

## Non-invasive diagnosis of *H pylori* infection: Evaluation of serological tests with and without current infection marker CIM

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### Abstract

**AIM:** To evaluate the performance of commercially available immunochromatographic (ICT) and immunoblot tests covering the current infection marker CIM and conventional ELISA for the diagnosis of *H pylori* infection in adult dyspeptic patients.

**METHODS:** Consecutive non-treated dyspeptic patients undergoing diagnostic endoscopy were tested for *H pylori* infection by culture, rapid urease test, and histology of gastric biopsy specimens. Serum from 61 *H pylori* infected and 21 non-infected patients were tested for anti-*H pylori* IgG antibodies by commercial ELISA (AccuBind™ ELISA, Monobind, USA), ICT (Assure® *H pylori* Rapid Test, Genelabs Diagnostics, Singapore), and immunoblot (Helico Blot 2.1, Genelabs Diagnostics, Singapore) assays. ICT and immunoblot kits cover CIM

among other parameters and their performance with and without CIM was evaluated separately.

**RESULTS:** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of ELISA were 96.7%, 42.8%, 83.1%, 81.8%, and 82.9%, of ICT were 90.1%, 80.9%, 93.2%, 73.9%, and 87.8%, of ICT with CIM were 88.5%, 90.4%, 96.4%, 73.0%, and 89.0%, of immunoblot were 98.3%, 80.9%, 93.7%, 94.4%, and 93.9%, and of immunoblot with CIM were 98.3%, 90.4%, 96.7%, 95.0%, and 96.3%, respectively.

**CONCLUSION:** Immunoblot with CIM had the best performance. ICT with CIM was found to be more specific and accurate than the conventional ELISA and may be useful for non-invasive diagnosis of *H pylori* infection.

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**Key words:** *H pylori*; ELISA; Immunochromatographic test; Immunoblot; Current infection marker

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### INTRODUCTION

*H pylori* causes peptic ulcer disease<sup>[1,2]</sup> that can be cured by antimicrobial treatment<sup>[3-5]</sup>. 'Test and treat' strategy that involves non-invasive testing without endoscopy and eradication therapy in young patients<sup>[6]</sup> is effective in management of dyspepsia<sup>[7-9]</sup>. This calls for a simple, reliable and non-invasive diagnostic test for *H pylori* infection in clinical practice. At present there is no single test for *H pylori* that can be used as the 'gold standard'<sup>[10]</sup>.

Culture, rapid urease test, and histology require endoscopic biopsy of gastric mucosal tissue that is expensive, inconvenient for the patient and available only at specialized centers. Moreover, because of a patchy distribution of *H pylori* in the gastric mucosa, biopsy tissue examination may yield false negative results<sup>[11]</sup>. Serological tests that detect anti-*H pylori* IgG antibodies are non-invasive, less expensive, not influenced by sampling error, and less likely to be confounded by suppression of *H pylori* infection by colloidal bismuth, proton pump inhibitors, or antibiotics<sup>[12]</sup>. Serological tests are widely used<sup>[13]</sup> but they cannot differentiate a current infection from a past exposure<sup>[10]</sup>. Performance of serological tests depends on the antigen preparation used<sup>[12,14]</sup>, and as *H pylori* strains differ among geographic locations, local validation of the test is necessary<sup>[13,14]</sup>. A recent development in *H pylori* diagnosis is a commercial immunochromatographic test (ICT) and an immunoblot test covering the current infection marker CIM. CIM is an antigenic protein synthesized by recombinant DNA technology. It is homologous to a conserved secreted protein of *H pylori*. According to its manufacturer (Genelabs Diagnostics, Singapore), presence of anti-CIM IgG antibody is highly predictive for active *H pylori* infection. If so, it should be helpful for diagnosis of *H pylori* infection where facility for endoscopy is not available. Several studies have found the immunoblot test Helico Blot 2.0, its newer version Helico Blot 2.1, and ICT kit Assure<sup>®</sup> *H pylori* Rapid Test, all of which are able to detect anti-CIM antibodies, effective for diagnosis of *H pylori* infection in adults and children<sup>[15-20]</sup>. Bangladesh is a developing country with a high prevalence of *H pylori* infection and peptic ulcer<sup>[21,22]</sup>. Different tests have been evaluated for diagnosis of *H pylori* infection in Bangladesh<sup>[23-26]</sup>. Two studies involved in-house immunoblot assays<sup>[25,26]</sup>. To the best of our knowledge standardized commercial immunoblot or ICT tests with CIM have not been evaluated in Bangladesh. The aim of this study was to evaluate the performance of three commercially available serological tests based on three methods to find a reliable serological test for non-invasive diagnosis of *H pylori* infection in adult dyspeptic patients: ELISA (AccuBind<sup>™</sup>, ELISA, Monobind, USA), ICT (Assure<sup>®</sup>, *H pylori* Rapid Test, Genelabs Diagnostics, Singapore), and immunoblot (Helico Blot 2.1, Genelabs Diagnostics, Singapore). ICT and immunoblot assays cover CIM. The performance of ICT and immunoblot tests was evaluated separately with and without CIM to see whether CIM improves performance of these tests.

## MATERIALS AND METHODS

### Patient selection

Consecutive adult dyspeptic patients attending the Department of Gastrointestinal, Hepatobiliary and Pancreatic Diseases (GHPD) of Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) for diagnostic endoscopy during June 2004 to January 2005 were selected. Informed consent was obtained from each patient before endoscopy, and sample collection and approval of the Ethical Review

Committee of BIRDEM was taken prior to initiation of the project work. Patients who underwent partial or complete gastrectomy, or those with a prior *H pylori* eradication therapy, or those who were treated with any antibiotics, colloidal bismuth compounds, proton pump inhibitors, or H2 receptor blocker within the last four weeks were excluded.

### Endoscopy and gastric biopsy

Endoscopy was done with an Olympus EVIS 160 video endoscope (Olympus Optical Company, Japan) after an overnight fast. Six gastric biopsies (three from antrum and three from corpus) were taken from each patient.

### Collection of serum

After endoscopy 3 mL of venous blood was collected from each patient. Serum was separated after 1 h and kept at -70°C until serological tests were performed.

### Culture

Two gastric biopsy specimens, one from the antrum and one from the corpus, were taken up into Stuart's transport media. Cooled samples were transported to the *H pylori* Laboratory of Laboratory Sciences Division of International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B) within 3-4 h where culture was done as described previously<sup>[27]</sup>. Positive cultures were identified by colony and Gram stain morphology and positive catalase, oxidase, and urease tests.

### Rapid urease test (RUT)

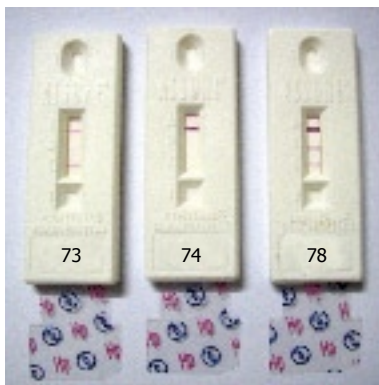
Two gastric biopsy specimens, one from the antrum and one from the corpus, were taken up in Christensen's urea agar media (pH adjusted at 7.0) in screw-capped bottles. A change of color from yellow to pink by any specimen within 2 h was considered as positive.

### Histology

Two gastric biopsy specimens, one from the antrum and one from the corpus, were fixed in 10% formalin in separate containers and were sent to the Histopathology Laboratory of Ibrahim Medical College. Samples were embedded in paraffin wax, cut at 5 µm thickness and were stained by modified Giemsa and hematoxylin & eosin (HE) dye. *H pylori* was identified from its characteristic appearance and distribution. The histopathologist (Nasim Ahmed) was unaware of patients' clinical conditions and other test results.

### 'Gold standard' definition of *H pylori* infection

As there is no single 'gold standard' test for *H pylori*, an operational 'gold standard' definition of *H pylori* infection was used. The definition was as follows: Patients with positive culture result were considered as infected. In the case of a negative culture, patients positive by both RUT and histology were considered as infected. Patients negative by all three gastric biopsy specimen based tests were considered as non-infected. Patients negative by culture and positive by either RUT or histology were considered as indeterminate<sup>[16,17]</sup>.



**Figure 1** Photograph of serum ICT by Assure® *H pylori* Rapid Test. Left: ICT positive; Middle: Negative; Right: ICT with CIM positive (73, 74, and 78: Patients' identification numbers).

### ELISA

ELISA test for anti-*H pylori* IgG antibody was performed using a commercial test kit, AccuBind™ ELISA (Monobind, USA), according to instructions of the manufacturer.

### ICT

ICT test was performed by the commercial test kit Assure® *H pylori* Rapid Test (Genelabs Diagnostics, Singapore) according to the instructions of the manufacturer. When control (A) and test line (C) were visible the ICT test was regarded positive, when control (A), test (C), and CIM line (B) were visible it was regarded ICT with CIM positive. When only control line (A) was visible, the test was regarded negative (Figure 1). When control line (A) was absent or control line (A) and CIM line (B) present but test line (C) absent the test was regarded to be invalid.

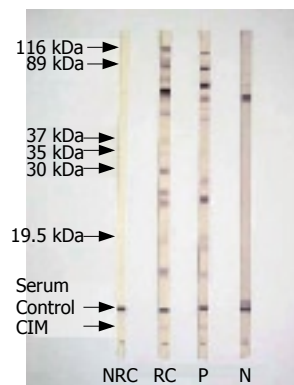
### Immunoblot

Immunoblot test was done using the commercial kit Helico Blot 2.1 (Genelabs Diagnostics, Singapore). Helico Blot 2.1 consists of a Western Blot made from bacterial lysate of *H pylori* strain ATCC 49503 and a recombinant antigen called CIM<sup>[18]</sup>. The test strip contains *H pylori* antigens with molecular weights of 116 kDa (CagA), 89 kDa (VacA), 37 kDa, 35 kDa, 30 kDa (Urease A), and 19.5 kDa as separate lines. CIM is located at the bottom of the strip as an independent band. The test was done and interpreted according to instructions of the manufacturer (Figure 2). The manufacturer's recommended criteria for determining *H pylori* positivity by Helico Blot 2.1 was as follows: (1) 116 kDa (CagA) positive, where CagA has to be present with at least one of the following bands - 89 kDa (VacA), 37 kDa, 35 kDa, 30 kDa (UreA), or 19.5 kDa, or with CIM, (2) presence of any one band at 89 kDa, 37 kDa, or 35 kDa, with or without CIM, (3) presence of both 30 kDa and 19.5 kDa band with or without CIM.

ELISA, ICT, and immunoblot tests were carried out at the Immunology Laboratory of the Department of Immunology of BIRDEM.

### Calculation of performance

Samples from 62 patients who were infected and 21 patients who were non-infected according to 'gold standard' definition were subjected to serological testing. Eight



**Figure 2** Photograph of serum Immunoblot test by Helico Blot 2.1. kDa: kilo Dalton; CIM: Current infection marker; NRC: Non-reactive control; RC: Reactive control; P: Positive Immunoblot with CIM; N: Negative.

patients were indeterminate and excluded. Results of serological tests were plotted against *H pylori* infection status, and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy with 95% confidence interval were calculated using standard formula.

## RESULTS

### Patients' characteristics

A total of 82 patients were included in the study. They were aged between 18 to 75 years with a mean age of 47.6 years and standard deviation 14.0 years. Patients' characteristics are shown in Table 1.

### *H pylori* positivity by different tests

Of the 82 patients, 53 (64.6%) were culture positive, 40 (48.8%) were rapid urease test positive, 31 (37.8%) were histology positive, 71 (86.5%) were ELISA positive, 59 (71.9%) were ICT positive, 56 (68.2%) were ICT with CIM positive, 64 (78.0%) were immunoblot positive, and 62 (75.6%) were immunoblot with CIM positive.

### Performance of serological tests

Sensitivity, specificity, PPV, NPV, and accuracy of serological tests were calculated from data presented in Table 2, and the calculated performance is shown in Table 3. ELISA was very sensitive but its specificity was low. Sensitivity and NPV of ICT were lower but specificity, PPV, and accuracy were much higher than ELISA. CIM decreased sensitivity of ICT slightly but increased its specificity, PPV, and accuracy. Sensitivity of immunoblot was higher than ELISA or ICT. Its specificity was higher than ELISA and same as ICT. Its PPV, NPV, and accuracy were much higher than ELISA and ICT. CIM increased specificity, PPV, NPV, and accuracy of immunoblot without altering its sensitivity (Table 3).

## DISCUSSION

Since its discovery many tests have been designed for diagnosis of *H pylori*<sup>[12]</sup>. But no test is accurate enough to be the 'gold standard'<sup>[10]</sup>. Serological tests are widely used for non-invasive diagnosis<sup>[13]</sup> but a positive serological test does not mean active infection<sup>[10]</sup>. To find a reliable non-invasive test for *H pylori*, we evaluated commercially available conventional ELISA, and new ICT and immunoblot assays covering the recombinant CIM.

Table 1 Characteristics of selected patients (n = 82)

Characteristics	n	Percentage
Age group (yr)		
≤ 30	11	13.4
31-40	15	18.3
41-50	21	25.6
51-60	22	26.8
≥ 60	13	15.9
Sex		
Male	49	59.8
Female	33	40.2
Smoking habit		
Smoker	25	30.5
Non-smoker	57	69.5
Alcohol intake		
Take alcohol	3	3.7
Do not take alcohol	79	96.3
Diabetes		
Diabetic	67	81.7
Non-diabetic	15	18.3
Endoscopic diagnoses		
Normal	20	24.4
Gastritis	24	29.3
Duodenitis	8	9.8
Peptic ulcer	25	30.5
Gastric carcinoma	4	4.8
Reflux esophagitis	1	1.2

The commercial ELISA that we evaluated was very sensitive but less specific (Table 3). Performance of the ELISA kit varies in different populations. Laheij *et al* reviewed a range of sensitivity of 57%-100% and a range of specificity of 31%-100% for different commercial kits in different populations<sup>[28]</sup>. A study in the Netherlands evaluated eight commercial ELISA tests and found sensitivities of 93%-98% and specificities of 95%-98%<sup>[29]</sup>. Using a further commercial kit, a study from Bangladesh found sensitivity, specificity, PPV, and NPV of 100%, 13.6%, 54.8%, and 100%, respectively<sup>[24]</sup>.

Performance of ICT with CIM evaluated in this study was better than two other commercial ICT kits evaluated in Chinese patients<sup>[30]</sup> and similar to another commercial ICT kit evaluated in African-American, Caucasian, and Asian population with <sup>13</sup>C urea breath test as the 'gold standard'<sup>[31]</sup>. Its performance in our study was lower than a study that evaluated this kit in Thai children<sup>[19]</sup> and similar to a study that evaluated this kit in Portuguese children<sup>[20]</sup>.

Performance of immunoblot with CIM in our study was similar to reports that evaluated the same kit in adult French population<sup>[16]</sup> and Portuguese children of different age groups<sup>[17]</sup>. Its specificity was lower in our study than a study in Japanese and American population<sup>[18]</sup>. Lepper *et al* compared different immunoblot kits and found sensitivity, specificity, PPV, and NPV 62.8%-95.9%, 85.7%-100.0%, 97.2%-100.0%, and 37.7%-82.4%, respectively<sup>[32]</sup>.

Among the serological tests evaluated, immunoblot with CIM had the highest sensitivity, specificity, PPV, NPV, and accuracy (Table 3). It thus may be useful for non-invasive diagnosis of *H pylori* infection. Though conventional ELISA was highly sensitive it may not be reliable for diagnostic purpose in these patients due to its low specificity. Immunoblot is costly, takes longer time, and requires laboratory set-up and a trained staff. Though

Table 2 Comparison of serological tests with *H pylori* infection (n = 82)

Test	<i>H pylori</i> infection <sup>1</sup>	
	Infected (n = 61) n (%) <sup>2</sup>	Non-infected (n = 21) n (%) <sup>2</sup>
ELISA		
Positive (n = 71)	59 (83.0)	12 (17.0)
Negative (n = 11)	2 (18.2)	9 (81.8)
ICT		
Positive (n = 59)	55 (93.2)	4 (6.8)
Negative (n = 23)	6 (26.1)	17 (73.9)
ICT with CIM		
Positive (n = 56)	54 (96.4)	2 (3.6)
Negative (n = 26)	7 (26.9)	19 (73.1)
Immunoblot		
Positive (n = 64)	60 (93.8)	4 (6.2)
Negative (n = 18)	1 (5.6)	17 (94.4)
Immunoblot with CIM		
Positive (n = 62)	60 (96.8)	2 (3.2)
Negative (n = 20)	1 (5.0)	19 (95.0)

<sup>1</sup>Infected: Culture positive or culture negative but both RUT and histology positive; non-infected: All three tests negative; <sup>2</sup>Percentages are over row total.

ELISA is cheaper than immunoblot, it also requires laboratory set-up and a trained staff. ICT with CIM had sensitivity and NPV lower than immunoblot or ELISA, but its specificity, PPV, and accuracy were higher than ELISA. Considering sensitivity, specificity, PPV, and accuracy, ICT with CIM yielded good performance (Table 3). ICT is a rapid test, easy to carry out, and can be done at the physician's chamber or in the field level where well-equipped laboratory and trained staff is not available.

In conclusion, immunoblot with CIM had the best performance. Though the sensitivity of ICT with CIM was slightly lower, due to its high specificity and accuracy it would be a more reliable diagnostic tool than conventional ELISA. As ICT with CIM has good performance and it is cheap, easy and rapid, it may be useful for non-invasive diagnosis of *H pylori* infection in adult dyspeptic patients of Bangladesh.

## ACKNOWLEDGMENTS

We wish to thank Genelabs Diagnostics, Singapore, for kindly providing Assure<sup>®</sup> *H pylori* Rapid test and Helico Blot 2.1 test kits free of cost.

## COMMENTS

### Background

A reliable non-invasive test for *H pylori* is essential in clinical practice. Commercial serological tests based on enzyme linked immunosorbent assay (ELISA), immunochromatographic test (ICT) and immunoblot are widely used. But they are unable to distinguish between active infection and a previous contact. Gastric biopsy based tests can indicate active infection but they are inconvenient for the patients and not available everywhere. A recent development is commercial ICT and immunoblot tests with CIM that may indicate active *H pylori* infection. In this study we evaluated performance of commercial ELISA without CIM and ICT and immunoblot with CIM for pre-treatment diagnosis of *H pylori* infection.

### Research frontiers

CIM is an antigenic protein synthesized by recombinant DNA technology.

Table 3 Performance of serological tests

Test	Performance (95% Confidence interval) in percentage				
	Sensitivity	Specificity	PPV <sup>1</sup>	NPV <sup>2</sup>	Accuracy
ELISA	96.7 (88.6-99.4)	42.8 (21.8-65.9)	83.1 (72.3-90.9)	81.8 (48.2-97.7)	82.9 (73.0-90.3)
ICT	90.1 (79.8-96.3)	80.9 (58.0-94.5)	93.2 (83.5-98.1)	73.9 (51.5-89.7)	87.8 (78.7-93.9)
ICT with CIM	88.5 (77.7-95.2)	90.4 (69.6-98.8)	96.4 (87.6-99.5)	73.0 (52.2-88.4)	89.0 (80.1-94.8)
Immunoblot	98.3 (91.2-99.9)	80.9 (58.0-94.5)	93.7 (84.7-98.2)	94.4 (72.7-99.8)	93.9 (86.3-97.9)
Immunoblot with CIM	98.3 (91.2-99.9)	90.4 (69.6-98.8)	96.7 (88.8-99.6)	95.0 (75.1-99.8)	96.3 (89.6-99.2)

<sup>1</sup>PPV: Positive predictive value; <sup>2</sup>NPV: Negative predictive value.

According to its manufacturer, presence of anti-CIM IgG antibodies indicates active *H pylori* infection. It is interesting because usually active infection cannot be diagnosed from serum IgG response to an organism.

### Innovations and breakthroughs

Though the commercial kits used in this study may not represent the methods, from this study we can see both ICT and immunoblot with CIM have better performance than conventional ELISA evaluated, and CIM improves performance of both ICT and immunoblot kits. We can also see that performance of CIM varies depending on the method in which it is used. It performed better in immunoblot than in ICT.

### Applications

This study will facilitate diagnosis of *H pylori* infection.

### Peer review

In this clinical report, Dr. Rahman *et al* evaluated the performance of serological tests with current infection marker and ELISA for the non-invasive diagnosis of *H pylori* infection. Informative results were obtained from their study which could benefit other physicians. Therefore, this study is of importance.

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