Office Bacteriology in Children

Louis W. Menachof, MD
Santa Rosa, California

Office bacteriological procedures can be a valuable part of a physician's diagnostic armamentarium when utilized within the framework of sound clinical judgment. This is particularly true in children, where infectious diseases represent the major portion of illnesses. The techniques are simple and inexpensive. The expertise necessary is rapidly and easily acquired. The results can be gratifying and serve as a stimulant for greater involvement in this technique.

Acute infectious diseases represent the major share of childhood illnesses. Although the diagnosis of most infectious diseases is made on clinical grounds only, a more specific etiologic diagnosis based on culture results can often be made by the judicious use of office culture techniques. While there are pitfalls in the techniques and interpretations of culture data, these can be kept to a minimum. Only a reasonable effort directed at gaining some expertise in this area is required. The end result of being closer to a specific etiologic diagnosis can be a more intelligent approach to therapy.

The most common diseases for which culture specimens are obtained include tonsillopharyngitis, urinary tract infection, and skin and soft tissue infections. This paper will discuss techniques and interpretations of cultures, together with pitfalls which may be encountered. Brief clinical comments will be included where relevant to the discussion.

Throat Culture

Tonsillopharyngitis, ie, infection involving tonsils and/or pharynx, can be caused by either viral or bacterial agents. Other than Corynebacterium diphtheriae and Neisseria gonorrhoeae, the only bacterium of importance in this disease is group A beta hemolytic streptococcus. Assuming that diphtheria and gonorrhea are not clinical considerations, a throat culture is taken for only one purpose, to determine the presence of significant numbers of group A beta hemolytic streptococci for which antibiotics are indicated. The absence of significant numbers of this organism indicates a probable viral etiology without indication for antibiotic therapy.

The patient with streptococcal tonsillopharyngitis often presents with an illness characterized by sudden onset, high fever, toxicity, and sore throat, often accompanied by headache and abdominal discomfort. Significant rhinorrhea, cough, or respiratory symptoms are so unusual in streptococcal tonsillopharyngitis that their presence makes this diagnosis unlikely. If significant numbers of strep are grown from a patient with significant rhinorrhea, cough or respiratory symptoms, con-

From the Community Hospital, Santa Rosa, California. Dr. Menachof is Assistant Clinical Professor, Ambulatory and Community Medicine and Pediatrics, University of California, San Francisco. Requests for reprints should be addressed to Dr. Louis W. Menachof, Community Hospital, 3325 Chanate Road, Santa Rosa, Calif 95402.

sideration should be given to the probability that the strep represents a carrier state, while the illness is actually caused by a viral agent. An occasional exception may be found in cases where a streptococcal infection is superimposed on a preceding runny nose or cough unrelated to the streptococcal infection.

Examination of the throat of a patient with streptococcal tonsillopharyngitis may or may not show exudation but characteristically shows a cellulitis of the tonsillar and adjacent structures, particularly the soft palate and uvula. A characteristic petechial or hemorrhagic rash of the soft palate and uvula is often seen. The presence of exudation on the tonsils, in the absence of cellulitis of the tonsils and adjacent structures, is almost always viral in origin. Infectious mononucleosis is a good example of this. However, even after 15 years of experience, this author's accuracy with clinical diagnosis remains approximately 75 percent, so culture, therefore, remains the best guide to diagnosis.

Technique

(1) Swab the tonsillopharyngeal area directly with a disposable sterile cotton applicator and inoculate heavily over approximately 20 percent of a five percent sheep's blood agar plate. (See Figure 1)

(2) Heat the streaking loop until the wire is red, then cool. (It will cool quickly if the loop is jabbed into the agar.)

(3) Streak the surface of the agar with the loop in the direction designed to progressively thin the inoculum. Since there are usually additional organisms grown from the throat beside beta hemolytic streptococci, beta hemolysis may be suppressed in the competition for nutrient in the initial, heavily swabbed area but will show clearly in the thinned area.

(4) Cut the agar at an angle with the loop in the heavily swabbed area. Beta hemolysis may show well surrounding this cut since streptolysin activity is greater at the depths of the agar plate.

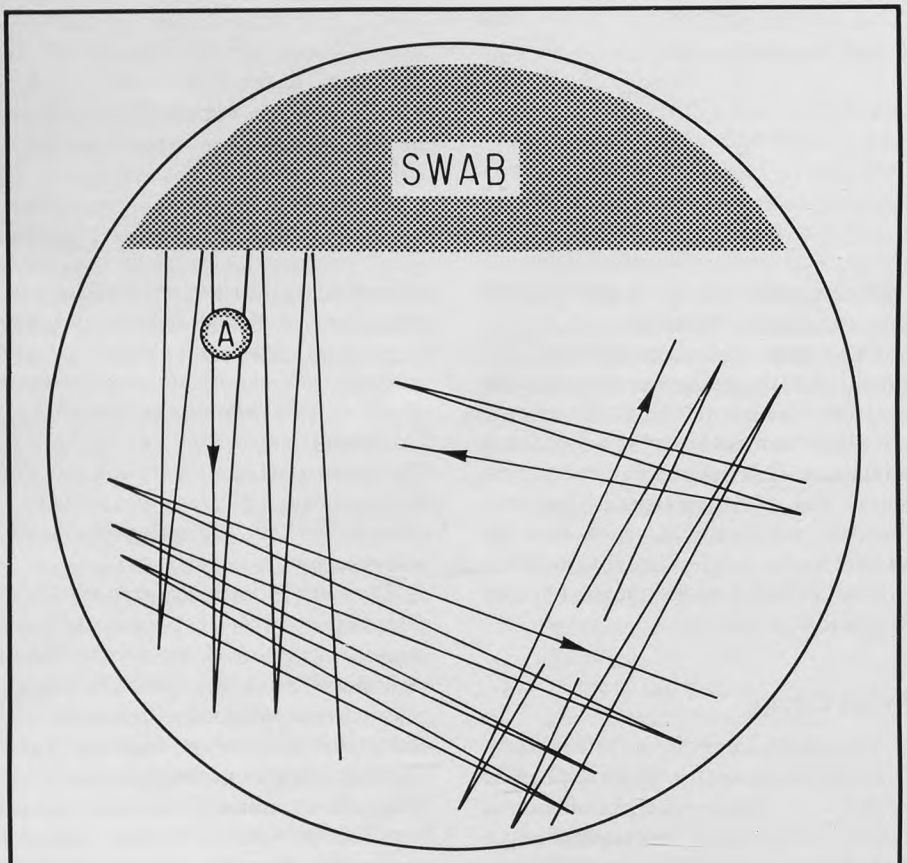

Figure 1. Technique for throat culture containing five percent sheep's blood agar

(5) Place the "A" disc (containing bacitracin) in the streaked area adjacent to the swabbed area where beta hemolysis is expected to be sufficient to detect the area of inhibition characteristic of group A.

(6) Following identification (label the plate and not the lid) the plates are then placed (lid down) in the incubator at 35 to 37 degrees until the end of the working day when they may be transferred to CO_2 for optimum growth.

Interpretation and Discussion

Accurate identification of group A beta hemolytic streptococci can be easily achieved by any physician. A good method of gaining expertise is to make quick rounds in the bacteriology laboratory of a hospital each morning and to look at various cultures on appropriate media. Laboratory personnel are anxious to help, and, in a short period of time, rapid and accurate identification by gross appearance can be mastered. Beta hemolytic streptococci are identified by a small-sized colony with distinct, clear hemolysis on sheep's blood. Beta hemolytic organisms are identified as belonging to group A by the zone of inhibition to beta hemolysis which the bacitracin-containing A discs impart. Since non-group A beta hemolytic streptococci are only rarely associated with clinical tonsillopharyngitis, isolation of this organism is not an indication for antibiotic therapy.

Beta hemolysis may often be obscured by other organisms in areas of heavy growth and, for this reason, the heavy inoculum must be thinned out with the loop so that beta hemolysis can be recognized. Since many normal throat cultures will demonstrate small numbers of beta streptococci, it is important to estimate roughly the percentage of beta streptococci in comparison to the overall number of organisms grown. In most cases of significant acute streptococcal illness requiring treatment and of the heavy carrier who may be at risk to others, throat cultures demonstrate large numbers of streptococci.[1] In general, al least 25 percent of colonies isolated in the thinned-out areas are beta hemolytic streptococci in streptococcal pharyngitis. An acute febrile illness is unlikely to be caused by the streptococcus if

the culture does not contain at least this number of streptococci. Studies using similar criteria for diagnosis and treatment demonstrated that only one of 53 ill children who had negative throat cultures and who, therefore, were not treated, developed an increase in ASO titer.[2] Although these studies have not been performed using the streptozyme test, some preliminary experiments indicate that the results will be similar. Selective blood agar media, designed to encourage identification of beta hemolytic strep by inhibiting growth of other organisms, have been recommended but have the disadvantage of failing to quantify relative numbers of organisms.

While I would not withhold antibiotic therapy from a seriously ill child with suspected streptococcal tonsillopharyngitis, it has been my experience that most children with this illness do not require immediate therapy and are able to wait comfortably overnight for culture results before the initiation of therapy. There are several advantages to this approach. First, there is evidence that starting antibiotic therapy early in the course of this disease may suppress natural antibodies. This may mean that following therapy the child remains susceptible to recurrence of infection with the same type of streptococcus.[3] Secondly, unnecessary exposure to antibiotics can be avoided since the greater share of tonsillopharyngitis is caused by viral agents. Thirdly, young parents, for the most part, no longer expect or demand immediate antibiotic therapy. A few words of explanation as to the advantages of awaiting a culture result are more often interpreted by parents as being representative of thoughtful and expert care. In addition, most physicians feel better about the quality of their work when a selective approach to antibiotic therapy is used.

Urine Culture

The diagnosis of urinary tract infection, by definition, refers to the presence of significant bacteriuria. The problems involved in determining significant bacteriuria are many, including method of collection, time interval between collection and examination, and numbers of organisms or colonies on culture. A complete discussion of these factors can be gained from other sources[4] and is beyond the scope of this paper. If the physician believes that pyuria is an inaccurate indication of infection and that gram stain and methylene blue stain of the urinary sediment have intrinsic errors, he then needs some method of quantifying bacterial growth to indicate the presence of urinary tract infection. Although there are limitations to the utilization of statistical chance as the criterion for diagnosis, it has been established[5] that less than 10,000 colonies per ml represents absence of infection and greater than 100,000 colonies per ml represents infection. The intermediate numbers are subject to individual clinical interpretation, with perhaps an indication for repeat cultures using clean voided, catheterized or suprapubic urine specimens.

Various methods of quantifying bacterial growth in urine have been utilized including pour plates, miniature agar plates (testuria), dip inoculum methods, and chemical indices demonstrating color changes. This author has found that direct culture of urine onto an agar biplate containing five percent sheep's blood and eosin-methylene blue (EMB) agars, utilizing a calibrated loop, has several advantages.

Technique

(1) Voided urine specimens may be obtained in any clean container. In older children, a midstream specimen may be obtained. Acceptability of voided urines in clean containers requires that the urine be cultured within ten minutes of voiding. This is not difficult when office personnel are convinced of the necessity of this requirement. Urine samples from infants may be obtained with a collection bag, as long as the infant is observed closely enough to detect urination within several minutes.

(2) A loop calibrated to deliver 0.001 ml is placed into the urine specimen and removed. Examine the loop to be certain that it contains the urine. All loops should be heated and cooled prior to use.

(3) Immediately deposit the contents of the loop with a single linear motion on the surface of the agar on one side of the biplate. (See Figure 2)

(4) Reinsert the loop in the urine and repeat the procedure on the opposite side of the biplate.

(5) This calibrated loop should be used only to deliver the desired volume of urine.

(6) A streaking loop (same as used for throat cultures) is then used to thin the inoculum in order that the individual colonies can be counted. This is done by streaking perpendicular to the linear line of urine deposited by the calibrated loop. This procedure is repeated on the opposite side.

(7) Biplates can be made inexpensively by any hospital or commercial microbiology laboratory and have the advantage that they conserve incubator space. However, individual agar plates containing these media can be used if biplates are not available.

Interpretation and Discussion

If the inoculum of urine is 0.001 ml, the number of colonies counted on either side multiplied by 1,000 equals the number of bacteria per ml of urine. These figures are based on the theory that each bacterium develops into one colony. While this may not be completely accurate, since some of the bacteria close together may develop into a single colony, it is reliable enough when considered in combination with the clinical picture. Since it is unusual for children without chronic urinary tract disease to have mixed infections, the presence of more than one organism should alert the physician to the probability of contamination. All of the usual gram negative organisms will grow well on EMB agar and can be readily identified grossly. Enterococcus, a relatively common urinary tract pathogen, is difficult to identify on EMB agar because the colony is small and frail, but it will grow well and can be easily identified on blood agar. In most instances, interpretation of cultures can be done after overnight incubation, particularly when grown in a CO_2 environment.

Bacteriological means of identification of urinary tract infection are ideally suited to the pediatric age group. The possibility of urinary tract infection is considered most often in children presenting with unexplained fever or in asymptomatic children with equivocal or abnormal urinary sediment. In my experience, urine cultures done for these purposes lead to a negative result in over 90 percent. Therefore, the physician need only be able to recognize absence of colonies in or-

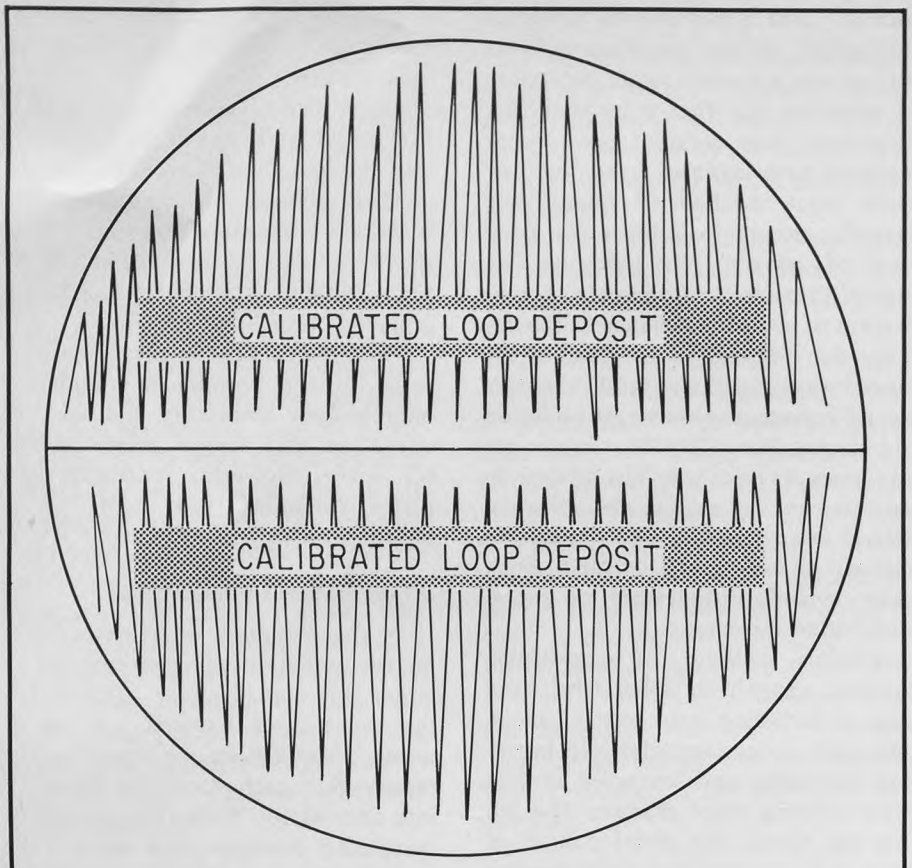

Figure 2. Technique for urine culture. Biplate containing five percent sheep's blood agar and EMB agar

der to be at least 90 percent correct in interpretation. Of the less than ten percent positive cultures in children, approximately 90 percent will be Escherichia coli. This allows the same physician to be nearly 99 percent accurate if he can identify E. coli. With only the minimal effort involved in visiting the microbiology laboratory, any physician can become quickly proficient in recognizing the common pathogens causing urinary tract infection in children. If any question exists as to the identification of an organism or if sensitivity studies are desirable (very rarely in children), the culture plate is available for instant consultation with laboratory personnel.

Once the diagnosis of urinary tract infection is made in a child, the choice and duration of antimicrobial therapy is probably not as important as the follow-up to determine which children require further evaluation because of continuing or recurrent infection. Office culture techniques allow for regular culture follow-up at minimal cost to the patient, since repeated physician examinations are rarely indicated in the long-term follow-up of childhood urinary tract infection. It is my practice to follow an acute urinary tract infection in a child for one year with urine cultures done one week following diagnosis while on therapy; one week following discontinuation of therapy; three, monthly cultures; and follow-up cultures every three months up to one year. A total of eight cultures during one year is equivalent in cost to approximately two office visits.

Miscellaneous Cultures

Although the greatest number of office cultures in children will involve throat or urine, the physician has available to him the expertise and equipment to expand culture techniques to many other areas, depending on the clinical situation and information to be gained. At times, identification of the organism causing impetigo or abscess may be indicated, particularly when it has failed to respond to initial antibiotic therapy, or when the original lesion is thought to be staphylococcal and the initial use of a more expensive penicillinase-resistant antibiotic is being considered.

Staphylococcal disease in newborns has increased since routine use of hexachlorophene washes in the nursery has been discontinued. Isolation of staphylococci from newborn skin lesions has diagnostic and therapeutic value to the physician. In addition, it is important in determining the source of the organism, since antibiotic sensitivity patterns of such staphylococci can often suggest a direct relationship between hospital-acquired staphylococci and clinical disease. The culture technique is identical with that used for throat culture. From both personal studies and recently published work,[6] one can safely assume that an impetigo lesion yielding only staphylococci in pure culture will be resistant in vitro to penicillin in greater than 85 percent of instances, whether it be a community or hospital-acquired organism. Identification of staphylococci grossly on five percent sheep's blood agar is not difficult. Staph colonies are at least 20 times larger than the tiny strep colonies. Staph colonies are granular in consistency and are easily picked up when a loop is streaked across the agar surface, in contrast to the slippery strep colonies which are pushed ahead of the loop without being picked up. When picked up on the loop in this manner, the yellow-orange color of most staph colonies will be apparent even though they may look white on the blood agar plate. Staph colonies bubble when hydrogen peroxide is dropped on them while strep colonies do not. When these criteria are used, confident identification will come quickly.

Instant availability of culture media, including EMB agar for identification of gram negative bacteria, can encourage most physicians to obtain more specimens for culture, thus adding a new dimension to their diagnostic armamentarium. Other diseases in which culturing has been shown to be of practical value in selected cases include purulent conjunctivitis, otitis media following myringotomy and chronic sinusitis. While some of these require special media or techniques, the expertise is easily obtained by someone who has the desire to expand his knowledge of office bacteriology.

An important word of caution is required. Any laboratory test, including

culture results, should never be interpreted without consideration of the clinical situation and must only be interpreted within this limitation. For instance, the growth of a pure culture of staphylococcus from the throat of a child with an acute pharyngitis is almost never significant and the patient should not receive antibiotic therapy on that basis alone. The presence of staphylococci along with streptococci in an impetigo lesion in rarely significant and will usually clear when only the streptococci are treated with penicillin. There are numerous examples where adherence only to the culture result will cause erroneous assumptions and improper treatment.

Incubators

Any incubator is acceptable which will reliably maintain the temperature of 35 to 37 degrees in which common bacteria grow best. These are readily available, occupy little space and are inexpensive. Table 1 lists the equipment and cost involved in carrying out the office bacteriology procedures discussed in this paper.

Table 1. Equipment for Office Bacteriology Laboratory

1. Incubator		$95.00
2. Loop for streaking		3.00
3. Loop, calibrated to deliver 0.001 ml		19.00
4. Five percent sheep's blood agar plates	@	.30
5. Eosin-methylene blue agar plates	@	.30
6. Alcohol lamp		1.50
7. "A" discs with dispenser (50)		1.75
8. CO_2 container (optional)		2.08

Since most bacteria with which we are concerned are aerobic or facultatively anaerobic, growth is enhanced by CO_2 content of four to eight percent. In addition, beta hemolysis is also enhanced at this CO_2 content because streptolysin S is more active in a lowered oxygen environment produced by the increased CO_2 content. While various means of producing this CO_2 content can be utilized, we have demonstrated that Alka-Seltzer and water in a plastic container* will produce a CO_2 content of this magnitude simply, reliably, and inexpensively. This CO_2 container, holding ten agar plates, comfortably fits into a small office incubator. All cultures taken are placed in the office incubator until the end of the working day, when they are placed in CO_2 for overnight growth. Although enhancement of growth by CO_2 allows more reliable interpretation when cultures are read in the morning after 16 to 18 hours incubation, it is not a requirement. If a culture is too young for adequate interpretation, leaving it to incubate longer will allow for further growth and easier interpretation.

References

1. Catanzaro FJ, et al: The role of the streptococcus in the pathogenesis of rheumatic fever. AM J Med 17:749-756, 1964
2. Moffet HL, et al: Group A streptococcal infections in a children's home. I. Evaluation of practical bacteriologic methods. Pediatrics 33:5-10, 1964
3. Brock LL, et al: Studies on the prevention of rheumatic fever: The effect of the time of initiation of treatment of streptococcal infections on the immune response of the host. J Clin Invest 32:630-632, 1953
4. Stamey TA: The diagnosis of bacteriuria. In Urinary Infections. Baltimore, Williams & Wilkins, 1972, ch 1, pp 1-30
5. Kass EH: Asymptomatic infections of the urinary tract. Trans Assoc Am Physicians 69:56-64, 1956
6. Ross S, et al: Staphylococcal susceptibility to penicillin G. JAMA 229:1075-1077, 1974

*One half foil-wrapped Alka-Seltzer tablet added to ten cc water in a Tupperware container (Catalog No. 140)