SHORT ARTICLES

IDENTIFICATION OF HAEMOPHILUS DUCREYI IN THE CLINICAL LABORATORY

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SUMMARY. Some of the characteristics of 42 clinical isolates of Haemophilus ducreyi are reported. Only six of the 42 strains were able to grow on horse-blood agar. All strains gave a positive oxidase test with tetramethyl-p-phenylenediamine and a negative result with dimethyl-p-phenylenediamine. All of 15 test strains were negative in the porphyrin test. Tests for haemin requirement were inconclusive because of difficulties encountered in obtaining growth on a basal medium.

INTRODUCTION

The diagnosis of chancroid is usually based upon the clinical features and exclusion of other conditions by adequate laboratory tests. Although direct smears taken from the ulcers show the presence of Ducrey's bacillus, difficulties are often encountered in culture and a definite diagnosis is not easily established. Other procedures such as a skin test (Ito-Reenstierna reaction), biopsy and autoinoculation, are unsatisfactory or impracticable.

Haemophilus ducreyi is a fastidious organism; it does not grow on simple nutrient media. Optimal growth occurs at 33°C. Although new isolation and identification procedures have been developed, some properties of the species are not clearly defined and its nutritional requirements, biochemical reactions and morphological and colonial characteristics are still debated. For example, rabbit blood and sheep blood support the growth of H. ducreyi, whereas it is said that human, horse and ox blood do not (Reymann, 1947). The reason for this selectivity is unknown. Discrepant results of the oxidase test have been reported by Reymann (1949a), Kilian (1976) and Sottnek et al. (1980). The X-factor requirement that is usually demonstrated by growth around a paper strip or disk impregnated with haemin is an unreliable procedure with H. ducreyi. Few basal media support the growth of the organism. The problem can be avoided by the use of the porphyrin test (Kilian, 1974), but this requires special equipment and is not yet a routine procedure.

We investigated some properties of clinical isolates of H. ducreyi to determine tests that might be used as a basis for screening and presumptive identification of the bacterium.

MATERIALS AND METHODS

Bacterial strains. The 42 test strains of H. ducreyi were isolated in the Instituto Bacteriológico Câmar Pestana, mostly from patients of the Dispensário Central de Higiene Social de Lisboa. The patients were all males and aged 17–57 years, with single or multiple ulcerative lesions almost always of the prepuce; some of them had inguinal adenitis.

Isolation procedures. Material from the genital ulcers was cultured on the following media: clotted rabbit blood (Teague and Deibert, 1920), rabbit-blood agar prepared from Columbia Blood Agar Base (Difco Laboratories, Detroit, Michigan, USA) with 20–30% defibrinated
rabbit-blood added, as described by Cruickshank et al. (1975), and chocolate agar [10\% heated, defibrinated horse-blood in Columbia Agar Base Difco, enriched with 1\% (v/v) Polyvitex (bioMérieux, Marcy-l’Etoile, Charbonnières-les-Bains, France) and vancomycin chloride hydrate (Eli Lilly, Indianapolis, Indiana, USA) 3 \mu g/ml (Hammond et al., 1978a).

The cultures were incubated at 33–34°C in a candle jar with a humid atmosphere and 5–10\% CO\(_2\). For subcultures, the colonies were crushed in a mortar or between two sterile glass slides (Reymann, 1949b).

**Horse-blood agar growth test.** The ability of the *H. ducreyi* isolates to grow on horse-blood agar within 2 days was tested on medium prepared from Columbia Blood Agar Base, with 5\% horse blood. The plates were incubated at 33–34°C with 5–10\% CO\(_2\).

**Oxidase activity** was determined by a modification of the technique of Kovács (1956) with NN-dimethyl-p-phenylenediamine oxalate and NNN′N′-tetramethyl-p-phenylenediamine dihydrochloride (BDH Chemicals Ltd, Poole, Dorset).

**Growth-factor requirement.** Tests included the following: culture on a Tryptose semisolid basal medium with and without haemin (haematin hydrochloride, BDH) 250 \mu g/ml; culture on supplemented GC Medium (Difco) around a haemin-impregnated paper strip (Taxo Haemophilus Differentiation Strips, BBL, Cockeysville, Maryland, USA); and the porphyrin test.

The basal semisolid medium was a modification of that used by Ajello et al. (1956) containing Tryptose (Oxoid) 0-8\%, sodium chloride 0-9\% and Oxoid Agar No. 1 0-15\%. Cultures on the same basal medium without haemin and with 5\% rabbit serum were done simultaneously. GC Medium Base (Difco) was supplemented with dextrose 0-1\%, glutamine 0-01\% and cysteine 0-05\% (Sottnek et al., 1980).

The porphyrin test was performed according to the technique of Kilian (1974). 5-Aminolevulinic acid hydrochloride (Aldrich-Europe, Beerse, Belgium) was used as substrate. Strains of *Haemophilus parahaemolyticus* isolated from clinical material in Instituto Bacteriológico Câmara Pestana were used as positive controls.

**Virulence testing.** The virulence of the isolates for rabbits was tested by intradermal injection by the method of Hammond et al. (1978b).

**RESULTS**

We isolated *H. ducreyi* more frequently on chocolate agar supplemented with Polyvitex and vancomycin than on any of the other media used. Long chains of bacilli consistent with the morphology of *H. ducreyi* were seen by gram staining of cultures on Teague-Deibert medium of material from 13 of 20 patients. The organism was isolated on chocolate agar from 42 of 74 patients and on rabbit blood agar from 11 of 27 patients. Scanty to abundant growth of small, convex, grey-yellowish, semi-opaque colonies appeared usually after incubation for 2 days. The colonies could be pushed intact across the surface of the medium. Variability in their size and opacity gave the impression of a mixed culture. After prolonged incubation, some colonies enlarged to 2 mm in diameter and became more opaque.

Microscopically, *H. ducreyi* appeared as pleomorphic gram-negative rods when grown on solid media and as long chains of bacilli and even bundles of chains when grown on semisolid media. Horse-blood agar supported the growth of only six of the 42 strains tested. One of these strains was virulent for the rabbit.

The oxidase test was positive with the tetramethyl and negative with the dimethyl compound. It was not possible to demonstrate the requirement for X factor either on supplemented GC Medium Base (Difco) or on Tryptose semisolid medium, tested respectively with 10 and 14 isolates. However, the Tryptose semisolid medium supplemented with rabbit serum supported luxuriant growth. The porphyrin test was negative for 15 isolates tested.

The rabbit virulence test was positive for five of seven strains tested and the organism was recovered from the cutaneous lesion that developed in one of the rabbits.

**DISCUSSION**

Our experience confirms the statement that few strains of *H. ducreyi* grow on horse-blood agar (Stokes and Ridgway, 1980). The different results observed in the oxidase test with the two
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Compounds, tetramethyl- and dimethyl-\(p\)-phenylenediamine, may explain the conflicting reports of different investigators.

Our findings are consistent with those of Sottnek et al. (1980) who tested various basal media currently used for the demonstration of requirement for X factor and showed that only one supported the growth of all strains of \(H.\) ducreyi tested. In our study, the luxuriant growth obtained on the Tryptose medium supplemented with rabbit serum, and the failure of growth on the same medium supplemented with haemin only, suggests that \(H.\) ducreyi requires growth factors other than haemin, as was stated by Ajello et al. (1956). A second possible explanation of our results would be that we used an inadequate source and concentration of haemin; this is difficult to accept, because the commercially available strips were shown to have a sufficient content of haemin for the growth of \(H.\) ducreyi (Hammond et al., 1978c). We conclude that although the morphology and coherence of the colonies are helpful characteristics for the presumptive identification of \(H.\) ducreyi, and a negative porphyrin test is useful additional evidence, the other approaches that we have evaluated, such as the horse-blood-agar growth test and the two-substrate oxidase test, merit further development for the routine identification of the bacterium.

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