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Fluorescent Monoclonal Antibody Compared with Carbohydrate Utilization for Rapid Identification of *Neisseria gonorrhoeae*

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A commercially available fluorescein-conjugated monoclonal antibody (MAb) (Syva Co., Palo Alto, Calif.; Genetic Systems, Seattle, Wash.) against *Neisseria gonorrhoeae* was compared with a standard cystine Trypticase agar (CTA) sugar utilization method and with three rapid carbohydrate utilization tests, including the Minitek (BBL Microbiology Systems, Cockeysville, Md.), Neisseria-Stat (Richardson Scientific, Dallas, Tex.), and Neisseria-Kwik (Micro-Biologics, St. Cloud, Minn.) systems for the identification of *Neisseria* species. The MAb correctly identified all 86 clinical isolates of *N. gonorrhoeae*. Of these 86 isolates, 28 were found later (48 h after the initial inoculation) to be contaminated with non-*Neisseria* bacteria. In the other four test systems studied, the identification rates for pure and contaminated *N. gonorrhoeae* cultures were, respectively, as follows: CTA sugars, 88 and 32%; Minitek, 67 and 50%; Neisseria-Stat, 97 and 96%; and Neisseria-Kwik, 80 and 74%. The MAb did not identify any of the 50 nongonoccocal *Neisseria* isolates tested. The most expensive test system was the MAb, followed by the Neisseria-Kwik, Minitek, Neisseria-Stat, and CTA sugars systems. The MAb appears to be a rapid and accurate method to identify in vitro isolates of *N. gonorrhoeae*.

Rapid identification of *Neisseria gonorrhoeae* may be accomplished by several diverse methods. The methods include carbohydrate utilization with preformed enzymes or enzymes formed during growth (5, 8, 12, 15, 20) and immunological (4, 7, 10, 13), chromogenic (2, 19), and radiometric (16) procedures. Immunological procedures include coagglutination with polyclonal (11) (Phadebact Gonococcus Test; Pharmacia Diagnostics, Piscataway, N.J.) and murine monoclonal (9) (Gono Gen; New Horizons Diagnostics, Columbia, Md.) antibodies and fluorescein-conjugated polyclonal antibodies (6, 18) (Difco Laboratories, Detroit, Mich.). Fluorescent-antibody methods potentially permit rapid and accurate identification of *N. gonorrhoeae*. Here, we report an evaluation of a fluorescent monoclonal antibody (MAb) (Syva, Palo Alto, Calif.; Genetic Systems, Seattle, Wash.) for the identification of *N. gonorrhoeae* from culture as compared with standard and rapid sugar utilization tests.

MATERIALS AND METHODS

Organisms and media. A total of 136 fresh clinical isolates were randomly obtained from the Parkland Memorial Hospital Microbiology Laboratory and the Dallas County Health Microbiology Laboratory. Numbers and sources of *Neisseria* species and *Branhamella catarrhalis* isolates are listed in Table 1. The patient specimens were inoculated on modified Thayer-Martin medium and immediately incubated under 5% CO_2 at 35°C. The Thayer-Martin medium was made by the Dallas County Health Microbiology Laboratory. The gonococcal agar base and hemoglobin were obtained from Difco. Enrichment factors and vancomycin, colistin, and nystatin were obtained from the Texas Department of Health.

Pure and contaminated cultures. A pure culture was defined as the appearance of only a single colony type on Thayer-Martin agar. A contaminated culture was defined as the appearance of more than one colony type on Thayer-Martin agar. If more than one colony type was observed after the overnight incubation, the culture was not used in the study. Contaminated cultures were often noticed only after 48 h of incubation. Thus, when the supposedly pure cultures were used for inoculating the various test systems, it was not apparent upon visual inspection, Gram stain, or oxidase activity that such cultures were indeed contaminated. These latter cultures were used in the present study as contaminated cultures. Although no attempt was made to quantitate the number of organisms on contaminated plates, almost all contaminated cultures had more than 50 colonies spread uniformly throughout the streak of *Neisseria* organisms.

Preparation of standardized inoculum suspension. A concentrated bacterial inoculum was prepared from the cultures by placing several loopfuls of organisms from well-isolated colonies into 0.1 ml of sterile saline. From this heavy suspension (approximately McFarland standard number 7), the appropriate dilution for the particular test system inoculum was made.

Fluorescent MAb against *N. gonorrhoeae*. The MAb was obtained from Syva Co. Fresh isolates grown for 18 to 24 h on chocolate agar under 5% CO_2 were used in all tests. One loopful of the standardized bacterial suspension was placed in 0.1 ml of sterile saline and vortexed. One loopful of this suspension (approximately 10^9 CFU/ml) was then placed on a sterile microscope slide, air dried, and fixed with acetone (from a disposable ampule provided by the manufacturer). The fixed smear was then stained with the fluorescent conjugate by placing 30 μl on the smear and incubating it at room temperature for 15 min in a humidified atmosphere. The excess conjugate was partly removed by tapping the slide on a paper towel, and then the slide was gently washed for 10 s in distilled water and air dried. The stained smear was read with an epifluorescence Nikon microscope at a magnification of ×400. Negative and positive control slides (provided by the manufacturer) were stained and read as described above. A specimen was judged to be positive if

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TABLE 1. Sources of clinical isolates

Organism	Source (no.)
Neisseria gonorrhoeae	Oropharyngeal (23) Penile (35) Cervical (15) Rectal (13)
Neisseria meningitidis	Oropharyngeal (26) Urogenital (2)
Neisseria lactamica	Oropharyngeal (2)
Neisseria sicca	Oropharyngeal (3)
Neisseria subflava	Oropharyngeal (1)
Branhamella catarrhalis	Oropharyngeal (16)

there was a fluorescence of ≥2+ (bright apple-green color) in over 50% of the organisms seen under the magnification of ×400 in 10 fields. A specimen was judged to be negative if there was a fluorescence of <1+ with the same criteria described above.

Neisseria-Stat system. Neisseria-Stat materials and reagents were obtained from Richardson Scientific, Inc., Richardson, Tex. The system consisted of five tubes, four of which had dried carbohydrates (glucose, maltose, lactose, and sucrose). The fifth tube contained a disk with 4 to 6 mg of penicillin G. Also included was an inoculum buffer tube containing phenol red as an indicator. An inoculum corresponding to McFarland standard number 7 or greater was prepared from the standardized bacterial suspension by dilution in the inoculum buffer tube. Three drops of the inoculum were added to each carbohydrate-containing tube with a sterile Pasteur pipette. These tubes were incubated in a 37 to 39°C water bath for 3 h and read as positive if colored yellow and negative if colored red or orange.

Neisseria-Kwik system. The Neisseria-Kwik system (Micro-Biologics, St. Cloud, Minn.) consisted of a buffer tube and test trays (each tray had four test wells containing glucose, maltose, sucrose, and lactose and tray covers). The standardized bacterial suspension was used to make the inoculum in the buffer tube correspond to McFarland standard number 8. Four drops of this inoculum were added to each well of the corresponding tray. The inoculated tray was then enclosed with the plastic tray cover and incubated at 35 to 40°C for 4 h.

Minitek and CTA systems. The Minitek and cystine Trypticase agar (CTA) carbohydrates systems were obtained from BBL Microbiology Systems, Cockeysville, Md., and were used as previously described (12).

Quality control. *N. gonorrhoeae* ATCC 9828, *N. meningitidis* ATCC 13090, *N. lactamica* ATCC 23970, and *B. catarrhalis* ATCC 25240 were used with each test run as controls for the test systems.

Cost analysis. All media, reagents, and additional materials necessary to perform the tests were commercially obtained. For the cost study, the list prices of these supplies were used.

RESULTS

A comparison of the MAb, Minitek, CTA sugars, Neisseria-Stat, and Neisseria-Kwik systems for the identification of clinical *N. gonorrhoeae* isolates is shown in Table 2. The MAb correctly identified all 86 isolates, 28 of which were found to be contaminated with non-*Neisseria* species (predominantly gram-negative rods, enterococci, and viridans group streptococci) on the original chocolate agar plate the following day (48 h after the original inoculation). The Minitek system only identified 39 of 58 (67%) pure cultures correctly and 14 of 28 (50%) contaminated cultures correctly. CTA sugars correctly identified 51 of 58 (88%) pure isolates but only 9 of 28 (32%) contaminated cultures. The Neisseria-Stat system correctly identified 56 of 58 (97%) pure cultures and 27 of 28 (96%) contaminated cultures. The Neisseria-Kwik test was added later in the study, and thus only 49 isolates were studied under this system. With this system, 24 of 30 (80%) pure cultures and 14 of 19 (74%) contaminated cultures of *N. gonorrhoeae* were identified correctly.

TABLE 2. Comparison of five test systems for the identification of clinical *N. gonorrhoeae* isolates

System	No. (%) identified correctly[a] in:	
	Pure culture	Contaminated culture[b]
MAb[c]	58 (100)	28 (100)
Minitek	39 (67)	14 (50)
CTA sugars	51 (88)	9 (32)
Neisseria-Stat	56 (97)	27 (96)
Neisseria-Kwik	24 (80)	14 (74)

[a] Species identification was determined by the modified rapid fermentation test of Kellog and Turner (8) as modified by Brown (3). The totals for pure and contaminated culture isolates tested with the MAb, Minitek, CTA sugars, and Neisseria-Stat systems were 58 and 28, respectively, and the totals for pure and contaminated culture isolates tested with the Neisseria-Kwik system were 30 and 19, respectively.

[b] The original chocolate agar plate was found to be contaminated with one or more non-*Neisseria* organisms 48 h after the original plate inoculation and 24 h after the test system had been inoculated.

[c] Fluorescent MAb against *N. gonorrhoeae*.

The identification of *N. meningitidis*, *N. lactamica*, *N. sicca*, *N. subflava*, and *B. catarrhalis* by the four test systems (there were not enough of these isolates to evaluate the Neisseria-Kwik system at the time it was brought into the study) is shown in Table 3 (see footnote *a*, Table 3 for MAb results). The MAb did not identify any of these 50 nongonococcal isolates, since this antiserum displayed no cross-reactivity (no fluorescence) with these isolates.

The Minitek system and CTA sugars identified 96 and 93% of the *N. meningitidis* isolates, respectively. All isolates of *N. lactamica*, *N. sicca*, and *N. subflava* were correctly identified by the Minitek, CTA sugars, and Neisseria-Stat systems. The cost per identification for each of the five test

TABLE 3. Comparison of four test systems for the identification of *Neisseria* species other than *N. gonorrhoeae* and *B. catarrhalis*

Organism (no. of isolates)	No. (%) identified correctly[a] with:		
	Minitek	Neisseria-Stat	CTA sugars
Neisseria meningitidis (28)	27 (96)	28 (100)	26 (93)
Neisseria lactamica (2)	2 (100)	2 (100)	2 (100)
Neisseria sicca (3)	3 (100)	3 (100)	3 (100)
Neisseria subflava (1)	1 (100)	1 (100)	1 (100)
Branhamella catarrhalis (16)[b]	14 (100)	14 (100)	16 (100)

[a] The fluorescent MAb against *N. gonorrhoeae* did not identify any of the other *Neisseria* isolates or *B. catarrhalis* since there was no cross-reactivity with these organisms. Species identification was established by the modified rapid carbohydrate utilization test of Kellog and Turner (8) as modified by Brown (3).

[b] Of the 16 isolates of *B. catarrhalis*, 2 were contaminated with non-*Neisseria* organisms.

TABLE 4. Cost per identification by system

Equipment used	Cost ($) per test[a]:				
	MAb	Minitek[b]	CTA sugars	Neisseria-Stat	Neisseria-Kwik
Chocolate agar plate	0.30	0.30	0.30	0.30	0.30
CTA sugars (4 types)			2.34		
Microscope slide	0.07				
MAb-gonococcal	3.00				
Sterile swab	0.01	0.01		0.01	0.01
Disposable pipette				0.04	0.04
Pipette tips		0.08			
Carbohydrate disks		0.48			
Minitek plates		0.01			
Neisseria broth		0.62			
Disposable inoculating loop	0.03		0.03	0.03	0.03
Neisseria-Kwik tray, cover, and buffer					1.88
Neisseria-Stat reagent pack (4 sugars) and beta-lactamase pack				2.30	
Labor	1.20	1.50	0.90	0.90	1.50

[a] The times required to perform an identification (at a cost of $18.00/h for labor) were 4 min (MAb), 5 min (Minitek), 3 min (Neisseria-Stat), and 5 min (Neisseria-Kwik). The total costs per test were as follows: MAb, $4.61; Minitek, $3.72; CTA sugars, $3.57; Neisseria-Stat, $3.58; and Neisseria-Kwik, $3.76.
[b] For the Minitek system, the use of a humidor, dispenser, and pipettes were not included in the direct cost.

systems is given in Table 4. The derived final cost includes media, reagents, and additional materials required to perform the test, as well as labor based on a rate of $18/h. The final cost per identification for the test systems was found to be $4.61 (MAb), $3.72 (Minitek), $3.57 (CTA sugars), $3.58 (Neisseria-Stat), and $3.76 (Neisseria-Kwik).

DISCUSSION

We evaluated a new, commercially available, mouse MAb against *N. gonorrhoeae*. This system was compared with the standard CTA sugar procedure and with three rapid carbohydrate utilization tests capable of identifying *Neisseria* species. These standard and rapid fermentation systems studied displayed a wide range in their abilities to identify pure and contaminated cultures of *N. gonorrhoeae* (Table 2), with the Neisseria-Stat system clearly being superior, showing 97 and 96% correct identifications for pure and contaminated *N. gonorrhoeae cultures*, respectively.

Although the Minitek system accurately identified *Neisseria* species (>96%) other than *N. gonorrhoeae*, the identification rate of pure *N. gonorrhoeae* isolates was only 67% (Table 3). This discrepancy may be due to a vivid and sharp color change in the maltose-containing tube occurring with *N. meningitidis* isolates, whereas with many gonococcal strains, only a weak or variable change is common with glucose utilization. The resulting uncertainty in interpretation of the glucose reaction and the definitive maltose reaction seen with *N. meningitidis* isolates may partly account for this discrepancy with the Minitek system.

The fluorescein-conjugated MAb appeared to correctly identify all the *N. gonorrhoeae* isolates and was not found to react with any *Neisseria* species except *N. gonorrhoeae*. *N. gonorrhoeae* isolates which were contaminated (with gram-negative rods, enterococci, and viridans group streptococci isolated on the initial chocolate plate the next day) were also easily identified by the MAb. The high specificity seen with the Syva MAb may be partly due to the recognition by this antibody preparation of unique epitopes (the major outer membrane protein antigens IA and IB are recognized by the Syva MAb preparation [personal communication, Jim Darner, Syva Corp.]) present only on *N. gonorrhoeae* organisms.

Although other fluorescent-antibody preparations against *N. gonorrhoeae* may be obtained from certain research institutions such as the Centers for Disease Control (this particular reagent is available for nondiagnostic use only), with the exception of the Syva MAb preparation, only Difco Laboratories has a commercially available product. In contrast to the Syva MAb, the Difco product is a polyclonal rabbit antiserum. In a previous report evaluating this antiserum, Pollock (14) reported an 8% discrepancy rate between the Difco antibody and a rapid carbohydrate utilization system for gonococcal identification. A related paper by Thin et al. (18) showed that the Difco antibody identified 90.5% of *N. gonorrhoeae* culture isolates compared with a standard carbohydrate utilization method. Thin (17) also used this antibody preparation for the detection of gonococci in cervical smears and found a 7.4% disagreement between culture and fluorescent-antibody staining. Cross-reactivity with the Difco antiserum has been documented by Hare (6), who showed a fluorescence of 3+ for this antiserum and *N. meningitidis* groups A, B, C, D, and E. Absorption of this conjugate with group A meningococci removed the cross-reaction, but the resulting conjugate was too weak to detect gonococci.

In conclusion, the Syva MAb offers a rapid (30-min) method for the in vitro identification of *N. gonorrhoeae*, with no apparent cross-reactivity observed with other *Neisseria* species or *B. catarrhalis*. With additional studies, such a reagent may be found suitable for the direct detection of gonococci in clinical specimens.

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