A Comparison of Three Commercial Test Systems for Biotyping

*Haemophilus influenzae* and *Haemophilus parainfluenzae*

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Running title: Note

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ABSTRACT

Biotypes of *Haemophilus influenzae* and *Haemophilus parainfluenzae* were determined with three commercially-available biochemical test kits: the IDS RapID NH system, the NHI card and the API NH strip. The API NH strip performed best, correctly classifying the biotypes of 371 of 380 different challenge strains (97.6%).
*Haemophilus influenzae* and *Haemophilus parainfluenzae* are classified into distinct biotypes on the basis of ornithine decarboxylase, urease, and indole activity [4,8,10,11,16,18]. There exists a relationship between selected biotypes of these organisms and sites of colonization, association with specific infectious disease problems and, in the case of *H. influenzae*, capsular serotype and antimicrobial resistance profiles [1-4,6,7,12-14,16,17,19-22]. While molecular typing procedures may also serve many of these purposes, generally speaking, molecular typing techniques are more expensive, slower, not as widely available and less well established than biotyping with *Haemophilus* spp. For these reasons, circumstances arise in clinical microbiology laboratories today in which it is useful from either a clinical or an epidemiologic perspective for the laboratory to provide biotype information on isolates of both *H. influenzae* and *H. parainfluenzae*.

A previous investigation in our laboratory [15] evaluated three commercially-available biochemical-based test kits as means for establishing the species identification of organisms in the *Haemophilus* genus: the IDS RapID NH system (Remel, Lenexa, KS) and the Neisseria-*Haemophilus* Identification test (NHI card) and API NH strip, both from bioMérieux (Marcy l'Etoile, France). The intent of the current study was to examine the utility of these same three test systems for determining the biotypes of *H. influenzae* and *H. parainfluenzae*.

Two hundred-eight isolates of *H. influenzae* and 172 isolates of *H. parainfluenzae* were examined in this study. Organisms had been recovered from patients with various *Haemophilus* infections as part of two national surveillance studies aimed at assessing antimicrobial resistance rates with *Haemophilus* spp. (5,9) and from patients receiving care at the institutions of the authors. Prior to biotype characterization, isolates were stored at -70°C and then sub-cultured...
twice on chocolate agar containing 10 µg of NAD per mL (Remel) with plates incubated at 35°C in 5-10% CO₂ overnight.

The three biochemical test systems examined in this investigation, the IDS RapID NH system, the NHI card and the API NH strip, were used precisely as defined by the manufacturer. Biotype assignments derived from the three test systems were compared and when there was complete agreement between the three test systems, that biotype was taken as being correct. When discordant results were obtained with any of the three systems, conventional biochemical tests for ornithine decarboxylase, indole and urease activity were performed as a means of establishing an individual strain’s biotype as described by Killian (11).

The results obtained with the three biotyping systems examined in this study are depicted in Table 1. With isolates of *H. influenzae*, the API NH strip and the NHI card both correctly categorized the biotypes of 204 of 208 test strains (98.1%). In distinction, the IDS RapID NH system yielded correct results with only 48 of 208 test strains (23.1%). False positive ornithine decarboxylase results with biotype II and III strains of *H. influenzae* were responsible for all of the erroneous biotype assignments with the IDS RapID NH system.

Among the 172 strains of *H. parainfluenzae* examined in this study, biotypes were correctly classified in 167 cases (97.1%) with the API NH strip, in 157 cases (91.3%) with the NHI card and in 148 cases (86.1%) with the IDS RapID NH system (Table 1). All fifteen strains of *H. parainfluenzae* with erroneous biotype assignments with NHI card yielded false negative ornithine decarboxylase results with this system; 16 of the 24 discordant assignments with the IDS RapID NH system were the result of false positive ornithine decarboxylase results.

One limitation of the current study was use of isolates that had been stored at -70°C prior to testing rather than use of fresh clinical isolates, arguably more representative of the
circumstances in which these test systems would be used in routine practice. We used a convenience sample of stock isolates expressly for purposes of having at least small numbers of less commonly encountered biotypes of both *H. influenzae* and *H. parainfluenzae* in our sample of organisms to be tested. However, even then, we were unable to include any biotype VIII strains of *H. influenzae* or biotypes VI, VII or VIII strains of *H. parainfluenzae*. These biotypes occur very infrequently in clinical practice.

In summary, among the three tests systems examined in this study, the API NH strip performed best. In comparison, the NHI kit was comparable for biotyping strains of *H. influenzae* but inferior for strains of *H. parainfluenzae*. The IDS RapID NH system was inferior to both of the other test systems as a means for biotyping both *H. influenzae* and *H. parainfluenzae*. The vast majority of the categorization errors with the IDS RapID NH system with both organisms were due to false positive ornithine decarboxylase results. Of note, use of a smaller inoculum than that recommended by the manufacturer did not obviate this problem (data on file). These observations are consistent with one previously published report (4) and indicate that, until this problem is rectified by the manufacturer, the IDS RapID NH system cannot be advocated for use in biotyping either *H. influenzae* or *H. parainfluenzae*. 

REFERENCES


Table 1. Biotyping results obtained with three commercial test systems versus 208 strains of *Haemophilus influenzae* and 172 strains of *Haemophilus parainfluenzae*.

(table attached)

Footnote: a. Strains of *H. influenzae* biotype VIII and *H. parainfluenzae* biotypes VI, VII and VII were not available for inclusion in this study.
<table>
<thead>
<tr>
<th>Organism&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Biotype</th>
<th>Expected reaction with:</th>
<th>Number of Isolates</th>
<th>IDS RapID NH system</th>
<th>NH card</th>
<th>API NH strip</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number correctly biotyped</td>
<td>Number of errors with:biotyped</td>
<td>Number correctly biotyped</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Omithine</td>
<td>Urease</td>
<td>Indole</td>
<td>Omithine</td>
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<tr>
<td><strong>Haemophilus influenzae</strong></td>
<td>I</td>
<td>+                      +</td>
<td>23</td>
<td>23</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>II</td>
<td>-                      +</td>
<td>105</td>
<td>7</td>
<td>98</td>
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<tr>
<td></td>
<td>III</td>
<td>-                      +</td>
<td>63</td>
<td>62</td>
<td>0</td>
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<tr>
<td></td>
<td>IV</td>
<td>+                      -</td>
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<td>V</td>
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<td></td>
<td>VI</td>
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<td>5</td>
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<td>VII</td>
<td>-                      +</td>
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<tr>
<td><strong>TOTAL</strong></td>
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<tr>
<td><strong>TOTAL</strong></td>
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<td>148</td>
<td>20</td>
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