

Lectins in Diagnostic Microbiology: Use of Wheat Germ Agglutinin for Laboratory Identification of *Neisseria gonorrhoeae*

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A lectin slide agglutination test has been developed for the confirmatory identification of *Neisseria gonorrhoeae*. With wheat germ lectin as an agglutinin, 164 of 165 clinical isolates of *N. gonorrhoeae* gave a 3 to 4+ reaction within 6 to 8 min. Four gonococcal isolates, even though negative by the fluorescent-antibody method, gave strong positive reactions with the wheat germ lectin. Among 23 isolates of *Neisseria meningitidis* tested, which included representatives of serogroups A, B, C, D, X, Y, and Z, only one strain in group X gave a false-positive reaction. The nonpathogenic species of *Neisseria*, as well as *Branhamella catarrhalis*, all showed negative reactions with the wheat germ agglutinin. The novel method provides a simple, rapid, and inexpensive means for the laboratory diagnosis of gonorrhea and obviates the need for performing second-stage sugar fermentation studies or utilizing the more expensive fluorescent-antibody techniques.

In making a definitive diagnosis of *Neisseria gonorrhoeae*, most clinical laboratories use one of two methods: carbohydrate degradation tests or the direct fluorescent-antibody technique. Both of these confirmatory methods are less than optimal in terms of their sensitivity or specificity or both (3, 14, 16). The sugar utilization tests are the most widely used procedures for the identification of *N. gonorrhoeae* and require incubation periods ranging from 4 to 48 h, depending on the system being employed and the fastidiousness of the isolate.

It is evident from the number of recent publications suggesting modifications for improving the reliability of the sugar degradation tests that they still pose a significant problem for many diagnostic laboratories (2, 4, 10, 12, 17, 20, 21). In several comparative studies by different workers to determine the most reliable of the various types of sugar utilization tests, the numbers of positives obtained, even when the same type of test was used, varied as much as 30 to 35% (3, 14, 16).

Laboratories using the fluorescent-antibody technique have reported cross-reactions with other *Neisseria* species as well as negative reactions with isolates subsequently diagnosed as gonococci by alternate confirmatory procedures (7, 14). A second disadvantage to the fluorescent-antibody technique is the cost factor; an expensive conjugate is required, as well as a fluores-

cence microscope. In an effort to circumvent the foregoing problems inherent in current technology, we examined the feasibility of utilizing a direct and rather novel approach to the identification of *N. gonorrhoeae*, namely, the use of lectins as species-specific agglutinins.

As early as 1936, Summer and Howell (18) reported that concanavalin A (jack bean lectin) agglutinated certain species of mycobacteria. Thirty years later, Prokop and Köhler (15) described the selective agglutination of certain species of bacteria with a lectin extracted from the albumen gland of the edible snail (*Helix pomatia*). Subsequently, these same workers (11) described the specific agglutination for group C streptococci with the snail lectin as well as with a lectin derived from the seeds of *Dolichos biflorus*. Ottensooser et al. (13) also reported the specific agglutination of group C streptococci with a lectin obtained from soybeans (*Wisteria floribunda*).

In addition to bacteria, plant agglutinins have also been used to differentiate yeasts. Guillot et al. (6) tested 114 strains of the genus *Candida* with 28 different lectins and, from the data collected, suggested that lectin agglutination tests could form the basis for a new approach to the taxonomy of *Candida* species. Herman et al. (8), using 15 different lectins and 16 strains of the genera *Candida* and *Torulopsis*, observed patterns of agglutination which differed not only

between different species, but even between strains within the same species.

Despite the fact that these previous reports suggested the potential for using lectins as selective agglutinins for bacteria, relatively little work has been done in an effort to develop a practical diagnostic procedure for the clinical laboratory.

MATERIALS AND METHODS

Organisms used. For this study, 160 isolates of *N. gonorrhoeae* from clinical specimens were obtained from the City-County Board of Health Laboratories in Louisville, Ky. Five fluorescent-antibody "negative isolates" of *N. gonorrhoeae* were furnished by the Kentucky State Board of Health Laboratories, Frankfort. A total of 23 strains of *Neisseria meningitidis* were obtained from the following sources: 7 from the Center for Disease Control, including KC0954 (A), KC-792 (C), D-9287 (B), and 3 unnumbered; 3 from the Kentucky State Board of Health Laboratories; and 13 from several hospital laboratories in the Louisville area.

The following *Neisseria* species, as well as *Branhamella catarrhalis*, were obtained from the Center for Disease Control, Kentucky State Board of Health Laboratories, and local area hospital laboratories: two strains of *N. lactamica* (CDC no. NS-19 and KC-1323), five strains of *B. catarrhalis*, nine strains of *N. sicca*, two strains of *N. mucosa*, and one strain each of *N. flava* and *N. subflava*.

Although all isolates of *N. gonorrhoeae* furnished by the City-County Board of Health Laboratories in Louisville were identified by that facility, at least 50 isolates were randomly selected for sugar confirmatory tests. All other organisms used in this study were retested by our laboratory to confirm their correct species identification.

In addition to sugar utilization tests, all strains of *N. meningitidis* were serotyped with group-specific meningococcal antisera (Difco Laboratories).

Culture media. Modified Thayer-Martin medium was used for the primary isolation of *Neisseria* from clinical materials. Stock cultures of *N. gonorrhoeae* were maintained on chocolate agar slants under light mineral oil, which we found satisfactory for short-term storage of nongonococcal *Neisseria*. *B. catarrhalis* was maintained on Trypticase soy agar slants (BBL Microbiology Systems) under light mineral oil. Mueller-Hinton agar slants (BBL) with 1% carbohydrates and phenol red indicator were used throughout this study for the determination of sugar utilization patterns.

Preparation of WGA. Wheat germ agglutinin (WGA) was prepared by the method of Bloch and Burger (1) and stored at -20°C as a freeze-dried preparation.

Titration of purified wheat germ lectin. For each new lectin preparation, hemagglutination titers as well as *N. gonorrhoeae* agglutination titers were determined. Stock solutions of the lectins were prepared in phosphate-buffered saline (0.05 M disodium phosphate, 0.15 sodium chloride, pH 7.2). Hemagglutination titers (washed 2% suspension of human type A erythrocytes in 0.05 M phosphate-buffered saline

[PBS]) were carried out in a conventional manner. In performing gonococcal titrations, one drop of each twofold dilution of lectin was placed in a series of wells on a Boerner slide. One drop of a standardized suspension of *N. gonorrhoeae* was then added to each dilution well. The plate was placed on a Venereal Disease Research Laboratory rotary shaker for 5 min and read. The highest dilution of lectin that gave a 4+ agglutination reaction with *N. gonorrhoeae* was used as the working concentration for performing subsequent slide agglutination tests on unknown *Neisseria* cultures. Most of the wheat germ lectins prepared in our laboratory, as well commercially purchased WGA from Sigma Chemical Co., were comparable in their hemagglutination titers (1:256) as well as their gonococcal titers (1:64; 0.062 mg of lectin per ml).

Agglutination test procedure. Overnight growth of the "unknown" colonies to be tested were removed from the culture medium (Thayer-Martin or chocolate agar) with a cotton swab. The colonies were then emulsified in PBS (pH 7.2), and the turbid suspension was adjusted to approximate a no. 3 McFarland barium sulfate standard. One drop of the bacterial suspension was placed into each of two wells in a Boerner slide; to well no. 1, one drop of WGA was added, and to well no. 2 (control) a drop of PBS was added. The Boerner slide was placed on a Venereal Disease Research Laboratory rotary shaker for 5 min and read. If no autoagglutination was observed in the PBS control well, and a 2 to 4+ agglutination occurred in the lectin well, the test was considered positive for *N. gonorrhoeae*.

Carbohydrate inhibition tests. Because wheat germ agglutination has a specificity for *N*-acetylglucosamine, the sugar was used in agglutination-inhibition studies. Another sugar, D-galactose, was also tested as a predictable negative control. A 10-mg amount of the carbohydrate to be tested was added to 1 ml of the working concentration of lectin. The sugar-containing lectins were then used in the standard agglutination procedure described above.

RESULTS

Wheat germ lectin, at an appropriate concentration (0.062 mg/ml) selectively agglutinated (3 to 4+) a standardized suspension of *N. gonorrhoeae* (Table 1). With the exception of one strain of serogroup X meningococcus, all of the species of *Neisseria* gave negative agglutination reactions. Those species of *Neisseria* that showed autoagglutination in the PBS control demonstrated that same degree of agglutination in the presence of lectin. The autoagglutination cannot be avoided with certain strains of *Neisseria*, but we observed that it can be minimized by incubating the culture in a heavily moisturized environment.

In the routine performance of the wheat germ lectin agglutination test, we found that satisfactory and reproducible results could be obtained by simply adjusting the density of the bacterial suspension (antigen) to approximate the density

TABLE 1. *Agglutination of Neisseria species with wheat germ lectin*

Species	No. of strains tested	Growth on T-M medium ^a	Agglutination reactions	
			Total no. positive	Positive PBS controls
<i>N. gonorrhoeae</i>	165	+	164 (3 to 4+) ^b	—
<i>N. meningitidis</i> (groups A, B, C, D, X, Y, Z)	23	+	1 (group X) ^b	—
<i>N. meningitidis</i> (non-groupable)	1	+	—	—
<i>N. lactamica</i>	2	+	—	—
<i>B. catarrhalis</i> (formerly <i>Neisseria</i>)	5	(ca. 17%)	5 (2+) ^c	5 (2+)
<i>N. subflava</i>	1	—	—	—
<i>N. flavens</i>	1	—	—	—
<i>N. sicca</i>	9	—	—	—
<i>N. mucosa</i>	2	—	2 (2+)	2 (2+)

^a Ability to grow on Thayer-Martin (T-M) medium as reported by Hollis et al. (9) and by Thayer and Martin (19).

^b Among the strains of *N. meningitidis* tested, only one serogroup X gave a positive reaction.

^c *B. catarrhalis* and *N. mucosa* were considered negative because the degree of agglutination in the lectin well was the same as in the control well (2+).

of a no. 3 McFarland barium sulfate standard. Reasonable variations between an optical density of 0.35 to 0.75 (wavelength of 600 nm) did not significantly alter the degree of reactivity. It was also observed that variation in pH had little effect on reactivity in the neutral to alkaline range (pH 6.5 to 8.0). The results of the carbohydrate inhibition studies are shown in Table 2. It will be observed that the agglutination of *N. gonorrhoeae*, as well as the one reactive strain of serogroup X meningococcus, was inhibited by the addition of 10 mg of *N*-acetyl-D-glucosamine to 1 ml of the wheat germ lectin. These findings indicate that the sugar receptor sites on the gonococcal cell are probably residues of *N*-acetyl-D-glucosamine and that the agglutination phenomenon is not the result of some nonspecific interaction.

DISCUSSION

The results of this study show that a plant lectin can be used for the laboratory identification of an important pathogen. The agglutination reaction between wheat germ lectin and *N. gonorrhoeae* has made it possible to develop an identification procedure for the organism.

In the confirmatory diagnosis of gonorrhea, many clinical laboratory workers have reported problems with sugar utilization methods, as well as with the fluorescent-antibody techniques. Use of the wheat germ slide agglutination test would obviate many of the disadvantages associated with present procedures. The advantages of the lectin test are: (i) it is rapid, requiring only 6 to 7 min to perform, thereby eliminating the prolonged incubation periods required for the sugar

TABLE 2. *Carbohydrate inhibition of Neisseria agglutination with wheat germ lectin*

Organism	Carbohydrate ^a	Agglutinated by wheat germ lectin
<i>N. gonorrhoeae</i>	None	+
<i>N. gonorrhoeae</i>	<i>N</i> -Acetyl-D-glucosamine	—
<i>N. gonorrhoeae</i>	D-Galactose	+
<i>N. meningitidis</i> ^b	None	+
<i>N. meningitidis</i>	<i>N</i> -Acetyl-D-glucosamine	—
<i>N. meningitidis</i>	D-Galactose	+

^a Carbohydrates were added at a final concentration of 10 mg per ml of lectin solution.

^b The serotype of *N. meningitidis* used in this experiment was a wheat germ lectin-reactive group X isolate.

tests; (ii) it is economical and there is no need for second-stage media or expensive equipment, such as a fluorescence microscope; and (iii) based on the 209 tests performed, it is sensitive, reliable, and easy to read. Of the 165 strains of *N. gonorrhoeae* tested, 164 gave a positive reaction.

Despite the fact that one serogroup X gave a false-positive reaction, it does not pose a significant problem in terms of urogenital cultures. According to a study by Faur et al. (5), group X meningococci are rarely, if ever, isolated from the genitourinary tract. Of 118,000 genitourinary cultures of *N. gonorrhoeae*, only 34 meningococci were isolated, and none of these was found to belong to serogroup X. Thus, the probability of isolating this particular serogroup from a urogenital culture is extremely remote.

The demonstration that wheat germ lectin can be utilized in the rapid diagnosis of gonorrhea suggests the broad potential for employing different lectins in the selective agglutination

and identification of other clinically important bacteria to species level.

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