Rapid Growth of *Helicobacter pylori*

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The ability of six variants of a new charcoal medium and Skirrow’s medium to grow *Helicobacter pylori* in 3 and 5 days was studied using 20 different strains of *Helicobacter pylori*. The main admixtures for the charcoal media were serum, whole blood, and egg yolk emulsion. For this purpose, serum was significantly better and egg yolk emulsion significantly worse than whole blood. The addition of IsoVitalex resulted in significantly improved growth on the charcoal media. Skirrow’s medium showed very poor performance after three days of incubation and needed a long incubation time.

In the management of *Helicobacter pylori* infections, delay of culture and sensitivity-test results reduce the value of laboratory work. Some reports indicate that certain media may produce visible colonies of *Helicobacter pylori* within three days or less (1–4), but the shortening of incubation time has so far not been an issue in discussions of media preparation.

We have examined the potential for rapid growth of *Helicobacter pylori* using variants of our own nonselective standard charcoal medium (ST) and selective standard charcoal medium (SST). The media contain 2,3,5 triphenyl-tetrazolium-chloride as an indicator. In the present study, the performances of six different modifications of these media are compared with Skirrow’s medium (SM) (Oxoid, UK).

**Materials and Methods.** Six of the media used were variants of our standard medium, which is based on charcoal agar CM119 (Oxoid, UK). The seventh medium was Skirrow’s selective blood-based medium (CM271, SR069E) (Oxoid, UK). Compositions of the different media are shown in Table 1. Serum and blood used in the media were horse serum and laked horse blood.

Twenty consecutive clinical specimens which had first been cultured on ST medium were subcultured on the seven different media using a 0.25 McFarland suspension. One μl of the suspension was spread on each plate. Plates were incubated at 37°C under microaerophilic conditions in airtight plastic jars. All seven plates used for a strain had the same production day, and they were all incubated in the same jar. Plates older than three weeks were not used.

The number and diameter of *Helicobacter pylori* colonies were recorded on days 3 and 5. In order to assign equal value to the growth of all strains regardless of the actual number of colonies, we applied the percentage system previously used by Westblom et al. (1) to calculate relative number of colonies. According to this system, maximum growth (100 %) is defined as the highest colony count seen for each strain on any of the media. All other colony counts are expressed as a percentage of this maximum growth equaling the relative number of colonies.

Culture of *Helicobacter pylori* is done in order to produce a sufficient volume of bacteria for identification and sensitivity tests, so the volume is what should be compared. However, because the volume of bacteria is difficult to assess as colony height is difficult to measure properly, we used the bacteria-covered area as an alternative to volume.

Using average colony diameter and relative number of colonies, the relative size of bacteria-covered area was calculated for each plate. Relative bacteria-covered area of a plate is thus the relative number of colonies (N) multiplied by the area of an average colony (πr²). This variable accounts for the number of colonies as well as their size and is in our view the most useful comprehensive indicator for performance of media.

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Table 2: Contaminated plates in Ethiopia and Norway.

<table>
<thead>
<tr>
<th>Media</th>
<th>Ethiopia (n = 23)</th>
<th>Norway (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) of plates with &gt; 5 contaminated colonies</td>
<td>No. (%) of plates with any contamination</td>
</tr>
<tr>
<td>Blood agar</td>
<td>9 (39)</td>
<td>19 (25)</td>
</tr>
<tr>
<td>Standard</td>
<td>10 (43)</td>
<td>19 (25)</td>
</tr>
<tr>
<td>Selective standard</td>
<td>4 (17)</td>
<td>7 (9)</td>
</tr>
<tr>
<td>Skirrow's</td>
<td>1 (4)</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not determined.

Readings on the different plates were compared with those on the ST medium. A parametric (paired t test) and a nonparametric (Wilcoxon signed rank test) test were used for identification of significant differences, and the Bonferroni method was used as a confirmation test.

The ability of SST medium to inhibit contamination was studied using gastric biopsies from 75 patients in Vestfold Sentralysykehus, Norway, and 23 patients in Sidamo Regional Hospital, Ethiopia. Biopsies were ground with five drops of saline (Ethiopia) or serum bouillon (Norway) and spread on plain blood agar, ST medium, and SST medium, as well as on SM medium in Ethiopia. The bacteria were incubated under microaerophilic conditions for five days.

Results and Discussion. The main results are seen in Table 1. P values were found to be 0.0184 and 0.0084, respectively, for the day 5 diameter on medium using serum (medium V2) and the relative bacteria-covered area on day 3 for medium using blood (medium V1), when the paired t test was applied. With the Bonferroni method, corresponding Pk values were 0.1104 and 0.0504. With the Wilcoxon signed rank test, the P values of the same parameters were 0.0038 and 0.0019, respectively, which indicates Pk values well below 0.05 for both parameters. We therefore regarded them as significantly different from corresponding ST values. For all other parameters marked P or p in Table 1, P values were low enough with both the paired t test and the Wilcoxon signed rank test to make Pk < 0.05 with the Bonferroni method.

On ST medium, 93% of all colonies visible on day 5 could be seen well on day 3, with a mean diameter of 0.42 mm. The addition of IsoVitalex (BBL Microbiology Systems, USA) (medium V1) yielded a significant increase in colony diameter. Other studies have also found IsoVitalex to be beneficial in promoting growth (1, 7).