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Article

Article

Detection of Anti-CagA Antibodies in Sera of *Helicobacter pylori*-Infected Patients Using an Immunochromatographic Test Strip

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Abstract

The *cagA* gene of *Helicobacter pylori* that encodes an immunodominant CagA protein provokes severe mucosal damage and acts as a risk factor for the development of peptic ulcer disease and gastric cancer. Our aim is to develop an immunochromatographic test strip (ICTS) using our previously developed recombinant CagA (rCagA) protein and anti-rCagA monoclonal antibody (Mab) for the detection of anti-CagA antibodies in sera of infected patients. The rCagA was firstly conjugated to gold nanoparticle and placed into the conjugate pad. A nonconjugated rCagA and anti-rCagA Mab (CK-02) were immobilized on the test line and control line, respectively. Biopsy and serum samples from 30 *H. pylori*-infected patients were used. The presence of *cagA* gene in biopsy samples was first detected by PCR (Polymerase Chain Reaction), and 22 patients were found positive while 8 were negative. When serum samples were tested by our developed ICTS, 21 were positive for anti-CagA antibodies while 9 were negative. The serum samples were also tested by a commercial ELISA (Enzyme Linked Immunosorbent Assay), and when compared to the ICTS a sensitivity of 95% and a specificity of 100% were obtained. The ICTS can be used for rapid detection of CagA-positive *H. pylori* infection instead of expensive, time consuming and laborious invasive approaches.

Introduction

Helicobacter pylori infection is one of the most common infections worldwide with an estimated prevalence of >90% in developing countries and 40% in developed countries (1, 2). *Helicobacter pylori* infection generally leads to the development of chronic gastritis that might progress to gastric ulcer and cancer (3, 4). The *cagA* gene, one of the most important virulent factors of *H. pylori*, is found in 60–70% of the European strains (5), while it is found in >90% of East Asian strains (6). Yamaoka *et al.* (7) reported that in Japan 96.9% of *H. pylori* isolates analyzed by Polymerase Chain Reaction (PCR) was *cagA*-positive. Several investigators have shown that patients with peptic ulcer diseases were generally infected with *cagA*-positive *H. pylori* strains as compared to strains from gastritis patients (5, 8, 9, 10, 11, 12). In our previous studies we showed that the *cagA* gene was detected in 57.0% of gastritis, 71.4% of gastric ulcer and 86.6% of duodenal ulcer patients, and in another study 57.1% of gastritis and 92.9% of peptic ulcers were *cagA*-positive (13, 14). In a recent study, de la Cruz-Herrera *et al.* (15) showed that 70.7% of gastric cancer patients were seropositive (total IgG) for the CagA antigen. Thus, the search for *cagA*-positive *H. pylori* strains will be a more reliable method for evaluating the risk of development of peptic ulceration and gastric cancer. In addition, the detection of *cagA*-positive *H. pylori* infection can help in the evaluation of the treatment strategy. The *cagA* gene status can only be determined in biopsy samples by PCR (invasive method). Serology, however, is a noninvasive method that can detect serum anti-CagA antibodies (16–21). The immunochromatographic test strip (ICTS) is another serological test that can be used for the detection of such antibodies.

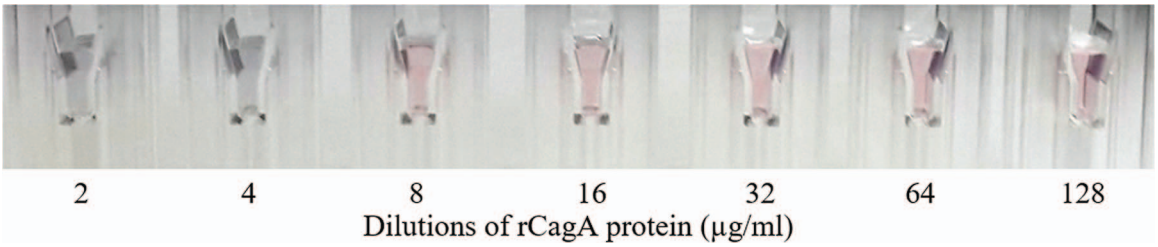

Figure 1. A 2-fold dilution of the rCagA protein to which an equal volume of gold nanoparticle was added. An optimum concentration of the protein to be used for gold conjugation was found 8 µg/ml as shown by the first dilution that did not change red color.

Several ICTSs are commercially available for the diagnosis of *H. pylori* infection; however, none of these ICTSs detects such anti-CagA antibodies. These qualitative tests were used either to detect *H. pylori* antigens in stool samples or to detect anti-*H. pylori* antibodies in blood samples (whole blood, serum and/or plasma) to aid in the diagnosis of *H. pylori* infection in adult patients with symptoms of gastrointestinal disorders (22). The advantages of the ICTS are being rapid, easy to use, sensitive, specific and cost-effective; it can be used at a health care unit without the need for complex equipment or experience, and it can be stored at room temperature (RT) for up to 24 months (23, 24).

Our aim is to develop an ICTS using our recombinant CagA (rCagA) protein and anti-rCagA monoclonal antibody (Mab) for the detection of anti-CagA antibodies in sera of *H. pylori*-infected patients. This test will help a great deal to determine the pathological potential of *H. pylori* strains in symptomatic subjects and correlate the presence of such antibodies with the clinical outcome. The test will also provide a predictive evaluation in particular for patients with gastritis for the progression to peptic ulceration and gastric cancer and eliminate the need for additional endoscopies in the follow up of eradication therapy.

Experimental

Gold-conjugated rCagA protein

The rCagA protein (GenBank accession no: KM102759) prepared previously in our laboratory by cloning the *cagA* gene 5′ conserved region of *H. pylori* strain was conjugated to 20 nm colloidal gold (BBI Solutions, UK) as described previously (25). The rCagA protein was expressed and purified as described in our previous study (26), and then buffer was exchanged with 5 mM phosphate buffer using Amicon Ultra-0.5 mL Centrifugal Filter (Merck Millipore, Germany) that filters the protein <50 kDa. Briefly, a 2-fold dilution of rCagA protein was made to which an equal volume of colloidal gold solution (pH 9.0) was added and incubated for 15 min at RT. The addition of 100 µL of 10% (w/v) NaCl solution resulted in a color change from brilliant red to blue. The first dilution that did not change color was taken as the optimum concentration of the protein to be used for gold conjugation. The optimum concentration of rCagA protein conjugated with gold nanoparticle was found to be 8 µg/mL (Figure 1). One milligram of this concentration was slowly added to 5 mL colloidal gold solution (pH 9.0) stirred vigorously and incubated for 45 min at RT. Then, 500 µL of 10% (w/v) bovine serum albumin (BSA) solution in 20 mM sodium borate (pH 9.0) was added and incubated at RT for 10 min. The conjugate was centrifuged at 10,000xg for 30 min at 10°C and then washed two times with 5 mL of 20 mM sodium borate (pH 9.0) containing 1% (w/v) BSA and 0.1% (w/v) sodium azide. The pellet was resuspended with 5 mL of washing buffer and stored at 4°C.

Direct dot blot assay

Direct dot blot assay was first used to confirm that gold-conjugated rCagA protein reacts with anti-rCagA Mab (CK-02) developed previously in our laboratory by hybridoma technology. A commercial anti-CagA Mab A-10 (Santa Cruz Biotechnology, USA) was used as internal positive control, and BSA was used as internal negative control. A 5 µL of each dilutions containing 0.5 µg protein was spotted onto the nitrocellulose membrane and incubated at RT for 15 min. The membrane was blocked by incubating into PBS-T containing 5% (w/v) BSA for 1 h at RT, then washed once using PBS-T for 30 s. Gold-conjugated rCagA protein solution was then added and incubated overnight at RT with shaking at low speed. The membrane was washed three times using PBS-T with shaking. A positive reaction was detected by the presence of red-colored dot.

Conjugate pad

The conjugate pad was prepared as described previously with minor changes (27). One milligram of gold-conjugated rCagA protein solution was centrifuged, and then the pellet was resuspended with 400 µL of 20 mM sodium borate buffer (pH 8.0) containing 8.75% (w/v) BSA, 8.75% (w/v) sucrose, 10 mM EDTA (Ethylenediaminetetraacetic acid), 0.6 M NaCl and 0.1% (w/v) sodium azide. The G041 conjugate pad (Merck Millipore, Germany) that was cut in size of 5 × 10 mm using a rolling trimmer was soaked into the conjugate solution and laid on a plastic surface. The conjugate pad was dried at 37°C for 3 h in a container with desiccant silica gel and stored at RT with desiccant to prevent rehydration.

Membrane

A 2 µL (1 µg) of rCagA protein in 5 mM phosphate buffer and 2 µL (0.35 µg) of anti-rCagA Mab (CK-02) in elution buffer of NAb Protein G Spin Kit (Thermo Scientific, USA) were dispensed on the membrane of Hi-Flow Plus 240 Membrane Card (Merck Millipore, Germany) that was cut in size of 5 × 60 mm as test line and control line, respectively, and immobilized by incubating at 37°C for 1 h in a container containing desiccant. The membrane was blocked using 2% (w/v) BSA in 5 mM phosphate buffer containing 0.05% (w/v) sodium dodecyl sulfate (SDS) at RT for 1 h on the shaker with low speed. Then, it was washed 2 times (1 × 30 s and 1 × 15 min) with 5 mM phosphate buffer containing 0.05% (w/v) SDS, dried at RT and stored at RT with desiccant.

Assembly of test strip

The prepared conjugate pad was firstly laminated between the sample pad section and the membrane on the Hi-Flow Plus 240 Membrane Card. Then, sample pad (C083) and absorbent pad (C083) (Merck Millipore, Germany), those were cut in size of 5 × 20 mm, were

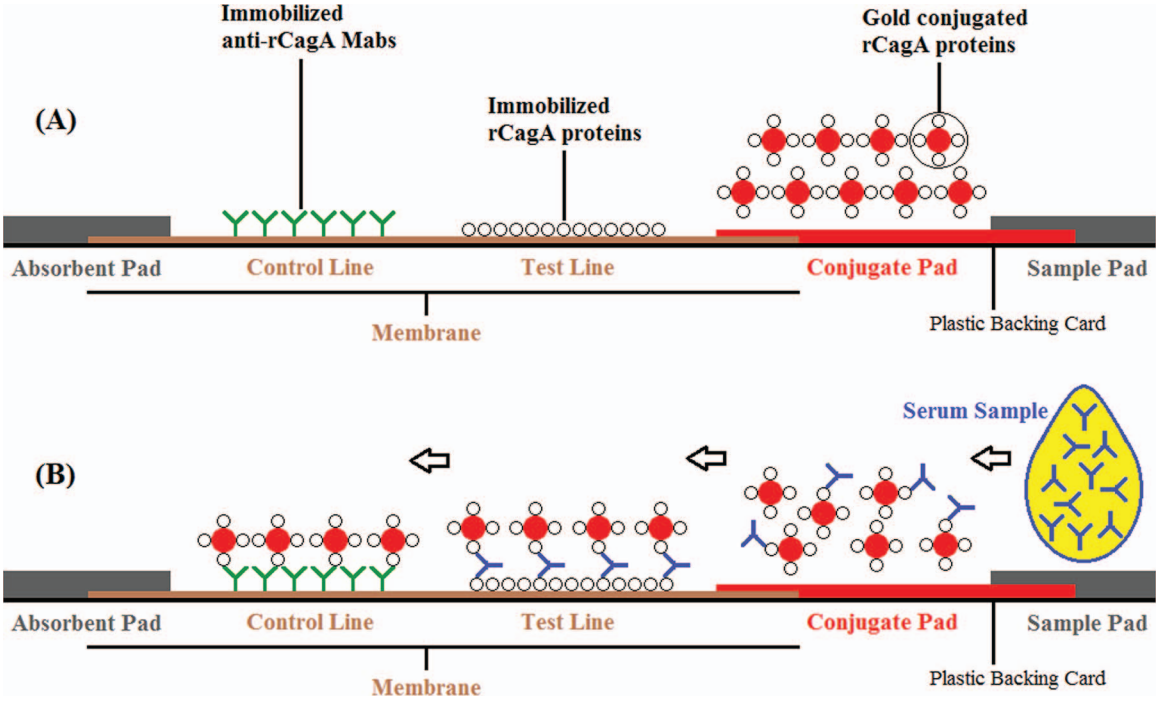

Figure 2. Schematic representation of the ICTS (for the detection of anti-CagA antibodies) showing the constituents of the strip before (A) and after (B) the addition of the sample.

adhered directly to the adhesive sections of the card. The assembled strip in size of 5 × 60 mm was housed in a plastic cassette for easier handling and protection. Ready-to-use test strip was stored in the presence of desiccant at RT.

Principles of the ICTS

The developed ICTS is a qualitative lateral flow immunoassay based on double-antigen sandwich-type format (Figure 2A). A drop of serum sample when added to the sample pad leads to a lateral flow of the sample fluid containing anti-CagA antibody toward the conjugate pad where it will bind to the gold-conjugated rCagA protein (detector reagent) and forms an immunocomplex. The complex continues to move toward the test line where it will irreversibly bind to the immobilized unconjugated rCagA protein (capture reagent) and forms a red color line (positive result). The more the anti-CagA antibodies present in the sample, the stronger the color of the test line. The flow of the sample fluid continues toward the control line where the remaining gold-conjugated rCagA protein binds to the immobilized anti-rCagA Mab (CK-02) and gives a red color line (Figure 2B). The control line always shows red color whether the sample is positive or negative, an indicator for a proper function of the test. The absorbent pad at the end of the strip absorbs the fluid through the membrane to ensure a continuous flow and thus maintains a clear background. Only the control line will be visible (negative result) if anti-CagA antibodies are lower than the detection limit of the test. The test is considered invalid if the control line shows no color and no results are interpreted after 1 h.

Samples

Gastric biopsies and serum samples were collected from 30 patients who attended the gastroenterology unit at Fatih University Hospital. A written consent was filled for each patient, and the study was approved by the university ethical committee. The presence of *H. pylori* (*ureC* gene) in biopsies and the *cagA* status were determined by PCR as described previously (12, 28).

Detection

Anti-CagA antibodies in serum samples were detected by the ICTS. A 100 µL of 1:5 diluted serum in 5 mM phosphate buffer was applied to sample application window of the ICTS using a micropipette. After 5 min, 20 µL of 5 mM phosphate buffer was added to wash membrane that has the slowest flow rate (capillary flow time of 240 s/4 cm), and the results were interpreted after 20 min incubation at RT. The presence of anti-CagA antibodies in serum samples was also detected by a commercial Enzyme Linked Immunosorbent Assay (ELISA) kit (Euroimmun, Germany) according to manufacturer instructions. The performance of the test strip was evaluated by comparing the results of our developed ICTS with those of the commercial ELISA. In addition, the relation between the presence of anti-CagA antibody and the clinical outcome was considered.

Results

Dot blot

The gold-conjugated rCagA protein was captured by both our Mab CK-02 and the commercial Mab A-10 (positive control) as shown in red-colored dots on the membrane (Figure 3). A clear background area (BSA) on the membrane was the negative control. Both controls confirmed that our Mab CK-02 reacted with the gold-conjugated rCagA protein specifically and the assay was done properly.

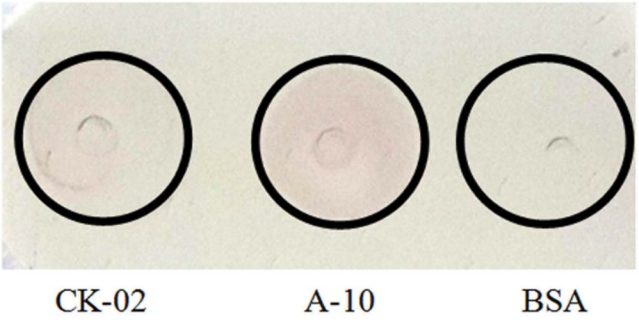

Figure 3. Direct dot blot assay that shows gold conjugated rCagA protein reacted with our Mab CK-02 (red-colored dot) on the membrane. A red dot was also seen at the commercial Mab A-10 site (positive control) while no color was seen on BSA site (negative control).

Figure 4. Test strip cassettes showing red-colored test line and control line following application of a strong and weak positive sample (1 and 2) and only red-colored control line following application of a negative sample (3). C represents control line; T, test line; S, sample application window.

PCR (Polymerase Chain Reaction)

Helicobacter pylori was positive in 19 patients with gastritis, 4 with gastric ulcer, 5 with duodenal ulcer and 2 with gastric cancer, and the *cagA* gene was detected in 14 of 19 patients with gastritis, 3 of 4 patients with gastric ulcer and all patients with duodenal ulcer but not in 2 patients with gastric cancer.

Performance of the ICTS

The developed ICTS was used to detect anti-CagA antibodies in sera of *H. pylori*-infected patients. To determine the sensitivity performance of the test a strong positive, weak positive and negative samples were used. A high-intensity red-colored test line was seen with the strong positive, a lower intensity red-colored test line was seen with the weak positive, and no colored test line was seen when the negative sample was used (Figure 4). The samples were defined as strong and weak according to optical densities obtained in the commercial ELISA.

Thirty patient sera when tested with our ICTS 21 were positive and 9 were negative. When these serum samples were tested with the commercial ELISA 22 were positive and 8 were negative (Table I). The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 95%, 100%, 100%, 89% and 97%, respectively. Table II shows the relation between the *H. pylori* infection cases and the presence of anti-CagA antibodies detected by our developed ICTS.

Table I. Comparison of Our Developed ICTS with the Commercial ELISA Kit for the Detection of Anti-CagA Antibodies in Sera of *H. pylori*-Infected Patients

		ELISA		
		Positive	Negative	Total
ICTS	Positive	21	0	21
	Negative	1	8	9
	Total	22	8	30

Table II. Anti-CagA Antibodies Detected by Our Developed ICTS in Sera of *H. pylori*-Infected Patients

Disease	CagA-positive	CagA-negative
Gastritis	13 (68%)	6 (32%)
GU	3 (75%)	1 (25%)
DU	5 (100%)	0 (0%)
GC	0 (0%)	2 (100%)
Total	21 (70%)	9 (30%)

GU represents gastric ulcer; DU, duodenal ulcer; GC, gastric cancer.

Discussion

The high prevalence of *H. pylori* infection worldwide makes the diagnosis of such infection a cumbersome task. It requires an invasive approach (endoscopy) for the collection of gastric biopsies. However, serology, a noninvasive approach, can help in the detection of such infection. Anti-CagA antibodies detected in sera of infected patients using the ICTS can be used for the identification of patients with higher risk or more severe disease. We have reported earlier that the presence of *cagA* was significantly associated with duodenal ulcer (89%) and with gastric cancer (90%) (12). Recently, Miftahussurur and Yamaoka (29) also reported that serological detection of anti-CagA antibodies correlate with further increases in risk of peptic ulcer and gastric cancer. They also indicated that CagA seropositivity was higher in patients with gastric cancer than in controls. Shiota *et al.* (30) reported that anti-CagA antibody detection can be used as a biomarker for gastric cancer. The majority of *H. pylori* strains in gastric ulcer (75%) and duodenal ulcer (100%) patients were also CagA-positive when the *H. pylori* infection cases and the anti-CagA antibodies detected by our ICTS were compared (Table II). However, two gastric cancer patients (100%) were negative for the anti-CagA antibodies. Thus, each infection cases, especially gastric cancer, are needed in large number to get enough statistical values for comparison. On the other hand, the relation between the *H. pylori* infection in patients and the presence of anti-CagA antibodies showed that CagA-positive *H. pylori* strains (70%) was significantly higher than the CagA-negative strains.

The gold-conjugated rCagA protein was used in the ICTS for the detection of anti-CagA antibodies in sera of *H. pylori*-infected patients. The control line coated with anti-rCagA Mab (CK-02) captured excess amount of detector reagents (gold-conjugated rCagA protein) regardless of the presence of target anti-CagA antibodies, and it was in red color for each test. The control line and the clear background that appeared on the reading window were the indicators of an internal positive and an internal negative procedural control, respectively.

The sucrose was used in conjugate solution as an essential ingredient to resolubilize detector reagents in the conjugate pad. When the conjugate pad was dried the sucrose covered detector reagents and protected the biological functions. It dissolved after the addition of the sample and released detector reagents into the flow of the sample fluid.

The membrane was blocked with BSA in phosphate buffer containing SDS to facilitate the migration of detector reagents. SDS provided a proper flow of sample on the membrane. On the other hand, the detector reagents did not move toward the capture reagents when the blocking buffer without the SDS was used.

Conclusion

We have developed a qualitative ICTS for the rapid detection (20 min) of anti-CagA antibodies in patient sera using our rCagA protein and anti-rCagA Mab (CK-02). This test showed high sensitivity and specificity in detecting such antibodies and gave an accuracy of 97% when compared with the commercial ELISA. The ICTS supports point-of-care testing without the need for complex equipment or experience and provides cost-effective and easy detection. Another advantage of such serological test is that it is not affected by the use of proton-pump inhibitor or antibiotics, ulcer bleeding and gastric atrophy which can give false negative results in tests that require gastric biopsy collection by invasive methods (31).

The test can support the diagnosis of CagA-positive *H. pylori* infection instead of laborious invasive approaches and eliminate the need for additional endoscopies in the follow up of eradication therapy. Since CagA protein plays an important role in the progression of the disease from gastritis to peptic ulceration and gastric cancer it will help to determine the CagA status in *H. pylori*-infected gastritis patients. Thus, it can also be used for the prediction of treatment strategy in order to block future progression to peptic ulceration and gastric cancer. According to our knowledge this is the first ICTS worldwide that detects anti-CagA antibodies in patient sera rather than whole anti-*H. pylori* antibodies.

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References

1. Perez-Perez, G.I., Rothenbacher, D., Brenner, H.; Epidemiology of *Helicobacter pylori* infection; *Helicobacter*, (2004); 9(Suppl 1): 1–6.
2. Salih, B.A.; *Helicobacter pylori* infection in developing countries: The burden for how long? *Saudi Journal of Gastroenterology*, (2009); 15: 201–207.
3. Sipponen, P.; Natural course of *Helicobacter pylori* gastric infection; *Italian Journal of Gastroenterology and Hepatology*, (1998); 30(Suppl 3): 270–272.
4. Sipponen, P., Marshall, B.J.; Gastritis and gastric cancer; western countries; *Gastroenterology Clinics of North America*, (2000); 29: 579–592.
5. Rudi, J., Kolb, C., Maiwald, M., Kuck, D., Sieg, A., Galle, P.R. *et al.*; Diversity of *Helicobacter pylori* vacA and cagA genes and relationship to VacA and CagA protein expression, cytotoxin production, and associated diseases; *Journal of Clinical Microbiology*, (1998); 36: 944–948.
6. Maeda, S., Ogura, K., Yoshida, H., Kanai, F., Ikenoue, T., Kato, N. *et al.*; Major virulence factors, vacA and cagA are commonly positive in *Helicobacter pylori* isolates in Japan; *Gut*, (1998); 42: 338–343.
7. Yamaoka, Y., Kodama, T., Kashima, K., Graham, D.Y., Sepulveda, A.R.; Variants of the 3′ region of the cagA gene in Helicobacter pylori isolates from patients with different *H. pylori*-associated diseases; *Journal of Clinical Microbiology*, (1998); 36: 2258–2263.
8. Navaglia, F., Basso, D., Piva, M.G., Brigato, L., Stefani, A., Dal Bo, N. *et al.*; *Helicobacter pylori* cytotoxic genotype is associated with peptic ulcer and influences serology; *The American Journal of Gastroenterology*, (1998); 93: 227–230.
9. Rudi, J., Kuck, D., Rudy, A., Sieg, A., Maiwald, M., Stremmel, W.; *Helicobacter pylori* vacA genotypes and cagA gene in a series of 383 *H. pylori*-positive patients; *Zeitschriftfür Gastroenterologie*, (2000); 38: 559–564.
10. Fox, J.G., Wang, T.C.; *Helicobacter pylori* infection: Pathogenesis; *Current Opinion in Gastroenterology*, (2002); 18: 15–25.
11. Ong, S.P., Duggan, A.; Eradication of *Helicobacter pylori* in clinical situations; *Clinical and Experimental Medicine*, (2004); 4: 30–38.
12. Saribasak, H., Salih, B.A., Yamaoka, Y., Sander, E.; Analysis of *Helicobacter pylori* genotypes and correlation with clinical outcome in Turkey; *Journal of Clinical Microbiology*, (2004); 42: 1648–1651.
13. Bolek, B.K., Salih, B.A., Sander, E.; Genotyping of *Helicobacter pylori* strains from gastric biopsies by multiplex polymerase chain reaction. How advantageous is it? *Diagnostic Microbiology and Infectious Disease*, (2007); 58: 67–70.
14. Salih, B.A., Abasiyanik, M.F., Ahmed, N.; A preliminary study on the genetic profile of cag pathogenicity-island and other virulent gene loci of *Helicobacter pylori* strains from Turkey; *Infection, Genetics and Evolution*, (2007); 7: 509–512.
15. de la Cruz-Herrera, C.F., Flores-Luna, L., Gutierrez-Xicotencatl, L., Chihu-Amparan, L., Sanchez-Aleman, M.A., Lazcano-Ponce, E. *et al.*; IgG2 response and low IgG titre specific to *Helicobacter pylori* CagA as serological markers for gastric cancer; *Journal of Medical Microbiology*, (2013); 62: 591–598.
16. Ricci, C., Holton, J., Vaira, D.; Diagnosis of *Helicobacter pylori*: Invasive and non-invasive tests; *Best Practice & Research: Clinical Gastroenterology*, (2007); 21: 299–313.
17. Yasuda, A., Uchida, T., Nguyen, L.T., Kawazato, H., Tanigawa, M., Murakami, K. *et al.*; A novel diagnostic monoclonal antibody specific for *Helicobacter pylori* CagA of East Asian type; *Acta Pathologica, Microbiologica et Immunologica Scandinavica*, (2009); 117: 893–899.
18. Klimovich, A.V., Samoylovich, M.P., Gryazeva, I.V., Terekhina, L.A., Suvorov, A.N., Klimovich, V.B.; Development of immunoreagents for diagnostics of CagA-positive *Helicobacter pylori* infections; *Helicobacter*, (2010); 15: 193–200.
19. Svarval, A.V., Ferman, R.S., Zhebrun, A.B.; Analysis of *Helicobacter pylori* infection prevalence in children in the contemporary period; *Zhurnal Mikrobiologii, Epidemiologii, I Immunobiologii*, (2012); 1: 83–88.
20. Jafarzadeh, A., Rezayati, M.T., Nemati, M.; *Helicobacter pylori* seropositivity in patients with type 2 diabetes mellitus in south-east of Iran; *Acta Medica Iranica*, (2013); 51: 892–896.
21. Lu, J., Wang, C.M., Xu, S.T., Song, L.L., Zhao, X.M., Wang, Q.Y. *et al.*; Role of *Helicobacter pylori* infection in the pathogenesis and clinical outcome of childhood acute idiopathic thrombocytopenic purpura; *Zhonghua Xueyexue Zazhi*, (2013); 34: 41–44.
22. Karakus, C., Salih, B.A.; Comparison of the lateral flow immunoassays (LFIA) for the diagnosis of *Helicobacter pylori* infection; *Journal of Immunological Methods*, (2013); 396: 8–14.
23. Posthuma-Trumpie, G.A., Korf, J., van Amerongen, A.; Lateral flow (immuno)assay: Its strengths, weaknesses, opportunities and threats. A

literature survey; *Analytical and Bioanalytical Chemistry*, (2009); 393: 569–582.

24. Ngom, B., Guo, Y., Wang, X., Bi, D.; Development and application of lateral flow test strip technology for detection of infectious agents and chemical contaminants: A review; *Analytical and Bioanalytical Chemistry*, (2010); 397: 1113–1135.
25. Yokota, S., Fujimori, O.; Preparation of colloidal gold probes. In *Methods of immunogold staining*. Soft Science Publications, Tokyo, (1992), pp. 29–47.
26. Karakus, C., Uslu, M., Yazici, D., Salih, B.A.; Evaluation of immobilized metal affinity chromatography kits for the purification of histidine-tagged recombinant CagA protein; *Journal of Chromatography B*, (2016); 1021: 182–187.
27. Zhang, G.P., Wang, X.N., Yang, J.F., Yang, Y.Y., Xing, G.X., Li, Q.M. *et al.*; Development of an immunochromatographic lateral flow test strip for detection of beta-adrenergic agonist Clenbuterol residues; *Journal of Immunological Methods*, (2006); 312: 27–33.
28. Salih, B.A., Guner, A., Karademir, A., Uslu, M., Ovali, M.A., Yazici, D. *et al.*; Evaluation of the effect of *cagPAI* genes of *Helicobacter pylori* on AGS epithelial cell morphology and IL-8 secretion; *Antonie van Leeuwenhoek*, (2014); 105: 179–189.
29. Miftahussurur, M., Yamaoka, Y.; Diagnostic methods of *Helicobacter pylori* infection for epidemiological studies: Critical importance of indirect test validation; *BioMed Research International*, (2016); 2016: 4819423.
30. Shiota, S., Matsunari, O., Watada, M., Yamaoka, Y.; Serum *Helicobacter pylori* CagA antibody as a biomarker for gastric cancer in East-Asian countries; *Future Microbiology*, (2010); 5(12): 1885–1893.
31. Wang, Y.K., Kuo, F.C., Liu, C.J., Wu, M.C., Shih, H.Y., Wang, S.S. *et al.*; Diagnosis of *Helicobacter pylori* infection: Current options and developments; *World Journal of Gastroenterology*, (2015); 21(40): 11221–11235.