Evaluation of the Phadebact Gonococcus Test in the identification of \textit{Neisseria gonorrhoeae} in a routine diagnostic laboratory

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SUMMARY The Phadebact Gonococcus Test, a coagglutination procedure for the confirmation of identity of presumptive \textit{N gonorrhoeae} isolates, was evaluated under normal working conditions in a routine diagnostic laboratory and compared with an immunofluorescence technique. Of 166 isolates of \textit{N gonorrhoeae} from urogenital, rectal, and pharyngeal sites, 164 gave a positive coagglutination reaction, and one of the two negative isolates gave a positive reaction on retesting after subculture. There were no cross-reactions with other organisms tested. This was in contrast with the immunofluorescence technique; with this method, three of 46 isolates of \textit{N meningitidis} fluoresced brightly, and a further 17 isolates of \textit{N meningitidis} gave reactions that were difficult to interpret.

The coagglutination test is rapid, simple and a more specific alternative to immunofluorescence. Colonies of presumptive \textit{N gonorrhoeae} (oxidase-positive, Gram-negative diplococci) may be identified by biochemical methods—for example, carbohydrate utilisation, enzymatic profiles, or serological techniques such as immunofluorescence (IFL), coagglutination (COA). The method of coagglutination of antibody-coated staphylococci has been applied to the identification and typing of several species of bacteria.\textsuperscript{1-3} This simple and rapid procedure was first described for the identification of \textit{N gonorrhoeae} in 1974.\textsuperscript{4} A commercial kit, the Phadebact Gonococcus Test, was introduced and has since been modified.\textsuperscript{5}

We compared this technique with IFL, another rapid method of identification, using a carbohydrate utilisation method for the confirmation of the identity of the organisms tested. In common with other workers, we have experienced some difficulties with IFL. Cross-reactions with other organisms, particularly \textit{N meningitidis}, occur and some isolates of \textit{N gonorrhoeae} do not fluoresce.\textsuperscript{6-8} Carbohydrate utilisation is the standard reference method.\textsuperscript{6,7} The traditional methods require overnight incubation, a pure inoculum and occasionally the test organism fails to grow, or degrade the sugars.\textsuperscript{6-10} Rapid carbohydrate degradation methods have been introduced but they require a large pure inoculum and at least four hours incubation for the accurate interpretation of results.\textsuperscript{8,11} Identification using enzymatic profile also requires four hours incubation.\textsuperscript{10}

The purpose of the study was to compare the two rapid methods of COA and IFL, and evaluate the use of the Phadebact Gonococcus Test under normal working conditions in the routine diagnostic laboratory.

Material and methods

**CLINICAL SPECIMENS**
Bacterial isolates were obtained from urogenital, rectal, and pharyngeal sites of patients attending the Department of Genito-Urinary Medicine of the General Infirmary at Leeds. Specimens were inoculated on to a selective medium* in the clinic, and incubated at 37°C in 5% CO\(_2\), usually for 24 to 48 h, although 30-7% of the cultures were incubated for a longer period, up to 96 h.

Colonies of oxidase-positive Gram-negative diplococci with the typical morphology of gonococci were tested. Colonies with the morphology more typical

*Based on Modified New York City medium:\textsuperscript{12} GC agar base (Oxoid) 10% saponin-lysed horse blood, dextrose (1 g/l), L-glutamine (100 mg/l), L-cysteine (11 mg/l), cocarboxylase (1 mg/l), ferric nitrate (0-2 mg/l), vancomycin (3 \(\mu\)g/ml), colistin (100 units/ml), amphotericin (1 \(\mu\)g/ml), trimethoprim (5 \(\mu\)g/ml).

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of meningococci were also included, together with two colonies of Gram-negative, oxidase-positive, coccobacilli which did not utilise glucose. These organisms were isolated on the selective medium during the course of the study; other isolates without the characteristics of gonococci were not tested.

CONFIRMATION OF IDENTITY OF ISOLATES
BY CARBOHYDRATE UTILISATION
Bottles of serum-free media (Flynn & Waitkins specification, Difco)13 were heavily inoculated with pure cultures, and incubated for 24 h or, if necessary, 48 h at 37°C in 5% CO₂, with the bottle caps loose. After removal from the incubator, the bottles were allowed to stand on the bench for 15 min before reading, to allow any CO₂ absorbed into the medium to dissipate; a true colour change, from pink to yellow, was then easily detected. Sugars tested were glucose, lactose, maltose, fructose and sucrose; isolates of N gonorrhoeae utilised glucose only.

IMMUNOFLUORESCENCE TECHNIQUES
A thin film of bacteria was made in distilled water on a glass slide. From one to 10 colonies (all morphologically similar and presumptive N gonorrhoeae) were tested from each culture, depending on the amount of growth. The air-dried smear was fixed by gentle heat, covered by a working dilution of standardised fluorescein-conjugated gonococcal antiglobulin (Difco) and incubated for 10 min in a moist chamber at room temperature. The slide was then washed in phosphate buffer (pH 7·2), air-dried, and the smear examined under oil immersion (×100 objective), using a Nikon microscope with Epi-fluorescence attachment.

Positive and negative control smears (N gonorrhoeae and Enterobacter cloacae, respectively) were examined with each batch of tests, and fluorescence graded by visual comparison as negative, "spotted staining", weakly positive (+) and strongly positive (++ to ++++).14

COAGGLUTINATION TECHNIQUE
A heavy suspension (several colonies) was made in 0·2 ml distilled water in a glass tube plugged with cotton-wool, and heated for five min in a boiling water bath. The lyophilised gonococcal and control reagents were washed and resuspended in the buffer substance provided. For economy, a 5 mm bacteriological loop was used to dispense one loopful of each reagent onto the glass slide. One loopful of boiled suspension was then added to each reagent and mixed. The slide was rocked slowly for 2–3 min and observed for coagglutination. This was graded visually as negative, non-interpretable (a filamentous reaction with both test and control reagents), weak COA (+) or strong COA (++ to ++++).45

Results
The COA test gave no false-positive reactions, and none of the organisms isolated gave non-interpretable COA reactions. There were two false-negative COA reactions with primary isolates (Table 1). Both gave strongly positive IFL reactions, and one gave a positive (++) COA reaction when retested after subculture. The IFL test gave one false-negative reaction, the isolate giving a positive (+) COA reaction. However, there were three meningococcal isolates positive by IFL (three false-positives), and a further 17 of the 46 meningococcal isolates gave "spotted staining" fluorescence.

The manufacturers recommend that all COA tests should be performed on colonies incubated for less than 24 h. The effect of time of incubation on the results is shown in Tables 2, 3 and 4. All gonococcal isolates present in sufficient quantity to be tested at 24 h gave strongly positive COA reactions. The majority of the isolates were not tested until after 48 h incubation, and 6·3% then gave weak COA reactions. However, this proportion did not increase in isolates tested after longer incubation, up to 96 h, and the number of isolates tested after only 24 h incubation may be too small for a valid comparison to be made. The proportion of weak to strong IFL reactions of the gonococcal isolates was greater after 24 than after 48 h incubation, but overall, there was no effect of time of incubation on the IFL reactions of either the gonococcal or meningococcal isolates.

Table 1 COA and IFL reactions of Neisseria spp and Gram-negative coccobacilli isolated from different sites

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Site from which specimen was taken</th>
<th>Coagglutination</th>
<th>Immunofluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urethral/cervix</td>
<td>Rectum</td>
<td>Pharynx</td>
</tr>
<tr>
<td>N gonorrhoeae</td>
<td>139</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>N meningitidis</td>
<td>0</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>N lactamica</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Gram-negative coccobacilli</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*One isolate initially negative, was subsequently positive when tested after subculture.
studies of isolates experienced in the technique have been seen, staining fluoresces in meningococci and meningococcal isolates.6 9,15 and in N. gonorrhoeae to above, we confirm the antigenic variation with the IFL reaction. COA reactions of meningococcal isolates: variation with time of incubation

<table>
<thead>
<tr>
<th>Degree of IFL reaction</th>
<th>Time of incubation (h)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Weak (+)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Strong (++ to ++++)</td>
<td>20</td>
<td>88</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>95</td>
</tr>
</tbody>
</table>

One isolate initially negative, was subsequently positive when tested after subculture.

Table 3 IFL reactions of gonococcal isolates: variation with time of incubation

<table>
<thead>
<tr>
<th>Degree of IFL reaction</th>
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<tr>
<td></td>
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<td>48</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Weak (+)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Strong (++ to ++++)</td>
<td>16</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>95</td>
</tr>
</tbody>
</table>

*See text.

Discussion

We have previously used the IFL technique described above to confirm the identity of isolates of presumptive N. gonorrhoeae from all sources. Carbohydrate utilisation tests are performed on all pharyngeal isolates and in some other selected cases. The close antigenic relationship between gonococci and meningococci is well known,7 14 16 We have observed meningococcal isolates resembling gonococci in fluorescence, when the whole organism fluoresces brightly. More commonly, punctate staining of the periphery of meningococcal organisms has been seen, and we have used the term “spotted staining” to describe this.14 In practice, specimens from the Department of Genito-Urinary Medicine are processed by one person, who becomes experienced in the interpretation of results.

Other workers have evaluated the performance of the Phadebact Gonococcus Test, both on clinical isolates and laboratory stock cultures. Three studies6 15 17 have used two smears of the colonies to be tested, mixed with the two reagents on the glass slide, in the “Direct Colonies Method.” The prototype test was found to be sensitive but not specific by this method.6 Later studies15 17 found the test to be sensitive and specific; no cross-reactions were observed with other Neisseria spp or other organisms found on selective culture media. Non-interpretable results (positive COA with gonococcal and control reagents) were a problem, thought to be caused by serum globulins in the medium.15 In one study15 trypsin was added to the test system to overcome this; in the other17 a boiled aqueous suspension of the colonies was used in the test on organisms initially giving a non-interpretable COA reaction, and all these then gave clearly negative COA reactions.

The manufacturers now recommend that the “Boiled Colonies Suspension Method” be used with all isolates from serum-containing media. One further study18 has used this method, boiling the suspension of colonies for 20 min and using 10 μl amounts only of test and control reagents in all determinations. All 236 isolates of N. gonorrhoeae gave positive COA reactions. Of 78 non-gonococcal organisms tested, one isolate each of N. subflava and Moraxella osloensis gave false-positive results, and one of 21 isolates of N. sicca gave a non-interpretable COA reaction.

In our hands the COA test was highly satisfactory. The method using a boiled, aqueous suspension of colonies increases the performance time and requires more colonial growth than the “Direct Colonies Method”, but is still a more rapid test than IFL, and the specificity of the COA test is increased. No doubtful or non-interpretable reactions were observed. The COA test was more specific (100%) than IFL (94%) with 50 and 47 (respectively) of the non-gonococcal isolates giving negative reactions. The sensitivities of each test were comparable at 98.8% for COA and 99.4% for IFL. No expensive or specialised equipment is needed for the Phadebact Gonococcus Test, which was found to be rapid, simple to perform, sensitive, and specific.

We wish to thank Dr MA Waugh of the Department of Genito-Urinary Medicine, The General Infirmary at Leeds, for his assistance, Janekin Hyde for technical assistance and Pharmacia (Great Britain) Ltd, for provision of the reagents.

References

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