

Identification of *Streptococcus pneumoniae* Revisited

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The sensitivities and specificities of several different diagnostic assays for *Streptococcus pneumoniae* were assessed using 99 clinical isolates of *S. pneumoniae* and 101 viridans streptococci and were as follows: Pneumoslide, 99 and 87%, respectively; Directigen, 100 and 85%, respectively; Phadebact, 100 and 98%, respectively; deoxycholate drop test, 99 and 98%, respectively; deoxycholate tube test, 100 and 99%, respectively; optochin, 99 and 98%, respectively; and Gram Positive Identification Card, 90 and 96%, respectively. Identification of clinical isolates of *S. pneumoniae* should be confirmed using one or more diagnostic assays with well-documented high (e.g., $\geq 95\%$) sensitivities and specificities.

Despite the clinical importance and frequent isolation of *Streptococcus pneumoniae*, there is no one “gold standard” or reference method for its identification. Laboratory identification of this pathogen has been accomplished using one or more assays, including Gram stain morphology, colony morphology, and hemolysis on sheep blood agar, pyrrolidonyl arylamidase reactivity, optochin susceptibility, solubility in deoxycholate (bile), carbohydrate utilization, reaction with specific antisera, miniaturized manual systems such as the API 20 Strep system (bioMerieux Vitek, Inc., Hazelwood, Mo.), the automated Gram Positive Identification (GPI) Card (bioMerieux Vitek, Inc.) and DNA probes (Gen-Probe, San Diego, Calif.) (1, 2, 5–9, 15, 17, 19, 20).

Deoxycholate solubility has been associated with a sensitivity of $\geq 98\%$ and a specificity of 100% (6, 7, 17, 19). However, 10 strains of nonencapsulated, deoxycholate-insoluble (but optochin-susceptible) *S. pneumoniae* isolated from eye swabs were reported (13). Optochin susceptibility has been reported to be 90 to 100% sensitive and 99 to 100% specific (2, 6, 8, 19) although numerous studies have confirmed optochin-resistant isolates of the organism (2, 4, 10–12, 14, 18). At least one report exists of an optochin-susceptible viridans streptococcal isolate with an 18-mm zone of inhibition (4). The few published studies of the Pneumoslide (Becton-Dickinson Microbiology Systems, Cockeysville, Md.) (5, 7, 17), Directigen (Becton-Dickinson Microbiology Systems) (20), and Phadebact (Boule Diagnostics AB, Huddinge, Sweden) (6, 12, 19) serological methods for *S. pneumoniae* antigen identification have reported them to be close to 100% sensitive but often, if not always, to have a substantially lower specificity. Only two studies could be found concerning the relative accuracy of the GPI Card for the identification of *S. pneumoniae*. They reported (in 1984) sensitivities of only 71% (9) and 81% (1). The study reported herein was undertaken to reassess the current sensitivity, specificity, and predictive values associated with each of the more common and traditionally used identification methods for *S. pneumoniae*.

Identification of isolates. Fresh clinical isolates of both *S. pneumoniae* and viridans streptococci from various sites were each identified using three serological tests (Pneumoslide, Directigen, and Phadebact), both the drop and tube deoxycholate (10%; Remel, Lenexa, Kans.) solubility procedures, optochin (Taxo P; Becton-Dickinson Microbiology Systems) susceptibility, the GPI Card, colony morphology and hemolysis, and Gram stain morphology. All commercial tests were performed as specified in their package inserts.

An isolate was considered to be *S. pneumoniae* if it could be described as follows. (i) The isolate was alpha-hemolytic and mucoid or formed shiny young colonies and/or had a depressed center on sheep blood agar (4). If the isolate was not mucoid or with a depressed center, it had to be both bile soluble and optochin susceptible. (ii) The isolate formed gram-positive cocci in chains. (iii) The isolate was bile soluble or susceptible to optochin (2, 7, 8, 15, 17, 19). When discrepancies between the results of the identification methods occurred, the organisms were reisolated and the tests with discrepant results were repeated. However, the first result from each test was counted as the result of record.

Test performance. From 28 July 2000 until 5 March 2001, 99 clinical isolates of *S. pneumoniae* (57 [58%] from sputum and 26 [26%] from blood) and 101 isolates of viridans streptococci were tested. Test sensitivity ranged from a low of 89.9% with the GPI Card to a high of 100% for two of the three serological assays (Directigen and Phadebact) and the deoxycholate tube test (Table 1). The one isolate of *S. pneumoniae* with a false-negative optochin susceptibility result gave repeated zones of growth inhibition of 13 mm (with a cutoff of ≥ 14 mm). For 10 false-negative calls by the GPI Card, one isolate was not identified by the system and nine were misidentified (as *Streptococcus oralis* [six isolates] or as *Gemella* spp. [three isolates]) when first tested. In repeat testing with the GPI Card, five of the 10 isolates were correctly identified.

Test specificity ranged from only 77.2% for colony morphology to 99.0% for the tube deoxycholate procedure. There was only one false-positive result when the tube deoxycholate test was used, compared to 13, 15, and 23 false-positive results associated with the Pneumoslide, Directigen, and colony morphology tests, respectively. Of the two false-positive optochin susceptibility test results, both were confirmed by test repeti-

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TABLE 1. Performance of tests for the identification of *S. pneumoniae*^a

Test	No. of isolates for which result was:				Sensitivity ^c	Specificity ^c	PVP ^c	PVN ^c
	True positive	False positive	False negative	True negative				
Pneumoslide	98	13	1	88	99.0	87.1	88.3	98.9
Directigen	99	15	0	86	100	85.1	86.8	100
Phadebact	99	2	0	99	100	98.0	98.0	100
Deoxycholate drop	98	2	1	99	99.0	98.0	98.0	99.0
Deoxycholate tube	99	1	0	100	100	99.0	99.0	100
Optochin susceptibility	98	2	1	99	99.0	98.0	98.0	99.0
GPI Card ^b	89	4	10	94	89.9	95.9	95.7	90.4
Colony morphology	93	23	6	78	93.9	77.2	80.2	92.9

^a PVP, positive predictive value; PVN, negative predictive value.

^b Three of the viridans streptococcal isolates were repeatedly determined to have insufficient growth by the GPI Card, and the results for these isolates were not included in the calculation of test specificity.

^c Values are percentages.

tion to be well above the test's 14-mm threshold (18 and 19 mm for one isolate; 18 and 17 mm for the other). For the four false-positive GPI Card results, the probabilities of accuracy were 51, 60, 84, and 97%. The positive predictive value, or the probability that an isolate identified as *S. pneumoniae* was correctly identified, was only 80.2% for colony morphology and no higher than 88% for both the Pneumoslide and Directigen assays but was 98 to 99% for Phadebact, the two deoxycholate procedures, and the optochin susceptibility test.

Problem isolates were occasionally encountered. For example, a blood culture isolate was strongly and repeatedly positive for *S. pneumoniae* with all three antigen detection assays. However, it was repeatedly negative to both the drop and tube deoxycholate assays and resistant to optochin (zone size, ≤ 6 mm). This isolate grew well on chocolate agar but failed to grow even minimally on 5% sheep blood agar. The GPI Card identified the isolate as *Gemella* (specificity, 94%). The colony was moderately large and shiny but without a depressed center. The Gram stain was more consistent with a viridans streptococcus morphology than with that of a pneumococcus. This isolate was finally identified by the Streptococcus Laboratory at the Centers for Disease Control and Prevention as *Granulicatella adiacens*.

Workers in clinical laboratories need to be aware of the potential difficulties that may be encountered when using traditional methods for the identification of *S. pneumoniae* (4, 18). Many strains of non-pneumococcal alpha and nonhemolytic streptococcal species have capsular antigens similar to those of *S. pneumoniae* (3). In the present study, false-positive Pneumoslide and Directigen results were so common that neither of these assays can be recommended as the sole means by which isolates of *S. pneumoniae* can be accurately identified, despite their sensitivity ranges of 99 to 100%. The deoxycholate bile solubility assay has been reported to be among the most sensitive and specific assays for identification of *S. pneumoniae* (6, 7, 17, 19). In the present study, no false-negative and only one false-positive tube deoxycholate test result was observed. The direct agar drop deoxycholate test was almost as accurate. Because the latter assay can be more rapidly and easily completed at the bench on multiple isolates, it is preferred over the tube deoxycholate method as a routine identification assay. Optochin-resistant strains of *S. pneumoniae* have been reported (2, 4, 10–12, 14, 18). We found only one of

99 (1%) isolates of *S. pneumoniae* that had a zone size of <14 mm (zone size, 13 mm [result obtained twice]). Because the optochin susceptibility assay requires overnight incubation and the more rapid bile solubility test had a similar accuracy, the latter method is preferred as a primary means of identification of most routine isolates of *S. pneumoniae*. The sensitivity and the specificity of the GPI Card have not been well documented in recent years. The results of the present study indicate that a GPI Card identification of an isolate as either *S. pneumoniae* or one of the viridans streptococcal species should be confirmed using either bile solubility or the optochin susceptibility test because of the relatively low sensitivity and negative predictive value (PVN) associated with the GPI Card assay.

During the present study, the most accurate single assay was the tube deoxycholate test, followed very closely by the Phadebact antigen agglutination assay, the agar drop deoxycholate assay, and the optochin susceptibility test. Because of the 99 to 100% PVN values associated with the three antigen detection tests, a negative result for the presence of pneumococcal antigen using these assays accurately rules out *S. pneumoniae* but a positive result with Directigen and Pneumoslide reagents should be confirmed using bile or optochin. The final identification of an isolate either as *S. pneumoniae* or as a viridans streptococcus should involve, as a minimum, all of the following: colony morphology, hemolysis, and Gram stain morphology; either deoxycholate solubility, optochin susceptibility, or Phadebact coagglutination; and a knowledge of the specimen source (bile-insoluble isolates of *S. pneumoniae* were recovered from the eye [13]; nontypeable isolates were recovered from conjunctivitis outbreaks [16]; optochin-resistant isolates from the blood and middle ear have been reported [4, 10–12, 14, 18]). Occasional clinical isolates of either *S. pneumoniae* or viridans streptococci, as found during the present study, cannot be accurately identified without using additional biochemical, molecular, and/or serological assays.

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