

# Identification of *Neisseria gonorrhoeae* in the routine venereological laboratory

## Comparative study of coagglutination, direct immunofluorescence, and sugar fermentation reaction

T RUFLI

*From the University Skin Clinic, Kantonsspital, Basel, Switzerland*

**SUMMARY** The coagglutination (CoA) method for the identification of *Neisseria gonorrhoeae* colonies grown on selective culture media was used on 116 strains in a routine venereological laboratory together with the direct immunofluorescence (IF) test and the sugar fermentation reaction. Correlation of results between the CoA method and the direct IF test and between the CoA test and the sugar fermentation reaction was 95.7% and 97% respectively. The sugar fermentation reaction requires subcultures and is more time-consuming than the IF test; the latter needs elaborate technical equipment and experience. The CoA method however can be carried out with the primary culture, is technically easy to perform and to reproduce, and the result is available within minutes.

### Introduction

Culture of *Neisseria gonorrhoeae* has become much simpler since the introduction of the selective media by Thayer and Martin.<sup>1</sup> These media may be obtained commercially at reasonable prices and have proved efficient. The characteristic morphological criteria for the colonies grown on the selective media, in combination with Gram staining and oxidase reaction, allow a preliminary classification of the organisms as *Neisseria*. However, neisseriae other than *N gonorrhoeae* can occur in pharyngeal and rectal smears, for example *Neisseria meningitidis* and *Neisseria lactamica*, both of which are found with increasing frequency in urethral and cervical smears.<sup>2-6</sup> For this reason, a clear classification of neisseriae is needed in venereological investigations.

Dunn and Gordon<sup>7</sup> observed in 1905 the varying ability of the different species of *Neisseria* to degrade sugars. Since 1906 this sugar fermentation test has been used with indicator and sugar-containing media as the method of choice for the identification of the different neisserial strains.<sup>8</sup> The CTA medium<sup>9</sup> is frequently used today with sugar-impregnated filter discs serving as fermentation substrate. The ferment-

ation reaction in a sugar solution without renewed subcultivation (non-growth carbohydrate degradation) is technically demanding and requires highly purified sugar solutions. For these reasons this method is not used in the routine laboratory.<sup>10</sup>

The immunological identification of *N gonorrhoeae*, introduced by Deacon and co-workers,<sup>11</sup> has been used successfully.<sup>12-20</sup> The commercially available FITC-labelled antiserum produced by Difco Laboratories was used in these studies.

Danielsson and Kronvall<sup>21</sup> adapted the method of coagglutination (CoA), originally introduced by Kronvall<sup>22</sup> for streptococci, to *N gonorrhoeae* and found highly specific results with this organism. This method is based on the ability of certain strains of staphylococci to bind immunoglobulins non-specifically to their surface. Addition of the corresponding antigen causes a visible agglutination. Menck<sup>23</sup> successfully evaluated the now commercially available product (Phadebact® Gonococcus Test, Pharmacia AB) using laboratory strains of *N gonorrhoeae* and other *Neisseria* as well as many clinically isolated strains. Barnham and Glynn<sup>24</sup> also evaluated the Phadebact reagent for gonococci on a larger series of gonococci and other neisseriae.

In the present investigation, the identification of 116 consecutive strains of Gram-negative, oxidase-positive diplococci was carried out simultaneously by the direct immunofluorescence (IF) test, by the sugar

Address for reprints: Dr T. Ruffli, University Skin Clinic, Kantonsspital, CH 4031 Basel, Switzerland

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fermentation reaction, using the method of Stacey and co-workers,<sup>25</sup> and by the CoA method. The latter method aims merely at a rapid and easy identification of *N gonorrhoeae*.

### Patients and methods

During the period, 1 April to 15 June 1978, 116 strains of *Neisseria* (61 isolated from 57 male and 55 from 37 female patients at this clinic) were studied. Specimens taken from the patients were immediately inoculated on to Thayer-Martin selective medium<sup>1</sup> (modified by Martin *et al*<sup>26</sup> and produced by Orion Laboratories) and incubated at 36.5°C in a candle jar. The characteristic morphology of the colonies and the positive results of the oxidase reaction and Gram-staining (Gram-negative diplococci) were used as criteria for further investigation.

### SUGAR DEGRADATION

The ability of the organisms to degrade sugar was tested on a CTA medium (cystein-tryptic-agar 28.5 g/l; GC agar Difco 10 g/l; NaOH 1 m 10 drops and phenol red as indicator; reagents, Difco) using the method of Thayer.<sup>9</sup> Filter discs impregnated with glucose, maltose, and sucrose were placed on the agar. Sugar degradation was shown by the change of the indicator around the discs to yellow.

### IF TEST

The IF test was carried out with FITC-conjugated anti-*N gonorrhoeae* serum (Difco). A characteristic colony of the primary culture was suspended in physiological saline and spread on a slide, allowed to dry, and then fixed by heat. Incubation with the conjugate diluted 1/4 was undertaken at 37°C for 30 minutes. After being washed with PBS and distilled water, the preparation was mounted with Bacto (Difco) mounting-fluid and examined.<sup>16</sup>

### COAGGLUTINATION TEST

The CoA test was performed with the Phadebact<sup>®</sup> Gonococcus Test (Pharmacia AB). A few colonies of

TABLE I Sites of isolation of the 116 strains of *Neisseria gonorrhoeae*

Patients	Site of isolation				Total
	Urethra	Cervix	Rectum	Tonsils	
Male	52		2	7	61
Female	5	12	13	25	55
Total	57	12	15	32	116

*Neisseria* from the primary culture were suspended in a drop of the test solution containing killed staphylococci coated with anti-*N gonorrhoeae* IgG on a glass slide. A reagent containing staphylococci coated with non-specific IgG served as control. The suspensions were carefully mixed. The result may be read within a minute, a positive reaction consisting of a distinct coagglutination.

### Results

Of the 116 strains of *Neisseria*, 61 were isolated from male and 55 from female patients. Most specimens from men were obtained from the urethra; tonsillar and rectal swabs were taken only from known homosexuals. Seven of the 61 strains were isolated from the tonsils and two from the rectum. Of the 55 strains isolated from female patients, five were obtained from the urethra, 12 from the cervix, 25 from the tonsils, and 13 from the rectum (table I).

### IDENTIFICATION PROCEDURES

#### Sugar fermentation reaction

The sugar fermentation reaction could be evaluated in only 86 (74%) of the 116 strains; 15 strains could not be subcultured, owing to growth failure, and another 15 strains were contaminated, so that the overgrowth made evaluation impossible (table II).

#### IF and coagglutination tests

The results of the IF and CoA tests, irrespective of the results of the sugar fermentation reactions, correlated for 95.7% of strains; 80 strains gave a positive result and 31 a negative result with both the

TABLE II Comparison of results with direct immunofluorescence, coagglutination, and sugar degradation tests

Test results	No of strains	Results of sugar degradation			
		Dextrose	Dextrose and maltose	Contaminated	Subculture failed
Positive to					
IF and CoA	80	63	0	9	8
IF only	3	1	0	1	1
CoA only	2	1	0	1	0
Negative to					
IF and CoA	31	1	20	4	6
Total	116	66	20	15	15

IF = indirect immunofluorescence  
CoA = coagglutination

IF and CoA tests. The five conflicting results were found in one strain isolated from the urethra of a male patient, in three strains isolated from the rectum, and in one strain isolated from the tonsils. Results thus agreed in 68 (98.6%) of 69 strains isolated from genital specimens, in 31 (96.9%) of 32 strains isolated from tonsillar specimens, and in 12 (80%) of 15 strains isolated from rectal specimens.

Of the 66 strains which had been identified as *N gonorrhoeae* by the isolated dextrose fermentation reaction, 64 showed a positive result to the IF test but two strains did not react. The CoA test confirmed the identification of *N gonorrhoeae* in 64 (97%) of 66 strains.

### Discussion

Our results clearly show the difficulties met with in the routine venereological laboratory. The specific identification of *N gonorrhoeae* by its ability to ferment sugar in the subcultures is difficult as well as time-consuming and expensive. Its use is further limited by possible contamination and death of subcultures as well as by the occasional strains which have a decreased ability to degrade sugar. These difficulties are often stressed in published reports but actual numbers of failures are not mentioned.<sup>27</sup>

The direct IF method has proved to be reliable for identification.<sup>16 17</sup> The specificity of the commercially available conjugate is sometimes disputed.<sup>28 29</sup> With practice, however, the method is highly specific compared with the fermentation reaction. The IF method reduces reporting time by at least 24 hours, and the actual technique and the evaluation take no longer than two hours to perform. The method, however, requires skill, experience, and the technical equipment for fluorescent microscopy.

On the other hand, the CoA method does not require specialised technical equipment; the test kit is commercially available. The test takes only a few minutes to perform, is easy to interpret, and is also reproducible. Even dead organisms still show a reaction. The costs of the reagents are similar to those of other methods. In the present study the results of the IF and CoA tests correlated for 111 (95.7%) of the 116 strains tested.

This agreement is even higher when only strains from genital specimens (98.4%) and from pharyngeal specimens (96.9%) are compared with each other. Three of 15 strains isolated from the rectum showed divergent results. Compared with sugar fermentation as the method of reference, there was a similarly high correlation between the IF and CoA tests. Of 86 strains which could be evaluated for their ability to degrade sugar, 66 showed an isolated reaction to dextrose and of these, 63 showed positive

reactions to both the IF and CoA tests. Twenty strains which fermented dextrose and maltose all gave negative results with the IF and CoA tests. Of the five strains with divergent results to the IF and CoA tests, one strain with a positive IF and a negative CoA result and one strain with both a negative IF and CoA result showed an isolated dextrose fermentation. These results cannot be interpreted, since a false result to the IF as well as to the CoA test, and the fact that a strain of *N meningitidis* is unable to ferment maltose, may be responsible.<sup>30</sup> Further investigations, such as serological grouping of meningococci, were not carried out.

Menck<sup>23</sup> reported pseudo-CoA (autoagglutination) results to the Phadebact® Gonococcus Test with colonies of *Neisseria* grown on serum-containing media and very rarely on serum-free media. We used the serum-free media, Orion, throughout and had no pseudo-CoA results.

In the routine venereological laboratory investigations must aim at a rapid accurate diagnosis—either by identification or by exclusion of *N gonorrhoeae*—and be inexpensive in time and materials, avoiding subcultures if possible. The present study shows that the sugar fermentation reaction does not fulfil these aims. On the other hand, the IF method confirmed 97% of *N gonorrhoeae* strains identified by sugar fermentation, the CoA yielding the same percentage. The agreement between the CoA and IF tests of 95.7% is also very high. Both methods showed no false-positive results for *N meningitidis*. The CoA test has therefore the same high specificity as the IF test but has the advantage of being a simple and rapid technique.

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