

The laboratory diagnosis of *Haemophilus ducreyi*

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Chancroid is a sexually transmitted infection caused by *Haemophilus ducreyi*. This fastidious, Gram-negative coccobacilli dies rapidly outside the human host, making diagnostic testing using culture methods difficult. This genital ulcer infection is not common in Canada and, therefore, can often be misdiagnosed. The objective of the present paper is to provide practical approaches for the diagnosis of chancroid in Canadian patients where the prevalence of this infection is low. Issues related to sample collection, sample transport and available diagnostic tests are reviewed, and several alternative approaches are outlined. Although antigen detection, serology and genetic amplification methods have all been reported for *H ducreyi*, none are commercially available. Culture is still the primary method available to most laboratories. However, the special media necessary for direct bedside inoculation is often not available; therefore, communication with the diagnostic laboratory and rapid specimen transport are essential when chancroid is suspected.

Key Words: Chancroid; Culture methods; Diagnostic; Genital ulcer disease; *Haemophilus ducreyi*; STI

Haemophilus ducreyi is the causative agent of the sexually transmitted infection known as chancroid. On a global basis, chancroid is thought to be the most common cause of genital ulcer disease (GUD) (1-7). Other causes of GUD include *Treponema pallidum*, *Chlamydia trachomatis* serovars L1, L2 and L3, *Calymatobacterium granulomatis* and herpes simplex virus. Although chancroid occurs commonly in parts of Africa, Asia and Latin America (accounting for 20% to 60% of GUD infections), it is found only sporadically in North America, although outbreaks linked to the sale of sex for crack cocaine have been reported (1,7). The basis for this differing geographic distribution is unknown. In geographic locations where chancroid is endemic, the overlap in clinical symptoms among the most common GUDs (eg, syphilis, herpes, chancroid) makes diagnosis based on clinical symptoms unreliable. As reviewed by Lewis (8), the accuracy of clinical diagnosis for chancroid ranges from 30% to 80%, and coinfection of GUDs (most commonly syphilis and *H ducreyi*) has been reported in 10 of 81 individuals presenting with GUD (1,7). Furthermore, infection with *H ducreyi* increases the

Le diagnostic en laboratoire de l'*Haemophilus ducreyi*

Le chancre mou est une infection transmise sexuellement causée par l'*Haemophilus ducreyi*. Ce coccobacille gram négatif exigeant meurt rapidement à l'extérieur de l'hôte humain, ce qui complique les épreuves diagnostiques à l'aide de méthodes de culture. Cette infection à ulcères génitaux n'est pas courante au Canada. Elle peut donc souvent être mal diagnostiquée. Le présent article vise à fournir des démarches pratiques de diagnostic chez les patients canadiens, où la prévalence de l'infection est faible. Les enjeux reliés au prélèvement et au transport des échantillons ainsi qu'à l'accès à des épreuves diagnostiques sont examinés, et plusieurs possibilités sont soulignées. Bien que les méthodes de détection des antigènes, de sérologie et par amplification génétique aient toutes été déclarées en cas d'*H ducreyi*, aucune n'est commercialisée. La culture demeure la principale méthode disponible pour la plupart des laboratoires. Toutefois, dans de nombreux cas, le milieu spécial nécessaire pour procéder à une inoculation directe de chevet n'est pas disponible. Par conséquent, il est essentiel de communiquer avec le laboratoire diagnostique et de transporter rapidement l'échantillon en cas de présomption de chancre mou.

likelihood of acquiring and transmitting HIV. Coinfection is important to recognize because the response to antibiotic therapy for chancroid in patients with AIDS is less effective compared with therapy in patients without AIDS (1,5,7). The major problem associated with this infection (when it presents in patients in North America) is whether the physician recognizes that an infection may be chancroid and, as a result, requests the appropriate diagnostic tests (eg, low index of suspicion in nonendemic areas). The high likelihood of misdiagnosis of sexually transmitted infections presenting as genital ulcers in both endemic and nonendemic countries makes diagnostic testing for GUD critical (3,6-8). Genetic amplification methods, although recognized as more sensitive than culture for *H ducreyi*, are not commercially available. Hence, culture remains the primary diagnostic test performed by most microbiology laboratories for suspected cases of chancroid.

Given these constraints, the current paper is aimed at providing practical approaches for the diagnosis of chancroid in Canadian patients where the prevalence of this infection is low. When investigating GUD that is believed to be due to

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TABLE 1
Specimen collection and optimal diagnostic tests for suspect chancroid in Canadian health care facilities

Specimen	Optimal diagnostic test approach	Alternative test approach	Comments
Ulcer swab: Cleanse ulcer, swab base of lesion, take four swab samples. Dacron, cotton or calcium alginate swabs are acceptable for specimen collection	Swab 1 – Culture by bedside inoculation: • GC-HgS agar • MH-HB agar Swab 2 – PCR: • place dry swab in sterile tube, keep at 4°C during transit. If delay is long, freeze at –70°C until ready to send to a reference laboratory Swab 3 – place in viral transport media for herpes culture Swab 4 – make two slides; one for Gram stain, one for DFA for syphilis	Swab 1 – Culture: • place swab in Amies transport media and transport to laboratory in less than 4 h* Swab 2 – PCR: • place dry swab in sterile tube, keep at 4°C during transit • If delay is long, freeze at –70°C until ready to send to reference laboratory Swab 3 – place in viral transport media for herpes culture Swab 4 – • make 2 slides: 1 for Gram stain, one for DFA for syphilis	*Ensure laboratory has been notified to make media if sending swabs in transport media for culture
Bubo aspirate: Aspirate above bubo through intact skin. Note: If bubo is draining, use swab to collect material and process as per ulcer swabs	Bedside culture: • GC-HgS agar • MH-HB agar PCR: Place aspirate in sterile container, keep at 4°C during transit. If delay is long, freeze at –70°C until ready to send to reference laboratory	Place aspirate in sterile container and transport to laboratory in less than 4 h for culture PCR can be performed using same aspirate material	Culture from intact bubos is often negative; ulcer swabs are better for culture
Serum: Collect 10 mL blood in red stopper tube. Send to reference laboratory	Serology: • syphilis		

DFA Direct immunofluorescence assay; PCR Polymerase chain reaction

H ducreyi, include herpes simplex culture and syphilis testing (direct fluorescence antibody [DFA] or serum for rapid plasma reagin testing).

SPECIMEN CHOICE, COLLECTION AND TRANSPORT

Genital lesions (ulcer, bubo)

For suspect cases of chancroid, testing for herpes simplex virus and syphilis should also be performed (Table 1). Chancroid produces genital ulcerative lesions (soft ulcers that are painful) and may lead to bubo formation (swelling of inguinal lymph nodes) but it is not known to spread systemically. As such, diagnostic samples for organism or antigen detection include ulcer lesion material and/or bubo aspirates. Culture of the organism from the ulcerative genital lesions remains the 'gold standard' for the diagnosis of chancroid. However, even using the optimal combination of media, it is only about 80% sensitive. The specimen of choice for the diagnosis of chancroid is a swab that has been taken from the base of the genital ulcer. This is best collected by cleansing the area by flushing with sterile physiological saline, and then collecting material from the base of the ulcer using a calcium alginate, Dacron or cotton swab (no special swab type has been identified to be optimal). Although no suitable transport medium has been developed, *H ducreyi* can survive for 2 h to 4 h on swabs (5), so an

alternative method (though less desirable) is to take the swab sample and place it in transport media such as Amies. The sample is then sent as quickly as possible to the laboratory (8). For bubos, a needle and syringe should be used to aspirate pustular material from the bubo though normal tissue (eg, insert needle above bubo through normal tissue – this will prevent chronic leakage of the fluid and reduces contamination). Culture from intact bubos has even lower detection rates compared with culture of the ulcer base or culture from ruptured bubos.

Ideally for culture, the ulcer specimen should be inoculated at the bedside onto two media that are optimal for growth of *H ducreyi* (see section on culture methods below). For samples to be used for amplification testing, the ulcer swab specimen can be shipped dry in a sterile tube. If transit is prolonged, the polymerase chain reaction (PCR) sample should be frozen at –70°C. This is a valuable alternative if culture media is not available at the time the patient is seen (Table 1). For culture isolation of *H ducreyi*, ulcer material is optimal, followed by recently ruptured bubo exudate, while exudate from an intact bubo is least sensitive.

Serum

Currently, the reference laboratories do not have any good serological tests for acute diagnosis of chancroid (8). Blood that is collected into a red stopper tube can be used for syphilis serology.

DIAGNOSTIC TESTS

Microscopy

Although microscopy is useful if there is a high load of organisms present that show the characteristic Gram-negative coccobacilli in railroad or chaining arrangement, microscopy is of limited value because of low sensitivity (5% to 63%) and specificity (51% to 99%) (8).

Antigen detection

Direct immunofluorescent testing of ulcer material using an *H ducreyi*-specific monoclonal antibody appears to be useful (8,9). Hansen et al (10) developed an antigen detection assay to detect *H ducreyi* lipooligosaccharide (LOS) using an LOS-specific monoclonal antibody and an adaptation of the limulus amoebocyte assay. The sensitivity and specificity of these antigen detection methods are 89% to 100%, and 63% to 81%, respectively (8). However, the reagents for these two antigen detection methods are not commercially available. Ulcer specimens for DFA may be collected, air dried and fixed until a reference laboratory can be identified to perform DFA.

Culture

Culture remains the one test method available to most laboratories and is still considered the 'gold standard'. However, nucleic acid amplification methods are known to be more sensitive. For optimal recovery, more than one medium should be used (11,12). The easiest way to provide this combination of media is to have a split plate that contains the two types of media. Gonococcal agar supplemented with 2% bovine hemoglobin and 5% fetal calf serum, 1% Isovitalax supplement (BBL Microbiology Systems, USA) (Note: CVA [Gibco Laboratories, USA] was used previously, but is no longer available), 3 µg/mL of vancomycin (gonococcal agar supplemented with 2% bovine hemoglobin and 5% fetal calf serum), and Mueller-Hinton agar supplemented with 5% chocolate horse blood, 1% Isovitalax supplement and 3 µg/mL of vancomycin (Mueller-Hinton agar supplemented with 5% chocolate horse blood) have been shown to be an optimal combination (7,8). Modification of this technique by the substitution of 0.2% activated charcoal for fetal calf serum has proven to be equally effective, and cheaper (13). The function of the serum is likely not nutritional; instead, the albumen component is important in absorbing toxic components from the agar and/or pus of the specimen.

Some strains of *H ducreyi* have been reported to be sensitive to vancomycin and therefore would not grow on selective media containing this antibiotic (4). If this occurs, it may be necessary to make nonselective chancroid medium (eg, omit the vancomycin). However, for routine use, inclusion of vancomycin in the media is preferred.

All inoculated media should be incubated in 5% CO₂ at 33°C to 35°C (it is critical that the temperature does not exceed 35°C) in a humid environment. This can be achieved using a CO₂ jar or a candle jar containing damp towels. Ideally, bedside inoculation is optimal. However, because of the short shelf life and sporadic need for culture of *H ducreyi*, these media are not kept on hand at most clinics. When working up a suspect *H ducreyi*, immediately let the microbiology laboratory know so they have 2 h to 3 h to prepare the media (many laboratories may need even more time because they may not have media preparation facilities on site). The swab in transport

medium (eg, Amies or Amies with charcoal) needs to reach the laboratory within 4 h because *H ducreyi* will not survive well beyond this time frame (5). Be sure to collect additional swabs for dry transport for PCR.

Identification of *H ducreyi* growing from cultured specimens is not easy because the organism often cannot grow in the media used for routine biochemical testing. Furthermore, identification is not easy because *H ducreyi* is asaccharolytic. Identification is usually made by Gram stain showing Gram-negative coccobacilli that produce characteristic tan-yellow colonies that are highly self adherent and can be 'nudged' intact over the surface of the agar. Additional identification tests to consider include oxidase (positive for *H ducreyi*), catalase (negative for *H ducreyi*) and X factor nutritional requirement (*H ducreyi* requires X factor for growth and this can most easily be evaluated using the porphyrin test). For confirmation of species identification, the isolate can be sent to the National Microbiology Laboratory (Winnipeg, Manitoba) for *H ducreyi*-specific molecular tests (8,14,15).

Nucleic acid detection (with or without amplification)

A variety of genetic probe (14,15) and amplification (16,17) methods have been developed for culture confirmation or direct detection of *H ducreyi* in clinical samples. Of particular relevance is the Roche-developed multiplex-PCR test (Roche Diagnostics Canada) that simultaneously detects *H ducreyi*, *T pallidum* and herpes simplex virus (17). The PCR amplification data available to date indicate that this method is more sensitive than other currently available culture methods (1,7,8,17). However, none of these methods are currently commercially available. In Canada, the specimen should be referred to the National Microbiology Laboratory, where a multiplex-PCR test for *H ducreyi*, herpes simplex and *T pallidum* can be done.

Serology

Humoral immune responses have been shown for patients infected with *H ducreyi*. Detection of antibody production to various cell components including whole cell lysate, purified and recombinant outer membrane proteins and LOS have been reported (18-20). For all antigens evaluated, the response is often slow in developing, cross-reactive with other *Haemophilus* species, and may last a long time after the infection has cleared. The currently reported assays are most useful as epidemiological tools and have limited value as diagnostic tests for chancroid, especially in areas where chancroid is endemic (8). The sensitivity and specificity range of these serological assays is 55% to 100% and 23% to 96%, respectively. Reference laboratories do not have an effective serology test for diagnosis of acute infection with *H ducreyi* (8).

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Although susceptibility testing of *H ducreyi* has been performed using agar dilution (21) or E-test (7) methods, it is not performed routinely in diagnostic laboratories due to the lack of standardized methods or interpretive criteria and the difficulty in growing *H ducreyi*. Syndromic treatment for GUD is recommended, especially if laboratory diagnostic capacity is restricted (1,7). However, given the growing resistance of *H ducreyi* to antibiotics as evidenced by therapy failures (1,4,7), having clinical isolates for antibiotic therapy evaluation is important from an epidemiological perspective.

PROFICIENCY AND QUALITY ASSURANCE

Challenging the diagnostic laboratory by including genital ulcer specimens sent as swabs in transport media that have been prepared using stock cultures of *H ducreyi* is useful, and will identify any chancroid culture media availability or culture confirmation problems. However, stock isolates that have been passaged extensively in vitro are often easier to grow and identify compared with fresh clinical isolates. As a result,

these proficiency challenges may have limited value. Routine quality assurance should be performed on all media that is made available to diagnostic clinics for culture of suspect chancroid genital ulcer specimens to ensure proper functioning of selective antibiotics. If media is prepared upon request, the quality control should be performed in parallel with the specimen inoculation. If the media quality control fails, then diagnostic analysis will rely on the PCR results.

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