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DNA Probes for the Identification of *Haemophilus ducreyi*

LINDA M. PARSONS,* MEHDI SHAYEGANI, ALFRED L. WARING, AND LAWRENCE H. BOPP

Laboratories for Bacteriology, Wadsworth Center for Laboratories and Research, New York State Department of Health, Box 509, Albany, New York 12201-0509

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***Haemophilus ducreyi* ATCC 33922, a virulent, well-characterized strain, was used to construct a genomic library in a bacteriophage expression vector. Three DNA fragments were selected for use as probes on the basis of their ability to encode *H. ducreyi*-specific proteins, as demonstrated by reactivity with rabbit polyclonal antiserum. With DNA-DNA hybridization, the three probes, labeled with ^{32}P, reacted strongly with 16 strains of *H. ducreyi* obtained from a variety of sources. Thirty-seven other bacterial isolates, representing 33 different species and including organisms likely to be encountered in the urogenital tract, were also tested with the three probes. Twenty-eight of these isolates, including the genital pathogen *Neisseria gonorrhoeae*, showed no hybridization with the probes. In addition, herpes simplex virus-infected tissue culture cells and *Treponema pallidum*-infected rabbit testicular fluid were also completely nonreactive. Nine isolates, six belonging to other *Haemophilus* species and three belonging to *Pasteurella* species, reacted weakly with the probes when approximately 3.0×10^7 to 6.0×10^7 CFU was tested. When 10^5 to 10^6 CFU of these organisms was tested, the weak reactions could no longer be seen. Yet this number of *H. ducreyi* still reacted strongly. In fact, the three probes consistently detected 10^4 CFU of *H. ducreyi* in pure and mixed cultures and even produced a weak signal when only 10^3 CFU was present. It is clear from our results that use of these probes will greatly facilitate the laboratory diagnosis of this genital pathogen.**

Chancroid is a human sexually transmitted disease caused by *Haemophilus ducreyi* and characterized by painful genital ulcers. The primary ulcer is often followed by multiple lesions which result in ulcers of various ages that can extend the duration of the illness to 1 to 3 months if appropriate treatment is not given (37). More than 50% of the cases progress to inguinal lymph node involvement usually manifested by buboes (37). Without treatment, the bubo can persist for many months. Infections with other pathogens such as *Treponema pallidum*, herpes simplex virus type 2, *Chlamydia trachomatis* (lymphogranuloma venereum), and *Calymmatobacterium granulomatis* (granuloma inguinale) can result in ulcerations clinically indistinguishable from those caused by *H. ducreyi*. Thus, accurate diagnosis of the etiologic agent is very important, since antimicrobial treatment for these different sexually transmitted diseases must be specific.

Open chancroidal lesions may enhance transmission of human immunodeficiency virus. Recent studies in Africa have established a significant association between history of genital ulceration and human immunodeficiency virus seropositivity (24, 30, 35). Since chancroid has been reported to be the most common cause of genital ulcers in Africa (12), this disease has been correlated specifically with heterosexual transmission of human immunodeficiency virus (35). These studies, together with the clinical observation that untreated ulcers can persist for months, highlight the importance of an accurate diagnosis which will lead to appropriate therapy.

H. ducreyi is a fastidious, slow-growing gram-negative bacillus. Colonies grown on solid media vary in size, perhaps because of the peculiar cohesiveness of the organism, with larger colonies arising from more than one cell. Moreover, *H. ducreyi* remains tightly autoagglutinated when suspended in liquid. This cohesiveness has ruled out identification of the organism by serological agglutination tests, and difficulties in interpretation of fluorescent-antibody tests (due to bacterial clumping) have also been reported with both polyclonal (14) and monoclonal antibodies (33).

To maintain viability of the bacteria, *H. ducreyi* must be cultured within 4 to 6 h after removal from the patient. Even when inoculated immediately, the organism does not grow well on most laboratory media, and recovery rates from clinical specimens have traditionally been poor. In 1978, Hammond et al. (19) reported the development of a selective enrichment agar. Use of this medium or adaptations of it (36) have led to significantly higher recovery rates of *H. ducreyi* in recent years. However, isolation rates of only 60 to 70% are still the norm for patients with clinically diagnosed chancroid (26).

During the past decade, outbreaks of chancroid have been reported in both Canada and the United States (3, 4, 8, 9, 20, 21, 27). In New York City, outbreaks have been reported yearly beginning in 1981 (10), and the number of cases has been increasing each year since then. In 1987, over 62% (3,116 of 4,998) of the chancroid cases reported in the United States were from New York City (11).

In summary, the increasing incidence of chancroid is a matter of serious concern, not only because of the problems of chancroidal disease itself, but also because this disease has been associated with heterosexual transmission of human immunodeficiency virus in Africa. Difficulties in clinical and laboratory diagnoses can interfere with an accurate identification of chancroid or its etiologic agent. In the present report, we describe the development and testing of DNA probes specific for *H. ducreyi*. This is the first published report which describes the utilization of DNA hybridization for the identification of this genital pathogen.

MATERIALS AND METHODS

Construction of an *H. ducreyi* genomic library in lambda gt11. Chromosomal DNA was isolated from five different strains of *H. ducreyi* (ATCC 27721, 27722, 33921, 33922, and

* Corresponding author.

type strain 33940) by the procedure of Brenner et al. (5). The purity, concentrations, T_m values, and moles percent G+C contents of the DNA samples were determined by spectrophotometry and thermal denaturation with a Gilford 2400 spectrophotometer and a 2527 Thermoprogrammer (CIBA-Corning, Gilford Systems, Oberlin, Ohio).

DNA from ATCC 33922 was selected for library construction and was digested with the restriction endonuclease *Eco*RI (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The *Eco*RI fragments were fractionated on a Bio-Gel A-50m column (Bio-Rad Laboratories, Richmond, Calif.), as described by Huynh et al. (22). Fractions containing *Eco*RI fragments of approximately 7 kilobases (kb) and smaller were pooled and then ligated to dephosphorylated lambda gt11 *Eco*RI arms (Promega Biotec, Madison, Wis.). The recombinant phage were packaged by using the Packagene in vitro packaging system (Promega Biotec) and adsorbed to *Escherichia coli* Y1090.

Screening of the *H. ducreyi* genomic library. Polyclonal antisera against Formalin-killed *H. ducreyi* ATCC 33922 were produced during 6 months of biweekly injections in rabbits. With fluorescent-antibody testing, the highest titer obtained was 1:2,048. This high-titer antiserum was used for library screening following absorption with an *E. coli* lysate. Recombinant phage were screened for the production of *H. ducreyi*-specific antigens by using the Express-Blot Assay Kit (Bio-Rad Laboratories) according to the directions of the manufacturer. Positive plaques were purified and then retested in the Express-Blot assay before proceeding.

Subcloning *H. ducreyi* DNA inserts into a plasmid vector. Purified plaques of antibody-reactive phage were grown on agarose plates to near-confluent lysis, and the phage were allowed to diffuse into an overlay of L broth. This phage lysate was then used for DNA isolation as previously described (34). The purified DNA was digested with *Eco*RI, and the sizes of the *H. ducreyi* inserts were determined by agarose gel electrophoresis. DNA was transferred from the gels to nitrocellulose and hybridized with ^{32}P-labeled *H. ducreyi* ATCC 33922 chromosomal DNA at 60 to 65°C as described by Maniatis et al. (25). Filters were washed under stringent conditions (0.1× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate] and 0.1% sodium dodecyl sulfate at 65 to 70°C), and bound probe was then visualized by autoradiography. Three *Eco*RI fragments which hybridized with the probe were subcloned into dephosphorylated *Eco*RI-digested pUC13 and transferred to *E. coli* JM107 by transformation. Large-scale plasmid preparations were made from *E. coli* clones containing recombinant plasmids. The plasmids were then subjected to two successive purifications by ultracentrifugation on CsCl gradients.

Testing bacterial suspensions and purified DNA with the probes. The bacteria used for the hybridization tests are listed in Table 1. *H. ducreyi* isolates were grown on Casman agar with 5% rabbit blood or on Mueller-Hinton agar with 5% chocolatized horse blood, 5% fetal bovine serum, 1% IsoVitaleX, and 3 μg of vancomycin per ml (19, 36). *H. ducreyi* has a strong tendency to autoagglutinate. Therefore, when it was necessary to determine the number of CFU present, bacterial suspensions were grown for 24 to 48 h in hemin broth (1), and large clumps were allowed to settle out before use. Despite these precautions, some CFU may still have originated from more than one cell. This procedure was selected because, in our laboratory, sonication or excessive vortexing of the suspensions resulted in loss of viability of *H. ducreyi*.

For testing with the probes, bacterial suspensions were blotted onto nitrocellulose in the Minifold II Slot-Blotter (Schleicher & Schuell, Keene, N.H.) by using a previously described procedure (13). In some instances, the bacterial colonies were transferred directly to nitrocellulose from the agar plates. The bacteria which adhered to the filters during either procedure were lysed and the DNA was denatured as described by Maniatis et al. (25). Other samples, which included purified DNA obtained from the five ATCC strains of *H. ducreyi*, herpes simplex virus type 2-infected human embryonic lung cells, and *Treponema pallidum*-infected rabbit testicular fluid, were also applied to nitrocellulose, and the DNA was denatured as described above for bacterial suspensions.

Either whole plasmids containing the *H. ducreyi* DNA inserts or the inserts alone, which had been removed from the pUC13 plasmid vector by digestion with *Eco*RI and then recovered from an agarose gel by using the Gene Clean procedure (Bio 101, Inc., La Jolla, Calif.), were used as probes after being labeled with ^{32}P. Hybridization, washing, and autoradiography were performed as described above.

Lesion material from rabbits. The shaved backs of rabbits were injected intradermally with 10^7 CFU of *H. ducreyi*. The injection procedure was performed and lesion development was interpreted as previously described (15, 16, 18, 28). At 4 to 5 days following the injections, samples of exudate were removed from raised lesions and suspended in saline. A portion of each suspension was plated on Casman rabbit blood agar to recover viable organisms and applied to nitrocellulose membranes by using the slot-blot apparatus for testing with the probes.

RESULTS

***H. ducreyi* probes.** Three DNA inserts coding for proteins which were recognized by the *H. ducreyi* antiserum were selected for subcloning into a pUC13 plasmid vector (2.7 kb). Either the whole recombinant plasmids (pLP1, 1.5-kb insert; pLP4, 5.4-kb insert; and pLP8, 5.7-kb insert) or the gel-purified inserts were used as *H. ducreyi*-specific probes (Table 1 and 2; Fig. 1 and 2). Because fragment 8, the gel-purified insert from pLP8, did not hybridize to the gel-purified vector from pLP8 (pUC13), and vice versa, the inserts were considered free of residual vector DNA.

Specificity and sensitivity of the probes. Table 1 lists the organisms tested and the results obtained using the three probes. All strains of *H. ducreyi* reacted strongly with the probes (Fig. 1), as did the purified DNA from all five ATCC strains. Nine recent isolates of *H. ducreyi* cultured from patients in 1988 by the New York City Department of Health were tested in the slot-blot apparatus and also by blotting colonies directly from plates onto nitrocellulose. All were found to be positive in pure and mixed cultures (Table 1; Fig. 2). The other significant genital pathogens, *Neisseria gonorrhoeae*, herpes simplex virus type 2, and *T. pallidum*, were all completely nonreactive with the probes.

Weak cross-reactivity was seen with the other *Haemophilus* species and with three of the four *Pasteurella* species when 3.0×10^7 to 6×10^7 CFU was tested. Figure 1 illustrates the intensity of the homologous reactions versus the heterologous reactions. When suspensions of the cross-reacting organisms were diluted and probed, the weak reactions were no longer seen with 10^5 to 10^6 CFU or less, while this same number of *H. ducreyi* still reacted strongly.

The three probes consistently detected 10^4 CFU of *H. ducreyi* in pure or mixed culture and produced a weak signal when 10^3 CFU was present (Table 2).

TABLE 1. Use of pLP1, pLP4, and pLP8 as specific probes for *H. ducreyi*

Bacterial suspension	Results with probe[a]:		
	pLP1	pLP4	pLP8
Haemophilus ducreyi[b]			
ATCC 27721	++++	++++	++++
ATCC 27722	++++	++++	++++
ATCC 33921	++++	++++	++++
ATCC 33922	++++	++++	++++
ATCC 33940	+++	++++	+++
CDC 542	++++	++++	++++
CDC 844	++++	++++	++++
Nine clinical isolates from New York City, 1988	++++[c]	++++[c]	++++
Other organisms[d]			
Acinetobacter calcoaceticus subsp. *lwoffii* B277	–	–	–
Actinobacillus actinomycetemcomitans B1083	ND	ND	–
Actinomyces species strain B1228	–	–	–
Alcaligenes faecalis B38-78	–	–	–
Bacteroides fragilis ATCC 25285	–	–	–
Clostridium perfringens ATCC 13124	–	–	–
Corynebacterium diphtheriae C5703	–	–	–
Escherichia coli ATCC 25922	–	–	–
Gardnerella vaginalis ATCC 14018	–	–	–
G. vaginalis B1905 (atypical)	–	–	–
Haemophilus haemoglobinophilus B1701	ND	ND	Weak
H. influenzae, type A	Weak	Weak	Weak
H. influenzae, type B	Weak	Weak	Weak
H. influenzae, nontypeable, biotype III	ND	ND	Weak
H. influenzae, nontypeable, biotype V	ND	ND	Weak
H. parainfluenzae B1134	Weak	Weak	Weak
Lactobacillus acidophilus ATCC 4962	–	–	–
Moraxella osloensis B1596	–	–	–
Neisseria gonorrhoeae 116	–	–	–
N. lactamica B2159	–	–	–
N. meningitidis W135	–	–	–
Pasteurella haemolytica M6169	ND	ND	–
P. gallinarum B697	ND	ND	Weak
P. multocida B1221-76	ND	ND	Weak
P. pneumotropica M5354	ND	ND	Weak
Peptostreptococcus anaerobius A943	–	–	–
Proteus mirabilis	–	–	–
Pseudomonas aeruginosa ATCC 27853	–	–	–
Staphylococcus aureus ATCC 25923	–	–	–
Staphylococcus coagulase-negative strain B778	–	–	–
Streptococcus agalactiae B2545	–	–	–
S. bovis B1450	–	–	–
S. faecalis ATCC 29212	–	–	–
S. mutans B1254	–	–	–
S. sanguis II B1508	–	–	–
Yersinia enterocolitica ATCC 9610	ND	ND	–
Y. rohdei CDC 3022	ND	ND	–
Other samples			
DNA from all five *H. ducreyi* ATCC strains	++++	++++	++++
Herpes simplex virus type 2-infected cells	ND	ND	–
Treponema pallidum in rabbit testicular fluid	ND	ND	–

[a] The intensities of the bands (following 24 to 48 h of exposure to X-ray film) were compared and degrees of intensity were determined visually. Positive reactions ranging from 3+ to 4+ and weak reactions (very slight shadows, determined to be less than 1+) are seen in Fig. 1. –, No hybridization; ND, not determined.

[b] Approximately 1.6×10^6 CFU per well.

[c] Two of the nine clinical isolates from New York City were tested.

[d] Approximately 3×10^7 to 6×10^7 CFU per well. These isolates were obtained from the lyophilized collection of the Laboratories for Bacteriology, New York State Department of Health.

Probing lesion material from rabbits. Four strains of *H. ducreyi* (ATCC 33922; ATCC 33940; CDC 542; and a recent clinical isolate from New York City, ME 38) were used to inject rabbits intradermally. All four strains produced necrotic lesions. Within 2.5 to 3 weeks, the lesions had healed. From the exudates obtained from the lesions 4 to 5 days following injection of 10^7 organisms, only CDC 542 could be recovered by culture. However, by using fragment 8 as a probe, *H. ducreyi* DNA could be detected as a weak to 1+ signal in exudate from lesions produced by all four strains. The hybridization with the *H. ducreyi* probe was specific,

TABLE 2. Sensitivity of probes specific for *H. ducreyi*

No. of CFU of *H. ducreyi*/well	Results with probe[a]:	
	pLP8	Fragment 1, 4, or 8
Pure cultures[b]		
4.9×10^6	4+	ND
4.9×10^5	3+	ND
4.9×10^4	1–2+	ND
4.9×10^3	Weak	ND
1.4×10^7	ND	2–3+
1.4×10^6	ND	2–3+
1.4×10^5	ND	1–2+
1.4×10^4	ND	Weak–1+
1.4×10^3	ND	Negative
Mixed cultures[c]		
4.7×10^6	ND	3+
4.7×10^5	ND	2+
4.7×10^4	ND	Weak–1+
4.7×10^3	ND	Negative–weak
4.7×10^2	ND	Negative–weak

[a] The intensities of the bands (following 24 to 48 h of exposure to X-ray film) were compared and degrees of intensity were determined visually. ND, Not determined.

[b] ATCC 33922 was used.

[c] Similar results from two experiments were combined. In the first experiment, the mixed culture consisted of 74% *H. ducreyi* ATCC 33922, 21% *H. influenzae* B, and 5% *E. coli*; the second mixture consisted of 96% *H. ducreyi*, 4% *Proteus mirabilis*, and 0.1% *Staphylococcus epidermidis*.

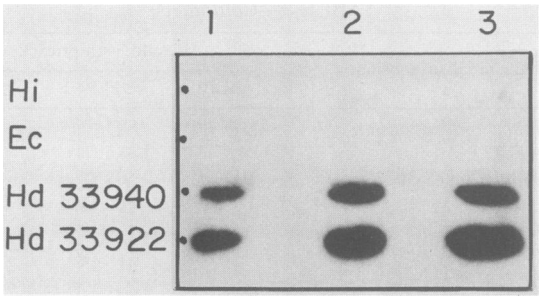

FIG. 1. Specificity of fragment 8 (5.7 kb) as a probe. The CFU per well for *H. influenzae* (Hi) and *E. coli* (Ec) were 1.2×10^7 (lane 1), 6.0×10^7 (lane 2), and 1.2×10^8 (lane 3). The CFU per well for *H. ducreyi* (Hd) were 3.2×10^5 (lane 1), 1.6×10^6 (lane 2), and 3.2×10^6 (lane 3).

since purified pUC13 used as a probe did not hybridize nonspecifically to the rabbit lesion samples.

DISCUSSION

In analyzing DNA from the *H. ducreyi* ATCC strains used in this study, we obtained T_m values (85.1 to 85.6°C) and G+C content values (37.9 to 39.0 mol%) which agreed with those previously reported. Kilian (23) had determined the average G+C content for two strains of *H. ducreyi* to be 37.8 mol%, and Piechulla et al. (29) had found average T_m and G+C content values of 85.1°C and 37.8 mol%, respectively, for two *H. ducreyi* ATCC strains.

The DNA probes we have constructed from ATCC 33922 are relatively specific for *H. ducreyi*. They react strongly with the DNA from the other ATCC strains and with all 16 *H. ducreyi* isolates tested. When 37 other bacterial isolates, including organisms likely to be encountered in the urogenital tract, were tested with the probes, 28 were completely negative and 9 (*Haemophilus haemolyticus*; *H. influenzae*, types A and B; *H. influenzae*, nontypeable, biotypes III and V; *Haemophilus parainfluenzae*; *Pasteurella gallinarum*; *Pasteurella multocida*; and *Pasteurella pneumotropica*) reacted weakly, even though a large number of organisms (10^7 CFU) was used. No reactions were seen with these organisms when 10^5 to 10^6 CFU was tested. In contrast, this number of *H. ducreyi* still reacted strongly with the probes.

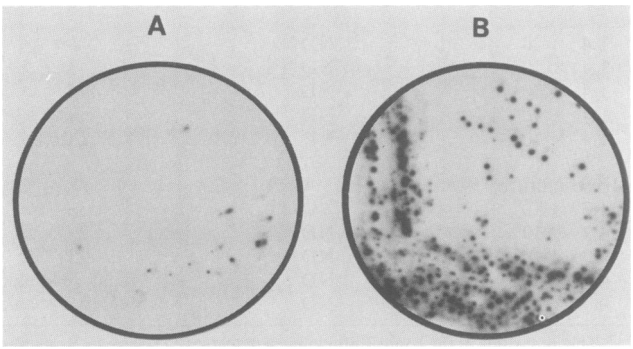

FIG. 2. Colony hybridization with pLP8 as probe. Bacterial colonies were transferred directly to nitrocellulose from agarose plates either as a mixed culture of *H. ducreyi* and other flora from a genital ulcer (ME 45) (A) or as a pure culture of *H. ducreyi* (ME 38) (B). Both strains were recovered from patients in New York City in 1988.

In testing bacterial suspensions and purified DNA with the probes, we have consistently seen strong positive reactions (Fig. 1 and 2) only with *H. ducreyi*. Moreover, these reactions have been seen with all strains of *H. ducreyi* tested.

The presence of areas of homology between the weakly reactive organisms and *H. ducreyi* is not surprising, since antigenic cross-reactivity between *H. ducreyi* and other *Haemophilus* species has been reported by others (32, 33) and has been seen in fluorescent-antibody tests performed in our own laboratory (Dianna Schoonmaker, personal communication). Also, the G+C contents of all members of the genus *Haemophilus* are similar (23). (However, DNA-DNA hybridization has shown that *H. ducreyi* is only slightly similar to all other *Haemophilus* species [0 to 6% related] [2, 7], and quinones extracted from bacterial membranes have been found to differ significantly between *H. ducreyi* and other *Haemophilus* species [6].) *Pasteurella* species may also share small areas of DNA relatedness with the cloned *H. ducreyi* sequences, since both *Pasteurella* and *Haemophilus* species are members of the family *Pasteurellaceae*.

By using any one of the three DNA probes, we were able to consistently detect 10^4 CFU of *H. ducreyi* in pure and mixed cultures. A weak signal was usually seen even when only 10^3 CFU was present. These results demonstrate both the sensitivity and specificity of the probes for *H. ducreyi*, as only this organism could be detected at these dilutions.

The probes were also able to detect four different strains of *H. ducreyi* in rabbit lesion exudate, while only one could be recovered by culture. *H. ducreyi* lesions do not ulcerate in rabbits but instead become dry and indurated and heal fairly quickly (16). Investigators have reported the recovery of *H. ducreyi* from rabbit lesions only at 24, 48 and 72 h postinfection (16, 18). These observations suggest that the organisms do not survive long in this particular host or tissue. In the present study, three of the four samples were obtained when the organisms were no longer viable, but sufficient DNA was still present so that detectable hybridization could occur.

Nucleic-acid hybridization using radiolabeling has previously been reported to be sensitive enough to detect homologous sequences in about 10^2 to 10^6 bacteria (17). Our ability to detect *H. ducreyi* is well within this range. However, increased sensitivity may be necessary in order to detect the low numbers of organisms which might be present in an original clinical specimen. The DNA probes which we have developed are potentially useful for this purpose. Detection of a small amount of *H. ducreyi* DNA can be achieved by amplification either of total DNA or of only the target sequence homologous to the probe. The most direct approach would involve amplification of the total DNA by growth of the organism. By this method, as few as 10 organisms could be amplified to a detectable level in 10 doubling times. However, if the organisms were no longer viable, the polymerase chain reaction, with specific primers and a thermostable DNA polymerase (31), could be used. Work is in progress in our laboratory to determine a method for successful amplification of *H. ducreyi* DNA in clinical specimens before testing with the probes.

In the meantime, the use of these specific DNA probes in laboratory diagnostic tests will ensure more rapid and accurate identification of *H. ducreyi*.

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LITERATURE CITED

1. **Abeck, D., A. P. Johnson, R. C. Ballard, Y. Dangor, E. A. Fontaine, and D. Taylor-Robinson.** 1987. Effect of cultural conditions on the protein and lipopolysaccharide profiles of *Haemophilus ducreyi* analysed by SDS-PAGE. FEMS Microbiol. Lett. **48**:397–399.
2. **Albritton, W. L., J. K. Setlow, M. Thomas, F. Sottnek, and A. G. Steigerwalt.** 1984. Heterospecific transformation in the genus *Haemophilus*. Mol. Gen. Genet. **193**:358–363.
3. **Becker, T. M., W. DeWitt, and G. VanDusen.** 1987. *Haemophilus ducreyi* infection in South Florida: a rare disease on the rise? South. Med. J. **80**:182–184.
4. **Blackmore, C. A., K. Limpakarnjanarat, J. G. Rigau-Perez, W. L. Albritton, and J. R. Greenwood.** 1985. An outbreak of chancroid in Orange County, California: descriptive epidemiology and disease-control measures. J. Infect. Dis. **151**:840–844.
5. **Brenner, D. J., A. C. McWhorter, J. K. L. Knutson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. **15**:1133–1140.
6. **Carlone, G. M., W. O. Schalla, C. W. Moss, D. L. Ashley, D. M. Fast, J. S. Holler, and B. D. Plikaytis.** 1988. *Haemophilus ducreyi* isoprenoid quinone content and structure determination. Int. J. Syst. Bacteriol. **38**:249–253.
7. **Casin, I., F. Grimont, P. A. D. Grimont, and M. Sanson-Le Pors.** 1985. Lack of deoxyribonucleic acid relatedness between *Haemophilus ducreyi* and other *Haemophilus* species. Int. J. Syst. Bacteriol. **35**:23–25.
8. **Centers for Disease Control.** 1982. Chancroid—California. Morbid. Mortal. Weekly Rep. **31**:173–175.
9. **Centers for Disease Control.** 1985. Chancroid—Massachusetts. Morbid. Mortal. Weekly Rep. **34**:711–718.
10. **Centers for Disease Control.** 1985. Sexually transmitted disease statistics. Issue no. 135. Centers for Disease Control, Atlanta.
11. **Centers for Disease Control.** 1987. Sexually transmitted disease statistics. Issue no. 136. Centers for Disease Control, Atlanta.
12. **Dangor, Y., S. D. Miller, F. da la Exposto, and H. J. Koornhof.** 1988. Antimicrobial susceptibilities of southern African isolates of *Haemophilus ducreyi*. Antimicrob. Agents Chemother. **32**: 1458–1460.
13. **Davis, L. G., M. D. Dibner, and J. F. Battey.** 1986. Basic methods in molecular biology. Elsevier Science Publishing, Inc. New York.
14. **Denys, G. A., T. A. Chapel, and C. D. Jeffries.** 1978. An indirect fluorescent antibody technique for *Haemophilus ducreyi*. Health Lab. Sci. **15**:128–132.
15. **Dienst, R. B.** 1948. Virulence and antigenicity of *Haemophilus ducreyi*. Am. J. Syph. Gonorrhea Vener. Dis. **32**:289–291.
16. **Feiner, R. R., and F. Mortara.** 1945. Infectivity of *Haemophilus ducreyi* for the rabbit and the development of skin hypersensitivity. Am. J. Syph. Gonorrhea Vener. Dis. **29**:71–79.
17. **Fung-Tomc, J. C., and R. C. Tilton.** 1988. Application of nucleic acid hybridization in clinical bacteriology, p. 238–244. *In* K. Wicher (ed.), Microbial antigenodiagnosis, vol. 1. CRC Press, Inc., Boca Raton, Fla.
18. **Hammond, G. W., C. J. Lian, J. C. Wilt, and A. R. Ronald.** 1978. Antimicrobial susceptibility of *Haemophilus ducreyi*. Antimicrob. Agents Chemother. **13**:608–612.
19. **Hammond, G. W., C. J. Lian, J. C. Wilt, and A. R. Ronald.** 1978. Comparison of specimen collection and laboratory techniques for isolation of *Haemophilus ducreyi*. J. Clin. Microbiol. **7**:39–43.
20. **Hammond, G. W., M. Slutchuk, J. Scatliff, E. Sherman, J. C. Wilt, and A. R. Ronald.** 1980. Epidemiologic, clinical, laboratory, and therapeutic features of an urban outbreak of chancroid in North America. Rev. Infect. Dis. **2**:867–879.
21. **Health and Welfare, Canada.** 1988. Chancroid outbreak—Winnipeg, Manitoba. Can. Dis. Weekly Rep. **14**:13–15.
22. **Huynh, T. V., R. A. Young, and R. W. Davis.** 1985. Constructing and screening cDNA libraries in lambda gt10 and lambda gt11, p. 49–77. *In* D. M. Glover (ed.), DNA cloning: a practical approach, vol. 1. IRL Press, Washington, D.C.
23. **Kilian, M.** 1976. A taxonomic study of the genus *Haemophilus* with the proposal of a new species. J. Gen. Microbiol. **93**:9–62.
24. **Kreiss, J. K., D. Koech, F. A. Plummer, K. K. Holmes, M. Lightfoote, P. Piot, A. R. Ronald, J. O. Ndinya-Achola, L. J. D'Costa, P. Roberts, E. N. Ngugi, and T. C. Quinn.** 1986. AIDS virus infection in Nairobi prostitutes: spread of the epidemic to East Africa. N. Engl. J. Med. **314**:414–418.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Museyi, K., E. Van Dyck, T. Vervoort, D. Taylor, C. Hoge, and P. Piot.** 1988. Use of an enzyme immunoassay to detect serum IgG antibodies to *Haemophilus ducreyi*. J. Infect. Dis. **157**: 1039–1043.
27. **New York City Department of Health.** 1987. Treatment of chancroid with ceftriaxone: New York City. City Health Information **6**:1–2.
28. **Odumeru, J. A., G. M. Wiseman, and A. R. Ronald.** 1984. Virulence factors of *Haemophilus ducreyi*. Infect. Immun. **43**:607–611.
29. **Piechulla, K., R. Mutters, S. Burbach, R. Klussmeier, S. Pohl, and W. Mannheim.** 1986. Deoxyribonucleic acid relationships of "*Histophilus ovis*/*Haemophilus sommus*," *Haemophilus haemoglobinophilus*, and "*Actinobacillus seminis*." Int. J. Syst. Bacteriol. **36**:1–7.
30. **Quinn, T. C., J. M. Mann, J. W. Curran, and P. Piot.** 1986. AIDS in Africa: an epidemiologic paradigm. Science **234**:955–963.
31. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
32. **Saunders, J. M., and J. D. Folds.** 1986. Immunoblot analysis of antigens associated with *Haemophilus ducreyi* using serum from immunized rabbits. Genitourin. Med. **62**:321–328.
33. **Schalla, W. O., L. L. Sanders, G. P. Schmid, M. R. Tam, and S. A. Morse.** 1986. Use of dot-immunobinding and immunofluorescence assays to investigate clinically suspected cases of chancroid. J. Infect. Dis. **153**:879–887.
34. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Simonsen, J. N., D. W. Cameron, M. N. Gakinya, J. O. Ndinya-Achola, L. J. D'Costa, P. Karasira, M. Cheang, A. R. Ronald, P. Piot, and F. A. Plummer.** 1988. Human immunodeficiency virus infection among men with sexually transmitted diseases: experience from a center in Africa. N. Engl. J. Med. **319**:274–278.
36. **Sottnek, F. O., J. W. Biddle, S. J. Kraus, R. E. Weaver, and J. A. Stewart.** 1980. Isolation and identification of *Haemophilus ducreyi* in a clinical study. J. Clin. Microbiol. **12**:170–174.
37. **Stüttgen, G.** 1981. Ulcus molle: chancroid. Grosse Verlag, Berlin.