Technical methods

Table 1 Determination of absorption maxima for the 18 hour Einarson gallocyanin-chromalum (GCA) and the rapid two minute 0-5 g% and 1-0 g% techniques

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>400</th>
<th>410</th>
<th>420</th>
<th>430</th>
<th>440</th>
<th>450</th>
<th>460</th>
<th>470</th>
<th>495</th>
<th>510</th>
<th>530</th>
<th>550</th>
<th>570</th>
<th>620</th>
<th>650</th>
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</thead>
<tbody>
<tr>
<td>18 hour GCA</td>
<td>15</td>
<td>23</td>
<td>26</td>
<td>28</td>
<td>31</td>
<td>31</td>
<td>32</td>
<td>33</td>
<td>37</td>
<td>38</td>
<td>42</td>
<td>43</td>
<td>43</td>
<td>37</td>
<td>28</td>
</tr>
<tr>
<td>0-5 g% 2 min</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>16</td>
<td>18</td>
<td>22</td>
<td>27</td>
<td>36</td>
<td>43</td>
<td>48</td>
<td>46</td>
<td>43</td>
<td>32</td>
</tr>
<tr>
<td>1-0 g% 2 min</td>
<td>8</td>
<td>8</td>
<td>15</td>
<td>15</td>
<td>16</td>
<td>21</td>
<td>25</td>
<td>29</td>
<td>38</td>
<td>48</td>
<td>56</td>
<td>64</td>
<td>54</td>
<td>58</td>
<td>46</td>
</tr>
</tbody>
</table>

Absorption maxima is 550 nm for all three techniques.

Table 2 Relative absorption of 20 nuclei read at 550 nm

<table>
<thead>
<tr>
<th>18 hour control</th>
<th>0-5 g% oxidised gallocyanin 17-5 g% chromic potassium sulphate</th>
<th>1-0 g% oxidised gallocyanin 17-5 g% chromic potassium sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minutes</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Mean</td>
<td>43.2</td>
<td>53.35</td>
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<td>SD</td>
<td>6.42</td>
<td>5.99</td>
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<tr>
<td>SEM</td>
<td>1.43</td>
<td>1.43</td>
</tr>
</tbody>
</table>

References


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Letters to the Editor

A fluorescent technique for demonstrating the chromatoid bodies and nuclei in cysts of Entamoeba histolytica from faecal deposits

The diagnosis of Entamoeba histolytica cysts depends on the observed criteria of size, number of nuclei and presence of chromatoid bodies (chromidial bar) which have blunt rounded ends. Whilst all of these may be clearly seen in freshly prepared deposits of faeces, samples of several days old may contain granular cysts, making the diagnosis more difficult.

Acridine orange can be used to stain the relevant DNA/RNA components of a cyst which when viewed under UV light show the chromidial bars as bright orange or green structures, and stain the nuclei green. This staining technique represents a useful alternative to Sargeant’s stain, as the cysts and their chromatid bars are readily visible on scanning the preparation under low power. This technique requires only 30 min staining time, the other staining techniques require the material to be stained for 24 h or longer for ideal results. It also gives satisfactory results on saline suspension of unconcentrated faeces, unlike the method of Sargeant.

The staining method is a modified version of that of Bertalanffy and Bickis, and is prepared as follows:

Stock solution: 0-1% acridine orange in distilled water.

For use: 1 ml stock acridine orange solution

- 0-5 ml acetic acid
- 8-5 ml Krebs-Ringer solution

This provides a working solution of pH below 4 (The actual pH is not critical providing it is below pH 4-5).

Technique: Faeces may be fresh suspension or concentrated by the standard formalin-ether technique.

An equal volume (approx two drops) of working acridine orange stain is added to the deposit and mixed well. After 30 min or longer at room temperature a drop of the deposit is placed on a slide with a coverslip and examined under a mercury vapour UV light source using a BG12 filter or an iodine quartz epifluorescent UV source, using a high power dark ground condenser to provide a black background.

The cysts are seen staining pale green with the chromatid bars staining bright orange or green and the nuclei stain green, against a black background. (No loss of
Cyst of Entamoeba histolytica stained with acridine orange. Tungsten illumination using dark ground condenser. ×400

fluorescence has been observed several weeks after preparation).

It is possible to use the high power dark ground condenser and a tungsten light source as the stain enhances the visual effect of the chromidal bar within the cyst.

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Bacteriologica! examination of removed cerebrospinal fluid shunts

This paper by Dr Bayston and his colleagues has been read here with interest and their method (A) should prove adequate as a groundplan for laboratories embarking on the detailed examination of internal prosthetic shunts, Hickman lines and similar devices suspected to be colonised. METHOD A resembles in many details the routine procedure adopted in this Hospital in 1969, since when some 600 complete ventriculo-caval shunts have been examined.

The understandable clinical euphoria which followed the successful implantation and function of ventriculo-caval shunts in 1957 had somewhat abated by 1960. By this time the author had examined several shunt systems “infected” by coagulase-negative staphylococci almost always accompanied by bacteraemia, and often by ventriculitis, caused apparently by the same organism. The investigational procedures were then relatively crude, none of us had had previous experience of such colonisation, and the author regrets that it was not until 1968 that he devised a more critical and searching method, early results of which were reported in 1970.

The complete shunt is placed in a sterile 6” (15-2 cm) Petri dish and sent immediately to the laboratory; it is first examined macroscopically and by plate microscope before the dish lid is opened. Any external pus or detritus is sampled separately with a swab moistened in sterile broth, and in all cases the whole length of the exterior of the shunt is similarly swabbed. Whenever practicable, the surgeon is encouraged to swab the tissue track immediately after shunt removal with a fine wire swab. The surface of each component of the shunt system is thoroughly treated with an iso-propanol swab and fluid aspirated from that site with a fine gauge needle and syringe. It is often necessary to flush the interior of the shunt with about 0.2 ml of sterile infusion broth. In most cases a sample of ventricular fluid, not taken through the proximal catheter, is collected, together with blood culture bottles inoculated at the time of surgery. All swabs and fluid are cultured aerobically and anaerobically onto blood-agar plates, onto MacConkey plates, and most of the residue is cultured in aerobic and anaerobic broth medium. A potent β-lactamase, either by Whatman or Merck, is added to all cultures when a β-lactam antibiotic has been administered to the patient. The β-lactamase is itself tested for sterility. A Gram film is made of each sample. The MacConkey plate is often valuable in distinguishing two or even three biotypes of Staphylococcus albus which may occasionally be concurrently present. All culture and subcultures are incubated for at least 7 days.

Finally, any thrombus or concretion, usually in the shunt lumen or round the tip of the distal catheter, is cut out, fixed in formal saline and thin sections prepared; these are stained by a conventional cytological method and by a histological Gram’s stain.

Dr Bayston et al are so very right when they point out the twin risks of “mixed infection” and of overdiagnosis in what they term the “conventional” method used in some inexperienced or unthinking departments, and they are to be thanked for demonstrating this so effectively.

It is a pleasure to acknowledge the high technical skills of Mr CH Frankcombe, who has shared the shunt investigations in this Hospital, and as always the unstinting support of our paediatric surgeons, Mr HB Eckstein and Mr DM Forrest and their surgical teams.

Reference


