Procedures for the Rapid Diagnosis of Perinatal Infections

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ABSTRACT

An increasing number of rapid diagnostic procedures are in use for the diagnosis of infectious diseases. This paper presents a review of the "state of the art" as it relates to the rapid diagnosis of perinatal infections. Emphasis is placed on techniques for the detection of microorganisms, microbial antigens, or diagnostic metabolic products.

Introduction

The primary function of the clinical microbiology laboratory is to provide relevant test results with accuracy and alacrity for the diagnosis and treatment of infectious diseases. Until recently, the types of diagnostic tests which have been developed have been greatly influenced by the "pure culture" dogma which pervades all microbiology. These tests require the isolation of a microorganism prior to determining its identification and relationship to a particular infectious process. Frequently, there is an extensive time lag between specimen procurement and the reporting of test results to the clinician. As a result, the diagnosis of many infectious diseases is, at best, retrospective.

A number of rapid diagnostic procedures have been developed to address this problem. This paper presents a review of the "state of the art" as it relates to the rapid diagnosis of perinatal infections. However, the same procedures can be used for the rapid diagnosis of infectious diseases in all patients. Emphasis is placed on non-growth dependent techniques which permit the rapid detection of microorganisms, antigens, or diagnostic metabolic products in clinical specimens.

Stained Smears

The microscopic observation of stained smears of body fluids, discharges, and tissue remains the most rapid and economical test available for the presumptive diagnosis of many infectious diseases.

For example, the Gram stain has at least presumptive diagnostic value when used to examine normally sterile body fluids such as cerebrospinal fluid (CSF) for the diagnosis of meningitis and neonatal sepsis and urine for the diagnosis of urinary tract infections and neonatal sepsis.
Cerebrospinal fluid should be centrifuged at 1000 to 1500 x g for at least 15 minutes and the sediment Gram stained and examined for the presence of bacteria, fungi, and inflammatory cells. The most commonly encountered bacterial incitants of meningitis in the perinatal period are gram negative rods, particularly *Escherichia coli* and group B streptococci. The inflammatory response is helpful for the differential diagnosis of meningitis. Whereas the leukocytic response in acute bacterial meningitis is usually polymorphonuclear, it is predominantly mononuclear and less intense in mycobacterial, fungal, viral, leptospiral, or protozoan meningitis. However, in the perinatal period, host cell-response may not be as typical as in later life. There is a tendency for gram positive bacteria to appear gram negative in partially treated cases of meningitis. Therefore, the Gram stained smear must be interpreted cautiously.

Direct microscopic examination of Gram stained smears of freshly collected urines for the presence of bacteria can be a valuable aid when evaluating a neonate for a urinary tract infection or sepsis. Optimally, urine should be collected by suprapubic needle aspiration of the bladder. A drop of well mixed, unspun, urine is placed on a slide with a Pasteur pipet and allowed to dry without spreading. The smear is fixed in methanol, stained, and examined for the presence of bacteria.

The value of Gram stained smears of gastric aspirates, collected just after birth, for the diagnosis of neonatal sepsis is somewhat controversial. Some reports suggest that granulocytes and bacteria may indicate imminent sepsis. Others contend that the presence of granulocytes and bacteria in the gastric aspirate may result from maternal infection or fetal stress of a noninfectious origin.

Conjunctivitis in the neonatal period is usually caused by *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, or *Staphylococcus aureus*. Microscopic examination of the discharge often yields a diagnosis. The diagnosis of inclusion conjunctivities caused by *C. trachomatis* can be made by demonstrating intracytoplasmic inclusions in Giemsa-stained smears. Gonococci and staphylococci are detectable in Gram-stained smears.

The direct microscopic examination of scrapings from various lesions, vesicular, amniotic, and cerebrospinal fluids and urine is helpful for the rapid diagnosis of herpes virus infections. Smears for direct examination can be stained with Papanicolaou or Giemsa stain and observed for characteristic multinucleated giant cells and intranuclear inclusion bodies. However, since these characteristics can also result from varicella-zoster and cytomegalovirus infections, only a presumptive diagnosis can be made.

Immunofluorescence staining has greatly improved our ability to detect rapidly the microorganisms and/or antigens in body fluids, discharges, and tissues. For example, Schmidt et al recently reported their experience in detecting herpes simplex virus and varicella-zoster virus in a variety of clinical specimens. They found a close correspondence between direct immunofluorescence staining and subsequent isolation in tissue culture for herpes simplex virus. They also demonstrated that direct immunofluorescence is more sensitive than culture for detecting varicella zoster virus.

Holland et al evaluated a commercially available direct immunofluorescent test kit for the rapid detection and identification of members of the *Bacteroides fragilis* and *B. melaninogenicus* group in clinical specimens. They concluded that the kit was reliable for detecting these bacteria in purulent material within one to two hours after specimen collection.

These are but a few examples of the usefulness of stained smears of clinical specimens for the rapid diagnosis of peri-
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nental infections. However, a note of caution must be made regarding this diagnostic procedure. The sensitivity and specificity of stained smears are very dependent on the experience of the personnel preparing and reading the smear and on the quality of the equipment and reagents used. This is particularly true for immunofluorescence staining.

Counterimmunoelectrophoresis

Counterimmunoelectrophoresis (CIE) is an immunoprecipitin technique which combines the principles of double immunodiffusion and electrophoresis and permits the rapid detection of antibodies or soluble microbial antigens in a variety of body fluids and tissue. Antigen and antibody are placed in opposing wells cut in an agar gel which is then subjected to an electric current. Under suitable conditions of buffer pH and diffusion media, antigens are negatively charged and migrate in an electric field towards the anode, whereas antibodies are less negatively charged and migrate in the opposite or “counter” direction as a result of endosmotic flow. If homologous antigen and antibody are present, they will combine and form a readily discernable precipitin line.

From the perspective of perinatal infections, the most important application of CIE is for the rapid diagnosis of meningitis and septicemia owing to group B streptococci and *E. coli*. Siegel and McCracken detected group B streptococcal antigen in cerebrospinal fluid, blood, urine, pleural fluid, or cord blood in 21 infants, 20 of whom had culture-proven group B streptococcal disease. In a study by Edwards and Baker, CIE of concentrated urine and serum permitted identification of all neonates with invasive group B streptococcal infection within hours of the onset of symptoms. Specimens from neonates with group B streptococcal surface colonization were negative by CIE. The authors reported concentrated urine to be the best single source of antigen. Using CIE, McCracken et al demonstrated a direct relationship between the concentration of *E. coli* K1 capsular antigen and the length of time it was present in serum and CSF of neonates with meningitis and clinical outcome.

While CIE permits rapid antigen detection often in patients who have been partially treated with antimicrobics, and therefore may have negative cultures, it has certain limitations, particularly for the diagnosis of meningitis. Cross reactions are known to occur between group B streptococci and *Haemophilus influenzae*, and *N. meningitidis* group B and *E. coli* K1. Artifacts owing to precipitated protein may appear around either the antigen or antibody wells. Additionally, a concentration of at least 10⁵ colony forming units per ml in CSF is necessary for antigen detection by CIE. Therefore, CIE may be only slightly more sensitive than a Gram stained smear of CSF and should always be used in conjunction with a smear and culture for the definitive diagnosis of meningitis.

Coagglutination

The coagglutination (CA) procedure makes use of the protein A surface component of strains of *S. aureus* (e.g., Cowan I strain) that bind the Fc portion of immunoglobulin G but leave the Fab fragment free to react with homologous antigen. It is most commonly employed as a rapid slide agglutination test and has been used to identify serologically *Streptococcus pneumoniae*, various Lancefield groups of β-haemolytic streptococci, *N. meningitidis*, *Salmonella* and *Shigella*, *N. gonorrhoeae*, and *H. influenzae* directly from primary isolation plates and broth media. Coagglutination also detects microbial antigens in CSF, urine, and serum. The procedure is rapid, reaction end points are reported within minutes, and antigens can be recognized in patients treated with antimicrobials.
However, nonspecific agglutination reactions and immunoserological cross-reactions have been observed. Commercial kits for the identification of β-hemolytic streptococci, N. gonorrhoeae, S. pneumoniae, and H. influenzae are available.

Webb et al reported CA to be comparable to CIE in detection of group B streptococcal antigen in CSF from infants with meningitis. These authors also found CA to be more sensitive than CIE in detecting group B streptococci in CSF after the initiation of antimicrobial therapy. Coagulation has the additional advantages of ease of use (no special equipment is needed) and availability of results within minutes after specimen collection.

Latex Agglutination

In the latex agglutination (LA) test, antibodies are attached to latex particles. In the presence of homologous antigen, agglutination of the sensitized latex particles will take place. Rapid slide agglutination tests for the identification of β-hemolytic streptococci and for the rapid detection of microbial antigens in body fluids are the most common applications of this test. Edwards et al developed a latex agglutination assay to detect type III group B streptococcal antigen in CSF of neonates and young infants. They detected antigen in all of 12 culture proven cases studied in which the CSF was obtained at the time of admission. Antigen was also detected in 54 per cent of 26 subsequent CSF specimens obtained after the initiation of antimicrobial therapy. Only four of these specimens were culture positive.

There are conflicting reports regarding the relative sensitivity of CIE, CA, and LA tests for antigen detection. Some investigators reported CA and/or LA to be more sensitive than CIE. Others found CIE and CA to be more sensitive than CA. These differences may be related to reagent quality and methodological variations.

The specificity of three tests is similar and cross reactions, which are method-independent, do occur. Tests for CA and LA are easier to perform, require no special equipment and reach reaction endpoints faster than CIE.

Radioimmunoassay

Radioimmunoassay (RIA) makes use of radiolabeled antigen or antibody for detection and quantitation of antigens and antibodies in test samples. For antigen detection and quantitation, radiolabeled antigen competes with unlabeled antigen, which may be present in a body fluid, for available binding sites on a specific amount of antibody. Labeled and unlabeled bound antigen are separated from the reaction mixture and the amount of radioactivity of the antigen-antibody complex is determined. The lower the radioactivity, the greater the concentration of unlabeled antigen in the test sample.

Radioimmunoassay is one of the most sensitive assay methods currently available for either antigen or antibody. It has been used to detect a wide variety of microbial antigens in body fluids. However, with the exception of viral hepatitis, RIA has not been widely applied for the routine diagnosis of infectious diseases. This lack of application is probably related to problems inherent in RIA, such as equipment and reagent expense, biohazards for personnel, licensure regulations, the paucity of commercially available, radiolabeled antigens and antibodies, and their relatively short shelf life.

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) is conceptually similar to RIA. An enzyme is coupled to an antigen or antibody and, if a corresponding reactant is present in the specimen under study, an enzymatically active antigen-antibody complex is formed. This complex remains in the test system through binding to a
solid phase. Addition of a specific chromogenic enzyme substrate results in the development of a quantifiable colorimetric reaction.

It is noted that ELISA is as sensitive and specific as RIA but does not require radioisotopes or expensive counters and the reagents are relatively stable. It has been used for the rapid diagnosis of a wide variety of infectious diseases. However, the true potential of ELISA as a rapid diagnostic method will only be realized if standard test procedures can be developed and consistently reliable sources of reagents become commercially available.

**Limulus Amebocyte Lysate Test**

There are a number of indirect methods which have been used to indicate the presence of an infectious disease without identifying the etiologic agent. The *Limulus* amebocyte lysate (LAL) test is the most sensitive method available for the rapid detection of bacterial endotoxins. The test is based upon the observation that the amebocyte lysate, obtained from *Limulus polyphemus*, the horseshoe crab, forms a gel in the presence of endotoxin. To perform the test, a sample of body fluid is mixed with amebocyte lysate in a test tube. If endotoxin is present in the body fluid, a gel or clot will form within one hour. The LAL test has shown good correlation with the presence of gram negative bacteria in cerebrospinal fluid and urine. It has also been used for the diagnosis of gram negative keratitis and endophthalmitis gonococcal urethritis and for the detection of endotoxin in the blood of patients with gram negative septicemia. However, some investigators reported poor correspondence between LAL tests for septicemia owing to gram-negative bacteria and blood culture results. Additionally, the LAL test cannot be used for the detection of gram positive bacteria.

Because the LAL test is rapid, sensitive, easy to perform, and is relatively inexpensive, it may prove to be a valuable diagnostic test. To date, it has not been approved by the Food and Drug Administration for clinical use.

**Cerebrospinal Fluid Lactic Acid**

The observation that CSF lactic acid levels increased in bacterial meningitis was made in 1925 by Killian. Lactic acid levels can be measured chromatographically (vide infra) or enzymatically (available commercially in kit form). The enzymatic test requires approximately 15 minutes to complete; chromatography is more time consuming and requires more sophisticated equipment and greater technical training (vide infra).

There are varying reports regarding the specificity of CSF lactic acid levels in the diagnosis of bacterial meningitis. Measurement of CSF lactic acid levels has been reported to be a rapid and reliable diagnostic test for the early diagnosis of untreated and partially treated cases of bacterial meningitis and for differentiating septic from aseptic meningitis. Moreover, a decreasing lactic acid level has also been shown to have prognostic value. However, Hurd et al and Komorowski et al found elevated lactic acid levels in patients with fungal meningitis. The latter authors also reported elevated levels in patients with central nervous system ischemia and necrosis and in patients with brain tumors. They did find the test useful in differentiating septic from aseptic meningitis and in following patients with fungal meningitis. Lannigan et al analyzed lactic acid levels in 493 spinal fluids submitted from 434 adult patients with various conditions involving the central nervous system. Fifty fluids had elevated lactic acid levels of which 19 were cases of infective meningitis of varying etiology. The 443 specimens with normal lactic acid levels included three cases of infective meningi-
tis, of which two were cryptococcal and one was bacterial.

What may be concluded from these studies is that the determination of CSF lactic acid levels appear to have both diagnostic and prognostic value for bacterial (including tuberculous) and fungal meningitis. Elevated lactic acid levels do not appear to occur with viral meningitis. However, elevated levels are not specific for meningitis; results must be considered along with the clinical history and physical examination of the patient and standard CSF parameters such as leukocyte count and protein and glucose concentrations.

Gas-Liquid Chromatography

Chromatography consists of a group of analytical methods for the separation of compounds on the basis of certain physical properties of the individual substances. The process is carried out by selectively partitioning the components of a mixture between a mobile and stationary phase. In gas-liquid chromatography (GLC), a test sample containing volatilized components is introduced into a carrier gas (mobile phase) at the inlet of a chromatography column. The gas continuously flows through the column and the volatilized components of the test sample become distributed between the mobile phase and a liquid phase (stationary phase) present in the column. As a result of the action of the carrier gas flow and the differential distribution of the volatilized components depending on their affinity for the stationary phase, the components become separated at the column outlet and are recorded as peaks on a chromatogram.

Microorganisms are composed of and produce a wide variety of chemical substances which can be extracted, volatilized, and analyzed by GLC. Gas-liquid chromatography of spent culture media has found wide application in the clinical microbiology laboratory for the identification of anaerobic bacteria and other microorganisms. It has also been used for the direct analysis of clinical specimens such as body fluids as a means of rapidly elucidating the etiologic agent of infectious diseases. For example, Craven et al showed that GLC analysis of CSF permitted the differentiation of viral, tuberculous, and cryptococcal meningitis. LaForce et al reported that patients with meningitis owing to S. pneumoniae and H. influenzae showed fatty-acid and carbohydrate profiles that were clearly different from those seen in normal cerebrospinal fluid.

Gas-liquid chromatography results can be available within a few hours after specimen collection or microbial isolation. An additional advantage is that prior antimicrobial treatment does not interfere with test results. This diagnostic method does require specialized equipment and an individual experienced with GLC techniques and with interpretation of GLC data. An excellent monograph on the application of gas chromatography in microbiology and medicine was written by Mitruka.

Quality Control

It is essential that there be careful quality control of the procedures discussed. Prior to use, the sensitivity and specificity of all the reagents must be ascertained for each lot number by testing new lot numbers in parallel with ones currently being used. Positive and negative controls must be included with each test series.

Summary

The procedures for the rapid diagnosis of perinatal infections discussed in this review vary as to their diagnostic potential, ease of performance, and cost. All address the basic problem in clinical microbiology of providing the clinician with rapid results. The increasing utilization of
procedures, such as CIE, RIA, ELISA and GLC for the rapid diagnosis of infectious diseases, is evidence that twentieth century technology is finding its way into the clinical microbiology laboratory. However, the direct microscopic observation of clinical specimens remains the most rapid, accessible, and economical test available for the diagnosis of infectious diseases.

References


