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Comparison of Three Commercial Test Systems for Biotyping *Haemophilus influenzae* and *Haemophilus parainfluenzae*▽

Erik L. Munson† and Gary V. Doern*

Division of Medical Microbiology, Department of Pathology, University of Iowa, Carver College of Medicine, Iowa City, Iowa 52242

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The biotypes of *Haemophilus influenzae* and *Haemophilus parainfluenzae* isolates were determined with three commercially available biochemical test kits: the IDS RapID NH system, the *Neisseria-Haemophilus* identification test (NHI card), and the API NH strip. The API NH strip performed best, correctly classifying the biotypes of 371 of 380 (97.6%) different challenge strains.

Haemophilus influenzae and *Haemophilus parainfluenzae* are classified into distinct biotypes on the basis of ornithine decarboxylase, urease, and indole activities (4, 8, 10, 11, 16, 18). There exists a relationship between the 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 a 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 present 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. The organisms had been recovered from patients with various *Haemophilus* infections as part of two national surveillance studies aimed at assessing antimicrobial resistance rates for *Haemophilus* spp. (5, 9) and from patients receiving care at the institutions of the authors. Prior to biotype characterization, the isolates were stored at −70°C and then subcultured twice on chocolate agar containing 10 μg of NAD per ml (Remel) with plates incubated at 35°C in 5 to 10% CO_2 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 described by the manufacturers. The 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 activities 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 shown 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 for 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.

For the 172 strains of *H. parainfluenzae* examined in this study, the 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 15 strains of *H. parainfluenzae* with erroneous biotype assignments with the NHI card yielded false-negative ornithine decarboxylase results with this system; 16 of the 24 discordant assignments with the IDS RapID NH system were attributable to false-positive ornithine decarboxylase results.

One limitation of the current study was the use of isolates that had been stored at −70°C prior to testing rather than 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 the purpose of having at least small numbers of less commonly encountered biotypes of both *H. influenzae* and *H. parainfluenzae* in the sample of organisms to be tested. However, even then, we were unable to include any biotype VIII strains of *H. influenzae* or biotype VI, VII, or VIII strains of *H. parainfluenzae*. These biotypes occur very infrequently in clinical practice.

In summary, of the three tests systems examined in this study, the API NH strip performed best. In comparison, the

* Corresponding author. Mailing address: Clinical Microbiology Laboratories, C606-GH, University of Iowa Hospital and Clinics, 200 Hawkins Drive, Iowa City, IA 52242. Phone: (319) 356-2591. Fax: (319) 384-9854. E-mail: gary-doern@uiowa.edu.

† Present address: Wheaton Franciscan and Midwest Clinical Laboratories, Wauwatosa, WI 53226.

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

Organism[a]	Biotype	Expected reaction with:			Total no. of isolates	IDS RapID NH system				NHI card				API NH strip			
		Ornithine	Urease	Indole		No. of isolates correctly biotyped	No. of isolates with errors for:			No. of isolates correctly biotyped	No. of isolates with errors for:			No. of isolates correctly biotyped	No. of isolates with errors for:		
							Ornithine	Urease	Indole		Ornithine	Urease	Indole		Ornithine	Urease	Indole
Haemophilus influenzae	I	+	+	+	23	23	0	0	0	22	1	0	0	23	0	0	0
	II	−	+	+	105	7	98	0	0	105	0	0	0	102	2	0	1
	III	−	+	−	63	1	62	0	0	62	1	1	0	62	1	0	0
	IV	+	+	−	5	5	0	0	0	5	0	0	0	5	0	0	0
	V	+	−	+	5	5	0	0	0	4	1	0	0	5	0	0	0
	VI	+	−	−	5	5	0	0	0	5	0	0	0	5	0	0	0
	VII	−	−	+	2	2	0	0	0	1	1	0	0	2	0	0	0
Total					208	48	160	0	0	204	4	1	0	204	3	0	1
Haemophilus parainfluenzae	I	+	−	−	64	58	3	4	0	54	10	0	0	61	1	3	0
	II	+	+	−	58	57	0	0	1	53	5	0	1	58	0	0	0
	III	−	+	−	22	6	16	0	0	22	0	0	0	22	0	0	0
	IV	+	+	+	2	1	1	1	0	2	0	0	0	2	0	0	0
	V	−	−	−	26	26	0	0	0	26	0	0	0	24	2	0	0
Total					172	148	20	5	1	157	15	0	1	167	3	3	0

[a] Strains of *H. influenzae* biotype VIII and *H. parainfluenzae* biotypes VI, VII, and VII were not available for inclusion in this study.

NHI kit was comparable to the APE NH strip 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 (unpublished data). 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 recommended for use in biotyping either *H. influenzae* or *H. parainfluenzae*.

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