Identification of *Neisseria gonorrhoeae* from Primary Cultures by a Slide Agglutination Test

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Hen antigenococcal lipopolysaccharide hen serum was used in a simple slide agglutination test for the identification of *Neisseria gonorrhoeae* from primary isolates.

Conventional techniques of colony morphology, microscopic examination, biochemical reactions, and fluorescent antibody still remain the principal diagnostic criteria for identification of *Neisseria gonorrhoeae* (2-4, 8). These techniques are costly, time consuming, and delay reporting time. Previously we described the use of hen antigenococcal lipopolysaccharide serum (anti-LPS serum) in a rapid slide test for the identification of *N. gonorrhoeae* (7). These studies indicated that the anti-LPS serum was useful for the identification of *N. gonorrhoeae* in secondary (purity) cultures and provided preliminary evidence that the antiserum could also be used in the identification of *N. gonorrhoeae* from primary cultures. This report is an extension of our previous publication and investigates the feasibility of using anti-LPS serum for the identification of *N. gonorrhoeae* from primary isolates in a routine diagnostic laboratory.

The majority of gonococcal strains used in this study were isolated from urethral, cervical, rectal, and pharyngeal specimens received from the Ottawa Veneral Disease Clinic on Jembec plates containing New York City transport (NYC) medium (1, 6). These specimens were incubated at 36°C for 24 h in 10% CO₂. Gonococcal-like oxidase-positive colonies were subcultured on NYC medium for further identification by Gram stain and carbohydrate utilization. All plates not showing suspect gonococcal colonies were reincubated for 24 h and reexamined before being discarded. Some specimens were received in Stuart transport medium (5) and were plated on NYC medium, incubated at 36°C in 10% CO₂, and examined after 48 h as described above.

Suspect gonococcal colonies were selected from the primary isolation plates with the aid of a stereoscopic microscope and tested by a slide agglutination test employing anti-LPS serum (7). Briefly, a plain glass microscope slide was

sectioned into two squares with a grease pencil. A drop of a 1:4 working dilution of the anti-LPS serum was placed on the left section, and a drop of Formalinized saline buffer (Sorensen phosphate-buffered saline, pH 7.2, with 0.5% Formalin) was placed on the right side. The suspect colonies were emulsified in the control buffer and serum, the slide was rocked gently for a few seconds, and agglutination was read with the naked eye. No grading of positive agglutination was done, but it was noted that heavier gonococcal suspensions from confluent growth (e.g., urethra) agglutinated more rapidly than did lighter suspensions obtained from sparse growth or single colonies from other body sites. After completion of the agglutination, the slides were air-dried, heat-fixed, and Gram stained.

Of the 730 specimens examined, 680 contained *N. gonorrhoeae* and 50 contained *N. meningitidis*, as identified by carbohydrate utilization tests. With the agglutination test, 670 of the 680 gonococcal strains were identified as *N. gonorrhoeae*, giving a 98.5% correlation between the two methods (Table 1). The remaining 10 strains which were either negative or autoagglutinated in the buffer were found to be mixed cultures of *N. gonorrhoeae* and other organisms, as eventually confirmed by conventional techniques. This was also apparent after examining the agglutination slides by Gram stain. The contaminating organisms were most commonly found in specimens from pharyngeal and rectal sites where other gram-negative organisms are often present. The 10 strains, once purified, agglutinated with the anti-LPS serum.

The best agglutination results were obtained with 24-h cultures isolated on NYC medium. Occasionally isolates appearing after 48 h of incubation were difficult to emulsify and had to be mixed with buffer and glycerol (7). Nonviable cultures or oxidase-treated primary cultures still maintained their ability to agglutinate in the

TABLE 1. Identification of *N. gonorrhoeae* in primary isolates by the slide agglutination test compared with conventional criteria

Specimen	Source	No. of primary isolates confirmed by:		% Correlation
		Conventional criteria	Agglutination	
<i>N. gonorrhoeae</i>	Cervix	193	191	98.9
	Pharynx	37	34	91.9
	Rectum	102	98	96.1
	Urethra	348	347	99.7
<i>N. meningitidis</i>	Pharynx	50		100

anti-LPS serum.

The results of this study indicate that hen anti-LPS serum can be used in a slide agglutination test for the rapid identification of *N. gonorrhoeae* in primary cultures. Cultures that show autoagglutination should be further checked by other confirmatory criteria (3). Routine diagnostic laboratories should find this technique an acceptable alternative for the identification or confirmation of *N. gonorrhoeae* by other presently used criteria such as carbohydrate utilization or fluorescent antibody. It should also be noted that the Jembec transport system may be read within 24 h, whereas the Stuart transport medium and routine plating are read after 48 h of incubation. Thus, a transport system showing growth of *N. gonorrhoeae* by 24

h and the rapid anti-LPS serum agglutination test could improve reporting time, reduce expenditures, and help serve as an effective control measure for gonorrhea.

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