Induction of IL-8 secretion by cagA negative isogenic mutant of H pylori

Discussion
These studies with the isogenic cagA negative mutant strain of H pylori demonstrate that deletion of the CagA protein does not effect the ability of H pylori to induce IL-8 secretion from gastric epithelial cells. The CagA protein is, therefore, not functionally involved in this response but is a marker for strains with proinflammatory properties. As all of the wild-type CagA positive strains we have studied to date induce epithelial IL-8 secretion,\(^5,10\) the bacterial factor(s) stimulating this inflammatory response is clearly closely associated with the expression of cagA. The function of the CagA protein remains to be determined.

The CagA protein shows considerable size variation in different strains of H pylori, ranging from 128 to 150 kD.\(^3\) Intragenic repeat sequences are the basis of this heterogeneity.\(^1\) Studies with native\(^4\) and recombinant CagA protein\(^15\) have clearly demonstrated the high immunogenicity of this protein both locally and systemically. It is important to consider the role of this antigen specific response in the immunopathogenesis of chronic gastritis.

If the inflammatory epithelial response generated by type I strains is important for bacterial nutrient supply, one bacterial survival strategy may be to avoid immunological recognition of bacterial inflammatory factors and tissue damaging agents such as the cytotoxin. The highly immunogenic CagA protein may therefore be an "immunological decoy" diverting host responses away from these functionally important bacterial factors.

A practical single sample dry latex agglutination test for Helicobacter pylori antibody detection

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Abstract
Assessment of a single serum sample for Helicobacter pylori antibodies is frequently requested in routine diagnostic laboratories. Current enzyme linked immunosorbent assay (ELISA) kits are not ideal for testing small numbers of serum samples and some have low sensitivities, specificities or large grey zones. A panel of 90 serum samples from patients who had presented for routine upper endoscopy was used to compare three kits for the detection of H pylori antibodies: (1) Pyloriset Dry, total antibody latex agglutination, Orion Diagnostica, Espoo, Finland; (2) Pyloriset enzyme immunoassay (EIA), IgG ELISA, Orton; and (3) Hel-p, IgG ELISA, Amrad, Kew, Victoria, Australia. Diagnosis of H pylori positivity was made if culture results and either rapid urease test or histopathology were positive. The sensitivity, specificity, positive and negative predictive value for each test was as follows: Orion: latex 93.3%, 95.6%, 95.5%, 93.3%, respectively; Orion: EIA-G 84.4%, 97.8%, 97.4%, 84.4%, respectively; and Amrad: EIA-G 100%, 88.9%, 90%, 100%, respectively. The latex test performed better than the ELAs with respect to sensitivity and specificity.

Keywords: Helicobacter pylori, serology, diagnostic test.
Helicobacter pylori is now established as the cause of type B active chronic gastritis. Methods first described to investigate H. pylori used endoscopy and biopsy of the gastric antrum. Biopsy samples can be investigated for H. pylori colonisation by culture, microscopy, or by urease tests to measure pH changes in the presence of urea. Biopsy is invasive and therefore not practical in prolonged follow up or in epidemiological studies.

Non-invasive methods to diagnose H. pylori include a breath test and serological tests. Various serological methods have been used including the complement fixation test, the bacterial agglutination test, the passive haemagglutination test, the haemagglutination assay, immunoblot techniques, and enzyme linked immunosorbent assays (ELISAs). All of these tests have demonstrated a correlation between reactivity and the presence of H. pylori in the gastric antrum. Thus, serology is a simple and practical tool to assess H. pylori infection.

The sensitivity and specificity of these serological tests are still under investigation. The preparation of antigen varies with the different techniques and may account for some of the reported differences between assays. ELISA has been the technique of choice in most laboratories because of its speed, low cost, simplicity, and reproducibility. A latex agglutination test has potential advantages over ELISA kits for many laboratories that wish to offer a H. pylori antibody test for small numbers of serum samples, as single tests can be done without the need for running numerous controls and reference serum samples. This study compares the performance of the Orion Diagnostica Pylokit dry latex agglutination test (Espoo, Finland) against two conventional enzyme immunoassay (EIA) tests using a panel of serum samples from patients with defined H. pylori disease status.

Methods
The assays involved in the comparison were as follows: Amrad Hel-p EIA, Amrad, Kew, Victoria, Australia; Orion Diagnostica Pylokit EIA; and Orion Diagnostica Pylokit total antibody dry latex agglutination test.

Forty five H. pylori positive and 45 H. pylori negative serum samples were tested with each assay. Assays were performed over two days and strictly in accordance with the manufacturer's guidelines. Both EIA kits had similar protocols and both took 150 minutes to complete, excluding dilution, dispensing and washing times. The Orion kit had the advantage of being performed at room temperature compared with 37°C for the Amrad kit. The latex test involved diluting each serum 1 in 4 with dilution buffer, pipetting 40 µl of the diluted sample onto the circle of the test card and mixing. The card was then rotated and tilted in a circular motion for three minutes, during which time evidence of agglutination was observed.

Subjects previously presenting for routine upper endoscopy had venous blood collected and stored on ice. Serum was separated within one hour of collection and stored at −20°C. During endoscopy three antral biopsy specimens were obtained for microbiology (phase contrast microscopy and culture), histopathology and rapid urease test. Diagnosis of H. pylori positivity was made if culture results and one or more of the other tests were positive. Clinical data were recorded on all subjects. Patients were excluded from the study if they were unable to give informed consent, presented an undue risk for endoscopy, had evidence of previous gastric surgery (other than simple oversewing of ulcer), or were on antimicrobial therapy, including bismuth preparations, within the previous three months. The sensitivity, specificity, positive predictive value and negative predictive value were calculated as previously described.

Results
Clinical details of the patients included in the study are presented in table 1. The mean age was 49.8 years (range 19 to 87 years) and the male: female ratio was 39:51. The criteria for assigning a serum as positive or negative was on the basis of the culture result and one other diagnostic test. This was used as the gold standard by which the three serology methods were compared. Table 2 shows the sensitivity, specificity and positive and negative predictive values for these kits.

The Amrad EIA was the only kit to include a "grey zone" in quantitative assays. This result was defined as an optical density (OD) reading between 0.1 (mean positive control OD) and 0.3 (mean positive control OD) and was deemed indeterminate for H. pylori antibody status. The manufacturer advised that patients returning this result should be examined using an alternative procedure. In this study 19% of our results were equivocal with this kit. To compare the Amrad sensitivities and specificities with the other kits, we used a cut off value of 0.2 times the mean of the positive controls, as described in their instructions for the performance of epidemiological studies. Using this method, of the 17 equivocal results,
two became positive on recalculation and the rest negative.

Discussion
All three methods performed well on a small but microbiologically defined panel of serum samples. The manufacturers of commercial serology kits must maintain a balance between sensitivity (true positives) and specificity (true negatives). The Amrad EIA kit had excellent sensitivity but had the lowest specificity of the three tests. The Orion EIA had the best specificity but the lowest sensitivity. The Orion dry latex test had the best compromise with very good sensitivity and specificity.

The Orion dry latex total antibody test was simple and quick to perform. Most serum samples gave clear cut results but one serum showed non-specific agglutination. When retested with more vigorous mixing this serum was read as negative. The major advantage of the dry latex test is the ability to test an individual serum. Each test card contains three test wells. The card may easily be cut in three to perform one test at a time. This has cost and convenience advantages over the EIA tests in that a full set of positive and negative controls as well as reference serum samples do not have to be run to test a single serum. However, automated EIA tests are more convenient for running large numbers of samples.

Based on the results of this study, the Orion Pyloriset dry latex total antibody test is recommended for laboratories which receive small numbers of requests for H pylori antibody serology.


Corynebacterium aquaticum septicaemia in a neutropenic patient

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Abstract
Corynebacteria are a well recognised cause of sepsis in the immunocompromised patient. Corynebacterium aquaticum, however, is rarely seen in the clinical setting, being an environmental organism associated with fresh water. A septicaemic episode caused by this organism in a 74 year old neutropenic woman with an indwelling central venous catheter is reported. It is postulated that the source of the organism was untreated stored rainwater which she used for showering.

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Keywords: Corynebacterium aquaticum, central venous catheter, septicaemia.

In the immunocompromised patient, corynebacteria are often implicated as a cause of sepsis. In particular, Corynebacterium jeikeium (CJK) and Corynebacterium Group D2 are frequently associated with catheter related sepsis and urinary tract infections, respectively.12 Corynebacterium aquaticum is an environmental organism associated with fresh water.3 It has been reported in association with neonatal meningitis and urinary tract infection, septicaemia in an elderly patient with diabetes, and relapsing peritonitis with continuous ambulatory peritoneal dialysis.4,5 The literature, however, is sparse and virtually confined to patients with some form of immunosuppression. Here, we describe a septicaemic episode caused by this organism in a neutropenic

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