*C.H.D.***	*Control*	*DSD****	*Total*	*Pediatric Clinic*	*Pediatric Office*
+	+	88	22	12	54	+	+	32	21	11
+	−	18	7	3	8	+	−	12	9	3
−	+	80	14	6	60	−	+	16	7	9
Positive		186	43	21	122	Positive		60	37	23
Negative		831	135	159	537	Negative		292	140	152
Total		1017	178	180	659	Total		552	277	275
% Positive		18.3	24.2	11.7	18.4	% Positive		10.9	13.2	8.5

* Filter Paper Strip.
** County Health Department.
*** Dacron swab with desiccant.

Classification according to group and type can not be made by observing the hemolytic zone or colonial form of the streptococcus. Even with the new augmented culture medium with neomycin and with, or without, transport on a Filter Paper Strip or a Dacron swab, a serologic procedure is required for specific identification of group and type. The proportion of Group C streptococci (1/14 of all strains isolated after transport on inert material) is in conformity with the percentage of Streptococcus equisimilis reported by Evans[14] to be associated with clinical illness. In a streptococcal outbreak in a school, Poskanzer *et al.*[15] classified a third of all beta-hemolytic streptococci as Group C, suggesting this group may have contributed to the course of the epidemic. In the study of augmented culture medium, a third of the isolates were Group C and G. These findings may contribute to the interest in beta-hemolytic streptococci reported by visual recognition rather than serologic grouping. As a fifth of all positives were type 12 beta-hemolytic streptococci of Group A, a study appears feasible with respect to organisms associated with acute glomerulonephritis in the same population groups for which the Filter Paper Strip and Dacron-tipped swab techniques were employed.

SUMMARY

Two new laboratory techniques for recovery and identification of beta-hemolytic streptococci are reviewed. One of these is an augmented culture medium containing nucleic acid, maltose and neomycin in addition to the classic agar base. Increased use of throat culture has followed a field trial of this augmented medium, with which pediatricians experienced no difficulty in distinguishing beta-hemolysis in the recognition of streptococci. The other technique, identification of streptococci with fluorescent antibody, affords a rapid means of grouping and typing on direct throat smears in those locations in which the equipment and specific fluorescein-tagged antisera are available.

Results are reported on the recovery of beta-hemolytic streptococci after transport on inert material (a Dacron-tipped throat swab in a tube with desiccant and on a Filter Paper Strip in a kit). Field trials in pediatric clinics and offices resulted in recovery of 90.5% of all positives using the Filter Paper Strip and 50.5% of the positives when swabbings were plated as controls from paired throat cultures on 1,017 children. The Filter Paper Strip technique is recommended as a reliable means of bringing the laboratory to the pediatrician. Simi-

lar trials of the Dacron swab with desiccant accounted for 80% of the positives from paired throat cultures on 552 children, with 73% positives from control cultures. The Dacron-tipped swab with desiccant technique was ready for reference laboratory study.

In the field trials of the Filter Paper Strip and Dacron-tipped swab techniques, the beta-hemolytic streptococci were isolated and classified by group and type. Of 193 positives, 82% were Group A, the rest were Group B, C or G; one-fifth were Group A, type 12. Percent by group and type is essentially the same as that reported in an earlier study of the same population. The proportion of Group C and G streptococci is high, one-seventh of the total. The proportion of Group A, type 12 organisms appears to warrant study of possible association between streptococci and glomerulonephritis in these population groups.

The new augmented culture medium, the rapid grouping and typing with fluorescent antibody, and the recently developed technique for transport on inert material enhance the value of laboratory aids to the clinician in recovery and in the identification of beta-hemolytic streptococci from throat cultures.

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