Selective medium for isolating Arcanobacterium haemolyticum

*Arcanobacterium haemolyticum* is a facultatively anaerobic Gram positive bacillus previously known as *Corynebacterium haemolyticum*. It is most commonly isolated from the upper respiratory tract of patients with pharyngitis, but has also been isolated from skin lesions and occasionally from systemic infections. Isolation of *A haemolyticum* from healthy subjects is rare. The only medium previously described for the isolation of *A haemolyticum* is an enriched agar containing human blood or horse blood. After 48 hours of incubation on this medium *A haemolyticum* produces colonies which, characteristically, have a central pit and are surrounded by a zone of complete haemolysis. Despite the use of enriched media, isolation of *A haemolyticum* can be difficult as the organism is slow growing and is easily masked by commensal flora. The organism may therefore be a more common cause of pharyngitis than is currently recognised. We have developed a selective medium suitable for its isolation.

A study of the antimicrobial susceptibilities of *A haemolyticum* we found that all 26 strains examined were resistant to mupirocin (minimum inhibitory concentrations > 128 mg/l). Mupirocin is highly active against commensal staphylococci and streptococci. Astremon and amphotericin B were used to inhibit the growth of Gram negative bacteria and yeasts, respectively. The complete medium consisted of a blood agar base (Oxoid No 2) containing 5% horse blood, 8 mg/l mupirocin (Beecham), 4 mg/l astremon (Squibb) and 1 mg/l amphotericin B (Sigma).

Strains from the National Collection of Type Cultures and clinical isolates of *A haemolyticum* grew well on the selective medium and produced characteristic colonies with narrow zones of complete haemolysis and a central pit. The efficacy of this medium for the isolation of *A haemolyticum* from clinical specimens was investigated during February and March 1989. All throat swabs received by Chelmsford Public Health Laboratory were inoculated on to the selective medium and on to conventional horse blood agar. The inoculated media were incubated for 48 hours at 37°C in an anaerobic atmosphere containing 10% carbon dioxide. Both media were examined for characteristic colonies of *A haemolyticum*. Identification was confirmed biochemically.

A haemolyticum was isolated from nine of 673 specimens (table). Isolation was much better with the selective medium as only two of the nine isolates were obtained on conventional blood agar. The selective medium greatly reduced the growth of commensal organisms, thus permitting easier recognition of *A haemolyticum*. In the group aged 11–20 years the organism was isolated from eight (6.3%) of the 126 specimens. A similar specific age association has been noted by others. The selective medium would therefore be of most value for the culture of throat swabs from teenagers or young adults. Lancefield group A streptococci were isolated from 18 (14.3%) of the 126 specimens from patients aged 11–20 years, so *A haemolyticum* seems to be a relatively important pathogen in this age group.

Erythromycin is the antibiotic of choice for treatment. With the recent concern over erythromycin resistance in Lancefield group A streptococci, however, it may not be the initial choice for the empirical treatment of pharyngitis. Precise identification of the infecting organism would seem desirable. Use of a selective medium such as that described would clearly facilitate recognition.

**Isolation of Arcanobacterium haemolyticum from throat swabs of patients in various age ranges**

<table>
<thead>
<tr>
<th>Age range (years)</th>
<th>Number of specimens</th>
<th>Number of isolates</th>
<th>Blood agar</th>
<th>Selective medium</th>
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<tr>
<td>0-10</td>
<td>215</td>
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<td>11-20</td>
<td>126</td>
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<td>21-30</td>
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<td>&gt;31</td>
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<tr>
<td>Unknown</td>
<td>65</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Total</td>
<td>673</td>
<td>2</td>
<td>9</td>
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Colorimetric determination of human albumin

Many methods, based on a variety of principles, have been described for the measurement of human albumin. Increases in serum albumin are almost exclusively due to dehydration. Decreases are seen in patients with (i) excessive protein loss due to kidney damage or severe haemorrhage or burns, (ii) impaired or genetic disorders, (iii) inadequate protein intake seen in malnutrition and (iv) gastrointestinal disorders with malabsorption, vomiting, or diarrhoea (before dehydration).

Methods for the quantitative measurement of serum or plasma albumin fall into four categories—namely, salt fractionation, electrophoresis, dye-binding and immununochemical. Dye-binding techniques are currently the most widely used in routine clinical chemistry as they are readily automated, inexpensive, simple and give reproducible results. Data from the United Kingdom External Quality Assurance Scheme for General Clinical Chemistry indicated that 98% of participating laboratories measure serum albumin by either bromocresol green (BCG) or bromocresol purple (BCP) dye-binding methods; of these, BCG methods remain the most widely used. A lack of specificity of BCG for albumin, however, has led the International Federation of Clinical Chemistry Expert Panel on Proteins' and other authors 4-7 to recommend that the method should only be used for screening purposes. It has been reported that not only does BCG overestimate low albumin concentrations, but that it is also underestimated concentrations in the high normal range.

The following assay (patent-pending) uses the specificity of the reduction of BSPT (2- (2'-benzoethiazolylo)-5-styryl-phthalidyl)-tetrazolium chloride to its coloured formazan, in the presence of a reducing agent, electron carrier, and human albumin and benefits from simple colorimetric detection.

To 50 ul serum add 1 ml buffered colour reagent containing 0.1% (w/v) thyroxin-N-methyl phenazine methyl sulphate, and dithio-threitol. After incubation for two minutes at room temperature the absorbance is read at 590 nm.

The procedure is linear over the albumin range 1–80 mg/ml. No interference was found with transferrin, bilirubin, or heparin and the method compared well with traditional dye- binding and immununochemical assays.

<table>
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<tr>
<th>BENSON</th>
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<td>Jones CE</td>
<td>HAMMOND</td>
<td>Diagnostic Enzymology Group,</td>
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<td>P</td>
<td>F</td>
<td>PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury,</td>
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**MATTERS ARISING**

Throat culture from patients with meningococcal meningitis

Cartwright and Jones suggest that throat culture can be useful in the diagnosis of meningococcal meningitis when the patients have taken antibiotics before admission into hospital. This approach seems valid in the

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light of the inability of most of the antibiotics that are used for the treatment of meningococcal infections to serve as prophylactic agents. We performed throat cultures on various populations in Cairo, Egypt, where group A meningococcal disease is endemic. Most cases occur in school-age children, a population that we found had a 3.8%, carrier rate. Only one of the 58 patients positive by culture of cerebrospinal fluid for agents other than Neisseria was a group A meningococcal carrier. Group A meningococci, however, were isolated from 55%, of 380 patients who were culture positive for this organism and from 30%, of 46 patients who were culture negative but shown to have meningococcal meningitis by stain or detection of specific antigen in cerebrospinal fluid.

We therefore concur that culture of patients’ throats can contribute to laboratory diagnosis. Jewes et al argued that culturing the throats of contacts was not useful for diagnosis due to a lack of correlation in serotype between isolates from contacts and index cases.1 We found that the rate of group A carriage in the contacts of group A patients (15%) was four times that in school children, suggesting that monitoring this population could also be helpful in diagnosis of cases.

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Lamina propia mast cells in ulcerative colitis

We were interested to see the response from Dr Crow to our paper on mast cells and eosinophils in Asian and Caucasian patients with ulcerative colitis1 and would agree that formalin fixed material is not ideally suited to demonstration of mast cells. In our experience, however, carefully controlled use of the Astra blue technique is at least generally acceptable in this context.1 It should, of course, be remembered that our study was of a comparative rather than absolute enumerative type and the probable lowering of counts for both groups would therefore still clearly show differences between them where there is no reason to presume that staining would differ between the groups. It might be of interest to run other techniques on our tissues, and if time permits we will consider this.


Dr Crow comments

Benfield et al found that there was no significant difference in the numbers of rectal mucosal mast cells between groups of Asian and Caucasian patients with ulcerative colitis. Unfortunately, the Astra blue technique used to stain the mast cells in this case would have seriously underestimated the numbers of such cells in intestinal mucosa fixed in formalin-saline and any differences which might be present would be masked. If there is only formalin fixed material available for study then the long (five to seven day) toluidine blue or tryptan toluidine blue techniques will at least partly overcome the blockage to staining induced by formalin and will give a more realistic count. Evidence from other tissues, however, suggests that fixation in basic lead phosphate, isotonic phosphate solution, or Carnoy’s fixative followed by long toluidine blue staining will show up even more mast cells and hence even this staining technique must be regarded as doubtful in formalin-fixed tissues unless it has been validated against one of the mast cell fixatives mentioned, for the tissue in question.


BOOK REVIEWS


Peter Millard’s book is eye-catching and has a text clearly laid out and supported by largely excellent photographs with splendid diagrams and line drawings. Many pathology books present the subject in a too detailed and boring manner but this is clearly not Millard’s style. The first impressions, therefore, is that this book is clearly going to be a hit. Reluctantly, after using it for several weeks I think it a splendid effort but, nevertheless, still a miss.


This book, by two experienced American gastrointestinal pathologists, sets out to offer information on all aspects of gastric and duodenal disease including histological, epidemiological, clinical, and pathophysiological data, with the emphasis on diagnostic and microscopic pathology. The coupling of stomach with duodenum was decided because of the common pathophysiology of some gastric and duodenal diseases, such as peptic ulcer disease. The exclusion of oesophageal disease seems somewhat arbitrary, therefore, as the principle of common pathophysiology would also seem to apply. The participation by one of the authors in a previously published monograph on this subject is the probable explanation.

The book succeeds in some of its aims and in particular the chapters on anomalies, hyperplasias, and benign epithelial tumours and carcinoid (neuroendocrine) tumours were very good and well referenced. On the debit side there was little current information on Campylobacter pylori and this discussion of malignant lymphomas was largely on the basis of the Rappaport classification. There were a number of typographical errors and the quality of many of the illustrations, particularly the photomicrographs, was poor.

In summary, while good in parts, this book does not stand out in a competitive marketplace. It is also expensive.

DW DAY
Throat culture from patients with meningococcal meningitis.

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