Evaluation of Granada Agar Plate for Detection of *Streptococcus agalactiae* in Urine Specimens from Pregnant Women

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The Granada agar plate (GAP; Biomedics SL, Madrid, Spain) was evaluated for the detection of group B streptococci (GBS) in urine specimens from pregnant women submitted for testing for asymptomatic bacteriuria and was compared with blood agar (BA [Columbia agar with 5% sheep blood]; bioMérieux, Marcy l’Etoile, France). The GAP detected 103 out of 105 GBS, whereas BA detected only 50. Use of the GAP could be a good method for the detection of GBS in urine specimens from pregnant women.

Group B streptococcus (GBS [*Streptococcus agalactiae]*) is a relevant cause of neonatal infection worldwide. The research on this microorganism in women at the end of pregnancy is based on the fact that women with vaginal and/or rectal colonization have a higher probability of severe perinatal infection (7).

In 2002, the Centers for Disease Control and Prevention (CDC) issued some guidelines for the prevention of perinatal GBS infection. Those guidelines state that the mere presence of GBS in urine, irrespective of its concentration, is a reliable marker of heavy genital colonization (8, 11) with increased risk of premature birth and other obstetric problems (3). Therefore, women with any urine concentration of GBS during pregnancy should receive chemoprophylaxis at labor. In these cases, vaginal or rectal screening in weeks 35 to 37 is not necessary (11).

A urine specimen culture is currently recommended for all pregnant women at the end of the first term or beginning of the second term to detect possible asymptomatic bacteriuria (2, 6). The urine specimen is cultured in a nonselective medium, usually blood agar (BA).

The objective of our study was to evaluate the Granada agar plate (GAP) for the detection of GBS in urine specimens of pregnant women by comparing it with BA. Urine specimens taken routinely to detect asymptomatic bacteriuria were investigated.

From January to August 2003, 834 urine specimens from pregnant women in the Móstoles Hospital area (Madrid, Spain) were received for asymptomatic bacteriuria testing. The urine specimens were plated onto two different media: BA medium (Columbia agar with 5% sheep blood; bioMérieux, Marcy l’Etoile, France) and GAP medium (Biomedics SL, Madrid, Spain). The BA plates were incubated at 37°C for 24 h. By protocol, the urine samples used for detection of asymptomatic bacteriuria in pregnant women were cultured in BA and MacConkey agar in our laboratory. As the main objective of our study is to compare the advantages of the GAP to those of BA by using the same sample to determine the existence of asymptomatic bacteriuria and the presence of GBS, we did not utilize an enrichment medium. Assuming that the use of a broth would yield higher rates of isolation of GBS in urine samples, the GBS carriers would be identified by examination of the vaginal and rectal samples taken in the last days of pregnancy, according to the protocol of the CDC (11).

Beta-hemolytic, catalase-negative colonies grown on BA that showed characteristics consistent with GBS were agglutinated with latex particles coated with anti-GBS antibodies (Oxoid, Basingstoke, Hampshire, England) for identification. Also, urine specimens were plated onto colistin-nalidixic acid agar plates (Columbia agar with 5% sheep blood; bioMérieux), which were read afterwards to determine counts of GBS.

Four urine specimens were plated on each GAP, one per quadrant. The plates were incubated anaerobically at 37°C and were examined for GBS at 24 and 48 h. The GAP contains a differential medium which identifies GBS based on the production of an orange pigment by this microorganism (1). GBS colonies were identified on the basis of pigment production and were later confirmed by anti-GBS antibody agglutination.

The media were assessed by two different individuals. One person observed the presence of GBS in BA, and the other person observed the presence of GBS in the GAP medium. Neither observer knew the other’s results until both had finished the daily evaluation of each medium.

The results obtained with the two media as well as manageability and costs were compared.

GBS was detected with one of the two media in 105 of the 834 urine specimens cultured. The GAP detected 103 of the 105 GBS, whereas the BA detected only 50. Table 1 summarizes the total numbers of true and false positives and true and false negatives detected with each medium and the sensitivity and specificity values for the two culture media.

Comparing the detection rates obtained with the two media indicates that the GAP medium is obviously a good medium for detecting GBS in urine specimens, with a markedly higher sensitivity than that of BA.

The specificity of the GAP (100%) eliminates the need of further latex agglutination confirmation, with corresponding savings of time and money.
One of the main advantages of the GAP is the easy visual identification of GBS (orange-colored colonies) even when the specimen contains a small number of GBS ($10^2$ to $10^3$ or $10^3$ to $10^4$ CFU/ml of urine) or when GBS is mixed with other microorganisms (whitish colonies which usually correspond to enterobacteria or staphylococci), with no specialized personnel being required for its identification.

In only two cases where GBS was present in urine did the GAP test fail to detect it, as no pigmented colonies were observed. In these two cases, the urine specimens were replaced, and this time, growth of GBS was detected. The likely explanation is that the GAP requires some stringent preservation conditions, as it is very sensitive to environmental conditions (at room temperature, the starch in the GAP medium is split by the amylase in horse blood) (9, M. De La Rosa-Fraile, Letter, J. Clin. Microbiol. 41:4007, 2003). Therefore, the initially negative results were probably due to poor preservation of the corresponding GAP plates. For this reason, it is important to control the possible deterioration of this medium by plating a control strain of GBS onto it each day.

All urine specimens that were positive with the GAP were so at 24 h of incubation, except in two cases in which the pigment became visible after 48 h (probably due to medium deterioration). It is our opinion, therefore, that a 24-h incubation is enough.

A disadvantage of the GAP medium is its relatively short shelf life, which is due to the easy degradation of some of its components and its extreme sensitivity to changes in its storage conditions (5). Therefore, the GAP should be used within a short period of time and be adequately refrigerated until use to ensure full efficiency of the test.

A possible disadvantage of the GAP is the need of anaerobiosis for incubation as well as the inherent increased costs in both time and money. Nevertheless, other investigations have confirmed that anaerobiosis is not necessary for pigment production and that the medium is also efficient covered merely with a coverslide (10).

Pigment production requires the colony to be beta-hemolytic (De La Rosa-Fraile, letter). Since approximately 3% of GBS colonies do not produce pigment (4), this pigment will not be produced or readily detected in BA or with the GAP.

Comparing the results obtained with BA and the GAP, the sensitivity of the BA test was clearly lower than that of the GAP. The explanation for the poor results obtained with BA is based on the following:

(i) In urine specimens with low GBS concentrations ($10^2$ to $10^4$ CFU/ml of urine) at identification, the presence of GBS went unnoticed because of the presence of other colonies, such as staphylococcal colonies. Specifically, in our study 30 urine specimens had bacterial counts between $10^2$ and $10^3$ CFU/ml, and in two cases the counts were between $10^3$ and $10^4$ CFU/ml.

(ii) Occasionally, GBS was overgrown by gram-negative rods (usually Escherichia coli), enterococcus, or staphylococcus, which made its detection difficult or impossible. In our study, high counts ($10^4$ to $10^5$ or $>10^5$ CFU/ml) of enterobacteriaceae, enterococci, and staphylococci were observed in 14, 6, and 3 cases, respectively.

(iii) The purpose of this urine culture is to search for potentially asymptomatic bacteriuria (indicated by $>10^4$ CFU/ml of urine in pure culture). The identification of GBS in these urine specimens would require great effort because of the extra time required (all beta-hemolytic colonies with characteristics consistent with GBS should be investigated). This could account for the low-sensitivity results obtained with BA. Compared with the GAP, identification with BA is immediate (the presence of pigmented colonies is enough).

When GBS was present in high concentrations in urine (higher than $10^4$ CFU/ml of urine), the efficiency of testing with BA was similar to that with the GAP.

Comparing costs, the GAP is more expensive than BA ($2.54 versus $0.74 per unit for the GAP and BA, respectively, per the Hardy Diagnostics catalog), but this initial disadvantage can be overcome by using one GAP for four urine specimens (as in our study), and therefore, costs can be significantly reduced.

One relevant advantage of the GAP is that women who had GBS detected in their urine specimens would be spared from further vaginal and/or rectal sampling, as such women would undergo mandatory chemoprophylaxis at labor on the basis of CDC guidelines (11). Therefore, the patient would not undergo further testing, and savings could be obtained in terms of both time and money.

In conclusion, based on sensitivity, specificity, and ease and speed of identification of GBS, the use of the GAP could be a good method for detecting GBS in urine specimens from pregnant women.

REFERENCES

<table>
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<tr>
<th>S. agalactiae detection medium</th>
<th>True positive</th>
<th>False positive</th>
<th>True negative</th>
<th>False negative</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<td>GAP medium</td>
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<td>729</td>
<td>55</td>
<td>47.6</td>
<td>100</td>
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*Values were calculated with the following equations: sensitivity = (number of true positives)/[(number of true positives) + (number of false negatives)] and specificity = (number of true negatives)/[(number of true negatives) + (number of false positives)].
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