

Isolation and Rapid Identification of *Haemophilus ducreyi*

PAUL HANNAH¹ AND J. R. GREENWOOD^{1,2*}

Public Health Laboratory, County of Orange, Santa Ana, California 92702,^{1*} and Division of Epidemiology, School of Public Health, University of California, Los Angeles, California 90024²

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During a 2-month period, 62 strains of *Haemophilus ducreyi* were isolated from 168 genital lesions and 2 lymph node aspirates. Of these strains, 22 were found on both chocolate agar and fetal bovine serum agar supplemented with vancomycin, 29 were found only on chocolate agar, and 9 were found only on fetal bovine serum agar. Two additional strains were isolated on sheep blood agar. All of these isolates were correctly identified with the RapID NH system (Innovative Diagnostic Systems, Inc., Decatur, Ga.) a new identification kit that has a database for *Haemophilus*, *Neisseria*, and other genera that include fastidious gram-negative bacilli.

Haemophilus ducreyi, the etiological agent of chancroid, is infrequently isolated in clinical or public health laboratories in the United States and Canada. Although there have been several reports in recent years of localized chancroid outbreaks from which *H. ducreyi* has been successfully isolated (11, 12), most reported cases are usually diagnosed on the basis of clinical observation or results of a Gram-stained smear (7). We report methods used for the successful isolation of 62 strains of *H. ducreyi* during a recent chancroid outbreak in Orange County, Calif. (5). In addition, because *H. ducreyi* isolates frequently present difficulties in definitive identification (11, 13), we evaluated a newly developed rapid-identification system, the RapID NH system (Innovative Diagnostic Systems, Inc., Decatur, Ga.) for its ability to correctly identify *H. ducreyi*. This 4-h system has a database for *Haemophilus*, *Kingella*, *Moraxella*, *Neisseria*, and additional genera that include fastidious gram-negative bacilli.

MATERIALS AND METHODS

Specimen collection. Genital ulcers with typical chancroid characteristics were cleansed with physiological saline. A sterile cotton swab was then moistened in sterile phosphate-buffered saline, pH 7.2, and used to sample the cleansed ulcer base (16). The swab was placed in a sterile screw-capped test tube (13 by 100 mm), which was transported to the laboratory.

Isolation. Within 10 min of collection, the following primary isolation media were inoculated: fetal bovine serum agar supplemented with 3 µg of vancomycin per ml (FBSA + V) (16), chocolate agar (CA) (Clinical Standards, Carson, Calif.), and 5% sheep blood agar (heart infusion agar base; Difco Laboratories, Detroit, Mich.). The order in which the plates were inoculated was alternated for each specimen. Each inoculated plate was immediately placed in a candle extinction jar

containing a water-saturated gauze pad (1, 11), and the jars were incubated at 34 to 36°C. The plates were examined daily under a dissection microscope for colonies characteristic of *H. ducreyi*.

Identification. On the basis of colonial morphology and results of Gram staining, some colonies were presumptively identified as *H. ducreyi*; these colonies were subcultured for purity onto a rabbit blood agar plate, which was incubated as outlined above. When pure subcultures of 20 representative isolates were obtained, they were sent to F. O. Sottnek, Centers for Disease Control, Atlanta, Ga., for confirmation by conventional techniques and were also used to prepare inocula for identification with the RapID NH system.

RapID NH system. The RapID NH system consists of a small plastic tray with 10 test cavities. Substrates are included for the following tests: phosphatase activity, reduction of nitrate to nitrite, hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (ONPG), hydrolysis of the amide substrates prolyl-*p*-nitroanilide and γ-glutamyl-*p*-nitroanilide, oxidation of resazurin, acidification of glucose and sucrose, production of indole from tryptophane, hydrolysis of urea, utilization of ornithine, and hydrolysis of penicillin. Inocula for the system are prepared by harvesting growth from 18- to 24-h plate cultures into a 1-ml tube of RapID NH system inoculation fluid (KCl-CaCl₂-FeCl₃ in distilled water). After the inocula are brought to the proper turbidity (McFarland no. 3 standard), a Pasteur pipette is used to transfer the entire contents of the tube to the upper right-hand corner of the test panel. When the test panel is gently tilted forward to an angle of approximately 45°, all of the test wells are inoculated at once. Panels are incubated for 4 h at 35 to 37°C in an air incubator, the appropriate reagents are added, and the reactions are scored.

Control organisms. In addition to presumptively identified *H. ducreyi* isolates, the following fastidious gram-negative bacteria were also tested by the RapID NH system: *H. ducreyi* strains 138 and 411 (kindly supplied by W. Albritton, Centers for Disease Control), five strains of *Neisseria gonorrhoeae* (Orange County Health Department [OCHD]), two strains of

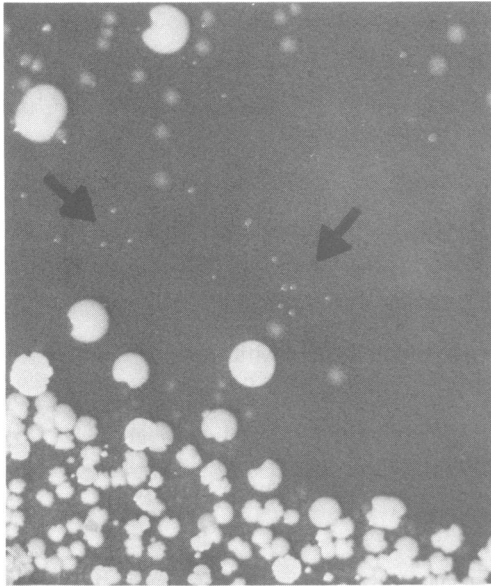


FIG. 1. Primary culture on CA plate. Arrows point to 48-h-old *H. ducreyi* colonies.

N. mucosa (OCHD), six strains of *N. lactamica* (OCHD), eight strains of *N. meningitidis* (OCHD), six strains of *H. influenzae* biotype 1 (OCHD), one strain of *H. influenzae* biotype 4 (OCHD), five strains of *H. parainfluenzae* (OCHD), two strains of *H. aphrophilus* (OCHD), and two strains of *Gardnerella vaginalis* (OCHD). All of the control organisms had been identified by the methods of Greenwood et al. (8) or Gröschel and Portnoy (10).

RESULTS

Over a 2-month period, 170 specimens were submitted to the laboratory for identification of *H. ducreyi*. Of these specimens, 168 were from genital lesions and 2 were from lymph node aspirates. A total of 62 (36%) were positive for *H. ducreyi*. On both FBSA + V and CA, colonies were generally pinpoint size at 24 to 48 h but increased in size to 0.5 and 1.0 mm after 4 to 5 days of incubation. On FBSA + V, a *H. ducreyi* colony appears as a translucent, entire colony that can be moved as an entire colony across the surface of the plate with an inoculating loop. On CA plates, a colony appears as a somewhat yellowish heaped colony that can also be moved as an entire colony. Figure 1 shows the typical morphology of *H. ducreyi* colonies on CA.

The most efficient isolation medium used in this study was CA: 51 of the 62 isolates were recovered on it. Of the isolates, 29 were recovered only on CA, and no typical *H. ducreyi* colonies were observed on FBSA + V. Nine *H. ducreyi* strains, however, were recovered only on FBSA + V. Thus, we had to use both CA and FBSA + V to recover 96% of the *H. ducreyi*

isolates. It is also interesting that two strains (4%) were recovered only on 5% sheep blood agar plates.

Table 1 lists the biochemical reactions of 101 strains of fastidious gram-negative bacilli tested by the RapID NH system. All of the *H. ducreyi* isolates confirmed by the Centers for Disease Control were also correctly identified by the RapID NH system. In addition, none of the non-*H. ducreyi* gram-negative bacilli included as control organisms was incorrectly identified as *H. ducreyi*. With the following exceptions, all of the control organisms were correctly identified by the RapID NH system: two strains of *N. lactamica* were incorrectly identified—one as *N. mucosa* and one as *G. vaginalis*, two strains of *G. vaginalis* yielded no species identification, and one strain of *N. mucosa* was identified as a member of the *N. subflava-N. sicca* group.

DISCUSSION

Although chancroid and *H. ducreyi* (sometimes as Ducrey's bacillus) have been recognized in the literature for approximately 90 years, relatively little knowledge about chancroid epidemiology or the basic microbiology of *H. ducreyi* has accumulated. A basic reason for this problem has been the inability of laboratories to routinely isolate *H. ducreyi*. Although numerous medium formulations and isolation procedures can be found in the literature (1-4, 11, 12, 16), many of the techniques have not been critically evaluated. Confounding this problem is the fact that the number of clinical chancroid cases is insufficient for comparison of existing isolation procedures or development of new ones. Recently, however, Sottnek et al. (16) have been able to compare several basal media for the ability to enable the isolation of *H. ducreyi* from genital ulcers. On the basis of results for 17 isolates, they reported rabbit blood agar with vancomycin and FBSA + V to give the highest isolation rates. They also reported CA supplemented with 3 µg of vancomycin per ml to be slightly less efficient. In contrast, we found more *H. ducreyi* isolates on commercial CA than on the FBSA + V. Several explanations for this difference are possible. *H. ducreyi* colonies on CA have a unique morphology and in low numbers might be picked with slightly greater efficiency than are low numbers of colonies on FBSA + V. Additionally, the CA we used contained no vancomycin but did contain freshly chocolate blood rather than a 1% hemoglobin suspension. Despite these differences, a significant point of our findings is that *H. ducreyi* can be isolated on commercially prepared media. It is important to note, however, that almost 12% of the isolates would not grow on CA. Strain-related nutritional differences have been noted

TABLE 1. Biochemical reactions of 101 fastidious gram-negative bacilli tested by the RapID NH system

Organism	No. tested	No. positive for ^a :										
		PO ₄	NIT	ONPG	PRO	GGT	RES	GLU	SUC	IND	URE	ORN
<i>H. ducreyi</i>	64 ^b	64	64	0	0	0	0	0	0	0	0	0
<i>H. influenzae</i>												
biotype 1	6	6	6	0	0	0	1	5	0	6	5	6
biotype 4	1	1	1	0	0	0	0	1	0	0	1	1
<i>H. parainfluenzae</i>	5	5	5	5	0	0	2	5	5	5	5	0
<i>H. aphrophilus</i>	2	2	2	2	0	2	1	2	2	0	0	0
<i>N. mucosa</i>	2 ^c	0	1	0	2	0	1	2	2	0	0	0
<i>N. lactamica</i>	6 ^d	0	0	5	6	0	0	4	1	0	0	0
<i>N. gonorrhoeae</i>	5	0	0	0	5	0	1	3	0	0	0	0
<i>N. meningitidis</i>	8	0	0	0	5	8	8	8	0	0	0	0
<i>G. vaginalis</i>	2 ^e	1	1	2	2	0	0	2	0	0	0	0

^a PO₄, Phosphatase activity; NIT, nitrate reduction; PRO, proline aminopeptidase activity; GGT, γ -glutamyl aminopeptidase activity; RES, resazurin reduction; GLU, glucose utilization; SUC, sucrose utilization; IND, indole production; URE, urease activity; ORN, ornithine decarboxylase activity.

^b Including two reference strains.

^c One strain was incorrectly identified as a member of the *N. subflava*-*N. sicca* group.

^d Two strains were incorrectly identified: one as *G. vaginalis*, the other as *N. mucosa*.

^e No identification for either strain was obtained.

previously (16) and are an important consideration when attempting isolation of *H. ducreyi*. Therefore, our findings suggest that at least two types of isolation media should be used for maximal recovery of *H. ducreyi*.

Because of taxonomic inconsistencies (13) and descriptive reports based on gram-positive organisms (6, 14), identification of *H. ducreyi* has always proven to be as difficult as isolation itself. However, in his classic study of the genus *Haemophilus*, Kilian (13) was able to better delineate *H. ducreyi* and provide differential features useful for identification purposes. Unfortunately, many of the media and techniques used by Kilian are not routinely available in most clinical laboratories. Our results indicate that the RapID NH system appears to offer an accurate and rapid alternative to conventional *Haemophilus* identification methods. Although the system does not identify X and V growth factor requirements, it still appears to accurately identify *H. ducreyi* and differentiate among the other *Haemophilus* spp. tested. In addition, although we only examined a limited number of strains, we also obtained excellent identification of *N. gonorrhoeae* and *N. meningitidis*. Inaccuracies were noticed, however, when testing other *Neisseria* species and *G. vaginalis*. Significantly, none of these species was incorrectly identified as *N. gonorrhoeae* or *H. ducreyi*. The

RapID NH system errors might be related to the limited data in the database for species such as *G. vaginalis*. Only positive results for ONPG hydrolysis are recorded in the database, and almost 50% of the strains previously tested are negative for this feature (9).

Isolation of *H. ducreyi* on commercial CA and subsequent identification by the RapID NH system will allow most microbiology laboratories to increase their capability to diagnose chancroid. We hope that the system will enable additional information on true chancroid incidence to be accumulated and the epidemiology of *H. ducreyi* infection to be elucidated.

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