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### Reproducible test for detecting *Helicobacter pylori* in frozen samples

Retrospective investigations of *Helicobacter pylori* in stored samples might help us to understand the aetiological role of *Helicobacter pylori* in different lesions with which it has been associated.<sup>1</sup> *Helicobacter pylori*, however, is a fastidious micro-organism, and it is difficult to store it without pronounced loss of viability.<sup>2</sup> Some investigators have studied the presence of *Helicobacter pylori* in stored samples using stained sections,<sup>3</sup> but no one has evaluated the reproducibility of a method to detect *Helicobacter pylori* in stored samples.

The aim of this study was to evaluate prospectively the accuracy of a simple and rapid urease test and culture techniques for the detection of *Helicobacter pylori* in infected frozen gastric biopsy specimens.

A total of 52 antral gastric biopsy specimens were included in the study. The biopsy specimens were ground in 1 ml of 20% sterile glucose chilled transport medium with the pH adjusted to 6.8. A fraction of each specimen was processed for culture and rapid urease test assay. The remaining parts of the homogenised specimens were divided into three groups and stored at -70°C. The groups were reanalysed at different times (two, four, and six months after endoscopy) by again using the rapid urease test and culture.

The culture was performed on Skirrow's *Campylobacter* agar (Difco Laboratories, Detroit, Michigan), trypticase soy agar (Difco) supplemented with 7% horse blood, 1% IsoVitalax, and with or without antibiotics (10 mg/l vancomycin, 5 mg/l trimethoprim lactate, and 2500 IU/l polymyxin B), chocolate agar, and Thayer-Martin agar (BBL) with 1% IsoVitalax and 2% haemoglobin. Plates were incubated at 37°C for up to seven days in a Campy Pak (BBL) generating microaerophilic or a *Campylobacter* gas generating Kit (Oxoid Ltd, London, England).

The rapid urease test used in a modification of that used by Arvind *et al.*,<sup>4</sup> and recently validated in our laboratory,<sup>5</sup> can be performed with a very small amount of homogenised tissue incubated at 55°C.

Culture of the fresh homogenised specimens identified *Helicobacter pylori* in 42 of the 52 biopsy specimens tested, a positive rapid urease test being observed in all 42 that were positive by culture. The rapid urease test performed after two, four, and six months of freezing was also positive in all the samples that were positive before storage. Although

the rapid urease test was 100% reproducible at any time, a decrease in the activity of the enzyme was observed.

The culture of the stored specimens that were *Helicobacter pylori* positive in the fresh analysis identified the micro-organism in 14 of 16 (87%) specimens after two months, 14 of 15 (93%) after four months, and 10 of 11 (91%) after six months. The viability determined by the number of colony forming units was reduced from 20% to 80% after freezing. This loss, however, was related to the initial number of colonies rather than the time of freezing.

None of the 10 *Helicobacter pylori* negative samples in the fresh examination gave a positive culture or rapid urease test when analysed at two, four, or six months.

These results indicate that despite freezing and greatly reducing the viability of the micro-organism, the urease enzyme remains sufficiently active after six months of freezing to give positive results at 55°C. We conclude that the modified rapid urease test is more accurate than culture in identifying *Helicobacter pylori* in frozen gastric mucosal specimens and, therefore, it is the most convenient method for retrospective studies.

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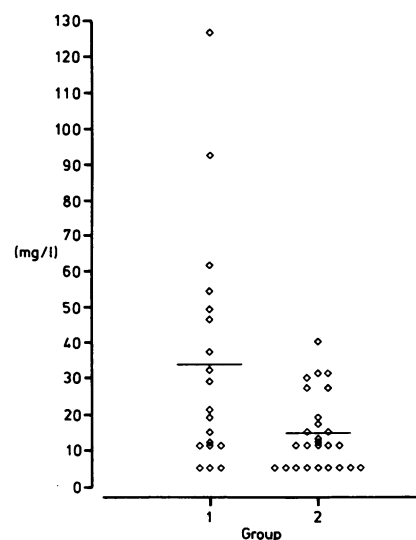
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### Acute phase response and deep lower limb venous thrombosis

The acute phase response with fever, a neutrophil leucocytosis, and increased concentrations of plasma proteins may be induced by different infectious, ischaemic, immunological, malignant, or traumatic stimuli. One of the most practical methods for monitoring it is to quantify the serum C-reactive protein (CRP) concentration.<sup>1</sup> The purpose of the present work was to compare the acute phase response in different types of deep lower limb thrombosis.

Forty five patients with deep lower limb thrombosis verified by ascending phlebography were studied. These patients did not have



Maximum serum C-reactive protein concentration in all cases of acute deep lower limb venous thrombosis. Group 1, iliofemoral, group 2, tibial or popliteal thrombosis. The horizontal lines denote means of serum CRP. The difference between the medians was significant  $p = 0.02$  (Mann-Whitney U-test).

any other cause for the acute phase response. Treatment protocol at our hospital includes the combination of heparin and coumarine and only coumarine after discharge.

Peripheral blood leucocyte counts on admission were determined with an automated calculator, and axillary temperatures and CRP concentrations were monitored throughout. Serum CRP was determined by an immunoturbidometric method (Orion Diagnostica, Espoo, Finland). The detection level for CRP at our hospital varied from < 10 mg/l to < 15 mg/l during the period concerned, but in those cases a serum CRP value of 5 mg/l was used for statistical purposes. Data were compared with the non-paired Student's *t* test, Mann-Whitney U-test, and Pearson's correlation analysis.

The 19 cases with iliofemoral thrombosis (group 1) were older than the 26 with the tibial or popliteal thrombosis (group 2), and the serum CRP response was higher in the former, but no such difference was seen in leucocyte responses or axillary temperatures (table). Age itself did not seem to explain the differences in CRP response between these two groups when examined by Pearson correlation analysis ( $r = 0.24$ ,  $p = 0.24$  in group 1;  $r = 0.24$ ,  $p = 0.33$  in group 2). Axillary temperature, CRP, and leucocyte responses were similar in the 22 men and 23 women. Serum CRP peaked over two days in all except four cases.

Analysis of the individual responses showed more than one third (35%) of the 26 patients in group 2 to have no CRP response (figure), while only three had a temperature of

### Characteristics of 45 patients with deep lower limb venous thrombosis

	Tibial/popliteal (n = 26)		Iliofemoral (n = 19)		Mean difference	95% confidence interval	t test	p value
	Mean	(SD)	Mean	(SD)				
Age (years)	52.6	14.7	66.5	15.6	13.9	4.8 to 23.1	3.1	0.004
Leucocytosis*	8.7	1.9	8.6	1.6	- 0.1	- 1.2 to 1.0	- 0.2	0.86
Tax†	37.0	0.4	36.9	0.3	- 0.1	- 0.4 to 0.1	- 1.0	0.34

\* - maximum of peripheral blood leucocyte counts,  $\times 10^9/l$ .  
† - maximum of axillary temperature, °C.