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Validation of the saliva-based *H. pylori* test, HeliSAL™, and its use in prevalence surveys

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SUMMARY

The saliva-based *H. pylori* test, HeliSAL™, is insufficiently accurate for use in the clinical setting. However, its ease of use and non-invasiveness make it attractive for population-based studies of the epidemiology of *H. pylori*. We validated HeliSAL™, and comment here on its usefulness in prevalence surveys. One hundred and ninety-six patients receiving endoscopy at a clinic in New Zealand provided saliva samples for *H. pylori* assessment, which were compared to CLOtest (Delta West Pty Ltd, Western Australia) as a gold standard measure. Nineteen percent were truly *H. pylori* positive, 41% were positive according to HeliSAL™. Test sensitivity was 74% and specificity was 67%. While HeliSAL™ is not well suited for the clinical diagnosis of *H. pylori* infection, it may be useful for large-scale prevalence surveys because, provided it is validated locally, mathematical adjustment can be made for misclassification. Being inexpensive, non-invasive, and easily stored and handled, HeliSAL™ may be a valuable tool for studies of the epidemiology of *H. pylori*.

INTRODUCTION

A number of recent articles have presented validation data on the saliva-based antibody test for the presence of *H. pylori*, HeliSAL™ (Cortecs Diagnostics Ltd, London). While a wide variation in the accuracy of the test has been reported, investigators seem adamant that although the test is attractive in its simplicity and non-invasiveness, its utility in the diagnosis of individuals remains to be proven [1–11]. The test is relatively cheap, but increasingly the more accurate urea breath test is becoming available on a cost-effective basis. One area in which HeliSAL™ may prove useful, however, is in population-based prevalence studies, particularly in epidemiology, such as the ecological comparison of prevalence in two large groups [8]. While the accuracy of a test is crucial in the diagnosis of a condition or in the assessment of a marker for disease, there is greater scope in the population-based setting to adjust for known test inaccuracies in the reporting of rates and their comparisons [12]. In large-scale studies of *H. pylori* the saliva-based test is a particularly attractive alternative to serum-based tests, for as well as eliminating the need to employ trained personnel to take blood, there is the possibility that saliva sampling might provide a better response rate than serum sampling in studies using volunteers [3].

Detection of antibodies to *H. pylori* with the HeliSAL™ kit relies on an antibody-specific antigen. The accuracy of HeliSAL™ has been previously investigated in clinical populations in the United Kingdom and in Canada (Table 1) in comparison with breath test, histology, urease test, or combinations thereof, all of which may be considered gold standards

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Table 1. *Previously reported studies of the sensitivity and specificity of salivary testing for antibodies to* H. pylori: *gold standard type, validation results and comparisons of accuracy with the current study*

Studies	Standard	Sample size	% Infected	Sensitivity (%)			Specificity (%)		
					χ^2‡	*P*-value		χ^2‡	*P*-value
United Kingdom									
Cripps et al. 1993 [2]	Histology	147	35	80	0·28	0·60	84	8·36	< 0·01
Phull et al. 1993 [4]	UBT	48	50	79	0·03	0·86	100	9·67	< 0·01
Moayeddi et al. 1994 [5]	Histology/urease	79	41	82	0·50	0·48	92	5·44	0·02
Guard et al. 1994†	Urease	58	47	79	0·03	0·87	100	12·26	< 0·01
Clancy et al. 1994 [3]	Histology/urease	134	27	89	1·81	0·18	94	23·67	< 0·01
Christie et al. 1996 [8]	Histology/culture/urease	86	38	88	1·25	0·26	71	0·18	0·67
Reilly et al. 1997 [10]	Urease/UBT/histology	300	46	84	1·35	0·25	70	0·25	0·61
Canada									
Lahaie et al. 1995 [6]	Breath test	256	n.s.	76	††		97	††	
Fallone et al. 1996 [1]	Histology	106	50	66	0·25	0·62	74	0·58	0·45
Loeb et al. 1997 [9]	Histology	157	30	86	1·02	0·31	58	1·63	0·20
Simor et al. 1997 [11]	Culture/histology	195	49	81	0·39	0·53	75	1·88	0·17
Unspecified									
Lin et al. 1995 [7]	Histology	151	n.s.	81	††		72	††	

* Significant at the 5% level.
** Significant at the 1% level.
† These results are quoted by the manufacturers.
†† Insufficient information was provided by the authors for the calculation of comparisons.
‡ Test for the comparison of the sensitivity/specificity of the reported study with the current study.
n.s. = not specified.

for *H. pylori* infection [13]. The range of values of specificity is greater than the range for sensitivity, and there is not necessarily any consistent relation between the two. Presuming that all of these studies were carried out in accordance with the manufacturer's recommendations, there remains substantial and presumably non-random variation in the accuracy of the test. There are at least three factors likely to be responsible for that marked variation. Firstly, there is concomitant variation in the accuracy of the 'gold standard' techniques, for there is no true gold standard for infection [14]. Secondly, antibodies measure past infection, while most gold standards measure current infection, so results may vary depending on the inclusion of people likely to have recently lost or gained infection [15]. Thirdly, the accuracy of any antibody-based test may vary from population to population due to the existence of different *H. pylori* strains with varying detectability [16]. The validation studies of HeliSAL™ use an antigen derived from a population in the United Kingdom, and while the populations in previous validation studies are likely to be similar with respect to ethnicity and ancestry, there is the potential that they harboured different strains of *H. pylori*.

A validation study of HeliSAL™ carried out in a clinical population in New Zealand is presented here. Two previous validation studies revealed that the accuracy of HeliSAL™ varied according to the level of antibody considered indicative of infection [5, 8]. In both cases, the level suggested by the manufacturer did not provide the optimum test accuracy. By varying the level of antibody at which a person is considered to be infected with *H. pylori*, sensitivity can be increased at the expense of specificity and *vice versa*. The optimum accuracy of the test in the current study was determined and compared with the manufacturer's own validation findings.

METHODS

Participants were selected from patients attending Christchurch Hospital Gastroenterology Unit for gastroscopy between 25 July 1996 and 17 October 1996. Patients outside the age range of 20–75 years were excluded. All patients returning for a gastroscopy to ascertain the eradication of *H. pylori* or to ascertain the presence of an ulcer after attempted *H. pylori* eradication were excluded. Any patient whose diagnosis implied malignancy was excluded.

The presence of *H. pylori* infection was determined using the urease-determinant 'CLOtest' (Delta West

Pty Ltd, Western Australia) in gastric mucosal biopsy samples from normal-looking tissue from the antrum, along the greater curvature. The CLOtest was performed immediately after gastroscopy by the attending Registrar. The test consisted of a slide containing agar gel with urea, a pH indicator and a bacteriostatic agent. When the sample was placed in the gel, a magenta or orange colour change indicated the presence of *H. pylori*, based on the presence of urease activity. This reaction can take up to 24 h, but the manufacturer states that 75% of true positive results change colour within 20 min, 85% by 1 h, 90% by 3 h, and 95% over 24 h (Delta West Pty).

Immediately after gastroscopy, patients provided a saliva sample by placing a cotton swab under their tongue until it was soaked through with saliva. Saliva samples were sealed in the plastic tube and stored between 4 and 10 °C until being transported to the Department of Microbiology, University of Otago, for analysis within 2 weeks of the tests.

The HeliSAL™ assay (Roche Diagnostics Ltd, London) was used to determine the presence of antibodies to *H. pylori* in saliva samples taken from participants. The procedure has been described elsewhere [9].

Plastic microwell plates were coated with antigen extracted from *H. pylori*. The microwells were incubated at room temperature for 30 min with control solution and the participants' saliva samples, in duplicate. Salivary IgG antibodies to *H. pylori* present in the samples became bound to the antigen on the microwells. After washing to remove unbound material, a biotinylated second antibody to human IgG was incubated at room temperature for 30 min with the antigen-anti-*H. pylori* complex.

Unbound anti-human IgG was removed by washing with buffer solution, and a third incubation for 15 min at room temperature with streptavidin horseradish peroxidase (HRP) enzyme conjugate was carried out. Excess enzyme conjugate was removed by washing with buffer solution and a substrate solution containing tetramethylbenzidine (TMB) and hydrogen peroxide was added. In the presence of HRP enzyme, TMB gave a blue colour, which turned yellow on incubation for 30 min in the dark and intensified on the addition of the stop solution. The intensity of the colour was proportional to the amount of salivary *H. pylori* IgG antibody in the sample. Absorbance values for the colour intensity were read at 450 nm.

In accordance with the manufacturer's recommendations, if the average of the two values for each sample was greater than or equal to one, the patient was considered *H. pylori* positive. If the average was less than 0·8, the patient was considered *H. pylori* negative. All other values of the average ELISA unit reading were considered equivocal. The laboratory technician was blinded to all information about the source of individual samples, including their true *H. pylori* positivity.

Ethical approval to approach and test patients was obtained from the Southern Regional Health Authority Ethics Committee (Otago).

Exact binomial confidence intervals [17] were calculated for the sensitivity and specificity of the HeliSAL™ test. The sensitivity and specificity of HeliSAL™ in this validation study was compared with previously reported validation studies using a χ^2 test on 1 degree of freedom, and Fisher's Exact test when any expected value was less than 5 [18].

Previous reports indicated that the accuracy of HeliSAL™ varies significantly with age [10]. Age-specific test attributes were assessed using a χ^2 test for heterogeneity of the odds ratio between HeliSAL™ and CLOtest.

To assess various cut-off points for *H. pylori* positivity, the manufacturer's recommendations were simplified to avoid equivocal results [1, 8, 11]. Observed sensitivity (and specificity) was calculated by determining the proportion of true positives (and true negatives) for each ELISA reading between 0·0 and 8·0 in steps of 0·1 units. Specificity was plotted against sensitivity in order to establish the cut-off ELISA reading for the optimum performance of the test in this study.

RESULTS

Eight hundred and one patients attended Christchurch Gastroenterology Clinic during the study period. Two hundred and thirty-four patients were outside the required age-range and 34 had investigations that revealed malignancy. A further 10 patients were excluded because of their participation in a study of non-ulcer dyspepsia and *H. pylori*, and 177 patients did not receive a CLOtest. One hundred and fifty patients received a conclusive CLOtest but no saliva test, because the clinician was unable to complete one in the time allotted to the gastroscopy. One hundred and ninety-six eligible patients were given saliva tests. Thirty-seven of these patients (18·9%) were *H. pylori* positive by CLOtest, and their median age was 54 years.

Nine saliva samples yielded equivocal HeliSAL™ results. Seventy-six of 187 (40·6%) samples yielding

Table 2. *Age-specific sensitivity and specificity of HeliSAL™ in a clinical population in New Zealand*

Age range (years)	Sensitivity (95% CI)	No. truly positive	Specificity (95% CI)	No. truly negative
20–39	75·0 (21·9, 98·7)	4	71·4 (53·5, 8408)	35
40–59	61·1 (36·1, 81·7)	18	67·2 (53·5, 78·6)	58
60–69	83·3 (36·5, 73·2)	6	56·3 (37·9, 73·2)	32
70–75	100·0 (51·7, 100·0)	6	71·4 (51·1, 86·0)	28

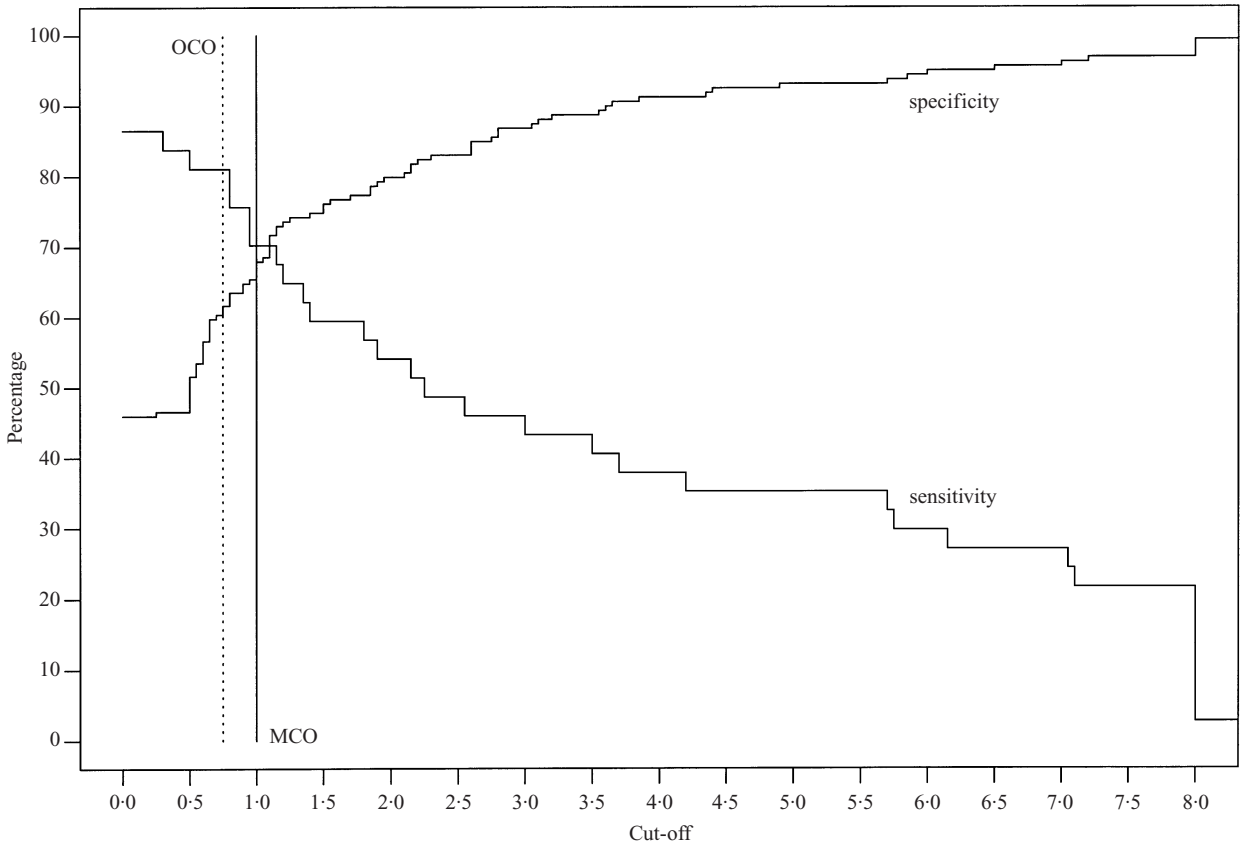

Fig. 1. Receiver-operator characteristic curve for HeliSAL™ comparing the manufacturer's suggested cut-off for *H. pylori* positivity with the optimum cut-off determined in the current study. OCO, Optimum cut-off; MCO, Manufacturer's cut-off.

an unequivocal result were *H. pylori* positive by HeliSAL™ test. Although sensitivity appeared to increase with age (Table 2), there was no statistically significant variation in sensitivity ($P = 0{\cdot}90$) or specificity ($P = 0{\cdot}92$) by age among the 196 samples.

The sensitivity observed in this study was not significantly lower than that reported in any other studies (Table 1). The observed specificity was significantly lower than the specificity reported in all but two studies from the United Kingdom. There were no statistically significant differences between the specificity in the current study and that of any of the Canadian studies (Table 1).

The manufacturer's designated cut-off ('MCO', 1·0 ELISA units, sensitivity = 73·5%, specificity = 66·7%) and the cut-off for the optimum performance of the test given the results of the validation study ('OCO', 0·75 EU, sensitivity = 81·0%, specificity = 61·6%) is plotted in Fig. 1. The cut-off that simultaneously maximized test sensitivity and specificity resulted in a higher sensitivity but lower specificity than was observed for the manufacturer's cut-off for this data. The maximum achievable sensitivity and specificity for these data when both parameters were at least 50% were 81·0% (EU = 0·75) and 82·4% (EU = 2·25) respectively.

DISCUSSION

Previous reports of the accuracy of the saliva-based antibody test for *H. pylori*, HeliSAL™, indicate that it is not sufficiently accurate for use in a clinical setting for the diagnosis of current *H. pylori* infection in individuals [8, 10]. However, its ease of use, and non-invasive application, make it attractive for use in population-based prevalence surveys in epidemiology [8]. While HeliSAL™ performed poorly in this validation exercise, epidemiological methods exist for adjusting *H. pylori* prevalence estimates, obtained using imperfect tests, provided that sufficient validation data is available [12]. For example, provided that the sensitivity and specificity relevant to the test population are known and are based on sufficiently large numbers, an accurate measure of prevalence can be estimated by adjustment even when sensitivity and specificity are as low as 60 or 70% [12].

We validated HeliSAL™ against the urease-based CLOtest in a clinical population in New Zealand. In this setting, the test did not perform as well as it has in previous validation studies in the United Kingdom, but performed similarly to some studies carried out in Canada. In particular, the specificity was far lower than previously reported. HeliSAL™ accuracy did not vary significantly by age in terms of either sensitivity or specificity, despite previous reports of poor test performance in those under 50 years of age [10]. Small numbers of truly positive individuals may have reduced this study's power to detect a difference by age.

The earliest validation of the HeliSAL™ test, compared it to another antibody-based (serum) test [3], and therefore likely overestimated its accuracy, as similar issues pertaining to the misclassification of *H. pylori* positivity apply to all antibody-based tests. The results obtained in that validation are, subsequently, the most favourable reported to date (Table 1). Elsewhere, the comparison of HeliSAL™ with a serum test yielded better results than comparison with histological methods [1].

Previous studies have used gold standard measures differing from each other and from the standard used in this study. The gold standard used in this study is presumed to have indicated current infection accurately.

If spontaneous eradication of *H. pylori* infection occurred prior to the gold standard tests, yet antibodies remained elevated, it is possible that infections measured simultaneously by ELISA and CLOtest could yield different results. It is certainly unlikely to occur at a sufficient rate to cause all 33% of the false positive test results identified here, as spontaneous eradication rates are estimated at about 1–2% per year [19]. If spontaneous eradication of *H. pylori* caused the low specificity reported here, it should also have caused similarly low specificity in previous validations of HeliSAL™, and of other antibody-based tests.

In the study conducted by Fallone et al. [1] a number of factors might have affected the comparison of HeliSAL™ with histological outcome. As in Simor et al. [11], there is no mention of the exclusion of patients with previous *H. pylori* eradication treatment. This group may have experienced lowered antibody levels while infection remained, or more likely, raised antibodies with a recently eradicated infection, as antibody levels persist for some 3–6 months after eradication [20].

Antigenic variation exists between strains of *H. pylori* [16], and antibody-based tests do not use standardized antigen preparations [21]. An antigen derived from one population may not perform well in a population where a different strain of *H. pylori* predominates [22]. However, the performance of a number of antibody-based serology tests from the United Kingdom and the United States has been investigated simultaneously in a single population [21]. Despite antigenic variation between strains of *H. pylori*, humans appear to develop antibodies that are identifiable by the antigens commonly found in most antibody-based serology tests [21]. The antigen upon which HeliSAL™ is based may well be derived from a different strain of *H. pylori* than that which predominates in New Zealand. Regardless of whether or not the variation in test accuracy is due to antigenic properties, there is clearly a need to validate antibody-based tests in the population in which they are to be used.

The optimum accuracy of the HeliSAL™ test in one previous study occurred using a cut-off (for level of measured antibody indicating infection) substantially different from that suggested by the manufacturer [8]. In the present study, the manufacturer's cut-off for determining *H. pylori* positivity did not provide the optimum sensitivity and specificity in this data set. Although the sensitivity of the test using the optimum cut-off was similar to those sensitivities previously reported, the specificity was still lower than reported in most previous studies. The manufacturer's cut-off may not provide the optimum test performance

in all populations, another reason for conducting a comprehensive validation study before using HeliSAL™ to determine the presence of *H. pylori* infection.

HeliSAL™ has a lower sensitivity and specificity than other antibody-based tests, the urea breath test, and invasive methods of determining *H. pylori* infection. However, as a tool for population-based epidemiology, a test requiring only a saliva sample is an attractive option. While it is possible to defray the inaccuracy of HeliSAL™ in population-based prevalence surveys using estimates of the sensitivity and specificity of the test, it is clear that HeliSAL™ must be validated separately in each population it is used in.

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