JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1992, p. 2725–2727
0095-1137/92/102725-03$02.00/0

Vol. 30, No. 10

Identification of *Streptococcus pneumoniae* with a DNA Probe

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Received 13 April 1992/Accepted 30 June 1992

The Accuprobe *Streptococcus pneumoniae* Culture Identification Test (Gen-Probe, Inc.) was evaluated with 172 isolates of *S. pneumoniae* and 204 nonpneumococcal isolates. The sensitivity and specificity of the Accuprobe test were 100%. Optimum results were obtained when four or more discrete colonies were selected for testing. The Accuprobe test was determined to be an accurate and rapid method for identification of *S. pneumoniae*.

Streptococcus pneumoniae is a major respiratory pathogen in adults and children. Despite the wide use of effective antibiotics, pneumococcal disease ranks among the 10 leading causes of death in the United States (5). *S. pneumoniae* is the most common cause of community-acquired pneumonia, the second most common cause of meningitis, and a leading cause of otitis media in children. The emergence of penicillin-resistant strains has made the management of pneumococcal infection difficult and the need for rapid and accurate identification more important.

Laboratory diagnosis of pneumococcal infection is usually performed by microscopic examination and culture of clinical specimens. Rapid identification of *S. pneumoniae* can be performed with a primary isolation plate by using the emerging technology of DNA probe assays, which have been simplified for use in the clinical laboratory.

The Accuprobe Culture Identification Test (Gen-Probe, Inc., San Diego, Calif.) utilizes a chemiluminescent acridinium ester-labeled DNA probe which hybridizes with target rRNA in solution. To evaluate the performance of the Accuprobe test, 172 strains of *S. pneumoniae* (target) and 204 miscellaneous bacteria (nontarget) were tested.

Test strains were either fresh clinical isolates or reference strains obtained from six clinical laboratories in diverse geographical locations. Reference strains were stored at −70°C. Isolates of *S. pneumoniae* were cultured from blood, spinal fluid, and the respiratory tract. Miscellaneous bacteria representative of those found in the respiratory tract were selected as the nontarget organisms. All bacteria were subcultured on appropriate agar media and incubated 24 to 48 h under suitable atmospheric conditions prior to testing.

Organisms were identified as *S. pneumoniae* on the basis of typical cellular and colonial morphologies, alpha-hemolysis on Trypticase soy agar with 5% sheep blood, sensitivity to optochin, bile solubility, and reaction in the Pneumoslide (BBL Microbiology Systems, Cockeysville, Md.) and Phadebact (Karo Bio Diagnostics, Huddinge, Sweden) serological tests. The API Rapid STREP kit (Analytab Products, Plainview, N.Y.) and serotyping (performed at the Centers for Disease Control) were used to test any streptococcal strains demonstrating discrepant results. Nontarget miscellaneous bacteria were identified by standard methods (1).

The Accuprobe *S. pneumoniae* Culture Identification Test was performed in accordance with the manufacturer's instructions. Samples were prepared by adding 50 μl of specimen diluent to a reaction tube containing lyophilized acridinium ester probe. Four or more discrete colonies were transferred to the reaction tube by using a 1-μl sterile, disposable calibration loop, and the tube was incubated for 5 min at 35°C to release the rRNA of the sample. In the hybridization step, 50 μl of diluent was added to the bacterial suspension and incubated for 15 min at 60°C to allow the rRNA to associate with the labeled probe. To degrade the unhybridized probe, 300 μl of selection reagent was added and the reaction tube was vortexed and incubated for 5 min at 60°C. The reaction tube was allowed to cool for 5 min at room temperature. The chemiluminescence of the hybridized probe was measured with a Gen-Probe Leader 1 luminometer. A positive result was indicated by a response of more than 50,000 relative light units (RLU). *S. pneumoniae* (ATCC 6305) and *Streptococcus bovis* (ATCC 9809) were run with each test as positive and negative control organisms, respectively.

A total of 376 target and nontarget organisms were evaluated (Table 1). The Accuprobe test correctly identified all 172 *S. pneumoniae* strains, including both encapsulated and nonencapsulated strains. The RLU values ranged from 57,768 to 2,872,570. Negative results were obtained with all 204 nontarget organisms tested. The RLU values ranged from a low reading of 338 with *Streptococcus agalactiae* to a high reading of 23,748 with a viridans streptococcus. All of the readings were significantly below the cutoff value of 50,000 RLU. The sensitivity, specificity, and positive and negative predictive values of the *S. pneumoniae* Accuprobe assay were 100%.

Three strains of streptococci were optochin resistant, nontypeable, and weakly positive by the Pneumoslide and Phadebact tests and were identified as *Streptococcus mitis* by the API Rapid STREP. These isolates, which were bile soluble and gave a positive response with the Accuprobe test, were reported as "probable *S. pneumoniae*" by the Centers for Disease Control. Fenoll et al. (4) have demonstrated that a specific pneumococcal DNA probe could be used to identify isolates showing atypical results by conventional tests.

Identification of *S. pneumoniae* in the clinical microbiology laboratory can be accomplished in many ways. Direct examination of clinical material has its limitations (3, 6). Presumptive and confirmatory tests for culture confirmation have also been reported to give discrepant results. For these

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TABLE 1. Results of the Accuprobe test for *S. pneumoniae*

Organism	No. of isolates tested	RLU[a]
S. pneumoniae	172	57,768–2,872,570
Viridans streptococci		
S. sanguis I	2	625–770
S. sanguis II	12	578–9,742
S. mitis	5	523–1,868
S. salivarius	1	624
S. mutans	1	600
Nutritionally variant sp.	1	1,884
Viridans group spp.	68	752–23,748
Group D enterococci		
E. faecalis	5	510–881
E. faecium	4	609–800
E. casseliflavus	2	647–676
E. mundtii	1	620
Enterococcus spp.	3	2,101–2,430
Group D streptococci		
S. bovis	2	618–1,985
Streptococcus spp.	2	876–3,249
Beta-hemolytic streptococci		
Group A spp.	13	612–3,323
Group B spp.	18	338–2,399
Group C spp.	15	552–6,980
Group F spp.	8	534–9,865
Group G spp.	9	673–9,189
Nongroupable spp.	4	618–8,653
Nonstreptococcal organisms		
Aerococcus spp.	2	2,784–3,199
Leuconostoc spp.	2	622–677
Pediococcus sp.	1	666
Coagulase-negative staphylococcus	1	2,606
Micrococcus spp.	2	2,218–2,427
Staphylococcus aureus	1	2,943
Arcanobacterium haemolyticum	1	885
Corynebacterium spp.	5	762–2,859
Lactobacillus sp.	1	717
Neisseria gonorrhoeae	1	673
Neisseria lactamica	1	717
Neisseria mucosa	2	1,683–2,561
Moraxella catarrhalis	2	1,544–3,886
Eikenella corrodens	1	2,265
Haemophilus influenzae	4	839–1,535
Haemophilus parainfluenzae	1	1,182

[a] RLU values higher than 50,000 were considered positive results.

reasons, many laboratories perform more than one physiological or serological test.

The optochin susceptibility test has been very reliable in differentiating pneumococci from other alpha-hemolytic streptococci (9). However, 5% of the strains of *S. pneumoniae* may be completely resistant or give small zones of inhibition to optochin. A drawback of the optochin test is its requirement for subculture and overnight incubation.

The bile solubility test may be used alone or in conjunction with the optochin test. Some rough strains of pneumococci may appear to be insoluble in bile or give equivocal results. When this occurs, a second identification test should be performed. Colonies which are bile soluble but have a small zone of inhibition around the optochin disk are considered to be *S. pneumoniae*. A false-positive bile solubility result will occur more often when the test is performed directly on colonies on the agar surface rather than on those in broth medium (5).

The API Rapid STREP kit requires 4 to 24 h for identification of viridans streptococci and enterococci. This commercial system, like other streptococcal identification kits, is not designed for identification of pneumococci, and they usually require supplemental tests to identify them.

The Quellung reaction with polyvalent antiserum is the most accurate and specific test for identification and typing of pneumococci (5). This procedure is no longer used routinely in the diagnostic laboratory. Other serological tests, such as latex agglutination and coagglutination, provide simpler and more rapid serological identification of *S. pneumoniae* from culture (7, 9). However, pneumococcal strains lacking a polysaccharide capsule cannot be identified by serological tests.

False-positive results may occur because of cross-reactions with viridans streptococci, which possess an antigen similar to pneumococcal C polysaccharide (8). Equivocal serological reactions may occur when older colonies or mixed cultures are used. Additional tests must be performed when results are noninterpretable.

The Accuprobe *S. pneumoniae* test is a rapid and highly accurate culture confirmation assay. It eliminates equivocal results observed with antibody-based reagents and allows more accurate identification of both typeable and nontypeable strains. Reagent costs to perform an Accuprobe assay are approximately $3.69 compared with $3.00 to perform both an optochin disk test ($0.32) and a Phadebact coagglutination test ($2.68) for each isolate. The Accuprobe procedure takes approximately 40 min to perform, with 10 min of hands-on time compared with 3 min for agglutination and 1.5 min for a disk test, according to College of American Pathologists workload values. The increased cost may be justified when the total operational costs for patient management are considered.

Colonies of *S. pneumoniae* can be detected in mixed cultures with the Accuprobe test; however, optimal sensitivity of the test is best achieved when four or more discrete colonies are chosen. Direct presumptive identification of *S. pneumoniae* in positive blood cultures by the Accuprobe test has also been successfully demonstrated (2). The Accuprobe test is a practical alternative to confirmatory tests requiring subculture and eliminates the need for multiple identification methods.

We thank Rodney Nagai and Deirdre Trainor for technical assistance.

This study was supported in part by Gen-Probe, Inc.

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