New Selective Medium for the Isolation of Neisseria gonorrhoeae

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GC-Lect, a new selective medium for the isolation of Neisseria gonorrhoeae which contains five antimicrobial agents, was evaluated with stock cultures and with 500 clinical specimens. With stock cultures, vancomycin-resistant Staphylococcus epidermidis that grew on modified Thayer-Martin medium (MTM) was inhibited on the new medium. Also, vancomycin-susceptible strains of N. gonorrhoeae were much less inhibited on the new medium than on Martin-Lewis agar or MTM. With oropharyngeal cultures of healthy volunteers, Capnocytophaga species were frequently isolated on MTM from two of three manufacturers but were completely inhibited on GC-Lect. In the clinical study, visible growth of N. gonorrhoeae occurred within 24 h in 72% of the positive cultures on GC-Lect, compared with only 52% on the reference medium. A total of 50 positive cultures were obtained with GC-Lect, compared with 49 obtained with MTM. The selectivity of GC-Lect was superior, with only 19 cultures producing growth of normal flora, compared with 78 cultures on MTM after 24 h of incubation. The selectivity was especially improved on GC-Lect with regard to yeasts (2 versus 30 cultures) and gram-positive cocci (5 versus 31 cultures).

The isolation of Neisseria gonorrhoeae from rectal, oral, and cervical sites is complicated by the presence of normal flora. Selective media have greatly improved the isolation of N. gonorrhoeae from these sites (7, 8). Martin and Lewis improved selectivity by increasing the vancomycin concentration and using a more stable antifungal agent (4).

Vancomycin-susceptible strains of N. gonorrhoeae may be inhibited by the amount of vancomycin present in conventional selective media (1–3, 5, 6, 8, 10, 11). Such strains may account for as many as 30% of gonococcal isolates in certain geographic areas (11).

Using a unique combination of five antimicrobial agents, a new medium, GC-Lect, has been developed and patented by Becton Dickinson Microbiology Systems, Cockeysville, Md. (BDMS). This medium contains only 2 μg of vancomycin per ml and is designed to produce visible growth of N. gonorrhoeae in 24 h, with better inhibition of normal flora, especially yeasts, gram-positive cocci, and Capnocytophaga species. Both clinical and laboratory studies were conducted to compare the growth and recovery of N. gonorrhoeae on and the selectivity of GC-Lect with those of modified Thayer-Martin medium (MTM).

MATERIALS AND METHODS

Media. BBL brand GC-Lect agar was obtained as a prepared plated medium from BDMS. GC-Lect medium consists of GC II agar base, bovine hemoglobin, IsoViteX enrichment (all from BDMS) and the following selective agents (per liter): lincomycin, 1 mg; vancomycin, 2 mg; colistin, 7.5 mg; trimethoprim, 5 mg; and amphotericin B, 1.5 mg. The reference medium for the clinical study was MTM obtained from Prepared Media Laboratories, Ltd. (PML, Tualatin, Ore.). Laboratory evaluations included MTM from BDMS, Difco Laboratories (Detroit, Mich.), Scott Laboratories, Inc. (Fiskenville, R.I.), GIBCO Laboratories (Madison, Wis.), and Remel (Lenexa, Kans.) and Martin-Lewis and Chocolate II agar media from BDMS.

Test cultures. In addition to American Type Culture Collection (Rockville, Md.) cultures, the following organisms were used in the laboratory study. A penicillinase-producing N. gonorrhoeae (PPNG) strain, no. 454, and a vancomycin-resistant Staphylococcus epidermidis strain (Scotland) were obtained from the North Carolina Health Department. Three vancomycin-susceptible N. gonorrhoeae strains (1185, 2900, and 4844) were obtained from B. W. Catlin, Medical College of Wisconsin. Two fastidious strains of N. gonorrhoeae (8658 and 10062) were obtained from the University of Göteborg, Göteborg, Sweden. All test strains were cultivated on Chocolate II agar (BBL) for 18 to 24 h in 5% CO2 at 35°C. The cultures were maintained as suspensions in Trypticase soy broth (BBL) with 15% glycerol, adjusted to a 0.5 McFarland standard, and stored at −70°C.

Culture methods. For quantitative recovery, cultures of N. gonorrhoeae ATCC 35201, 43069, and 43070 were thawed and diluted to obtain 100 to 300 CFU per 0.1 ml of inoculum. A Minitek System pipette (BDMS) was used to deliver a 0.1-ml inoculum onto the test media. The inoculum was spread evenly over the agar surface with a glass spreader. Plates were incubated in 5% CO2 at 35°C for 18 to 24 h. A Lab-Line Colony Counter (model 1590) was used to enumerate the colonies. Colony size was measured by using a stereomicroscope with an ocular micrometer (American Optical Corp., Buffalo, N.Y.). Colony sizes were scored from + (colonies smaller than 0.1 mm in diameter) to 4 (colonies larger than 1.0 mm) by 0.3-mm increments. Recovery on spread plates was recorded as a percentage of the MTM II (BBL) control (see Table 1, footnote b).

For qualitative recovery, cultures were thawed, 0.01 ml was placed onto each of the test media, and the inoculum was streaked for isolation. Plates were incubated as described above and inspected for growth at both 24 and 48 h. Recovery was then scored (see Table 2, footnote a).

Throat cultures. Throat cultures from 18 healthy volunteers were inoculated on GC-Lect and MTM from various manufacturers by rolling the swab on about one-third of the agar surface and then streaking for isolation. Plates were incubated in 5% CO2 at 35°C. Growth was scored at 24 and 48 h.

Clinical study. A total of 500 specimens were collected
from patients in the Spokane County Health District in Spokane, Wash., over a 2-month period. Sets of plates were directly inoculated from a single swab obtained from a cervical, oral, rectal, or urethral site as indicated by clinical protocols. Each set of media contained one GC-Lect plate and one MTM plate. In 244 sets, the MTM plate was inoculated first, and in 256 sets, the plate was inoculated first. The patients were assigned to the nurses in a random fashion. Inoculated plates were incubated in 5% CO₂ at 35°C within one-half hour of specimen collection.

**Bacterial identification.** Colonies of oxidase-positive, gram-negative diplococci obtained from the primary plates were confirmed as *N. gonorrhoeae* by Direct Fluorescent Antibody Technique (Difco). The Gono-Check II (Dupont Co., Wilmington, Del.) test also was performed on colonies from genital cultures showing less than a strong fluorescence and on all colonies from oral and rectal cultures. All tests were performed according to the instructions of the manufacturer. The growth of normal flora was recorded as to the type and relative number of colonies.

### RESULTS

**Laboratory study.** The colony size and recovery scores for the three ATCC strains of *N. gonorrhoeae* are shown in Table 1. Recovery and colony size, except for strain 35201, were comparable on GC-Lect and MTM from GIBCO, and both were superior to those on MTM from Difco, Scott, and Remel. The colony size of 35201 (an arginine, hypoxanthine, and uracil [AHU] auxotroph) was greatest on GC-Lect. The other two ATCC strains, 43069 and 43070, have been used by the Centers for Disease Control for evaluating MTM for many years and are presumed to be heterotrophic.

Recovery of fastidious strains of *N. gonorrhoeae*, including two from Sweden (8658 and 10062), the PPNG strain (454), and one of the vancomycin-susceptible strains (1185), is shown in Table 2. Recovery of the two Swedish strains was best on GC-Lect, with little or no growth on MTM from three of four manufacturers. Recovery of the PPNG strain was comparable on GC-Lect and on two of the four MTM sources evaluated. The vancomycin-susceptible strain grew only on GC-Lect, although only at the 1+ level. MTM II from BDMS was not evaluated for recovery of *N. gonorrhoeae* in comparison with GC-Lect because both media are manufactured from the same GC agar base and supplements and therefore have identical nutrient properties.

The selectivity of GC-Lect compared with that of MTM from four manufacturers is also shown in Table 2. GC-Lect produced superior inhibition of *Capnocytophaga ochracea*, *Candida albicans*, and the vancomycin-resistant (MIC, 3 to 4 μg/ml) *Staphylococcus epidermidis*.

### TABLE 1. Recovery of *N. gonorrhoeae* on GC-Lect versus recovery on MTM from various manufacturers

<table>
<thead>
<tr>
<th><em>N. gonorrhoeae</em> strain</th>
<th>Colony size* (recovery*) on:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GC-Lect</td>
</tr>
<tr>
<td></td>
<td>Difco</td>
</tr>
<tr>
<td>ATCC 43070</td>
<td>Size 4 (4)</td>
</tr>
<tr>
<td>ATCC 43069</td>
<td>Size 4 (3)</td>
</tr>
<tr>
<td>ATCC 35201</td>
<td>Size 3 (3)</td>
</tr>
</tbody>
</table>

* Colony size: 4, >1.0 mm; 3, 0.7 to 1.0 mm; 2, 0.4 to 0.7 mm; 1, 0.1 to 0.4 mm; +, <0.1 mm.

* Recovery: 4, within 90% of control (BBL MTM II); 3, within 75% of control; 2, within 50% of control; 1, within 25% of control; +, less than 25% of control.

### TABLE 2. Recovery of fastidious *N. gonorrhoeae* and inhibition of normal flora on GC-Lect versus recovery on MTM from various manufacturers

<table>
<thead>
<tr>
<th>Organism type and strain</th>
<th>Growth* on:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>GC-Lect</td>
</tr>
<tr>
<td></td>
<td>Difco</td>
</tr>
</tbody>
</table>

| *N. gonorrhoeae*          | 8658 | 4 | 1 | 1 | 2 | 0 |
| 10062                    | 4 | 0 | 0 | 3 | 0 | 0 |
| 454                      | 3 | 1 | 3 | 3 | 1 | 0 |
| 1185                     | 1 | 0 | 0 | 0 | 0 | 0 |

Normal flora

- *C. ochracea* ATCC 33595: 0 2 2 2 2
- *C. albicans* ATCC 60193: 0 3 3 3 3
- *S. epidermidis*: 0 2 1 1 4

* Growth into quadrant 4; 3, growth into quadrant 3; 2, growth into quadrant 2; 1, growth into quadrant 1; 0, no growth.

* Vancomycin-resistant strain.

The growth of vancomycin-susceptible *N. gonorrhoeae* strains on GC-Lect compared with that on other BBL media is shown in Table 3. Two of the strains failed to grow on MTM II and Martin-Lewis media but produced a 1+ growth on GC-Lect and a 2+ growth on Chocolate II agar. The third strain grew on all four media but showed the least growth on Martin-Lewis agar.

GC-Lect and MTM from BDMS, GIBCO, and Scott were evaluated for isolation of *Capnocytophaga* species from normal throat cultures of 18 volunteers. *Capnocytophaga* species were differentiated from other normal flora on the basis of Gram staining and characteristic (spreading) colonial morphology. No growth of *Capnocytophaga* species occurred on GC-Lect and MTM from GIBCO. Of the 18 cultures, 15 were positive for *Capnocytophaga* species on the MTM II from BDMS, and 11 of 18 were positive on MTM from Scott.

**Clinical study.** The results of the clinical evaluation with 500 specimens are presented in Tables 4 and 5. Table 4 shows the number of *N. gonorrhoeae* isolated relative to incubation time. On GC-Lect, 72% of the positives were detected in 24 h, compared with 52% for MTM. In addition, one isolate was detected only on GC-Lect, which was the second plate inoculated of the set. The GC-Lect plate showed growth throughout the streaked area of the plate. This would be in the too-numerous-to-count or 4+ range. Vancomycin MICs were not determined. In general, the colony size of *N. gonorrhoeae* on GC-Lect at 24 h was equivalent to that on the MTM after 48 h.

After 24 h of incubation, 78 specimens showed growth of normal flora on MTM, compared with only 19 on GC-Lect (Table 5). Yeastlike organisms were isolated from only two...
TABLE 4. Isolation of N. gonorrhoeae from 500 clinical specimens

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>Cumulative no. (%) of positives with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GC-Lect</td>
</tr>
<tr>
<td>24</td>
<td>36 (72)</td>
</tr>
<tr>
<td>48</td>
<td>46 (92)</td>
</tr>
<tr>
<td>72–96</td>
<td>50 (100)</td>
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</tbody>
</table>

* Prepared medium obtained from PML.

specimens on GC-Lect, compared with 30 on MTM. Inhibition of gram-positive cocci was also better, as shown by their isolation from 31 specimens on MTM, compared with only 5 on GC-Lect.

DISCUSSION

Selective media for the isolation of N. gonorrhoeae that are currently available have several drawbacks. The trend in the development of these media has been increased selectivity, resulting in some cases in decreased recovery of N. gonorrhoeae (1, 5, 6).

GC-Lect agar was developed to provide increased selectivity without sacrificing the ability to detect visible growth of N. gonorrhoeae in 24 h, and this was demonstrated in a clinical evaluation with 500 specimens and in a laboratory evaluation with stock cultures.

In the clinical study, 72% of the GC-Lect cultures were positive in 24 h, compared with only 52% of MTM cultures. Since there were no readings on weekends and holidays, the 24-h positive rate may actually have been higher. In the laboratory study, GC-Lect was shown to produce better growth of fastidious N. gonorrhoeae strains than MTM from several manufacturers. In particular, two Swedish strains and an AHU auxotroph (ATCC 35201) grew poorly on the MTM from four manufacturers. The PPNG strain grew poorly on MTM from two of the four manufacturers tested.

For inhibition of gram-negative bacteria, vancomycin has been used almost exclusively in selective media for isolation of N. gonorrhoeae. In New York City medium the vancomycin concentration is 2 μg/ml, in MTM it is 3 μg/ml, and in Martin-Lewis medium it is 4 μg/ml (7). By contrast, GC-Lect uses a combination of 2 μg of vancomycin per ml with 1 μg of lincomycin per ml.

With the emergence of vancomycin-susceptible strains, it has been recommended that a nonselective medium be inoculated in addition to one of the selective types (1, 10). However, it has been reported that gonococci were isolated more frequently on a selective medium than on the same medium without antibiotics (8). Using three strains of vancomycin-susceptible N. gonorrhoeae, GC-Lect agar was shown to be much less inhibitory than MTM or Martin-Lewis agar. New York City medium has been shown to improve the isolation of vancomycin-susceptible N. gonorrhoeae (3). However, this medium is transparent and therefore does not show the characteristic colonial morphology of chocolate-based media, is difficult to prepare, and contains expensive and variable components such as horse serum and dialysate of bakers' yeast (Saccharomyces cerevisiae).

Although GC-Lect agar contains only 2 μg of vancomycin per ml, the combination with lincomycin provides better inhibition of staphylococci and other gram-positive cocci. In the clinical study, only five cultures on GC-Lect agar were positive, compared with 31 on MTM. In the laboratory evaluation, a vancomycin-resistant S. epidermidis was completely inhibited on GC-Lect agar but grew on MTM from all manufacturers.

The inhibition of gram-negative bacteria, except for Proteus species, is accomplished by colistin, and this antibiotic is used at a 7.5-μg/ml concentration in all the media tested. Trimethoprim is used for the inhibition of Proteus species in all of the selective media in concentrations ranging from 3 to 5 μg/ml. In our study, the inhibition of gram-negative rods was somewhat improved on GC-Lect agar, with 12 specimens showing growth (versus 17 with the reference medium).

For the inhibition of yeasts, particularly C. albicans, nystatin is used in Thayer-Martin medium and MTM. This antibiotic is very labile, and some laboratories actually use MTM to isolate Candida species. Martin and Lewis recommended the use of anisomycin for inhibition of yeasts (4). Although this antibiotic is very effective, it is extremely expensive and is currently only used in Martin-Lewis agar (originally called improved Thayer-Martin agar). In the present evaluation, GC-Lect agar, containing amphotericin B, showed excellent inhibition of yeastlike organisms, with only two specimens showing growth (compared with 30 on MTM).

With the increased culture of pharyngeal specimens, the growth of Capnocytophaga species becomes a potential problem in certain geographic areas. Because of its ability to spread over the surface of the plate by using its gliding motility, it can obscure the growth of pathogenic Neisseria species that may be present (9).

In the clinical evaluation, Capnocytophaga species were not isolated on either GC-Lect or the reference MTM. This may be due to the lower nutrient content of the base medium used for the reference MTM. In the laboratory study, Capnocytophaga ochracea ATCC 33595 was shown to grow on MTM produced by four manufacturers, but was completely inhibited on GC-Lect. For healthy volunteers, Capnocytophaga species were isolated from 83.3% on MTM II (BBL) and from 61% on MTM from Scott Laboratories, whereas none were isolated on GC-Lect or MTM from GBCO.

Our results show that GC-Lect offers significant advantages over existing selective media for the isolation of N. gonorrhoeae. It produces more rapid growth of N. gonorrhoeae and better inhibition of normal flora, particularly yeasts, gram-positive cocci, and Capnocytophaga species. A recent study (9) confirmed the improved selectivity of GC-Lect compared with MTM. In that study there was no difference between these media in the time required for isolation of N. gonorrhoeae. However, the MTM used was obtained from BDMS.

LITERATURE CITED

1. Bonin, P., T. T. Tanino, and H. H. Handsfield. 1984. Isolation of Neisseria gonorrhoeae on selective and nonselective media in a...