Laboratory Diagnosis of Bacterial Meningitis

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INTRODUCTION

Bacterial meningitis is the most common and notable infection of the central nervous system, can progress rapidly, and can result in death or permanent debilitation. Not surprisingly, this infection justifiably elicits strong emotional responses and, hopefully, immediate medical intervention. The advent and widespread use of antibacterial agents in the treatment of meningitis have drastically reduced the mortality caused by this disease. However, both the morbidity (0.2 to 6 cases per 100,000 population per year) and the mortality (3 to 33%) of untreated and inappropriately treated bacterial meningitis in the United States remain high (91, 128, 129, 161). The majority of patients with bacterial meningitis survive, but neurological sequelae occur in as many as one-third of all survivors (especially newborns and children) (128, 129). Bacterial meningitis is much more common in developing countries than in the United States. For example,

morbidity in Brazil and some parts of Africa has been reported to be 300 to 400 cases per 100,000 population during epidemics (161).

This review is a brief presentation of the pathogenesis of bacterial meningitis and a review of current knowledge, literature, and recommendations on the subject of the laboratory diagnosis of bacterial meningitis. Readers should consult other references and reviews for laboratory and clinical information concerning viral (20, 24, 51, 91), slow viral (81, 115, 116, 159), fungal (24, 51, 69, 108), spirochetal (27, 51, 91), parasitic (24, 51, 91), mycobacterial (24, 33, 51, 108), and chronic (33, 69, 108) central nervous system infections, which are beyond the scope of this review.

ANATOMICAL CONSIDERATIONS IN BACTERIAL MENINGITIS

Meningitis is inflammation of the meninges, the thin anatomical structure (three layers or "membranes") that intimately and delicately covers the brain and spinal cord (Fig.

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FIG. 1. Major anatomical features of the central nervous system. The continuous darkly shaded areas represent the subarachnoid space which surrounds the brain and spinal cord and which is filled with CSF. Reproduced from Ray et al. (120) with permission of the publisher.

1 and 2). Specifically, meningitis is an infection within the subarachnoid space, a space between the middle and innermost layers. The three layers of the meninges are briefly described as follows.

(i) The dura mater (Latin: dura, "hard"; mater, "mother"), the outermost layer, is composed of tough, nonelastic, dense connective tissue and adheres to the skull and vertebral column (Fig. 2). The dura mater is covered on its innermost surface by squamous epithelial cells.

(ii) The arachnoid (Greek: arachnoeides, "like a cobweb"), the middle layer, is composed of dense collagenous and elastic connective tissue, adheres to the dura mater, and has delicate spiderweb-like projections (trabeculae) which connect it to the third layer, the pia mater (Fig. 2). The arachnoid and its trabeculae are covered with squamous epithelial cells.

(iii) The pia mater (Latin: pia, "tender"; mater, "mother"), the innermost layer, is composed of delicate collagenous and elastic connective tissue and is covered by squamous epithelial cells (Fig. 2). The pia mater is the only meningeal layer which contacts the central nervous system;

specifically, the pia mater (and, thus, the meninges) covers the surfaces of the brain and spinal cord.

Clinical microbiologists should be familiar with three anatomical spaces in the central nervous system, because the spaces are sites of distinct bacterial infections. Epidural abscesses occur in the epidural space (between the vertebrae and the dura mater). Subdural abscesses occur in the subdural space (between the dura mater and the arachnoid). Meningitis occurs in the subarachnoid space (between the arachnoid [including the trabeculae] and the pia mater). The subarachnoid space is the largest of the three spaces and is the main reservoir of cerebrospinal fluid (CSF).

Highly vascularized villi of the pia mater project into four ventricles (cavities) within the brain and are covered with ependymal epithelial cells. These projections are known as the choroid plexuses and are the sites at which the fluid component of the blood is modified (by secretion and absorption of certain solutes) and secreted into the ventricles (Fig. 1). This modified and secreted fluid is CSF. CSF circulates in the ventricles and the subarachnoid space around the brain and spinal cord and returns to the blood

FIG. 2. Major anatomical features of the meninges. The meninges surrounds the brain and spinal cord and is composed of three distinct layers. The subarachnoid space (between the arachnoid and the pia mater) is filled with CSF. Reproduced from Junqueira et al. (64a) with permission of the publisher.

circulatory system through subarachnoid villi that project into the superior sagittal sinus, which traverses the inner roof of the skull.

In adults, 400 to 600 ml of CSF is produced and recirculated each day. At any given time, the normal CSF volume is 10 to 60 ml in newborns and 100 to 160 ml in adults.

PATHOGENESIS OF BACTERIAL MENINGITIS

During the last several years, much has been learned about the pathogenesis of bacterial meningitis (50, 91, 129, 151). At any given time, the following is a brief presentation of current knowledge of the subject.

Colonization and Attachment

Some bacteria that cause meningitis have pili that allow the bacteria to attach to specific mucosal cells and, subsequently, to colonize mucosal surfaces of the nasopharynx. The distribution of specific mucosal and epithelial cell receptors probably determines the sites of colonization. This concept has been proposed most convincingly for *Haemophilus influenzae* (53) and *Neisseria meningitidis* (92, 140, 141).

Crossing Mucosal Barriers

The portals of entry for bacteria capable of causing meningitis and the mechanisms by which entry is gained are not well understood. The portals of entry probably are sites at which the bacteria actively (by direct invasion with or without damage to the host cells) or passively (by phagocytosis) enter subepithelial tissues and, subsequently, enter the blood circulation. *N. meningitidis* is known to be phagocytized by nasopharyngeal epithelial cells (140).

Entry into CSF

While in the blood circulation, the bacteria that cause meningitis must avoid being phagocytized by polymorphonuclear leukocytes and reticuloendothelial cells and must avoid being lysed by complement and specific antibody. Eventually, the bacteria enter the subarachnoid space and, thus, the CSF. The most likely portals of entry into the subarachnoid space are areas of minimal resistance such as choroid plexuses; dural venous sinuses; the cribriform plate; cerebral capillaries; sites of surgical, traumatic, or congenital central nervous system defects; or sites of parameningeal infection (e.g., epidural abscess) (91, 135, 151).

Bacterial Meningitis

The subarachnoid space and its CSF are relatively defenseless in stopping invasion by bacterial pathogens because of the CSF's paucity of phagocytic cells and low concentrations of complement and immunoglobulin. Unchecked invasion and multiplication of bacteria in the CSF result in meningitis. The pathophysiology of bacterial meningitis has been studied experimentally and is reasonably well understood (91, 129, 151). Inflammation of the meninges is initiated by the presence of bacterial lipopolysaccharide, teichoic acid, and/or other bacterial cell wall components in the subarachnoid space. The bacterial antigens stimulate monocytes to produce the cytokine interleukin-1 and stimulate macrophages, astrocytes, microglial cells, ependymal cells, and endothelial cells in the central nervous system to produce the cytokine tumor necrosis factor (cachectin). Tumor necrosis factor and interleukin-1 probably act synergistically to elicit inflammatory responses which manifest clinically as meningitis. A logical temporal sequence of such responses is as follows: chemotaxis and adherence of polymorphonuclear leukocytes to cerebral capillaries; damage to capillary endothelial cells; structural changes in the blood-brain barrier; cytotoxic parenchymal edema; increased intracranial pressure; decreased intracranial perfusion; cerebral infarction; and focal or diffuse brain damage.

CHANGES IN CELLULAR AND CHEMICAL COMPOSITIONS OF CSF DURING BACTERIAL MENINGITIS

The most important considerations in the management of a patient with acute bacterial meningitis are determining the most likely etiological agent and initiating immediate empirical antimicrobial therapy within 30 min of presentation. If possible, CSF and blood specimens for culture should be obtained prior to administration of treatment. Subsequently, the results of antigen detection tests and the analyses of CSF for protein and glucose concentrations and for cell count and cell differential can be beneficial in initially differentiating bacterial, viral, fungal, and mycobacterial forms of meningitis. The aforementioned inflammation-induced anatomical and physiological changes in the meninges are at least partially responsible for characteristic changes in the laboratory values of CSF from patients with bacterial meningitis. The loss of integrity of cerebral capillaries (and, thus, loss of integrity of the blood-brain barrier) results in leakage of protein into the CSF and increased migration of polymorphonuclear leukocytes into the CSF (129, 151).

Table 1 is a compilation of values of widely published and often used CSF parameters in healthy persons and in patients with meningitis (24, 48, 51, 52, 85, 91, 128, 161). The

TABLE 1. Laboratory values of components of CSF from healthy persons and from patients with meningitis^a

Source	CSF laboratory value			
	Protein (mg/dl)	Glucose (mg/dl) ^b	Leukocytes (per μ l)	Predominant cell type (%)
Healthy persons				
Newborns	15–170	34–119	0–30	
Adults	15–50	40–80	0–10	Lymphocytes (63–99) Monocytes (3–37) PMN (0–15)
Adult patients with:				
Bacterial meningitis	>100	<40	>1,000	PMN (>50)
Fungal meningitis	Increased	<30	Increased	Lymphocytes
Viral or aseptic meningitis	<100	Normal ^c	<500	PMN (early) and lymphocytes (late)

^a Data are commonly observed values. Notable exceptions to these values and overlap of values elicited by different etiological agents are not uncommon. Data were compiled from references 24, 48, 51, 52, 85, 91, 128, and 161. PMN, polymorphonuclear leukocytes.

^b The CSF glucose/serum glucose ratio usually is 0.6 (adults) or 0.74 to 0.96 (neonates and preterm babies). In patients with bacterial meningitis, the ratios usually are <0.5 (adults) and <0.6 (neonates and preterm babies).

^c Lower than normal glucose concentrations have been observed during some noninfectious disease processes and in some patients with viral meningoencephalitis due to herpesviruses, varicella-zoster virus, mumps virus, lymphocytic choriomeningitis virus, and enteroviruses.

values in Table 1 are guidelines and are usually characteristic of meningitis. However, the values elicited by different etiological agents often overlap by as much as 30 to 40% and might be relatively normal in some patients. Therefore, physicians should be extremely conservative in using CSF chemistry and cell counts alone to ascribe meningitis to a particular etiological agent. Analysis of CSF for other indicators of bacterial meningitis (e.g., endotoxin, lactate dehydrogenase, C-reactive protein, tumor necrosis factor, prostaglandin, total amino acid content, and interleukins 1, 2, and 6) also has been used. However, routine analysis of CSF for these molecules has not become widely performed or accepted because of lack of documented sensitivity or specificity, technical difficulty, cost, or lack of extensive clinical utility (5, 24, 39, 51, 89, 90, 95–97, 118, 119, 127).

ETIOLOGICAL AGENTS OF BACTERIAL MENINGITIS

The results of national surveillance studies have shown that both the etiological agents and mortality rates (0 to 54%) of bacterial meningitis depend on the season of the year and the age, sex, ethnic background, and geographic location of the patient (91, 130, 157). Table 2 shows the results of a 1986 multistate surveillance study of the etiological agents of bacterial meningitis (157). *H. influenzae* was the most frequent cause of bacterial meningitis (2.9 cases per 100,000

population) and, paradoxically, was associated with the lowest fatality rate (3%) of the five most frequent bacterial agents. On the other hand, *Listeria monocytogenes* was reported relatively infrequently (0.2 case per 100,000 population) but had the highest fatality rate (22%). Table 3 contains additional data from the aforementioned 1986 study and shows the distribution of etiological agents of bacterial meningitis in five commonly defined age groups. *Streptococcus* group B (*Streptococcus agalactiae*), *H. influenzae*, *N. meningitidis*, and *Streptococcus pneumoniae* were the leading causes of bacterial meningitis in neonates, young children, young adults, and adults and senior adults, respectively.

Certain elements of a patient's history (e.g., predisposing factors, medical condition, epidemiology, occupation, and immune status) can suggest specific bacterial agents of meningitis (Table 4) (61, 70, 91).

Unusual and rare bacteria that have been reported to cause meningitis include *Bacteroides fragilis* (102), *Achromobacter xylosoxidans* (99), *Gordona aurantiaca* (*Rhodococcus aurantiacus*) (113), *Lactobacillus* spp. (16), *Corynebacterium aquaticum* (11), *Streptococcus mitis* (13), *Staphylococcus aureus* (71), *Pasteurella multocida* (3, 56,

TABLE 2. Bacterial meningitis in the United States (1986)^a

Bacterium	No. (%) of cases	Incidence (cases/100,000)	Case fatality rate (%)
<i>H. influenzae</i>	964 (45)	2.9	3
<i>S. pneumoniae</i>	379 (18)	1.1	19
<i>N. meningitidis</i>	293 (14)	0.9	13
<i>Streptococcus</i> group B	122 (5)	0.4	12
<i>L. monocytogenes</i>	69 (3)	0.2	22
Other ^b	331 (15)	1.0	18

^a Data were obtained from a surveillance study by Wenger et al. (157) and are used with permission of the publisher.

^b Other bacteria include *Streptococcus* spp. other than group B, *S. aureus*, *E. coli*, *S. epidermidis*, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., and *Acinetobacter* spp.

TABLE 3. Etiological agents of bacterial meningitis in five age groups (1986)^a

Age group	% of cases ^b caused by:					
	<i>Streptococcus</i> group B	<i>L. monocytogenes</i>	<i>H. influenzae</i>	<i>S. pneumoniae</i>	<i>N. meningitidis</i>	Other ^c
0–1 mo	49	9	5	3	1	33
2 mo–4 yr	2		70	10	13	5
5–29 yr	2	2	8	17	42	29
30–59 yr	4	6	5	37	10	38
≥60 yr	3	14	4	48	3	28

^a Data were obtained from a surveillance study by Wenger et al. (157) and are used with permission of the publisher.

^b The percentages were extrapolated by us from the data in reference 157.

^c Other bacteria include *Streptococcus* spp. other than group B, *S. aureus*, *E. coli*, *S. epidermidis*, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., and *Acinetobacter* spp.

TABLE 4. Elements of a patient's history and physical condition that can suggest etiological agents of acute bacterial meningitis^a

Element	Possible bacterial etiological agent ^b
Epidemiology	
Summer and fall	Leptospire
Contact with dog or rodent urine	Leptospire
Nosocomial	GNR, <i>Staphylococcus</i> spp., <i>Candida</i> spp.
Family with meningitis	<i>N. meningitidis</i> , <i>H. influenzae</i>
Prior meningitis	<i>S. pneumoniae</i>
Associated infection	
Upper respiratory	<i>H. influenzae</i> , <i>S. pneumoniae</i> , <i>N. meningitidis</i>
Pneumonia	<i>S. pneumoniae</i> , <i>N. meningitidis</i>
Sinusitis	<i>S. pneumoniae</i> , <i>H. influenzae</i> , anaerobes
Otitis	<i>S. pneumoniae</i> , <i>H. influenzae</i> , anaerobes
Cellulitis	<i>Streptococcus</i> spp., <i>Staphylococcus</i> spp.
Surgery or central nervous system infection	
Cranial epidural abscess	Anaerobes, <i>Streptococcus</i> spp., <i>Staphylococcus</i> spp., GNR
Spinal epidural abscess	<i>S. aureus</i> , <i>Pseudomonas aeruginosa</i> , GNR
Shunt infections	<i>Staphylococcus</i> spp., GNR, <i>Streptococcus</i> spp.
Trauma	
Closed skull fracture	<i>S. pneumoniae</i> , GNR
Open skull fracture	<i>Staphylococcus</i> spp., GNR, <i>Enterococcus</i> spp.
CSF oto- or rhinorrhea	<i>S. pneumoniae</i> , GNR, <i>Staphylococcus</i> spp., <i>H. influenzae</i>
Underlying condition	
Alcoholism	<i>S. pneumoniae</i>
Leukemia or lymphoma	<i>S. pneumoniae</i> , GNR
Diabetes mellitus	<i>S. pneumoniae</i> , GNR, <i>Staphylococcus</i> spp.

^a Data were adapted from McGee and Baringer (91) with permission of the publisher.

^b GNR, gram-negative rods.

75, 107, 121), *H. influenzae* type f (57), and *Psychrobacter immobilis* (83).

COLLECTION, TRANSPORTATION, RECEIPT, AND STORAGE OF CSF

Universal precautions (i.e., barrier protection, hand washing, proper disposal of waste, prevention of generation of aerosols, etc.) apply to CSF (18). Readers are reminded that two cases of clinical laboratory-acquired *N. meningitidis* disease have been reported (19).

The identification of a bacterial pathogen is often essential to the physician in choosing appropriate antimicrobial therapy and in managing the infection control aspects of bacterial meningitis. For example, the American Public Health Association recommends 24-h respiratory isolation of patients with *N. meningitidis* or *H. influenzae* meningitis after the initiation of therapy, and prophylaxis of persons who have had close contact with such patients (1).

To initiate the definitive identification of a bacterium responsible for meningitis, CSF and blood culture specimens should be obtained from patients with clinical signs and symptoms of meningitis and should be transported to the laboratory without delay (154). CSF is hypotonic; therefore, neutrophils may lyse, and counts may decrease by 32% after 1 h and by 50% after 2 h in CSF specimens held at room temperature (139). *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* are fastidious organisms that may not survive long transit times or variations in temperature. Refrigeration may prevent the recovery of these organisms; therefore, CSF specimens should be stored at room temperature or in an incubator (37°C) if they cannot be processed immediately (68).

The processing of a CSF specimen is one of the few clinical microbiology procedures that must be done immediately. Results of rapid tests (Gram stain, antigen detection assays, *Limulus* amebocyte lysate [LAL] assays, etc.) and positive cultures must be conveyed to the physician caring for the patient (if possible) as soon as the results are available, and a permanent record of the communication should be made. Laboratorians should always record the date and time a specimen was received and the name of the person who was notified of the initial results.

Usually, three or more tubes of CSF are collected during a lumbar puncture procedure. The tubes should be numbered in sequential order with tube number 1 containing the first sample of CSF obtained. The CSF in tubes 1, 2, and 3 most often are examined for chemistry, microbiology, and cytology, respectively (10, 48, 72, 131, 163). However, the particular test(s) performed on tubes 2 and 3 is subjective and probably best determined by the staff of individual hospitals. Any contamination with skin flora and disinfectant should be minimal after the first tube of CSF is collected.

The probabilities of detecting microorganisms by staining and by culturing are related to the volume of specimen that is concentrated and examined (145). CSF volumes of 1 to 2 ml are usually sufficient to detect bacteria, but the isolation of fungi and mycobacteria requires a minimum of 3 ml (preferably 10 to 15 ml) of CSF for each culture (30). If only a small amount of CSF is received (a single tube) with requests for multiple assays (microbiology, chemistry, and cytology tests), the patient's physician should be consulted to determine the order of priority of the tests. The specimen might have to be shared among sections of the laboratory. If such a small volume of CSF must be shared, the specimen can be centrifuged. The sediment can be used for bacterial

culture and stain (to the exclusion of cytological examination), and the supernatant can be used for other tests as the volume allows.

CONVENTIONAL METHODS FOR PROCESSING AND CULTURING CSF

Concentration

The probabilities of detecting bacteria by culture and staining techniques are increased by concentrating the bacteria in a CSF specimen. The number of bacteria present in a CSF specimen from a patient with meningitis may be as few as 10 CFU/ml (154). In addition, approximately 50% of patients with meningitis receive antimicrobial therapy before CSF specimens are collected (29); antibacterial therapy may reduce the number of bacteria in the CSF by 10^2 - to 10^6 -fold (94).

Generally, when <0.5 ml of CSF is received into a microbiology laboratory, the entire unconcentrated specimen is used for microscopic examination and culture. When >0.5 ml of CSF is available for microscopic examination and culture, the specimen should be concentrated by centrifugation for at least 15 min at $1,500 \times g$ (60, 62, 94, 120). A centrifugal force of $10,000 \times g$ has been recommended to sediment bacterial CSF pathogens (136), but such force has been demonstrated to be unnecessary (94). A significant number of positive CSF specimens may be missed if the laboratorian uses a sterile pipette to remove the sediment from underneath the entire volume of supernatant (9). The supernatant should be decanted or carefully removed into a sterile tube, leaving approximately 0.5 ml behind to be used to suspend the sediment. Thorough mixing of the sediment after removal of the supernatant is essential. Mixing the sediment with the aid of a vortex mixer or forceful aspiration with a sterile pipette will be adequate to dislodge bacteria that may have adhered to the bottom of the tube following centrifugation. The sediment should be used to inoculate culture media and prepare smears for staining. If a grossly bloody specimen is received, smears for stains can be prepared before and after centrifugation to decrease the likelihood of erythrocytes obscuring bacteria in the sediment of a centrifuged specimen (30).

An alternative method of concentrating bacteria in a CSF specimen to be cultured is the membrane filtration technique (153). CSF (usually >2 ml) is filtered through a 0.45- μ m-pore-size, sterile, disposable filter. The "upstream" side of the filter is aseptically placed face down onto chocolate agar. Sterile forceps are used to move the filter every 24 h so bacterial growth beneath the filter can be detected. Murray and Hampton used CSF with and without antibacterial agents and seeded with bacteria to examine the effectiveness of the filtration technique (94). These workers found that the membrane filtration method provided culture results equivalent to those of centrifugation ($1,500 \times g$, 15 min) when antimicrobial agent-free CSF was cultured. However, when antibiotic-supplemented CSF was examined, the membrane filtration method was not as effective as centrifugation.

Culture

The media routinely used for bacterial culture of CSF are 5% sheep blood agar, enriched chocolate agar, and an enrichment broth (e.g., thioglycolate, Columbia, brucella, supplemented peptone, or eugonic). The culture plates should be incubated for at least 72 h at 37°C in an atmosphere

containing 5 to 10% CO₂. A candle jar can be used if a CO₂ incubator is not available. The enrichment broth, with the cap loosened, should be incubated at 37°C in air for at least 5 days. If the Gram stain demonstrates the presence of gram-negative rods resembling members of the family *Enterobacteriaceae*, a MacConkey agar plate can also be inoculated (9). If the Gram stain reveals organisms that morphologically resemble anaerobic bacteria or if the patient is known to have an underlying condition predisposing the patient to an anaerobic infection (such as chronic otitis media, a pilonidal sinus, or dermal sinus), an anaerobic blood agar plate can be added to the routine culture media, and the plate should be incubated at 37°C in an anaerobic atmosphere (9, 47, 102).

If possible, a portion of each CSF specimen should be stored temporarily at -70°C, room temperature, or 37°C for potential reculture. If antigen detection tests are anticipated, the specimen should be stored at $\leq 4^\circ\text{C}$ because bacterial polysaccharide antigens often tend to break down faster at room temperature and 37°C than at $\leq 4^\circ\text{C}$.

Cultures should be examined daily. Gram stain results of colonial or broth growth should be telephoned to a physician caring for the patient. Although bacterial concentrations of $\geq 10^7$ CFU/ml of CSF have been correlated with increased morbidity and mortality (42), quantitative culturing of CSF specimens is not a common or practical procedure. Growth of normal skin flora should raise suspicion of contamination, especially when there is minimal growth on the solid media or growth on a single plate or in the broth only. Culture plates with no growth may be discarded after 72 h, and negative enrichment broths may be discarded after 5 days of incubation. The authors of *Cumitech 14* suggest incubating negative cultures that have positive Gram stain findings for an additional 4 days before the cultures are discarded as negative (120).

Antimicrobial Susceptibility Testing

In general, complete antimicrobial susceptibility testing should be performed on all clinically relevant bacteria isolated from CSF.

H. influenzae should be tested for the production of β -lactamase by a chromogenic or acidometric assay (34, 93, 135, 147). In addition, an assay for the detection of chloramphenicol acetyltransferase may be used to assess the clinical utility of chloramphenicol (34, 100). *N. meningitidis* should be tested for β -lactamase production when the isolate is from a patient who is not responding well to antimicrobial therapy (93, 135, 154). *S. pneumoniae* initially should be tested by the oxacillin agar screen method to screen for frank resistance and relative resistance to penicillin (34, 100). The agar screen method detects both types of resistance but does not differentiate between them. If an isolate produces a ≤ 19 -mm zone of inhibition in the screen test, the isolate is either frankly resistant or relatively resistant to penicillin. Subsequently, dilution (MIC) testing should be performed to confirm resistance (relative resistance, MIC of 0.12 to 1.0 $\mu\text{g/ml}$; frank resistance, MIC of $>1.0 \mu\text{g/ml}$), because the prevalence of both types of resistance in the United States is so low that the predictive value of a resistant screen result is also low (34, 64). *S. pneumoniae* isolates from the CSF of patients with meningitis and that are confirmed by dilution testing to be relatively resistant to penicillin should always be reported as resistant, because such isolates probably will not respond clinically to penicillin (34).

RAPID METHODS FOR DETECTING BACTERIA AND COMPONENTS OF BACTERIA IN CSF

Microscopy

Samples of all CSF specimens should be stained with the Gram stain (or other comparable stain) and examined microscopically. Because the diagnostic usefulness of staining procedures depends on the concentration of bacteria in the CSF of patients with bacterial meningitis (10 to 10^9 CFU/ml), all CSF specimens of sufficient quantity should be processed to concentrate pathogens prior to microscopic examination and culture (42, 45, 60, 62, 94, 120, 154). A small degree of additional concentration for Gram stain purposes can be achieved by sequentially layering small amounts of previously concentrated CSF onto the same area of a microscope slide and allowing each amount to dry thoroughly (9). Obviously, this layering technique severely compromises rapid turnaround time and depends heavily on the unpredictable adherence of the specimen to the slide.

Gram stain. The Gram stain is a simple, rapid, accurate, and inexpensive method for detecting bacteria and inflammatory cells in CSF from patients with bacterial meningitis. Seventy-five to 90% of CSF culture-positive specimens are Gram stain positive (67, 80); the percentages decrease to 40 to 60% in patients who have received antimicrobial therapy prior to lumbar puncture (29, 63).

The Gram stain is generally accepted to be most reliable at detecting $\geq 10^5$ bacteria per ml of body fluid (21, 62, 126, 149). This fact has been demonstrated for CSF by La Scolea and Dryja (79), who showed that 25, 60, and 97% of CSF specimens with $<10^3$, 10^3 to 10^4 , and $>10^5$ CFU/ml, respectively, were positive by Gram stain.

Gram-stained CSF specimens must be examined carefully, diligently, and patiently. Only a few poorly staining bacteria may be present on an entire slide, and inflammatory cells, erythrocytes, stained protein, and precipitated stain may obscure the bacteria. The presence, number, and morphology of bacteria, inflammatory cells, and erythrocytes should be reported immediately.

The clinical utility of the Gram stain apparently depends on the bacterial pathogen. Bacteria have been observed in 90% of cases of meningitis caused by *S. pneumoniae* and *Staphylococcus* spp., 86% caused by *H. influenzae*, 75% caused by *N. meningitidis*, 50% caused by gram-negative bacilli, and $<50\%$ caused by *L. monocytogenes* and anaerobic bacteria (51). Some workers prefer to use basic fuchsin as the Gram counterstain to provide better staining of organisms such as *Haemophilus* spp. and *Fusobacterium* spp., which stain poorly with safranin (120).

The chances of observing bacteria in CSF can be increased by replacing conventional centrifugation with Cytospin centrifugation (Shandon Southern Products, Cheshire, England). Shanholtzer et al. found that concentration of 0.5 ml of CSF by Cytospin centrifugation increased the chances of observing organisms in Gram-stained CSF by up to 100-fold over the possibility with unconcentrated and conventionally centrifuged ($1,000 \times g$, 15 min) specimens (132). This increase is comparable to the concentration of 100 ml of CSF to a volume of 1.0 ml by conventional centrifugation. Those authors found that Cytospin-prepared smears not only demonstrated more bacteria but also maintained better leukocyte morphology than did conventional centrifugation.

Acridine orange stain. Stains other than the Gram stain can be used to screen smears of CSF for bacteria. Acridine

orange is a fluorochrome stain that can intercalate into nucleic acid. At a low pH (4.0), bacteria and yeasts appear bright red, and leukocytes appear pale apple green. In one study, the acridine orange stain was slightly more sensitive than the Gram stain (82.2% compared with 76.7%) and was capable of detecting bacteria at concentrations of $\geq 10^4$ CFU/ml, a concentration 10-fold lower than that detectable by the Gram stain (80). Work by Kleiman et al. suggests that a major advantage of acridine orange is that it is more sensitive than the Gram stain in detecting both intra- and extracellular bacteria in CSF from patients who have received antimicrobial therapy (73). Kleiman et al. found the Gram stain to be positive in 0 of 47 and the acridine orange stain to be positive in 45 of 47 (96%) CSF specimens obtained from patients who had been given antimicrobial agents for ≥ 18 h prior to collection of CSF. Another advantage of the acridine orange stain is a reduction in the time devoted to examining a CSF smear. This reduction results from the striking contrast between the bright bacteria and the dark background and the use of only $\times 400$ magnification to examine most smears. Acridine orange-positive smears must be Gram stained to verify the presence of bacteria and to determine the Gram reaction of the bacteria (120). Fortunately, acridine orange-stained smears can be Gram stained without prior decolorization of the acridine orange (87). A major disadvantage of the acridine orange stain technique is its requirement for a fluorescence microscope.

Wayson stain. The Wayson stain appears to be a simple and sensitive stain for screening smears of CSF for bacteria. The components of the stain are basic fuchsin, methylene blue, ethanol, and phenol. Daly et al. found the Wayson stain to be more sensitive (90%) than the Gram stain (73%) and to be as specific (98%) as the Gram stain (99%) in the detection of bacteria in smears of CSF (31). In Wayson-stained preparations, bacteria appear dark blue, proteinaceous material appears light blue, and leukocytes appear light blue and purple. In the opinion of Daly et al., the contrast between bacteria and background is more pronounced with the Wayson stain than with the Gram stain, which enables the laboratorian to spend less time examining CSF smears (31). However, Wayson-stained smears cannot be Gram stained. A second smear must be Gram stained when bacteria are detected in Wayson-stained smears of CSF.

Quellung procedure. The quellung capsular reaction is rarely used; however, it can be used to confirm the presence of organisms with a morphology typical of *S. pneumoniae*, *N. meningitidis*, or *H. influenzae* type b. In the quellung reaction procedure, antisera specific for the capsular polysaccharides of each of these three bacteria are mixed with separate portions of clinical specimens. Specifically, a drop of CSF, a loopful of specific antiserum, and saturated methylene blue can be mixed on a microscope slide, covered, and examined under an oil objective. The formation of antigen-antibody complexes on the surfaces of these bacteria induces changes in the refractive indices of their capsules. Microscopically, the capsule appears to be clear and swollen. The test requires highly specific antibody at high titer and a laboratorian with expertise in the method. Details concerning the methodology of the test can be found in the fifth edition of the *Manual of Clinical Microbiology* (40).

The rapid antigen detection methods discussed in the following section are used by most laboratories as a supplement to staining smears of CSF.

Methods of Detecting Bacterial Antigens

Counterimmunoelectrophoresis (CIE), coagglutination (COAG), and latex agglutination (LA) have been adapted for the rapid and direct detection of soluble bacterial antigens in CSF of patients suspected of having bacterial meningitis. These tests are widely used in clinical microbiology laboratories and can be important supplements to the culture and Gram stain of CSF specimens. Rapid antigen detection tests may provide true-positive results when culture and Gram stain results are negative for meningitis patients who have received antimicrobial therapy (46, 85, 148). In addition, the results of these rapid tests can prompt a physician to implement early and specific antimicrobial therapy rather than the broad-coverage therapy that is usually instituted until culture and antimicrobial susceptibility results are available, in 18 to 24 h.

The most common central nervous system pathogens to which antigen detection tests have been applied are *H. influenzae* type b, *S. pneumoniae*, *Streptococcus* group B, and *N. meningitidis* serogroups A, B, C, Y, and W135. Except for *Streptococcus* group B, these bacteria possess soluble, type-specific, capsular polysaccharide antigens that are released into surrounding body tissues and fluids as the bacteria proliferate (25). *Streptococcus* group B possesses a soluble type-specific cell wall polysaccharide antigen.

CIE, COAG, and LA are most efficient in detecting *H. influenzae* antigens. CIE is least efficient in detecting *Streptococcus* group B antigens, and COAG and LA are least efficient in detecting *N. meningitidis* antigens (44, 82). The minimum concentrations of bacterial antigen detectable by CIE have been reported to be 1 to 25 ng of *H. influenzae* type b antigen per ml to 500 to 14,000 ng of *Streptococcus* group B antigen per ml. The minimum concentrations of bacterial antigen detectable by LA and COAG have been reported to be 0.1 to 5 ng of *H. influenzae* type b antigen per ml to 50 to 100 ng of *N. meningitidis* antigen per ml (44, 82). The aforementioned wide ranges of minimal amounts of antigens detectable by CIE depend on reagent manufacturer, investigator, antisera, source of antisera, bacterial group or strain, and other variables (44).

Antigen detection methods are most productive when used to examine fluids obtained directly from an infected site where the bacteria are actively proliferating and shedding polysaccharide, e.g., CSF in cases of meningitis. However, if CSF is not obtainable, serum or urine may be tested. Solubilized capsular polysaccharides readily cross capillary endothelial cells and are excreted into the urine (25). Serum is often a poor specimen for detection of free capsular polysaccharide because the antigen can bind to antibodies and other serum proteins, be metabolized, and/or be removed by lymphocytes. Urine may serve as an alternative to CSF because urine can be obtained noninvasively and can be readily obtained and concentrated to enhance the possibility of antigen detection (46).

In bacterial meningitis patients successfully treated with antimicrobial agents, bacterial antigens are detectable in body fluids for many days after the CSF becomes sterile. *H. influenzae*, *N. meningitidis*, and *S. pneumoniae* antigens have been detected by COAG and LA in the CSF and serum for 1 to 10 days after the initiation of treatment with antimicrobial agents (54, 143, 146). Thirumoorth and Dajani used COAG and LA to detect higher concentrations of *H. influenzae* type b antigen in urine and serum than in CSF of patients who had received 1 to 3 days of treatment with antimicrobial agents (146). Kaldor et al. found that the *H.*

influenzae type b antigen titer in concentrated urine from children often increased on the second day of therapy and slowly decreased thereafter (65). *H. influenzae* antigen was detected in the urine as long as 18 days (mean, 10 days) after the initiation of therapy. With the use of COAG and LA, Riera reported the persistence of *H. influenzae* antigenuria to be a mean of 19.9 days in patients recovering from *H. influenzae* meningitis (123). Baker et al. used CIE to detect bacterial antigen in the urine of survivors of *Streptococcus* group B meningitis for as long as 75 days (mean, 22.4 days) after the initiation of appropriate therapy with antimicrobial agents (7).

In patients with bacterial meningitis and who have not been treated or who have received therapy for <24 h, CSF is the specimen of choice for the detection of bacterial antigens. Urine is probably the specimen of choice for bacterial antigens when patients have undergone treatment for >24 h (146).

Antigen detection methods can be hampered by nonspecific reactions, cross-reactions, and/or low concentrations of antigen in clinical specimens. Rheumatoid factor, blood, hemolyzed erythrocytes, and high concentrations of protein can cause nonspecific reactions in both COAG and LA tests (104, 106, 146). Pepsin, protein A, and EDTA are some of the substances used to reduce nonspecific reactions, but boiling specimens for 5 to 15 min is the most commonly used technique (101, 104, 137, 146, 160). Boiling can also be used to liberate bacterial antigen bound by CSF proteins. Unfortunately, meningococcal antigen is sensitive to heat and, therefore, might not be detectable by LA after specimens have been heated to 100°C (160). Cross-reactions between a commercial *H. influenzae* LA reagent and *S. pneumoniae*, *N. meningitidis* group C, *S. aureus*, and *Escherichia coli* have been reported (84). In addition, urine can contain urethral flora that can cause false-positive results due to antigenic cross-reactions (44). Spinola et al. have reported that 93% of children with negative rapid antigen urine tests prior to receiving *H. influenzae* type b immunization excreted *H. influenzae* antigen into their urine from 1 to 11 days postimmunization (138). In an appropriate clinical setting, these results could be misinterpreted as being falsely positive.

When rapid antigen tests are applied to urine, false-negative results may be obtained because antigen is often at a lower concentration in urine than in CSF. The concentrations of bacterial antigens can be artificially increased by concentration techniques. Some methods used for concentration of bacterial antigens in body fluids before testing include ethanol precipitation, membrane filtration, and the use of a polyacrylamide absorbent gel (Sigma Chemical Co., St. Louis, Mo.) (35, 111, 156). Urine specimens should be concentrated 20- to 50-fold. The most commonly used method of concentrating urine is use of a disposable ultrafiltration system (Minicon B15; Amicon Corp., Lexington, Mass.) (7, 41, 65).

CIE. CIE was once an important and rapid diagnostic method for the laboratory diagnosis of bacterial meningitis. In CIE, the application of an electric current to immunodiffusion agar accelerates the diffusion of antigen and antibody toward each other in the agar and enables any subsequent immunoprecipitation to be completed in 30 to 60 min. The introduction of commercially available COAG and LA reagents for the detection of CSF pathogens has made CIE a test performed in only a few laboratories.

CIE is less sensitive (by a factor of 10) than COAG and LA in the detection of bacterial antigens in CSF and urine (44);

TABLE 5. Sensitivities of commercial assays for detection of bacterial antigen in CSF and urine from patients with meningitis

Assay	Specimen	Avg % sensitivity (range)				Reference(s)
		<i>H. influenzae</i> type b	<i>S.</i> <i>pneumoniae</i>	<i>Streptococcus</i> group B ^a	<i>N.</i> <i>meningitidis</i>	
COAG						
Phadebact	CSF	85 (66–100)	76 (59–93)	72 (61.5–87)	67 (50–78)	17, 22, 26, 29, 37, 38, 55, 86, 133, 144, 148, 150, 155
	Urine	90 (85–95)	37.5 (25–50)	77 (62–92)	4.5 (0–9)	54, 144
LA						
Bactigen	CSF	94 (91–100)	100 ^b	100	56 (33–78)	8, 22, 26, 37, 55, 76, 86, 133, 148
	Urine	96	23	84	0	8
Directigen	CSF	82 (78–86)	81 (67–100)	73 (69–87)	74 (50–93)	8, 133, 148
	Urine	100	50	75 ^c (51–100)	0	8, 122
Wellcogen	CSF	89 (86–92)	81 (69–100)	91 (90–92)	55 (50–59)	6, 8, 23, 58, 117
	Urine	100	0	82 (61–100)	0	6, 8, 59

^a Sensitivities of the commercial assays for *Streptococcus* group B include data presented at the 86th Annual Meeting of the American Society for Microbiology, 1986; used with permission from the authors (45a).

^b All references evaluating Bactigen with *S. pneumoniae* (8, 26, 55, 148) report sensitivity of 100%.

^c Reference 122 reports sensitivities of 86% for unconcentrated urine and 100% for concentrated urine.

however, CIE has excellent specificity (44, 49). CIE is used only rarely today because it requires high-quality antisera, stringent quality control, special equipment, and an experienced laboratorian to obtain optimum sensitivity. In addition, CIE is cumbersome and slow when compared with COAG and LA.

As a testament to the decreasing utilization of CIE in the clinical laboratory, the procedure for performing the test has been omitted from the fifth edition of the *Manual of Clinical Microbiology* (40). Details of CIE methodology can be found in *Cumitech* 8 (2) and in the fourth edition of the *Manual of Clinical Microbiology* (44).

COAG and LA. COAG reagents are composed of suspensions of *S. aureus* (particularly Cowan strain 1) that contain the cell surface component protein A, a 12,000- to 43,000-molecular-weight protein that is covalently linked to the peptidoglycan of the bacterium. Immunoglobulin G molecules (directed toward the antigen of interest) adhere to protein A by the Fc end of the immunoglobulin G molecule; the immunoreactive Fab end remains free to react with specific antigen. In the presence of specific antigen, grossly visible agglutination of the staphylococci takes place. LA assays for antigen detection utilize latex polystyrene beads with immunoglobulin molecules nonspecifically adsorbed onto their surfaces. In the presence of homologous antigen, grossly visible agglutination of the antibody-coated latex beads occurs.

COAG and LA have several advantages over other assays in the rapid laboratory diagnosis of bacterial meningitis. The two tests are rapid (≤ 15 min), simple to perform, and do not require special equipment. The simplicity and fast turnaround time of these two tests make them suitable for use in the clinical microbiology laboratory on all shifts on a stat basis.

Well-trained personnel and proper quality control are important to ensure maximum sensitivity of the tests. The frequency of quality control testing is dependent on the guidelines of the agency that inspects the laboratory. The College of American Pathologists quality control standards for antisera require (allow) initial testing of each lot of test kits and subsequent testing every 6 months thereafter (4). Because of the clinical importance of rapid tests in the laboratory diagnosis of bacterial meningitis, laboratorians may be well advised to perform more frequent (more than every 6 months) quality control testing of CSF agglutination

tests. At the other extreme, the Joint Commission on the Accreditation of Healthcare Organizations insists that antisera be quality control tested each day of use (4).

Four bacterial antigen detection kits are commercially available in the United States. The kit based on COAG is the Phadebact CSF Test (Karo Bio Diagnostics, Huddinge, Sweden). The three kits based on LA are Directigen (Becton Dickinson Microbiology Systems, Cockeysville, Md.), Bactigen (Wampole Laboratories, Cranbury, N.J.), and Wellcogen (Wellcome Diagnostics, Research Triangle Park, N.C.). The sensitivities of test kits compared with culture are shown in Table 5. Differences in sensitivities are apparent; however, no kit appears to be superior to the others for the detection of all antigens. The data in Table 5 show that COAG and LA tests have difficulty detecting *N. meningitidis* antigens in urine (8, 54, 59, 144). *N. meningitidis* groups A, B, and C were isolated from the CSF of the patients whose urine specimens were tested in these studies. Suwanagool et al. have suggested that the relative inabilities of COAG and LA tests to detect *N. meningitidis* antigens in urine could be due to the absence of significant numbers of *N. meningitidis* in the urine during infection, the configuration and stability of the *N. meningitidis* antigens in the urine, and/or the specificity of the antibodies for the antigens (144). Almost all of the studies cited in Table 5 report that the kits have excellent specificity. Except for one report of the specificity of Phadebact being 88% for *N. meningitidis* antigen and another report of the specificity of Wellcogen being 81% for *Streptococcus* group B antigen, the references in Table 5 report specificities of 96 to 100%.

Antigen detection methods should never be substituted for culture and Gram stain. If only a small amount of CSF is received, Gram stain and culture should always have priority over antigen detection tests.

OTHER METHODS FOR DETECTING BACTERIA AND COMPONENTS OF BACTERIA IN CSF

EIA

Enzyme immunoassays (EIAs) for the detection of bacterial antigens in CSF use specific (primary) antibodies bound to a solid support such as a plastic microwell tray or tube or polystyrene beads. If homologous bacterial antigen is

present in a CSF specimen, the antigen will be bound by the immobilized primary antibody. An enzyme-labeled (secondary) antibody with specificity for the bacterial antigen of interest detects the antigen bound to the primary antibody. The addition of a substrate for the enzyme results in the production of a colored product if specific bacterial antigen was present in the CSF specimen and bound to the primary antibody.

EIAs have been evaluated for their abilities to detect *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* antigens in CSF (12, 37, 134, 142, 162). The sensitivities and specificities of these tests have been reported to be 84 to 100% and 89 to 100%, respectively. The tests can detect bacterial antigens in concentrations as low as 0.1 to 5 ng/ml. Currently, commercially available EIAs for the detection of bacterial antigens in CSF are available only in Europe (Behring, a biotin-avidin procedure; Pharmacia, a horseradish peroxidase procedure). EIAs generally take several hours to complete and require multiple controls. For these reasons, EIAs are better suited for testing specimens in a batch mode than for testing of individual CSF specimens as they are received into the laboratory, usually on a stat basis.

LAL Assay

The bloodlike circulating fluid of the horseshoe crab, *Limulus polyphemus*, is called hemolymph. The only circulating cells within the hemolymph are amebocytes. Under optimal conditions (36 to 38°C, pH 6.0 to 7.5), a lysate derived from *L. polyphemus* amebocytes will clot within 1 h when exposed to very small amounts of lipopolysaccharide (endotoxin), which is contained in the cell wall of all gram-negative bacteria (114, 149). The active component of bacterial lipopolysaccharide in this reaction appears to be the lipid A segment (pyrogen). Concentrations as low as 0.1 ng of lipid A per ml are capable of clotting amebocyte lysate (120). For the horseshoe crab infected by gram-negative bacteria, this clotting reaction serves as a defense mechanism against infection by isolating an infected limb. For humans, this reaction provides a very sensitive assay for the detection of endotoxin in medical products and in body fluids from patients with gram-negative bacterial infection.

There are three Food and Drug Administration-approved methods for the LAL assays (114). The simplest LAL assay is the gel endpoint method. This test is performed by incubating 0.1 ml of lysate with 0.1 ml of fluid specimen for 1 h at 37°C and inverting the mixture 180° to determine whether a clot has formed. The determination of whether a clot has partially or fully formed is subjective and can make endpoints difficult to read. Patients with untreated meningitis commonly have at least 10^5 CFU per ml of CSF (149). The turbidometric LAL assay involves the use of a spectrophotometer to measure the change in optical density that occurs during the gelation reaction. In the chromogenic substrate LAL assay, a synthetic color-producing substrate (which contains a chromogenic *p*-nitroanilide group) and a modified LAL assay are used. The formation of a clot as an endpoint is eliminated to a large degree and is replaced by the production of a yellow color. In all three LAL assays, the use of pyrogen-free laboratoryware is imperative.

The LAL assay is a very sensitive and specific assay for the detection of endotoxin in CSF. A correctly performed LAL assay can detect approximately 10^3 gram-negative bacteria per ml of specimen (149). Nachum reviewed 4,884 CSF specimens which had been examined by LAL assays and calculated the overall sensitivity and specificity of the

LAL assay (98). Compared with cultures for gram-negative bacteria, LAL assays have a sensitivity of 93% and a specificity of 99.4%. Bacteria that have been detected in CSF by LAL tests include *H. influenzae* type b, *N. meningitidis*, *E. coli*, *Pseudomonas* spp., *Serratia marcescens*, *Klebsiella pneumoniae*, and other gram-negative bacilli (98).

Two reports emphasize the simplicity of LAL assays. Ross et al. examined a bedside adaptation of the gel endpoint method for the diagnosis of gram-negative bacterial meningitis (125). This test was performed by house staff and medical students and showed approximately 98% agreement with the same test performed by laboratory personnel (125). Dwelle et al. simplified the gel endpoint assay to a microslide gelation test that had a sensitivity of 97.3% and a negative predictive value of 99.9% (39).

Not all reports give the LAL test the stamp of approval for diagnosis of gram-negative meningitis. McCracken and Sarff reported a sensitivity of 71% for the detection of neonatal gram-negative meningitis in CSF specimens with positive cultures and a false-positive rate of 14% in CSF specimens with negative cultures (90). These results led McCracken and Sarff to conclude that the LAL test was not sensitive enough to serve as a screening procedure for the diagnosis of gram-negative meningitis in neonates. The LAL test has not found widespread use as a diagnostic tool for meningitis because the test detects only gram-negative bacteria and does not differentiate between different gram-negative bacteria.

GLC

Gas-liquid chromatography (GLC) was first used in clinical microbiology for the identification of anaerobic bacteria. This technique facilitates the separation, quantitation, and identification of several (often trace) constituents of physiological fluids (77). Amines, alcohols, carbohydrates, and short-chain fatty acids are examples of microbial metabolites that are produced in body tissues and fluids and that can be detected by GLC. The sample to be analyzed is introduced into a heated injector port, where the sample is volatilized. The volatilized sample mixes with an inert carrier gas and advances through a heated column. The affinity of the gaseous components for the liquid phase determines the rate at which they advance along the column. A change in electrical signal is produced as the gases pass through a detector. Changes in electrical signals are amplified to deflect a pen recorder, which produces tracings that represent the retention time and the concentration of each gas.

The application of GLC for the detection and identification of microorganisms in CSF is still in the developmental stages. Craven et al. presented data that demonstrated that GLC can be used to differentiate among cryptococcal, tuberculous, viral, and parasitic infections of the central nervous system (28). Brice et al. used GLC techniques to establish chromatography patterns for the following five common bacterial agents of meningitis: *S. pneumoniae*, *H. influenzae*, *N. meningitidis*, *S. aureus*, and *E. coli* (15). Lipid, carbohydrate, and lipopolysaccharide components served as characteristic markers for the identification of these organisms. Brice et al. concluded that GLC might be a useful assay for the rapid laboratory diagnosis of bacterial meningitis. GLC has been reported to be potentially useful in the detection of bacteria in CSF. LaForce et al. found that CSF from patients with meningitis caused by *H. influenzae* and *S. pneumoniae* showed fatty acid and carbohydrate GLC profiles that were clearly different from those of normal

CSF (77). In addition, GLC profiles of *H. influenzae*- and *S. pneumoniae*-infected CSF were different from each other. These investigators suggested that, at least theoretically, prior treatment of a patient with antibacterial agents would not be expected to interfere immediately with GLC results because antibacterial agents would not alter fatty acid or carbohydrate components of infecting bacteria.

GLC has not been widely used for the diagnosis of bacterial meningitis for several reasons. The technique requires equipment that is relatively expensive, and the methodology is much more technically demanding than the antigen detection assays in use in most laboratories. Computer-assisted evaluation of results might be needed to aid in the interpretation of GLC results because misleading backgrounds and artifacts can cause difficulty in identification (36).

PCR

The polymerase chain reaction (PCR) is a primer-mediated, temperature-dependent technique for the enzymatic amplification of a specific DNA sequence (32, 109, 124). The technique is self-contained and easily automated because all reactions take place in a single vessel. The reaction mixture consists of (i) target DNA in the specimen, (ii) single-stranded oligonucleotide primers complementary to known sequences of the target DNA, (iii) a thermostable DNA polymerase from the bacterium *Thermus aquaticus* (*Taq* polymerase), and (iv) ample amounts of triphosphate forms of the four deoxyribonucleoside components of DNA (deoxythymidine, -cytidine, -adenosine, and -guanosine).

The technique is dependent on the repetitive cycling of three simple reactions (32). A reaction cycle begins when the temperature is raised to 94°C to denature the DNA into single strands. The temperature is then lowered to 55°C for attachment of the oligonucleotide primers to their complementary regions on the single-stranded target DNA (primer annealing). Primer extension occurs when the temperature is raised to 72°C and the *Taq* polymerase uses the primers as starting points and synthesizes double-stranded DNA from each single strand. If this three-reaction cycle is repeated 30 times, a segment of DNA can be amplified by a factor of 10^6 , usually in 3 to 4 h (32, 109, 124). Subsequently, nucleic acid probes are used to detect the products of PCR reactions and, thus, to detect the specifically sought organism in the original patient specimen.

The PCR has been used recently in the early detection of *N. meningitidis* in CSF from a patient with meningitis (74). The patient's blood cultures were positive for *N. meningitidis*, but culture, Gram stain, and acridine orange stain of CSF did not detect bacteria in the CSF. The CSF was purulent, with 48,000 polymorphonuclear leukocytes per μl . The patient had received intravenous penicillin 30 min before the CSF specimen was obtained. Use of the PCR and nucleic acid probes could have provided an early definitive diagnosis of meningococcal meningitis in this patient if the test had been performed on CSF when the patient was admitted to the hospital. The authors concluded that the PCR is a rapid method for the amplification of DNA and can be extremely useful in the early laboratory diagnosis of meningitis caused by *N. meningitidis* even when the patient has received prior antibiotic therapy. They also stated that, in principle, meningococcal meningitis could be excluded on the basis of a negative PCR result.

PRACTICAL CONSIDERATIONS

In the era of diagnostically related groups, federal reductions in dollars spent for Medicare and Medicaid, and continuing reductions in reimbursements from third-party payers, it is important for clinical microbiology laboratory personnel to select and use all diagnostic tools wisely. Rapid methods for the diagnosis of bacterial meningitis can provide life-saving results; however, the tests can also increase the laboratory's cost of processing and testing CSF specimens and, therefore, the cost of health care. In addition, and at least in pediatric cases, the results of rapid testing (other than the Gram stain) usually do not prompt physicians to alter empiric therapy of bacterial meningitis (78, 85). The Gram stain continues to be an accurate, inexpensive, and rapid method for the detection of bacterial pathogens in CSF. Bacterial culture is inexpensive and is still accepted as the "gold standard" for the diagnosis of bacterial meningitis.

The CSF from patients with bacterial meningitis (with the exception of neonates) is characterized by some abnormality in cell count or chemical composition, even when the patient has received partial or inappropriate antimicrobial therapy (14, 51, 66, 88, 91, 105, 128). Wadke et al. examined the usefulness of the Gram stain and culture in the laboratory diagnosis of bacterial meningitis (152). They found that 0 and 1 (0.07%) of 1,536 CSF specimens with <10 leukocytes per mm^3 of CSF were positive by Gram stain and culture, respectively. Therefore, Wadke et al. suggested that the two tests were not diagnostically useful in cases of bacterial meningitis with CSF cell counts of <10 leukocytes per mm^3 of CSF. Phillips and Millan performed a study similar to that of Wadke et al. and presented Gram stain recommendations similar to those of Wadke et al. (110). However, because Phillips and Millan, as well as other workers (43, 103, 112), found a higher percentage of positive culture results in bacterial meningitis patients with CSF cell counts of <10 leukocytes per mm^3 , they recommended culture of all CSF specimens.

The fifth edition of the *Manual of Clinical Microbiology* discourages direct antigen testing of CSF specimens with normal leukocyte count, glucose, and protein determinations (62, 149). Gilligan and Folds have stated that alternative rapid methods should be considered only for CSF specimens with negative Gram stains and with cell counts and chemistries consistent with bacterial meningitis (46). In addition, Marcon has used the presence of 50 or more leukocytes of any type per μl of CSF as suggestive of an infectious process and justification for bacterial antigen testing (85). Werner and Kruger recently examined leukocyte counts, glucose, and protein values and bacterial antigen testing results of CSF specimens from pediatric patients to determine criteria that could be used to limit antigen testing to specimens with a high likelihood of yielding positive antigen results (158). Glucose and protein values were not useful in predicting a specimen that would yield positive antigen results. A leukocyte count of at least 50 leukocytes per mm^3 correlated with all CSF specimens with a true antigen-positive test result. Use of the prerequisite criterion of 50 leukocytes per mm^3 would have reduced the number of antigen tests performed by 85%. Those workers stressed that good communication between laboratory personnel and physicians is imperative because patients with bacterial meningitis who have received partial treatment or who are immunocompromised sometimes have normal CSF cell counts.

An analysis of 2 years' use of an LA assay for bacterial antigen detection in CSF at Wake Medical Center in Raleigh,

N.C., provides much insight into the impact of rapid antigen detection on the care of patients with bacterial meningitis (49). The cost per positive patient was calculated to be \$638. In 34 of 35 patients with documented *H. influenzae* type b meningitis, a Gram stain of cytocentrifuged CSF sediment provided the correct diagnosis. In these cases the antigen detection test only provided confirmation of the Gram stain result. In addition, the authors discovered that LA test results did not affect changes in antimicrobial therapy. Physicians at that institution waited until culture confirmation and antimicrobial susceptibility testing results were available before modifying therapy.

Because physicians are reluctant to modify antimicrobial therapy until culture confirmation and because of the common practice of instituting empiric broad-spectrum antimicrobial therapy in patients with suspected bacterial meningitis, Marcon has made the wise (albeit radical) proposal that laboratories adopt a policy whereby all CSF specimens submitted for antigen testing would be held for 24 h awaiting positive culture results (85). Antigen testing would be performed only in clinically indicated, culture-negative cases.

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