Immunoperoxidase method for detection of immunoglobulins

We have appreciated the letter by Sells and Burton in this Journal (1980;33:98).

We agree that the sections lift and are lost during the immunoperoxidase reaction in spite of the use of adhesives such as amilpectin, albumin, and chrome alum. Furthermore, in our experience, it is very difficult to keep the sections on the slides when an ar-gyrophilic method is used after bleaching for melanin or employing Lee’s method for oestrogen receptors (Cancer 1979;44:1).

We have developed an adhesive which is very simple to prepare and is also resistant to proteolytic digestion. It consists of a polyurethane adhesive (Quick set, USM Chemical SpA, Milan) which is formed in two parts; 0.3 ml of each part (1+2) is diluted in separate tubes with 20 ml of acetone. The two solutions are mixed together when needed, and the slides are immersed in the freshly made solution. The adhesive dries immediately, and the sections stick without any further treatment. The mixed solution keeps for 2 hours at room temperature and for 4 hours at 4°C.

All possible staining procedures can be performed on these sections without any obvious interference by the adhesive.

Letters to the Editors

β-lactamase production by Campylobacter jejuni

There is very little information now available about the incidence of β-lactamase producing strains of Campylobacter jejuni among human isolates. Severin1 in Holland reported β-lactamase production by 57 (92%) of 62 human isolates by the chromogenic cephalosporin method. We therefore decided to undertake a retrospective study of the incidence of β-lactamase production among strains of C. jejuni isolated over a 12-month period from human stools. Two simple laboratory methods were used, the starch-iodine paper2 and the chromogenic cephalosporin substrate.3 In addition, minimum inhibitory concentrations (MIC) of ampicillin for each of the strains were determined.

Identical strains isolated from family outbreaks were included only once. A total of 76 strains of C. jejuni were studied, of which 73 (96%) had been stored in liquid nitrogen for varying periods not exceeding one year. Strains were inoculated on to Oxoid blood agar base with 7% lysed horse blood and incubated under appropriate conditions.4

Minimum inhibitory concentrations of ampicillin for all 76 strains were determined by 2 μl spot-inoculation on Oxoid Diagnostic Sensitivity Test agar incorporating doubling dilutions of ampicillin. These plates were then incubated for 48 hours under identical conditions. The inoculum was derived from an overnight culture of the test strain suspended in Bloodgrow (Medical Wire and Equipment Co (Bath) Ltd, Potley, Corsham, Wilts, UK) and incubated in 10% CO₂ atmosphere at 37°C and diluted so that there were 10⁹ to 10¹⁰ organisms/ml.

Of the 76 strains of C. jejuni tested for β-lactamase production, four (5.3%) gave positive results; identical results were obtained by both methods. The Figure shows the MIC of ampicillin of 72 strains was 25-0 μg/ml or less; all these strains were negative when tested for β-lactamase. The MIC of the four β-lactamase producing strains was 50-0 μg/ml or more.

Our finding of an incidence of 5-3% from human stools differs markedly from the 92% of Severin’s study. There may thus be a much higher prevalence of β-lactamase producing strains of C. jejuni in Holland. Severin also reports that 30 (48%) of 62 strains were sensitive to 5-0 μg ampicillin/ml, this figure being similar to our own findings, but sensitivities to higher concentrations of ampicillin were not given.

We noticed that it was difficult to interpret the colour change with the chromogenic cephalosporin substrate method because of the pigmented nature of the colonies when picked off agar.
medium containing blood. For this reason we preferred to test our strains after growth on blood agar base. Precise details of Severin’s chromogenic cephalosporin substrate method were not stated. To the best of our knowledge, antibiotic therapy had not been given to the four patients from whom β-lactamase producing strains were isolated. Clearly, the possibility of transfer of plasmids determining β-lactamase production between campylobacters and other intestinal bacteria deserves consideration.

EP WRIGHT
Public Health Laboratory, Luton and Dunstable Hospital, Lewsey Road, Luton LU4 0DZ
MARGARET A KNOWLES
Public Health Laboratory, Fazakerley Hospital, Liverpool L9 7AL

The Coulter Counter Model S. It has been our experience, in an unpublished study comparing 1500 duplicate pairs of haematocrit values done by the micro-haematocrit technique and by the Coulter Counter Model S, that the micro-haematocrit was one unit lower than the haematocrit as determined by the Coulter Counter Model S. We feel that this is probably due to excess EDTA, which overcompensates for the increased PCV created by excess plasma.

JOHN V PETRUCCI
Department of Pathology, School of Medical Technology, Mercy Hospital Inc, 301 Saint Paul Place, Baltimore, Md. 21202, USA

References

Reference standard for packed cell volume
In a recent issue (J Clin Pathol 1980;33:1) the International Committee for Standardization in Haematology presents a recommendation for a reference method for determining packed cell volume (PCV) of blood. I feel that there has been a significant omission in that the committee does not specify the proportion and type of anticoagulant to be used. Although trapped plasma may increase PCV, the effect of the anticoagulant may offset this by decreasing the volume of the individual red cell.

Brittin et al.1 studied the effect of excess disodium EDTA and demonstrated that excess EDTA shrinks red cells in proportion to the excessive concentration of anticoagulant. However, this error, due to excess anticoagulant, was not produced when the haematocrit was determined by

Over the period 25 January to 14 February 1980 in one rehabilitation ward of the geriatric service, 10 out of 14 women and 2 out of 4 men developed diarrhoea, accompanied in some cases by vomiting. The majority of patients on this ward occupy single rooms but there is a common day area. Three female members of staff also developed diarrhoea.

The average age (± SD) of the 12 symptomatic patients was 85.1 (± 6.7) years. Stool specimens from 11 of them were examined and salmonella, shigella, campylobacter, and enteropathogenic Escherichia coli were not isolated. Rotavirus particles were, however, seen on electron microscopy in 5 of the 11 (4-5%) cases; corona virus was seen in one. No virus-like particles were seen in stool samples obtained from the six asymptomatic patients.

These findings support the suggestion of Cubitt and Holzel that rotavirus should be considered as a possible cause of outbreaks of diarrhoea in elderly patients in longer stay wards.

I WANDLESS
J GRIMLEY EVANS
Department of Medicine (Geriatrics), and the Public Health Laboratory, Newcastle General Hospital

Pseudoleptospirales in blood culture
We noted with interest the observation by Rahman and MacIs1 that pseudoleptospirales could be identified when blood cultures from healthy humans were examined under dark-ground microscopy.

We have observed the presence of artefacts similar in all respects to those described by these authors when whole blood samples from normal, healthy guinea-pigs, hamsters, mice, and chickens have been submitted to direct dark-ground examination. Furthermore, the same type of spiral filaments have invariably been observed when fluid from freshly prepared or incubated suspensions of liver and kidney tissue from these same animals have been similarly examined. It would thus seem likely that such artefacts would be found in corresponding preparations from other animal species as well as man. Although these pseudoleptospirales can usually be fairly easily differentiated from the true leptospire by an experienced worker, we concur wholeheartedly with the view that a diagnosis of

Dr England replies as follows:
The publication by the International Committee for Standardization in Haematology Expert Panel on Blood Cell Sizing was intended as a reference standard for determining packed cell volume.

Dr Petrucci’s comments are, of course, quite valid, but the panel’s view is that conditions of anticoagulation, etc, are more relevant to the measurements of the PCV in routine practice. It is the panel’s hope to have a further publication on a selected method which would be more relevant to the routine application.

JM ENGLAND
Pathology Laboratory, Haematology Department, Watford General Hospital, Watford, Herts WD1 8HB

Rotavirus infection
We were very interested to read the paper by Cubitt and Holzel (J Clin Pathol 1980;33:306) about an outbreak of rotavirus infection in a long-stay ward of a geriatric hospital. We have recently seen a similar outbreak.

Reference

*Coulter Electronics, Inc, Hialeah, Florida, USA
Beta-lactamase production by Campylobacter jejuni.

E P Wright and M A Knowles

*J Clin Pathol* 1980 33: 904-905
doi: 10.1136/jcp.33.9.904-b

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