Urease tests for *Campylobacter pylori*: care in interpretation

Campylobacter pylori is associated with the presence of histological gastritis[1] yet its role in the pathogenesis of peptic ulcer disease and non-ulcer dyspepsia remains unclear. Detection of *C pylori* in antral or duodenal biopsy specimens usually entails histological or microbiological methods that may take up to seven days. The unusual characteristic of rapid urea hydrolysis by *C pylori*, indicating the presence of preformed urease, has been well described. There are now several reports of rapid urease testing (RUT) for the detection of *C pylori*, and the results of sensitivity and specificity at various incubation times are given in table 1.

We have previously reported that incubation at 50°C raises the sensitivity of the four hour RUT.[2] The aim of this letter is to report a new method for detecting *C pylori* (The Middlesex Hospital CP-Test) and to compare it with currently available tests.

Table 1 *Comparison of different sensitivities and specificities of various urease tests*

Source	Incubation time (hours)	Sensitivity	Specificity
Morris *et al*[4]	CLO-test (24)	100	100
Marshall *et al*[3]	CLO-test (24)	97	100
Das *et al*[5]	2% RUT (24)	59	86
Vaira *et al*[2]	2% RUT (4)	89	100

Table 2 *In vitro urease testing of C pylori (CP), proteus (P), and Klebsiella (K) strains*

Time (minutes)	2% RUT		6% RUT		CLO-test	
	Room temperature	50°C	Room temperature	50°C	Room temperature	50°C
5			6 10 (CP)	6 10 (CP)		7 10 (CP)
10			5 10 (CP)	5 10 (CP)		
20		7 10 (CP)			7 10 (CP)	
60	7 10 (CP)			4 10 (CP)		6 10 (CP)
	7 10 (P)	7 10 (P)	7 10 (P)	7 10 (P)	7 10 (P)	7 10 (P)
120		6 10 (CP)	4 10 (CP)		6 10 (CP)	
			6 10 (P)	6 10 (P)	6 10 (P)	6 10 (P)
1440	6 10 (CP)	5 10 (CP)	3 10 (CP)	3 10 (CP)	6 10 (CP)	6 10 (CP)
	5 10 (P)	5 10 (P)	5 10 (P)	5 10 (P)	5 10 (P)	5 10 (P)
	7 10 (K)	7 10 (K)	7 10 (K)	7 10 (K)	7 10 (K)	7 10 (K)

Table 3 *Sensitivity and specificity of methods for detection of C pylori*

	Histology	Culture	2% RUT	6% RUT (CP-test)	CLO-test
Sensitivity	100	70	94	94	58
Specificity	100	100	100	100	100

In vitro study

We tested three strains each of *Proteus mirabilis*, *P vulgaris*, *Morganella (Proteus) morgani*, and two strains of *Klebsiella aerogenes*, and two of *C pylori*. A dense suspension was made from an agar plate of each organism and doubling dilutions in physiological saline, taking 0·1 ml of each dilution for the inoculum; 0·1 ml of each dilution was also plated out and incubated to determine the concentration of bacteria in colony forming units (cfu)/ml in the original suspension and thus each dilution. Each dilution was inoculated into 2% RUT, 6% urea solution (6%-RUT), and CLO-test (Delta-West Ltd, Perth, Western Australia) slides and incubated either at room temperature or at 50°C. Tests were read at timed intervals up to 24 hours. The lowest sensitivity to *C pylori* was found with the 2% RUT, which detected only 10^7 cfu/ml at 20 minutes' incubation at 50°C. The CLO-test was positive after 20 minutes at room temperature with the same *C pylori* concentration of 10^7 cfu/ml, but at 50°C produced a positive result in five minutes. The highest sensitivity was obtained from the 6% RUT (CP-test) which gave a positive result within 10 minutes at both room temperature and at 50°C with the lower *C pylori* concentration of 10^5 cfu/ml. Other urease producing organisms at 10^7 cfu/ml did not give a positive urease test (false positive RUT) until incubation for more than one hour at either room temperature or 50°C (table 2).

In vivo study

Ninety mucosal biopsy specimens taken at endoscopy were evaluated by histological examination and culture and placed in 6% urea solution (CP-test) and 2% urea broth and incubated at 50°C and room temperature. CLO-testing of 50 biopsy specimens was also performed. Urease tests were examined at timed intervals up to 24 hours. Histological interpretation was obtained after Giemsa staining. Specimens were homogenised in 0·5 ml isotonic saline, one drop taken for immediate Gram stain and the remainder placed on blood agar with amphotericin (4 μg/ml). Plates were incubated in a microaerophilic environment at 37°C for six days. Marshall *et al* showed that 75% of specimens containing *C pylori* gave a positive CLO-test result after 20 minutes, increasing to 98% at 24 hours' incubation.[3] We found that the CLO-test incubated at room temperature gave a positive result in 50% of specimens containing *C pylori* after 20 minutes, rising to 55% at 90 minutes, and 58% at 24 hours. Incubation in 2% RUT at 50°C gave positive results in 70% of *C pylori* specimens containing *C pylori* at three hours, rising to 90% at four hours, and 94% at six hours. When incubating the CP-test either at room temperature or at 50°C, 75% were positive after 15 minutes, 90% after 20 minutes, and 94% after two hours (table 3). All specimens containing *C pylori* on microscopy showed histological evidence of gastritis.

We believe that the CP-test is the preferred method for detection of antral *C pylori*,

having a higher sensitivity and the same specificity (100%) as the CLO-test. Our results indicate that a positive CP-test before 20 minutes' incubation is strong evidence of *C pylori* infection and is an indication for treatment. Furthermore, if a urease test becomes positive after one hour of incubation then treatment should not be started immediately as other urease producing species may be responsible for the positive test, and confirmation for the presence of *C pylori* should be obtained either by histological examination or culture.

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Anti-human immunodeficiency virus (HIV) positive laboratory reagents

In June 1985 Jones *et al* drew attention to the potential infectivity for acquired immune deficiency syndrome (AIDS) of laboratory reagents used in the diagnosis of bleeding disorders; antibody to the human immunodeficiency virus (HIV) was detected in eight of 15 laboratory reagents prepared from human plasma. We examined 30 lots of human serum and six control samples of cerebrospinal fluid (CSF), from eight manufacturers, which are devised for the calibration of biochemical tests for evidence of contamination with HIV. None of the CSF serum control samples contained antibodies to HIV but nine lots of controls purchased between September 1984 and December 1986 from three manufacturers were found to be reactive in a commercial (Abbott) enzyme immunoassay (EIA). Eight of these results were verified in the Abbott "confirmatory" EIA for anti-HIV. One of the reagents was positive, another weakly positive, and two gave equivocal reactions when tested by immunofluorescence in H9 cells infected with HIV. HIV antigen was not detected in any of the antibody-containing lots by the Abbott EIA for HIV antigen(s) (table). One of the manufacturers told us that reverse transcriptase could not be detected in cultures of H9 cells inoculated with their serum control lots. Nevertheless, as donors infected with HIV contributed to the pools from which the serum controls were prepared, these reagents should be regarded as contaminated with HIV and therefore potentially infectious for laboratory personnel.

Table *Results of tests for HIV antibody and antigen in laboratory serum control reagents found positive for anti-HIV in preliminary screening enzyme immunoassay*

	HIV antibodies				
	Enzyme immunoassay				
*Serum control reagents**	*Screening (ratio: optical density/ cut off)*	*Confirmatory*		*Immunofluorescence assay (HIV-infected H9 cells as substrate)*	*HIV antigen(s)*
		Anti-core P24	*Anti-ENV GP41*		
1	5·99	+	+	+	0
2	5·02	+	+	Equivocal	0
3	3·19	+	+	Weak +	0
4	2·22	+	+	0	0
5	1·81	0	+	0	0
6	1·73	0	+	Equivocal	0
7	1·71	0	+	0	0
8	1·54	0	+	0	0
9	1·37	Sample insufficient		0	0

+, positive result; 0, negative result.
*Sera 1, 2, 4 and 6 from manufacturer I; 3, 8 and 9 from manufacturer II; 5 and 7 from manufacturer III.

Notwithstanding the lack of evidence of inadvertent infection of laboratory technicians with HIV and the possible inactivation of the virus during preparation of these controls, we take the view that reagents for laboratory use should be prepared from sera non-reactive for anti-HIV. We are assured by the manufacturers whose products we tested that they now follow this practice.

Within the past few weeks we have received a further consignment of eight lots of serum control samples, only one of which was repeatedly reactive in the Abbott EIA for anti-HIV. This lot, supplied by a manufacturer whose products we had not tested previously, was of low reactivity and found to be negative in the Abbott confirmatory test.

We would endorse the recommendation of the Centers for Disease Control, Atlanta, that human sera used as controls or reagents should carry a caveat to the effect that freedom from contamination with HIV cannot be guaranteed. Additionally, it should be stated whether the product has been tested for the presence of anti-HIV or HIV antigen, and, if the test has been done, the result should be given.

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Correction of plasma protein concentrations for haemodilution

My attention has been drawn to the fact that the formula used by my colleagues and me[1,2] to adjust the concentrations and plasma proteins for changes in haematocrit in vivo does not apply in vitro. I propose an amended formula and show its validity for in vitro applications (table).

Addition of a volume (V) of isotonic saline to a sample of blood treated with a suitable anticoagulant in vitro will not change the total volume of the red cells present (RBC),