Letters to the Editor

Is enrichment culture necessary for isolating Campylobacter jejuni from faeces?

There has been debate in your columns previously as to the necessity of including an enrichment stage when culturing human faecal samples for "thermophilic" Campylobacters. Between May and October 1985 19 of the 21 microbiology laboratories in Wales participated in a study under the auspices of the Welsh Standing Specialist Advisory Group in Microbiology to investigate this need.

Each laboratory used its own selective technique for both primary isolation and subculture after enrichment. Enrichment was performed as described previously. Only two of the participating laboratories used identical cultural techniques.

A total of 16 837 samples were cultured. One thousand and forty four (6.2%), range from 1.8-11.5%) yielded Campylobacters on direct culture and a further 166 after enrichment, representing an overall increase in yield on enrichment of 15.9%. The increase varied considerably between laboratories, from 3-2% in one to 85.7% in another. The laboratory which obtained the highest increase used commercially supplied ready poured plates for selective isolation. This observation led to a subsequent study comparing a ready poured medium with an identical freshly prepared one. Seven positive isolates were detected on the freshly prepared medium. Two of these failed to grow on the ready poured medium, and the remaining five grew less profusely.

Clearly, many variations in methodology exist in our region and this may be reflected nationwide. Furthermore, our results suggest that the different selective techniques are not all equally efficient. While enrichment may not be required for specimens cultured by optimal methods it would seem to be necessary in many cases in practice.

Doubt still exists as to the optimal selective technique for the isolation of Campylobacters from human faecal samples. Various workers have reported promising results with blood free media. Bolton et al have also been reassessing filtration methods. Until this question is resolved our experience suggests that microbiologists should continue to assess the need for an enrichment stage in the context of their own laboratories.

Before this investigation was undertaken just three laboratories in Wales routinely used an enrichment procedure while two others used it only on selected samples: as a result of the study a further 11 laboratories introduced it into their laboratory routine.

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Participating laboratories (Welsh Standing Specialist Advisory Group in Microbiology)

Royal Infirmary, Cardiff; University Hospital of Wales, Cardiff; Public Health Laboratory, Cardiff; Llandough Hospital, Penarth; East Glamorgan Hospital, Near Pontypridd; Bridgend General Hospital; Caerphilly District Minors Hospital; Prince Charles Hospital, Merthyr Tydfil; Singleton Hospital, Swansea; Neath General Hospital; Morriston Hospital, Swansea; Withybush Hospital, Haverfordwest; Bron glais Hospital, Aberystwyth; Ysbyty Gwynedd, Bangor; Ysbyty Glan Clwyd, Near Rhyd; Maelor Hospital, Wrexham; Public Health Laboratory, Swansea; Nevill Hall Hospital, Abergavenny; Llanelli General Hospital.

False positive results in the Toxoreagent test for Toxoplasma gondii in immunocompetent patients

The latex agglutination test (Toxoreagent, Eiken Chemical Company) is used in many laboratories to screen serum samples for antibodies to Toxoplasma gondii and has been claimed to give results comparable with those of the dye test when assessed qualitatively. The dye test is recognised as the serological reference test for toxoplasmosis. As a toxoplasma reference centre we are aware of positive results being reported in the latex agglutination test which cannot be confirmed by the dye test. It has also been reported that false positive latex agglutination test results may be associated with a rise in cytomegalovirus (CMV) specific IgM in heart transplant recipients.

We looked for false positive latex agglutination test results in sera from 552 non-immunocompromised patients with various clinical symptoms considered to be compatible with toxoplasmosis but no evidence that the immune system was depressed. Fifty four samples were referred as latex agglutination test positive but were negative when run in the dye test (titre < 8). The latex agglutination test reaction was confirmed in only five (9.3%) sera, while two (22.2%) sera reacted with unsensitised latex. A further 498 sera were tested in the latex agglutination test only after they had been shown to be dye test negative. Two of these samples gave a false positive latex agglutination test result, an incidence of 0.4%, although two further sera reacted with unsensitised latex. In total, serum from seven patients gave false positive latex agglutination test results, with titres of 32 to 64. Of these sera, four (57.1%) reacted in the CMV-IgM ELISA. None of the serum samples reacting in the CMV IgM ELISA had T gondii specific IgM when tested by ELISA.

These data indicate that the false positive results previously found in heart transplant patients with acute CMV infection can also be found in other non-immunocompromised patients. In transplant recipients the reaction seems to be IgM mediated but not associated directly with CMV specific IgM nor rheumatoid factor.

More than 49 of 54 (90%) of the sera submitted as being latex positive (= > 32) but found to be dye test negative were negative when retested in the latex agglutination test at the reference centre. The data suggest that there is some variation between laboratories in the way that this assay is performed and read. Adsorption with unsensitised latex will reduce but not remove the

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problem, although unsensitised latex is not provided in the kit. Positive latex results should be confirmed in the dye test and current CMV infection excluded in those patients whose serum samples give false positive results in the latex agglutination test. This is important as clinical manifestations of both CMV and toxoplasmosis have many similar features.

Further work to identify the antigens recognised by sera giving false positive reactions is in hand, using the Western blot technique.

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References

Limited value of AgNOR enumeration in assessment of thyroid neoplasms

Argyrophilic nucleolar organising region-associated proteins (AgNORs) have recently been shown to be of interest in a variety of different organs and disease states, including lymphomas, melanocytic lesions of the skin, and pleural mesothelioma. In these and other cases the enumeration of nucleolar structures has been shown to be of diagnostic value in differentiating benign from malignant disease, or in distinguishing between low and high grade malignancy. Accordingly, a study of AgNORs in thyroid tissue was undertaken to see if the technique could distinguish between benign and malignant lesions, particularly follicular neoplasms.

Thirty three specimens were examined and these included anaplastic carcinoma (n = 4), follicular carcinoma (n = 4), papillary carcinoma (n = 6), follicular adenoma (n = 6), and nodular colloid goitre (n = 13). The usual one step silver colloid reaction was run at room temperature for 35 minutes. Intra-cytoplasmic dots of silver deposit were counted in 100 cells. Counting was difficult in papillary carcinomas because there were clear nuclei with often only one large intranuclear deposit, presumably corresponding to the nucleolus. There was, however, consistency in all categories within cases.

The results are expressed in the figure.

Figure Scattergram showing mean number of AgNOR counts for each case in anaplastic, follicular, and papillary carcinoma and for adenoma and colloid goitre.

There was separation of AgNOR counts between anaplastic and both papillary and follicular carcinomas. The x2 test was used to assess significance between the pooled means of anaplastic carcinoma and both follicular and papillary carcinomas (p < 0.05). A similar difference was found between anaplastic carcinoma and adenoma (p < 0.05) and a more significant difference (p < 0.02) between follicular and papillary carcinoma and also papillary carcinoma and colloid goitre. There was also a considerable overlap between all carcinomas and colloid goitre. No other pairings showed a significant difference, particularly follicular adenoma and carcinoma. These findings are not as clear cut as in other studies where a clear distinction was obtained between high and low grade lymphomas, benign and malignant melanocytic lesions, and reactive mesothelium and mesotheliomas.

NORs are loops of ribosomal RNA and are therefore important in protein synthesis. It may be that follicular cells are in variable stages of proliferation or protein synthetic activity in both benign and malignant conditions. Certainly in the study of other endocrine tissues such as breast and prostate by means of the AgNOR method, discrimination between benign and malignant tissue has been relatively disappointing. This may be, in part, the result of rRNA gene amplification in stimulated non-malignant endocrine cells, leading to increased gene copies and hence higher NOR numbers than in “resting” cells. AgNORs have therefore shown little value in differentiating between benign and malignant follicular neoplasms in view of the “overlap” in numbers between these groups.

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References
4 Anonymous. NORs—a new method for the pathologist [Editorial]. Lancet 1987;i:1415.4

Coulter Plus IV leucocyte volume analysis instrument: sensitivity of blast identification in peripheral blood

Previous reports evaluating the clinical usefulness of the three population differential have examined various diseases. We studied the sensitivity of the Coulter automated differential in identifying blast cells in peripheral blood samples.
**Notices**

**Methods of Immunological Research and Diagnosis**
(An at-the-bench laboratory training programme).

The Ernest Witebsky Center for Immunology, University at Buffalo, State University of New York.

A two week program consisting of daily practical laboratory exercises personally carried out by participants, accompanied by lectures, demonstrations and discussions.

Contact James F Mohn, Director, The Ernest Witebsky Center for Immunology, 234 Sherman Hall, Buffalo, New York 14214.

Inquiries should be received by 10 March 1989 to ensure consideration for acceptance.

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**Corrections**

The letter: False positive results in the Toxoreagent test for *Toxoplasma gondii* in immunocompetent patients (*J Clin Pathol* 1988;41:1135–6) should have indicated the first author to have been JK Wood. We apologise for this oversight.

Similarly, an error was made in the legend to Fig. 1a in the laboratory technique: Detection of cytomegalovirus by in situ hybridisation and immunohistochemistry using new monoclonal antibody CLH2 (*J Clin Pathol* 1988;41:1005–9). The legend should have read: Detection of CMV infected cells by in situ hybridisation in a lung section.

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**FUTURE IMLS SYMPOSIA**

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<tr>
<td>4 March 1989 Southampton</td>
<td>Conception to cradle—fetal and neonatal diagnosis and monitoring</td>
<td>Dr D McLellan, School of Pharmacy, Portsmouth Polytechnic, Park Building, King Henry I Street, Portsmouth PO11 2DZ.</td>
</tr>
<tr>
<td>18 March 1989 Cambridge</td>
<td>Special coagulation review—educational seminar</td>
<td>Mr D M Reardon, Haematology Department, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QO.</td>
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<tr>
<td>March 1989 Nottingham</td>
<td>Microbiology/virology</td>
<td>Mr A Pawley, Virology Department, PHL, Queens Medical Centre, University Hospital, Nottingham.</td>
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<tr>
<td>8 April 1989 Sheffield</td>
<td>Haematology—in a lighter vein</td>
<td>Mr N R Porter, Haematology, Floor H, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF.</td>
</tr>
<tr>
<td>26 April 1989 Liverpool</td>
<td>Laboratory developments in paediatric biochemistry</td>
<td>Mr D Kilshaw, Pathology Department, Arrowe Park Hospital, Upton, Wirral, Merseyside L49 3PE.</td>
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<tr>
<td>2–9 September 1989 Coventry</td>
<td>IMLS Triennial Conference</td>
<td>Mr C G Smith, Pathology Department, Dudley Road Hospital, Summerfield, Birmingham B12 7QH.</td>
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<tr>
<td>17 March 1990 Cambridge</td>
<td>Special red cell review—educational seminar</td>
<td>Mr D M Reardon, Haematology Department, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QO.</td>
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<tr>
<td>30–31 March 1990 York</td>
<td>Microbiology/histology/cytology/immunology</td>
<td>Mr B Jones, Pathology Department, Royal Halifax Infirmary, Free School Lane, Halifax HX1 2YP.</td>
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<tr>
<td>April 1990 Durham</td>
<td>Blood Group Serology '90</td>
<td>Miss D L Trattles, Blood Transfusion Laboratory, Department of Clinical Pathology, General Hospital, Ayresome Green Lane, Middlesbrough, Cleveland TS5 5AZ.</td>
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<tr>
<td>13–16 September 1990 Sheffield</td>
<td>Microbe '90</td>
<td>Mr C J P Brazier, Public Health Laboratory, Northern General Hospital, Herries Road, Sheffield S3 7AU.</td>
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<td>September 1990 Birmingham</td>
<td>Cellular pathology</td>
<td>Miss L Grosvenor, Histopathology Department, General Hospital, Steelhouse Lane, Birmingham B4 6NH.</td>
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<tr>
<td>April 1991 Durham</td>
<td>Microbiology</td>
<td>Mr G P Hedley, Regional Transfusion Centre, Holland Drive, Barrack Road, Newcastle upon Tyne NE2 4NG.</td>
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<td>April 1991 Sheffield</td>
<td>Haematology—in a different vein</td>
<td>Mr N R Porter, Haematology, Floor H, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF.</td>
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<tr>
<td>September 1991 Birmingham</td>
<td>Microbiology</td>
<td>Mr T Johnson, Microbiology Department, Selly Oak Hospital, Raddlebarn Road, Selly Oak, Birmingham B29 6JD.</td>
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**September 1992**

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