

## Detection of *Helicobacter Pylori* Infection by Imported IgG ELISA Kits in Comparison with Iranian Home Made Kit

Yeganeh Talebkhan<sup>1</sup>, Marjan Mohammadi<sup>2</sup>, Ghader Khalili<sup>3</sup>,

Arghavan Sheykholeslami<sup>4</sup>, Naser Rakhshani<sup>5</sup>, Fereidoun Mahboudi<sup>6</sup>, Sadegh Massarrat<sup>7</sup>

<sup>1</sup> Researcher, Biotechnology Research Center, Pasteur Institute of Iran-National Biotechnology Network, Ministry of Health and Medical Education

<sup>2</sup> Assistant Professor, Biotechnology Research Center, Pasteur Institute of Iran-National Biotechnology Network, Ministry of Health and Medical Education

<sup>3</sup> Researcher, Immunology Department, Pasteur Institute of Iran

<sup>4</sup> Researcher, Digestive Disease Research Center, Tehran University of Medical Science, Shariati Hospital, Tehran, Iran

<sup>5</sup> Assistant Professor, Digestive Disease Research Center, Tehran University of Medical Science, Shariati Hospital, Tehran, Iran

<sup>6</sup> Associate Professor, Biotechnology Research Center, Pasteur Institute of Iran-National Biotechnology Network, Ministry of Health and Medical Education

<sup>7</sup> Professor, Digestive Disease Research Center, Tehran University of Medical Science, Shariati Hospital, Tehran, Iran

### ABSTRACT

#### Background

*Helicobacter pylori* (Hp) is a gram negative, spiral shaped bacterium which colonizes the gastric mucosa and induces gastroduodenal complications varying from mild gastritis with no clinical complications to peptic ulcer diseases and even gastric malignancies. The rate of Hp infection is 30-50% in developed countries whereas it has been rated up to 80% of the adult population in developing countries like Iran.

Hp infection can be detected by various diagnostic methods. Culturing biopsy specimens and Rapid Urease Test (RUT) are the most common and reliable tests which can manifest Hp infection through proper sampling but these methods are invasive ones due to the need for endoscopy procedure in isolation of biopsy specimen. Application of serological assays are being increasingly used for epidemiological studies and detecting systemic immune responses toward past Hp infection. ELISA assays are the most popular techniques particularly in cases with no previous treatment.

#### Materials and Methods

In this study we tested three imported IgG ELISA kits which are available for clinical diagnostics in detecting host sero-reactivity to Hp infection and compared them with a home made IgG ELISA kit. Histology and RUT were used as the gold standard tests for determination of Hp positive vs. Hp negative subjects using biopsy specimens from antrum. Sensitivity, specificity, accuracy and other required criteria were evaluated for each ELISA kit.

#### Results

According to the results the original criteria (Sensitivity and specificity) for each kit were as follows: BIOHIT (41.6%, 100%), Trinity (100%, 86.6%), Pishtaz (100%, 86.6%), Home made (100%, 92.6%).

#### Conclusions

Evaluation of these different IgG ELISA kits originating from different parts of the world and cross comparison of the results indicated that the cut off values should be refined for user country in order to obtain the highest sensitivity and specificity. These differences can be due to the vast geographic heterogeneity among Hp antigens. Furthermore, this study showed that home made ELISA kit can be substituted for imported ELISA kits due to its valid serological criteria.

**Keywords:** *Helicobacter pylori*, IgG ELISA, Serology, Screening

Govaresh/ Vol. 11, No. 2, Summer 2006; 120-125

Received: 13-07-2006

Edited: 10-08-2006

Accepted: 12-08-2006

Corresponding author: Biotechnology Research Center, Pasteur Institute of Iran. No. 69, Pasteur Blvd., Tehran, 13164, Iran.

Telefax: +98 21 66480780

E-mail: marjan@pasteur.ac.ir

### BACKGROUND

*Helicobacter pylori* (Hp) infects more than half the world adult population and is one of the rare

microorganisms that causes gastritis, ulcers, adenocarcinoma and even MALT lymphoma. The rate of its infection varies in different countries and even in different parts of one country. Epidemiological studies have revealed that nearly half of the world population and the majority of people in developing countries are infected with this bacterium.(1), Evidences indicate that infection occurs in the childhood and its rate is directly associated with low socio-economic status, poor hygiene, number of family members, contamination of drinking water supplies, etc.(2)

This bacterium colonizes the mucosal layer of the stomach and protects itself from the existing harsh acidic conditions. In this environment, it remains extra cellular and produces immune stimulatory antigens (3) which can pass through the protective gastric epithelial layer and cause the migration of inflammatory cells (through the classical pathway of complement activation) and adaptive immune cells.(4, 5), The activated cells proliferate in the gastric tissue in an attempt to eliminate the invasive bacterium. Due to their inefficiency in traversing the epithelial layer, they are unable to rid the host from the colonizing bacteria. Nevertheless, as activated cells, they produce destructive compounds which inadvertently damages the host gastric tissue. The sum of such defective cycle is chronicity of infection despite induction of host humoral immune responses, i.e. local and systematic antibody production. Although this response is incapable of protection, it is a very valuable tool for detection of infection and relapses following failure of drug treatment.(6, 7)

Serological detection of Hp infection is one of the major noninvasive methods which can be used in epidemiological studies, screening young Hp positive patients suffering from dyspepsia before performance of endoscopy and long term follow-ups subsequent to drug therapy or screening those with severe pathological findings especially in areas with low incidence of Hp infection.(8, 9, 10), Although serum levels of IgG and IgA against Hp surface antigens drop within 2 to 6 months following treatment but they will remain present up to one year after antibiotic treatment and do not

reveal the actual presence of bacterial infection.(11, 12), Thus, altogether use of serological tests especially in infants, children and disabled individuals is preferable over invasive techniques such as endoscopy and biopsy collection.(13)

The non invasive nature, low cost and easy applications are critical characteristics of serological assays in comparison to UBT using C14 or C13. Hence several imported commercial kits are currently used for detection of Hp infection in Iran.

The vast molecular heterogeneity among Hp strains isolated from different geographic regions worldwide necessitates the use of native antigens for such serological assays to avoid false results.(14, 15)

This is the first report evaluating and comparing the diagnostic accuracy, sensitivity and specificity of commonly used commercially imported IgG ELISA kits with a home made kit (based on local Hp antigens) for detection of Hp-specific serum IgG antibodies.

## MATERIALS AND METHODS

**Subjects.** Iranian Subjects recruited to Shariati Hospital as first degree relatives of confirmed gastric cancer (GC) cases were included in this study. The age group ranging from 40 to 65 composed of both genders (14 males and 18 females). These individuals underwent endoscopy procedure and were diagnosed without any abnormal macroscopic changes or any indication of peptic ulcer disease (PUD). Those who had received antibiotic therapy for eradication of Hp during last 6 months were excluded. Other exclusion criteria were use of proton pump inhibitors or H2 receptor antagonists during the last 2 weeks. Informed consent was obtained from every subject.

**H. pylori status.** Diagnosis of Hp infection was performed by histologic examination of five gastric biopsies from five locations of fundus (1), corpus(2), and antrum.(2), Rapid Urease Test (RUT) was performed on an additional antral biopsy specimen. All histology slides were H&E stained and suspicious ones were Giemsa stained, as well. The slides were scored for the presence of

Hp in several fields. Confirmed positive cases were those with three or more Hp-positive specimens by histology plus positive RUT results. Confirmed negative cases were those with no Hp-positive specimens (0/ 5 samples). Cases with one or two Hp-positive specimens were excluded from this study. The study was thus performed on 32 individuals including 15 and 17 confirmed positive and negative cases respectively.

**Serology.** The tested IgG ELISA kits are listed in Table 1. All tests were performed according to their manufacturers' instructions blinded to the status of Hp infection. Diagnostic accuracy of each kit was determined based on the status of histology and RUT.

**Home made ELISA assay.** This kit is approved by the National Reference Center and is pending for patent registration. Briefly, MaxiSorp 96 microwell plates (Nunc GmbH, Germany) were

coated with 5µg/ml Hp soluble antigenic fraction from a cocktail of Iranian Hp strains isolated from 5 patients suffering from different clinical manifestations such as gastritis and gastric cancer. The plates were blocked with 1% BSA in PBS (pH 7.2) for 1hr. Tested sera were diluted in PBS containing Tween20 (pH 7.2). The coated wells were washed (5 times) and the wells were incubated with diluted secondary antibody (HRP conjugated rabbit anti human IgG) (Dako, Denmark) for 1hr. The plates were then washed and the substrate was added and incubated at RT. The stop solution (1M H<sub>2</sub>SO<sub>4</sub>) stopped the colorimetric reaction and the plates were read at 450nm with a micro plate ELISA reader (BIOHIT, Finland).

**Statistics.** SPSS package (v. 11.5) was used for statistical analysis and refinement of the cut off values for each kit.

**Table 1.** ELISA kits used

Kits	Commercial name	Supplier
BIOHIT	<i>Helicobacter pylori</i>	BIOHIT PLC, Helsinki, Finland
Trinity	Capita™ H. pylori IgG ELISA	Trinity Biotech PLC, Bray, Ireland
Phishtaz	H. pylori IgG ELISA kit	Pishtaz Teb Diagnostic, Tehran, Iran
Home made ELISA	H. pylori IgG ELISA kit	Pasteur Institute of Iran, Tehran, Iran

## RESULTS

Of the 32 subjects included in this study, 46% (15/32) were diagnosed as Hp-positive and 17 individuals (53.1%) as Hp negative cases via histology and RUT. Values for sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), accuracy and efficiency of each test are listed in Table 2. The above criteria were calculated after exclusion of intermediate cases for each kit.

Cut off points were corrected for each kit according to the histological data. Based on these new values, the serology criteria were recalculated and are listed in Table 3.

Receiver Operator Characteristic Curve (ROC) was drawn for each test and the appropriate cut off value was determined. The optimal cut off values

(border range) in this studied group were as follows: 13 PIU (12-14) (Pasteur IgG Unit) for home made kit, 1.2 (1.1-1.3) ISR for Trinity ELISA kit, 9.5 (9-10) EIU for BIOHIT ELISA assay and 8.5 (7-10) for Pishtaz ELISA kit. These new cut off values could improve the serological criteria as demonstrated in Table 3.

## DISCUSSION

Serological approaches are non invasive tools widely used for determining Hp infection and ELISA assays are more popular than other approaches among clinicians and laboratory staff due to the ease and practicality of this technique and low cost of assays plus their high sensitivity. Evaluation of specificity and sensitivity of these tests results in disagreeing and even contradicting

**Table 2.** Comparison of serological indices with histology and RUT results

Kits	Serology result	Histology Result		Serology Criteria				
		Negative (n=17)	Positive (n=15)	Sensitivity %	Specificity %	PPV %	NPV %	Efficiency %
BIOHIT*	Positive	0	5					
	Intermediate	0	3	41.6	100	100	70.8	75.8
	Negative	17	7					
Trinity**	Positive	2	15					
	Intermediate	2	0	100	86.6	88	100	93.3
	Negative	13	0					
Pishtaz***	Positive	2	12					
	Intermediate	2	3	100	86.6	85.7	100	92.6
	Negative	13	0					
Home made****	Positive	1	15					
	Intermediate	3	0	100	92.8	93.75	100	96.5
	Negative	13	0					

\* The cut-off value for BIOHIT kit is 34-42 EIU (Enzyme Immuno Units)

\*\* The cut-off value for Trinity kit is 0.91- 1.09 ISR (Immune Status Ratio)

\*\*\* The cut-off value for Pisthaz kit is 5-10 U/ml

\*\*\*\* The cut-off value for home made kit is 8-12 PIU/ml (Pasteur IgG Unit)

**Table 3.** Corrected serology criteria based on histology and RUT results

Kits	Serology result	Histology result		Serology Criteria				
		Negative (n=17)	Positive (n=15)	Sensitivity %	Specificity %	PPV %	NPV %	Efficiency %
BIOHIT	Positive	0	12					
	Intermediate	1	1	85.7	100	100	88.8	93.3
	Negative	16	2					
Trinity	Positive	1	14					
	Intermediate	1	1	100	93.75	93.3	100	96.6
	Negative	15	0					
Pishtaz	Positive	2	12					
	Intermediate	1	3	100	87.5	85.7	100	92.8
	Negative	14	0					
Home made	Positive	1	15					
	Intermediate	0	0	100	94.11	93.75	100	96.9
	Negative	16	0					

results.(16), Low specificity maybe due to various reasons such as the presence or progress of atrophy and intestinal metaplasia (17, 18), antibiotic treatment of Hp infection or other infections may lead to reduced Hp load but does not affect antibody titers in serum for a long time, the presence of IgA against Hp instead of IgG in 2% of total cases (19) or incorrect sampling from gastric mucosal tissue during endoscopy procedure which can decrease the rate of Hp identification and isolation.

Discrepancies among the sensitivity of these assays can be due to the presence of a recent Hp infection which can lead to a negative Hp serology test whereas identification tests based on biopsy sampling are simultaneously positive.

Significant heterogeneity among isolated Hp strains from different geographic regions, cross reactivity with other enteric pathogens are some possible reasons for uncertain results in determining Hp infection using imported ELISA kits.(20, 21, 22), Therefore the sensitivity and specificity of an assay in a particular population may not necessarily be applied to another.

In Iran the rate of Hp infection is high among adult population and up to 85% of these subjects are Hp seropositive (23) which might be due to acquiring the infection in early stages of life and collection of Hp negative sera is quite challenging as faced during this study.

Although there are various *Helicobacter pylori* IgG ELISA assays available in the country which are imported from other countries with defined specificity and sensitivity, the existing significant molecular heterogeneity among Iranian and western Hp strains in some Hp conserved and non conserved genes (24, 25) emphasizes the need for use of native (local) antigens in developing ELISA assay for detecting seroreactivity toward this microorganism.

Evaluation of these kits and comparison with a home made kit demonstrated that BIOHIT (Finland) provides the best specificity but with low sensitivity. Trinity (Ireland), as another imported kit, provides 100% sensitivity similar to that for

home made ELISA kit, the latter of which had greater specificity. The last kit, Pishtaz ELISA, was similar to Trinity kit but produced more intermediate or equivocal results.

Several reports indicate variations in the accuracy of one ELISA kit when tested in different populations. For example, HM CAP (High Molecular Cell Associated Proteins) produced two different values for sensitivity and specificity of 100 and 98.7% when tested in the US (26), whereas these values were 81 and 71% in Netherlands (27) and 72.7% and 68.4% in the Chinese population.(28), Thus further supporting the heterogeneity among the infecting strains of *Helicobacter pylori* in different geographic regions with lower indices for East Asia as compared to the West. However, the ROC curves can be employed to refine the cut-off values and adjust it for the target population. Larger sample size is required in order to draw firm conclusions.

The authors hereby recommend the use of home made IgG ELISA assay in substitution for imported kits due to its use of local Hp antigens and the resulting high sensitivity and specificity.

## CONCLUSION

Evaluation of these different IgG ELISA kits originating from different parts of the world and cross comparison of the results indicated that the cut off values should be refined for user country in order to obtain the highest sensitivity and specificity. These differences can be due to the vast geographic heterogeneity among Hp antigens. Furthermore, this study showed that home made ELISA kit can be substituted for imported ELISA kits due to its valid serological criteria.

## ACKNOWLEDGMENT

*The development of home-made IgG ELISA kit was generously funded by the Medical Biotechnology Network, Ministry of Health and Medical Education of Iran.*

## References

1. Mitchell HM. The epidemiology of *Helicobacter pylori*. *Curr Top Microbiol Immunol* 1999; 241: 11-39.
2. Breuer T, Malaty HM, Graham DY. The epidemiology of *H. pylori*-associated gastroduodenal diseases. In: Ernst PB, Michetti P, Smith PD, editors. *The immunobiology of H. pylori: From pathogenesis to prevention* 1st ed. Philadelphia: *Lippincott-Raven*; 1997. p. 1-14.
3. Blom K, Lundin BS, Bolin I, Svennerholm A. Flow cytometric analysis of the localization of *Helicobacter pylori* antigens during different growth phases. *FEMS Immunol Med Microbiol* 2001; 30: 173-9.
4. De Francesco V, Zullo A, Rinaldi V, Hassan C, Ballanti P, Winn S, *et al.* Relationship between antral lymphocyte density and basal gastrin levels in patients with *Helicobacter pylori* infection. *Dig J Liver Dis* 2000; 32: 676-81.
5. Berstad AE, Hogasen K, Bukholm G, Moran AP, Brandtzaeg P. Complement activation directly induced by *Helicobacter pylori*. *Gastroenterology* 2001; 120: 1108-16.
6. Lerro P, Perrucci V, Morra I, Lombardo L, Gaido F, Ansaldi N. CagA seropositivity and the severity of *Helicobacter pylori* infection in dyspeptic children. *Acta Paediatr* 2000; 89: 1312-5.
7. Figueroa G, Troncoso M, Toledo MS, Acuna R. Application of serology to confirm the eradication of *Helicobacter pylori* in peptic ulcer patients. *Rev Med Chil* 2000; 128: 1119-26.
8. Huang JQ, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. *Gastroenterology* 1998; 114: 1169-79.
9. Axon ATR, Bell GD, Jones RH, Quine MA, McCloy RF. Guidelines on appropriate indications for upper gastrointestinal endoscopy. *Brit Med J* 1995; 310: 853-6.
10. Malfertheiner M, Megraud F, O'Morain C, Hungin AP, Jones R, Axon A, *et al.* Current concepts in the management of *Helicobacter pylori* infection-the Maastricht 2-2000 consensus report. *Aliment Pharmacol Ther* 2002; 16: 167-80.
11. Kosunen TU, Seppala K, Sarna S, Sipponen P. Diagnostic value of decreasing IgG, IgA, and IgM antibody titres after eradication of *Helicobacter pylori*. *Lancet* 1992; 339: 893-5.
12. Anderson LP. The antibody response to *Helicobacter pylori* infection and the value of serologic tests to detect *H. pylori* and for post-treatment monitoring. In: Stewart Goodwin C, Worsley BW, editors. *Helicobacter pylori biology and clinical practice* 1st ed. Boca Raton: *CRC Press*; 1993. p. 285-305.
13. Belkind-Gerson J, Basurto G, Newton O, Avila-Figueroa C, del Rio C, Garcia-Gaytan E, *et al.* Incidence of *Helicobacter pylori* infection in a cohort of infants in the state of Morelos. *Salud Publica Mex* 2001; 43: 122-6.
14. Hoshiya S, Tokunaga K, Tanaka A, Takahashi S. Serological diagnosis of *Helicobacter pylori* infection. *Nippon Rinsho* 2001; 59: 259-64.
15. Leung WK, Chow TP, Ng EK, Chan FK, Chung SC, Sung JJ. Validation of a new immunoblot assay for the diagnosis of *Helicobacter pylori*. *Aliment Pharmacol Ther* 2001; 15: 423-8.
16. Miwa H, Kikuchi S, Ohtaka K, Kobayashi O, Ogihara A, Hojo M, Nagahara A, *et al.* Insufficient diagnostic accuracy of imported serological kits for *Helicobacter pylori* infection in Japanese population. *Diag Microbiol Infect Dis* 2000; 36: 95-9.
17. Nedenskov-Sorensen P, Aase S, Bjormeklett A, Fausa O, Bukholm G. Sampling efficiency in the diagnosis of *H. pylori* infection and chronic active gastritis. *J Clin Microbiol* 1991; 29: 672-5.
18. Asaka M, Sugiyama T, Nobuta A, Kato M, Takeda H, Graham DY. Atrophic gastritis and intestinal metaplasia in Japan: Results of a large multicenter study. *Helicobacter* 2001; 6: 294-9.
19. Shirin H, Bruck R, Kenet G, Krepel Z, Wardi Y, Reif S, *et al.* Evaluation of a new immunochromatographic test for *Helicobacter pylori* IgG antibodies in elderly symptomatic patients. *J Gastroenterol* 1999; 34: 7-10.
20. Atherton JC, Peek RM Jr, Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. *Gastroenterology* 1997; 112: 92-99.
21. Atherton JC, Cao P, Peek RM Jr, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; 270: 17771-7.
22. Yamaoka Y, Kodama T, Kita M, Imanishi J, Kashima K, Graham DY. Relationship of *vacA* genotypes of *Helicobacter pylori* to *cagA* status, cytotoxin production, and clinical outcome. *Helicobacter* 1998; 3: 241-53.
23. Massarrat S, Saberi-Firooz M, Soleimani A, Himmelmann GW, Hitzges M, Keshavarz H. Peptic ulcer disease, irritable bowel syndrome and constipation in two populations in Iran. *Eur J Gastroenterol Hepatol* 1995; 7: 427-33.
24. Mohammadi M. Genotype and strains of Iranian *Helicobacter pylori* strains based on PCR-RFLP of conserved and non-conserved genes. *Bull Soc Pathol Exot* 2000; 93: 224-6.
25. Mohammadi M, Oghalaie A, Mohajerani N, Massarrat S, Nasiri M, Bennedsen M, *et al.* Prevalence of *Helicobacter pylori* vacuolating cytotoxin and its allelic mosaicism as a predictive marker for Iranian dyspeptic patients. *Bull Soc Pathol Exot* 2003; 96: 3-5.
26. Evans DJ, Evans DG, Graham DY, Klein PD. A sensitive and specific serologic test for detection of *Campylobacter pylori* infection. *Gastroenterology* 1989; 96: 1004-8.
27. Jensen AK, Anderson LP, Gaarslev K, Wachmann CH. Comparison of four-second generation kits for detection of IgG antibodies against *Helicobacter pylori* in adults. *Zentralbl Bakteriol* 1993; 280: 221-6.
28. Leung WK, Ng Enders KW, Chan FKL, Chung SC, Sung JJ. Evaluation of three commercial enzyme-linked immunosorbent assay kits for diagnosis of *Helicobacter pylori* in Chinese patients. *Diagn Microbiol Infect Dis* 1999; 34: 13-7.