

# Use of Rapid, Nonradioactive DNA Probes in Culture Confirmation Tests To Detect *Streptococcus agalactiae*, *Haemophilus influenzae*, and *Enterococcus* spp. from Pediatric Patients with Significant Infections

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Received 10 July 1990/Accepted 14 October 1990

**Acridinium ester-labeled, chemiluminescent DNA probe tests (Accuprobe; Gen-Probe, Inc.) for culture confirmation of *Streptococcus agalactiae*, *Haemophilus influenzae*, and *Enterococcus* spp. were compared with conventional identification techniques. The probe is a DNA oligomer that is complementary to the RNA of the target. The DNA-RNA hybrids are measured in a luminometer. The 40-min assay requires one reaction tube and the addition of three reagents. When two colonies were used to add a sample to the reaction tube, 325 of 327 isolates were detected by the probe. Isolates of 404 nonprobe target organisms did not hybridize with the probe.**

*Streptococcus agalactiae* is an important agent in acute bacterial infections of newborn infants (1). In infants and young children, *Haemophilus influenzae* is a major cause of meningitis, otitis media, epiglottitis, septic arthritis, occult febrile bacteremia, cellulitis, pneumonia, and empyema; occasionally it causes neonatal meningitis and sepsis. Group D streptococci are responsible for 5% of all positive blood cultures. Group D streptococci are also implicated as relatively frequent causes of neonatal sepsis; in this situation, *Enterococcus faecalis* is four times as common as *Streptococcus bovis*. Considered together, these pathogens cause substantial morbidity and mortality in the pediatric population.

Beta-hemolytic group B streptococci are presumptively identified by hemolysis, serologic identification of the group B antigen, hippurate hydrolysis, the CAMP test, or a combination of reactions to bacitracin and sulfamethoxazole-trimethoprim disks (3). Conventional biochemical presumptive identification procedures for *H. influenzae* include growth on chocolate agar (a medium enriched with X and V factors) at 37°C in a moist, 5 to 10% CO<sub>2</sub> atmosphere, no hemolysis on horse blood agar, a negative reaction for the porphyrin test, a positive reaction for fermentation of glucose, and a positive reaction for catalase (5). In addition, serotyping for capsular antigen (if the strain is encapsulated) with latex agglutination techniques often is used. Group D streptococci have traditionally been identified by the abilities to grow in a medium containing 40% bile and to cleave esculin. Hydrolysis of L-pyrrolidonyl-β-naphthylamide (PYR reaction) identifies nonhemolytic enterococcal strains. Broth containing 6.5% NaCl allows the growth of *E. faecalis*, *E. faecium*, and *E. durans*, the most frequently isolated pathogens from human infections (3). More recently, Facklam and Collins (4) and Ruoff et al. (10) have described alternate schemes for identifying enterococci to the species level. A number of reports have emphasized the limitations of many of these tests (3, 4, 5, 10).

The purpose of this study is to evaluate and compare three

culture confirmation DNA probes with conventional procedures for detecting pathogens from significant pediatric infections.

## MATERIALS AND METHODS

**Bacterial strains.** Strains used in this study were obtained from recent clinical specimens submitted to the clinical microbiology laboratory of Primary Children's Medical Center. If stored, specimens were frozen in skim milk or in 20% glycerol and trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) at -70°C. Subcultures of stock isolates were passaged three times on either sheep blood or chocolate agar plates (BBL), with each passage followed by incubation at 37°C in ambient air or in a 5% CO<sub>2</sub> atmosphere for 24 h. Reference strains obtained from the American Type Culture Collection were used to validate biochemical and serotyping results. Streptococcal strains were serologically grouped with the PathoDx Strep Grouping Kit (Diagnostic Products Corporation, Los Angeles, Calif.) or Streptex (Wellcome Diagnostics, Research Triangle Park, N.C.) when appropriate and were identified to the species level according to the physiological characteristics described by Facklam and associates (3, 4). These included hippurate hydrolysis, the CAMP test (beta-lysin disks from Remel, Lenexa, Mo.) or a combination of reactions to bacitracin (Taxo A; BBL) and sulfamethoxazole-trimethoprim (BBL) disks. Pyrrolidonyl-arylamidase activity was determined by the filter paper substrate test (E-Y Laboratories, San Mateo, Calif.). Tolerance to bile esculin and growth in 6.5% NaCl broth in addition to the API rapid strep test (Analytab Products, Plainview, N.Y.) and the AutoMicrobic System GPI card (Vitek Systems, Hazelwood, Mo.) were performed as previously described and according to manufacturer's instructions (3, 10). Organisms were identified as *Haemophilus* spp. if they had typical colonial morphology and cellular morphology as determined by Gram staining and appropriate hemolysis reaction on horse blood agar (BBL) and if they required V or X and V factors for growth. Serotyping (Difco Laboratories, Detroit, Mich.), determining the production of porphyrin from delta-aminolevulinic acid and biochemical

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characteristics (HNID; MicroScan, West Sacramento, Calif.), completed the identification of these strains (5). Other isolates were identified by conventional methods (7).

**Chemiluminescence assay.** In-solution hybridization assays having rRNA rather than DNA as the target provide more sensitivity, because there are thousands more copies of ribosomes than of DNA per cell (6). Recently, the hybridization protection assay has been developed, allowing the separation of the unreacted probe from the hybrid without the need of a solid support (2). Acridinium ester is used in this assay as the nonradioactive probe label. In solution, hybridization occurs first and is followed by the addition of a selection reagent, which hydrolyzes the acridinium ester on the free probe. The hybrid protects the acridinium ester bound to the hybridized probe. A luminometer is used in the final step to read chemiluminescence associated with a reaction involving acridinium ester bound to hybrid.

Accuprobe culture confirmation tests for *S. agalactiae*, *H. influenzae*, and enterococci were performed as described by the manufacturer (Gen-Probe, San Diego, Calif.). Briefly, 50  $\mu$ l of specimen diluent was combined with one- or two-organism colonies (*Enterococcus* spp. and *S. agalactiae*) in a lyophilized probe tube and incubated for 5 min; this was followed by the addition of 50  $\mu$ l of probe diluent and a 15-min incubation in a 60°C water bath. Next, 300  $\mu$ l of a selection reagent was added and, after a 5-min incubation in a 60°C water bath, the assay results were read on a luminometer (Leader 1 or Leader 250). Each test run included a positive and negative control sample. A positive result was a luminometer reading above the cutoff. This was determined by the manufacturer on the basis of readings obtained from in-house testing. Readings were found not to require adjustments when these probes were challenged with pediatric pathogens. A value below the cutoff was scored as negative. The Leader 1 calculates the results automatically and prints a hard copy test result for each sample tested.

## RESULTS

A total of 631 organisms isolated from significant pediatric infections were evaluated in this study. Both the *S. agalactiae* and the *Enterococcus* spp. Accuprobe assays correlated well with culture, with no false-positive and no false-negative test reactions. The strains tested included *S. agalactiae* ( $n = 100$ ), *Enterococcus* spp. ( $n = 100$ ), group D non-*Enterococcus* spp. ( $n = 30$ ), *Streptococcus pyogenes* ( $n = 10$ ), *Staphylococcus epidermidis* ( $n = 15$ ), *Micrococcus* spp. ( $n = 15$ ), and *Listeria monocytogenes* ( $n = 30$ ).

Table 1 shows the comparative culture and *H. influenzae* Accuprobe assay results for 331 challenge organisms isolated from significant pediatric infections. Overall, the DNA probe assay resulted in an excellent correlation with culture. Two nontypeable strains conventionally identified as *H. influenzae* were *H. influenzae* probe test negative but were positive with a *Haemophilus haemolyticus*-specific probe and were shown to be closely related to *H. haemolyticus* by DNA-DNA homology studies (11). Therefore, with conventional culture techniques as the standard of reference, two discordant results were observed.

For both *S. agalactiae* and *Enterococcus* spp. Accuprobe, the assay had sensitivity, specificity, and positive and negative predictive values of 100%; for the *H. influenzae* Accuprobe, the sensitivity, specificity, and positive and negative predictive values were 98.4, 100, 100, and 99.0%, respectively. The statistical parameters for strains isolated from significant pediatric infections indicate that these Accuprobe

TABLE 1. Culture and DNA probe (*H. influenzae* Accuprobe) test results for pediatric specimens from significant infection

Organism and serotype	No. of probe test results	
	Positive	Negative
<i>Haemophilus influenzae</i>		
a	2	0
b	63	0
c	2	0
d	1	0
e	8	0
f	16	0
Nontypeable	33	2
Other bacteria <sup>a</sup>	0	204

<sup>a</sup> Strains tested included *Haemophilus paraphrophilus* ( $n = 1$ ), *Haemophilus aphrophilus* ( $n = 1$ ), *Haemophilus parahaemolyticus* ( $n = 4$ ), *Haemophilus ducreyi* ( $n = 1$ ), *Haemophilus parainfluenzae* ( $n = 22$ ), *Neisseria meningitidis* ( $n = 25$ ), *Neisseria* spp. ( $n = 10$ ), *Moraxella* (*Branhamella*) *catarrhalis* ( $n = 10$ ), *Moraxella* spp. ( $n = 9$ ), *Kingella* sp. ( $n = 5$ ), *Capnocytophaga* sp. ( $n = 5$ ), *Escherichia coli* O157:H7 ( $n = 5$ ), *Escherichia coli* ( $n = 5$ ), *Klebsiella pneumoniae* ( $n = 5$ ), *Klebsiella oxytoca* ( $n = 5$ ), *Proteus vulgaris* ( $n = 3$ ), *Proteus mirabilis* ( $n = 3$ ), *Listeria monocytogenes* ( $n = 5$ ), *Staphylococcus aureus* ( $n = 5$ ), *Streptococcus agalactiae* ( $n = 5$ ), *Streptococcus pneumoniae* ( $n = 23$ ), *Yersinia enterocolitica* ( $n = 6$ ), *Eikenella corrodens* ( $n = 15$ ), *Pasteurella multocida* ( $n = 12$ ), *Vibrio* sp. ( $n = 1$ ), *Bordetella pertussis* ( $n = 11$ ), *Bordetella parapertussis* ( $n = 1$ ), and *Bordetella bronchiseptica* ( $n = 1$ ).

assays compare very favorably with routine culture methods.

## DISCUSSION

Identification of *S. agalactiae*, *Enterococcus* spp., and *H. influenzae* in the clinical microbiology laboratory can be accomplished in several ways. Conventionally, the simplest techniques involve key phenotypic characteristics and serotyping results (7). Because exceptions occur with each of these tests, it is advisable to interpret several results from several tests rather than just one (1, 4, 8).

The Gen-Probe Accuprobe culture confirmation assay requires approximately 40 min to perform with less than 10 min of hands-on time. The test is impressively simple. In this evaluation the procedure was 100% accurate in identifying isolates from significant pediatric infection for the *S. agalactiae* and the *Enterococcus* spp. probes. Accuracy for the *H. influenzae* probe was 99.4% if conventional culture techniques are considered the "gold standard." These DNA probe-based tests provide a practical and rapid alternative to any confirmatory test which requires subculturing. Also, these probes eliminate many of the problems now encountered in attempts to provide a more definitive identification. For example, 5 to 13% of group B streptococci are susceptible to bacitracin and therefore subject to misinterpretation as belonging to group A. A potential disadvantage is the cost of Accuprobe. If both serologic and physiologic assays are replaced by one DNA probe test, Accuprobe identification would then be similar to the expense of conventional identification procedures, yet it would be more rapid and not as labor intensive. However, if for epidemiologic or other reasons, serologic and physiologic tests or both remain a requirement of organism identification, Accuprobe could then increase the cost of isolate identification. This issue and the convenience of using a luminometer only one-fourth the size of the Leader 1 are currently being evaluated in our laboratory with regard to routine identification procedures.

However, for those laboratories in which academic issues are not a priority, the replacement of a battery of serologic tests and 1 to 14 physiologic tests requiring up to an additional 24 h (with at least 1 additional h of hands-on time) with a single, accurate test requiring 40 min to complete would be valuable.

#### ACKNOWLEDGMENTS

We thank Marguerite Watson and Ruth Henson for their excellent secretarial and editorial assistance. The expert support from Ira G. Rosen and Deirdre Trainor is gratefully appreciated. We thank the staff in the Microbiology Laboratory at Primary Children's Medical Center for their help in collecting specimens.

This evaluation was supported, in part, by a grant from Gen-Probe, Inc.

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