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MINI-REVIEW

Biomarkers and diagnostic tools for detection of *Helicobacter pylori*

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Abstract *Helicobacter pylori* is responsible for worldwide chronic bacterial infection in humans affecting approximately half of the world's population. *H. pylori* is associated with significant morbidity and mortality including gastric cancer. The infection has both direct and indirect impacts on economic and overall well-being of patients; hence, there is a great need for diagnostic markers that could be used in the development of diagnostic kits. Here, we briefly review general aspects of *H. pylori* infection and the diagnostic biomarkers used in laboratory tests today with a focus on the potential role of microfluidic systems in future immunodiagnosis platforms.

Keywords *Helicobacter pylori* · Infection markers · Diagnostic kits · Microfluidic systems

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Introduction

Helicobacter pylori infection is caused by a gram-negative organism that colonizes the mucous layers and the apical surface of human gastric epithelial cells (Alam et al. 2012; Khalilpour et al. 2014a). More than 90 % of population in developing and Eastern countries and half of the world population have been affected with *H. pylori* infection (Chan et al. 2006a; Malaty 2007a). The prevalence rate of infection during the first decade and by the sixth decade of life in industrialized countries (West Europe and North America) is approximately 10 and 60 %, respectively. In nonindustrialized countries (Africa, South America, Middle, and Far East), infection often occurs during childhood by intrafamilial transmission with the prevalence rate of 60–80 % that slightly increases with age. In contrast, in developed countries, the annual prevalence of acquisition of *H. pylori* infection is much lower (~1 %) and is estimated to be 30–40 % during adulthood (Rahim 2004).

H. pylori is related to peptic ulcer diseases (PUDs) such as, duodenal ulcer (DU) and gastric ulcer (GU), gastric cancer (GC), atrophic gastritis, and mucosa-associated lymphoid tissue lymphoma (MALT) (Fig. 1a) (Kusters et al. 2006; Lydyard et al. 2010). *H. pylori* infection may damage the tissue in five different ways. First, damage is caused by ammonium ions resulting from the urease activity and the production of phospholipase and vacuolating cytotoxin, which participate in the formation of a poor quality mucus layer. Second, gastric physiology is altered by enhanced acid production; the gastric cell dynamics are affected by interference in normal cell signaling events caused by the introduction of the CagA protein and peptidoglycan from *Helicobacter*. Third, release of free radicals from the granulocytes causes the damage. Fourth, autoantibodies are induced by *Helicobacter* and kill acid-secreting parietal cells. Lastly, the infection can alter the balance of cell division and apoptosis (Fig. 1b) (Eslick 2006; Lydyard

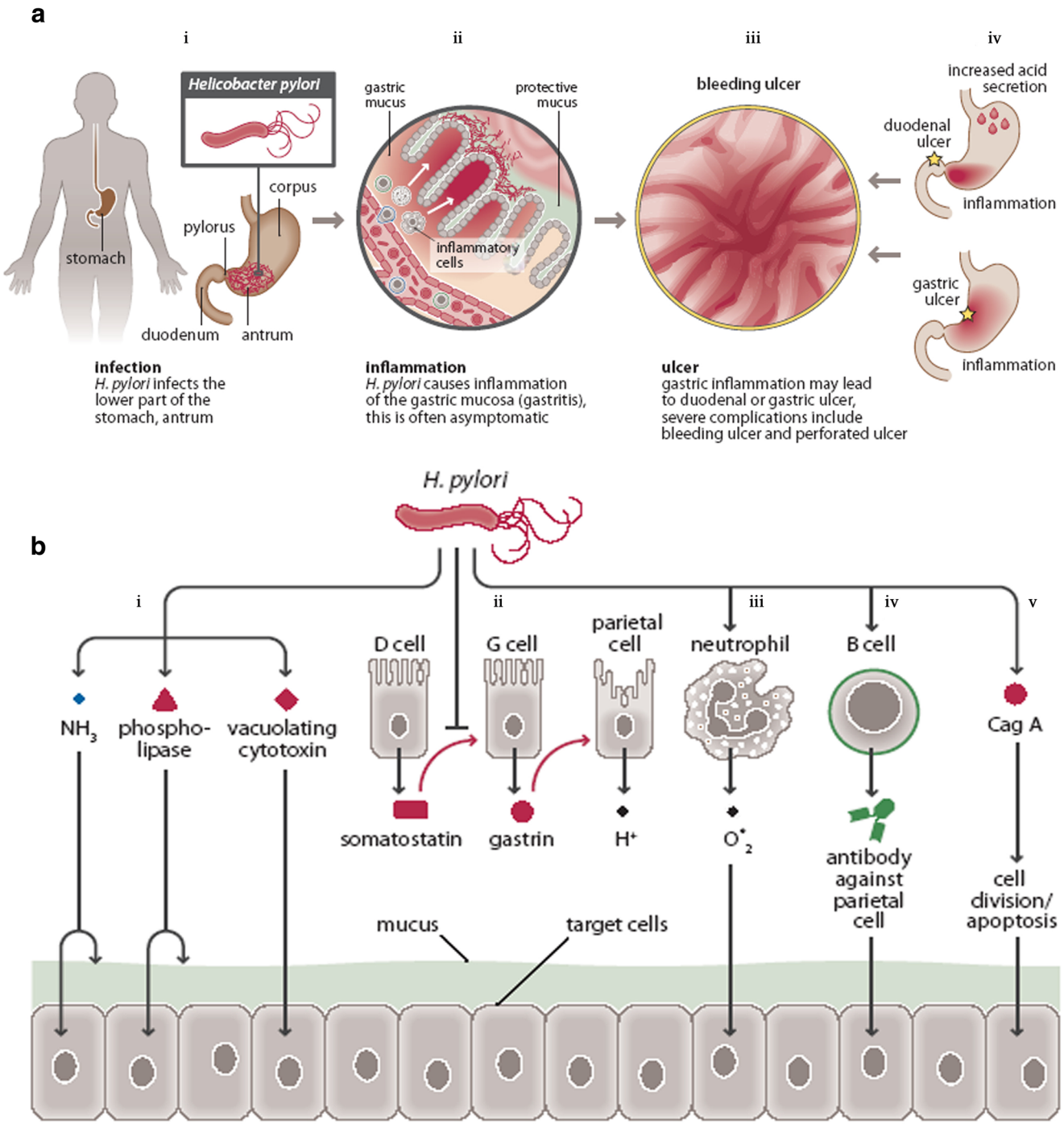


Fig. 1 General scheme of infection with *Helicobacter pylori*. **a** A picture of *H. pylori* (*i*), the location of the organisms in the human stomach (*ii*), a picture of the microscopic location of *H. pylori* in the mucus layer and on the surface epithelial cells (*iii*), and the location of a duodenal and gastric ulcer (*iv*). **b** Mechanisms of pathogenesis of *H. pylori*. Reprinted with permission from Lydyard et al. (2010)

et al. 2010). Although *H. pylori* is related to serious diseases, only a fraction of infected people will develop gastroduodenal disease during their lifetime as limited strains of *H. pylori* are highly pathogenic. In general, the development of chronic gastritis depends on the virulence of the *H. pylori* strain, host susceptibility, and environmental cofactors such as smoking and diet.

The major challenge in regard to treating diseases related to *H. pylori* infection is to understand the pathogenesis of the disease so that patients with a high risk of developing cancer can be recognized and the disease can be prevented or treated in the early stages. A variety of *H. pylori* antigens such as UreA, UreB, CagA, VacA, FlaA, FlaB, and HspB have been reported to elicit strong humoral immune responses, thus

making them potential candidates as biomarkers associated with *H. pylori* infection (Portal-Celhay and Perez-Perez 2006). A new range of markers have been made into commercial diagnostic kits and provide better efficiency in terms of accuracy and cost. Currently, the approved diagnosing systems for *H. pylori* infection are often based on serological diagnosis or polymerase chain reaction (PCR). Diagnostic kits that utilize stool antigens and carbon-labeled urease breath tests have opened new options for diagnosing active *H. pylori* infections, however, most of them are expensive and time consuming (Vaira and Vakil 2001).

This mini-review provides an overview of currently used biomarkers and highlights the potential biomarkers related to *H. pylori* infection. It also explores different immunoassays regarding the detection of anti-*H. pylori* and outlines future directions for the field.

Biomarkers

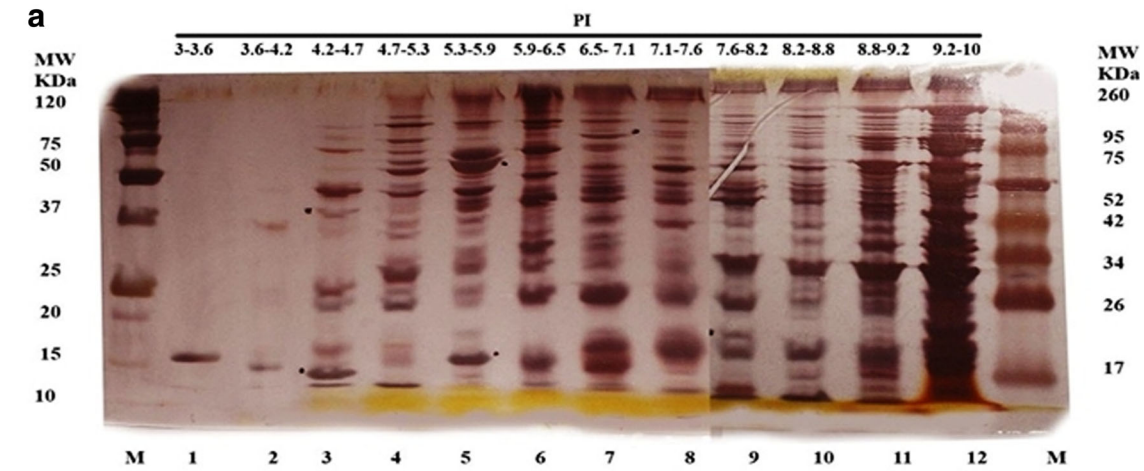
Biomarkers play major roles in molecular medicine in the identification, validation, diagnosis, and prevention of disease. Biomarkers can reflect biological activities that are relevant to disease and provide valuable information that may be useful diagnostically and therapeutically. There are various protein detection techniques that have been applied in biomarker discovery. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting are the most basic and traditional techniques used for separation of macromolecules. Currently, these techniques have been combined with high-throughput techniques. Different experimental methods, such as two-dimensional electrophoresis (2-DE), OFFGEL electrophoresis, high-performance liquid chromatography (HPLC), bioinformatics software, and mass spectrometry (MS)-based high-throughput proteomics have been employed for the discovery of essential proteins. A variety of *H. pylori* antigens were identified using 2-DE and mass spectrometry to elicit strong humoral immune responses making them potential candidates for biomarkers (Portal-Celhay and Perez-Perez 2006). The majority of studies have focused on the urease enzyme, VacA, heat shock protein, and outer membrane protein in order to quickly produce a diagnostic kit for the diagnosis of *H. pylori* infection (Michetti et al. 1999). Several clinical studies have suggested the application of multiple antigens with high sensitivity and specificity as an efficient combination for detecting *H. pylori* infection (Manes et al. 2005; Pelerito et al. 2006).

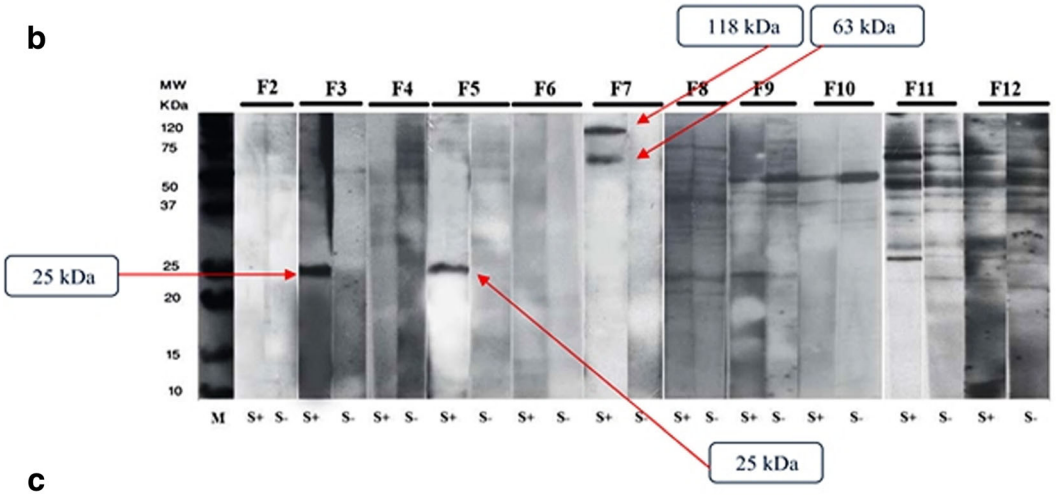
Virulence factors

Virulence factors are frequently responsible for causing disease in a host because they inhibit certain biological functions. Several virulence-associated genes of *H. pylori* have been reported to play a major role in the pathogenesis of *H. pylori.* Previous researches have focused on the urease enzymes, CagA, VacA, FlaA, HspB, FlaB, and outer membrane proteins in order to quickly produce a diagnostic kit for recognition of *H. pylori* infection (Cremonini et al. 2004; Khalilpour et al. 2013; Schumann et al. 2006; Zheng et al. 2002). Although some bacterial factors such as the heat shock protein, *H. pylori* adhesin (HpaA), and flagella have also been identified as pathogenic determinants, understanding the function of *H. pylori* cellular components in the pathogenesis of gastric disorders requires further investigation (Park et al. 2006). In addition, a few unidentified antigenic bands such as 18, 39.5, 33, and 34 kDa have been reported to be of good diagnostic value (Andersen and Espersen 1992; Galmiche et al. 2000; Keenan et al. 2000). For instance, Lin et al. (2007) detected seven proteins in duodenal ulcer and gastric cancer serum samples with relative molecular masses of 53 kDa (flagellin A), 53 kDa (flagellin B), 67 kDa (molecular chaperone DnaK), 61 kDa (urease β subunit), 74 kDa (flagellar hook-associated protein (FliD)), 76 kDa (flagellar hook protein), and 51 kDa (serine protease (HtrA)) (Lin et al. 2007). Khalilpour et al. identified four *H. pylori* infection markers including UreG, UreB, CagI, and pyrroline-S-carboxylate dehydrogenase using off-gel and mass spectrometry with high sensitivity and specificity (Fig. 2). Out of the four *H. pylori* infection markers identified by Khalilpour et al., UreG, CagI, and pyroline-S-carboxylate dehydrogenase were reported for the first time (Khalilpour et al. 2014a; Khalilpour et al. 2012; Khalilpour et al. 2013; Noordin et al. 2013).

After infection of the gastric or duodenal cells with *H. pylori*, a number of things occur in the tissue. First, the bacteria must penetrate the mucus layer and attach to the surface of the epitheliums (Fig. 3). Several proteins that are involved in this process also play a role in causing damage to the host's stomach. In some, but not all strains of *H. pylori*, the protein called cytotoxin-associated gene A (CagA) is expressed. This protein is related to the increased virulence and risk of gastric carcinoma. CagA is encoded by a gene placed in the Cag pathogenicity island (Cag PAI), which is found in more than 90 % of *H. pylori* isolated in Eastern countries and almost 60 % in Western countries (Chan et al. 2006b). The CagPAI is 40 kb in size and a complex of Cag genes including CagI, CagE, CagL, CagH, and CagM, which are a major virulence factor of *H. pylori* (Chan et al. 2006a; Cui and Shao 2007). *H. pylori* makes this protein via a type IV secretion system and physically injects it into the mucosal cells using its pilus (Fleming et al. 2009; Peek et al. 2010).

Vacuolating cytotoxin gene A (VacA) is another virulence factor that is produced during *H. pylori* infection. A unique exotoxin of *H. pylori* can cause vacuolation, disruption of endo-lysosomal activity, apoptosis in gastric cells, pore formation, and immunomodulation (Yan et al. 2005). A distinct





c

Fraction No.	Secondary antibody	Bands	Sequence Result					
			Protein ID	Accession Number	Theoretical Mass (Da)	protein score (cut-off score)	No. Peptides (MS-MS)	Theoretical pI
3[a]	IgG	25 KDa	cag island protein [*Helicobacter pylori* J99]	gil15611542	23228	222(>83)	7	5.11
5[b]	IgG	25 KDa	urease accessory protein (ureG) [*Helicobacter pylori* 26695]	gil15611134	22098	462(>83)	6	5.02
7[c]	IgG	63 KDa	urease UreB [*Helicobacter pylori* 26695]	gil5913962	61803	420(>82)	4	5.64
		118 KDa	proline/pyrroline-5-carboxylate dehydrogenase [*Helicobacter pylori* J99]	gil15611119	135778	179(>54)	6	6.22

[a]pI: 4.16-4.70. [b]pI: 5.33-5.90. [c]pI: 6.49-7.0

Fig. 2 Schematic of identification of antigenic proteins of *H. pylori*. **a** Gel profile (12 % SDS-PAGE) of OFFGEL fractions of antigens using the 3100 OFFGEL High Res Kit, pH 3–10. **b** Representative profile of IgG immunoblot of *H. pylori* antigen incubated with patient (S+) and control serum sample (S−). **c** Mass spectrometric identification of antigenic proteins of *H. pylori*. Reprinted with permission from Khalilpour et al. (2013)

allelic variant in two different parts of VacA has been expressed. The presence of alleles s1a, s1b, and s2 and the middle region (m) as the alleles m1 or m2 might lead to N-terminal signal region (s). The different structure of VacA has been related to the variations in the cytotoxin production as well as the clinical effects of the *H. pylori* infection (Faundez et al. 2002). The VacA protein is released outside of the *H. pylori* where it binds to the outer surface of a stomach cell and induces the vacuoles inside. The VacA protein also inserts itself into the mitochondrial membrane and induces apoptosis partly by forming pores in the mitochondrial membrane. This cellular destruction alerts the immune system to the fact that there is something occurring in the interior of the stomach.

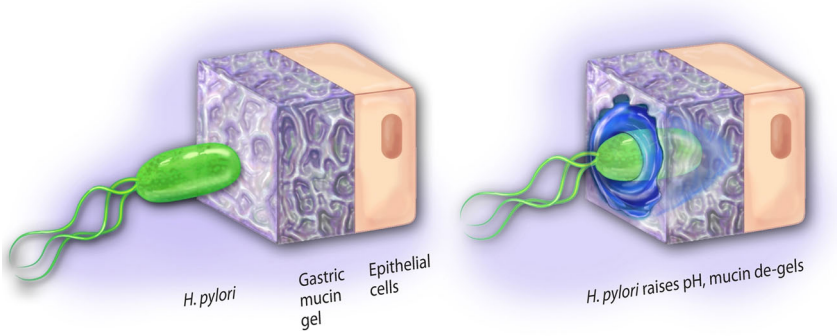


Fig. 3 Using clever biochemical strategy *H. pylori* liquefies stomach mucin barrier by increasing the pH of its surroundings to cross over to the epithelial cells and establish colonies, attack surface cells, and form ulcers. Reprinted with permission from Deretsky (2015)

Cytokines, produced by the injured cells send signals to the immune system, leading to tissue infiltration of immune cells.

Diagnosis of *H. pylori* infection

A number of tests with varying accuracy and sensitivity have been developed to diagnose *H. pylori* infection. These tests could be separated into invasive and noninvasive approaches, depending on whether an endoscopy is needed. The choice of test depends on factors such as the clinical situation, availability, cost, pretest probability of infection, prevalence of infection in the population, the use of antibiotics that may affect test results, etc. An invasive test is usually employed for children and anyone with symptoms that indicate a cause for serious concern (Hammett and Evans 1999; Mones et al. 2005). In an adult, conditions such as advanced age, anemia, persistent vomiting, a long history of symptoms, persistent weight loss or lack of appetite, gastrointestinal bleeding, or severe stomach pain that indicates an ulcer with complications might warrant this thorough examination (Fleming et al. 2009). This type of test not only diagnoses the amount of damage in the stomach but also allows the clinician to directly sample the tissue to test it for the presence of the *H. pylori* organism. Noninvasive tests can identify *H. pylori* in a sample, but do not reveal the amount of tissue damage (Fleming et al. 2009; Sugimoto et al. 2009).

Invasive tests

Histopathology staining remains one of the best methods for detecting *H. pylori* infection, and together with visual observation by endoscopy, it provides important information based on the pathological condition of the stomach (Makristathis et al. 2004). However, endoscopy is expensive, unpleasant for patients, and requires highly specialized operator to perform the test. In some cases, endoscopy is too costly to be used as a routine diagnostic test for gastric patients. In addition, sampling error may occur and cause false-negative results (Mitchell and Megraud 2002) that can lead to inaccurate diagnosis. Bacterial culture is one of the options for detecting the presence of *H. pylori*. Successful culture of the *H. pylori* is the gold standard for diagnosis. One of the advantages of culture diagnosis is the antibiotic susceptibility test which can be performed in cases of antibiotic resistance (Wadstrom et al. 1994). However, the media used for culturing *H. pylori* is costly, coupled with special conditions for maintenance, and the length of time necessary to obtain a result is slow compared to other methods (Perez-Perez 2000). The rapid urease test (RUT) is used to detect the *H. pylori* urease enzyme in gastric cells. A small biopsy sample is placed in a test vial containing the rapid urease test solution and incubated overnight at 37 °C. The sample is then observed for a change in color from yellow to pink which indicates a positive result (Yousfi et al. 1996). There are several versions of this test that are quite sensitive and specific for *H. pylori*, but they vary in the amount of time needed to get a result. This method is the most time-efficient and is affordable for use as a routine diagnostic procedure (Fleming et al. 2009).

PCR is the most commonly used molecular technique for the diagnosis of *H. pylori*, even though it has not been standardized (Brooks et al. 2004; Monteiro et al. 2001a). No attempts have been made so far to standardize either the sample preparation process or the PCR amplification itself. Laboratories use different "in-house" methods that often have varying sensitivity and reliability. The PCR has been performed successfully to identify *H. pylori* DNA in gastric tissue by amplifying antigenic genes such as the urease gene, adhesin genes, and 16S rRNA gene. The highly specific target for PCR diagnosis of *H.pylori* is the 16S rRNA gene. This gene is a popular target confirming *H. pylori* infection and has been previously used to help reclassifying the organism. Urease is another specific target for detecting *H. pylori* infection, and positive amplification of bacterium-specific DNA can be considered as a direct evidence of the presence of the pathogen (Tiwari et al. 2005). These genes are routinely obtained from clinical samples such as blood, biopsy tissues, feces, saliva, whole blood, and cultures for diagnostic purposes (Brooks et al. 2004; Chisholm and Owen 2008; Kim

et al. 2000; Monteiro et al. 2001b). However, the specificity and sensitivity of the diagnosis varies, according to different sample preparations and various laboratory conditions (Sugimoto et al. 2009). Monoplex, multiplex, and nested PCR methods are useful for specially identifying *H. pylori* in biological samples. Real-time quantitative (Q) PCR technique is a more developed technique compared to the other conventional methods for *H. pylori* clinical diagnosis. However, this technique requires endoscopy to obtain biopsy samples. Many primer sets have been reported for targeting *H. pylori*, most of which are targeted at the small subunit RNA nucleotide sequence that is highly conserved and contains species-specific regions (He et al. 2002). Other reported primer sets target the highly repetitive DNA sequences UreA, UreB, UreC, and VacA (He et al. 2002).

Noninvasive tests

An interesting test to detect *H. pylori* is the urea breath test. This method is the preferred noninvasive choice for *H. pylori* diagnosis before and after treatment. The urea breath test uses the fact that *H. pylori* yields urease, an enzyme that metabolizes urea into ammonia and carbon dioxide (CO_2). For this test, patients swallow a tablet that contains a small amount of 13C-labeled urea (Abdullahi et al. 2008; Fleming et al. 2009). 13C is a nonradioactive form of carbon that is slightly heavier than normal carbon. The 13-CO_2 is absorbed through the stomach lining and transported to the circulatory system. After 20 min, a breath sample is analyzed by a machine. Patients without *H. pylori* infections will produce little or no 13-CO_2, and the urea will be eliminated in the feces and urine. The urea breath test is known as the "gold standard" for in vivo detection of *H. pylori* infection and is also a good means of evaluating whether a course of *H. pylori* therapy has successfully eradicated the organism. However, this test may be less reliable for children due to the lower production of CO_2 in children compared to adults (Boncristiano et al. 2003; Wang et al. 2013).

The fecal antigen test is a noninvasive method of diagnosis of *H. pylori* antigens in the feces of patients suspected of having an active *H. pylori* infection (Andrews et al. 2003). Studies have proved the accuracy of the fecal antigen test named the *H. pylori* stool antigen (HpSA) test for detection of *H. pylori* infection (Iranikhah et al. 2013). The test is highly sensitive and precise and is especially good for diagnosing infection in children who are unable to perform a UBT. Nevertheless, some studies have shown increased false-negative results for the stool antigen test during proton pump inhibitor (PPI) treatment. PPIs such as omeprazole or lansoprazole are a group of drugs that work by reducing the amount of stomach acid made by glands in the lining of stomach. They are the most potent inhibitors of acid secretion available (Fleming et al. 2009; Kodama et al. 2012).

Infection with *H. pylori* excites a strong immune response that results in the production of antibodies. Antibodies specific to *H. pylori* in serum, whole blood, saliva, and stool can be detected using serological methods like enzyme-linked immunosorbent assay (ELISA) and Western blotting (Alkout et al. 1997; Faulde et al. 1991; Kimmel et al. 2000). A positive antibody test, along with dyspeptic symptoms may be convincible enough for a physician to prescribe antibiotic therapy to eradicate the *H. pylori* infection. However, a concern regarding blood tests is the remain of antibodies in the circulatory system for months or years after the elimination of infection which may result in misdiagnosis and improper treatment due to false-positive serological test result.

In general, invasive tests are more reliable than noninvasive tests for diagnosing an infection, as noninvasive tests sometimes fail to detect the organism in an infected person (false-negative) or incorrectly diagnose an uninfected person (false-positive) (Fleming et al. 2009).

Miniaturized and point of care diagnostic tools

The conventional invasive and noninvasive methods such as PCR, serological tests, and ELISA have some restrictions for their applications as they require long processing times, high quantity of costly reagents, and equipment. Thus, these processes are centralized and samples should be collected and transferred to a central lab to be processed (Bhattacharyya and Klapperich 2007; Eteshola and Leckband 2001). To address these limitations, miniaturized diagnostic tools including microfluidic-based systems have been developed. The smaller length scales, in comparison with conventional systems, reduce the diffusion time of reagents and the required time for assays. In addition, miniaturized systems require less reagents and sample size in comparison with conventional tests (Riahi et al. 2015). These miniaturized systems are also portable and can easily be multiplexed for parallel detection of various diseases. These characteristics make them ideal for designing point of care (POC) diagnostic tools.

Lateral flow tests, also known as Immunochromatographic assays, are simple, cheap, efficient, and easy to apply, which have been widely employed for designing POC diagnostics. A number of lateral assays have been developed for *H. pylori* detection (Hujakka et al. 2003; Khalilpour et al. 2014b). Yang and Seo (2008) compared the sensitivity and reliability of a rapid lateral assay with conventional ELISA system for detection of stool antigen (HpSA). They did not observe any significant difference between the two assays, while the lateral one was 20 times faster. The key challenge associated with lateral assays is their high background noise that can potentially reduce their limit of detection. Thus, polymer-based microfluidic systems which have a lower background noise have recently drawn significant attention for designing POC diagnostics (Gubala et al. 2011). Recent development of

microfabrication technologies that has significantly reduced the fabrication cost of polymer-based microfluidics has made their commercialization more probable.

Liquid handling in microfluidic systems has normally been achieved by the use of (1) centrifugal force, (2) electrical forces, (3) surface tension, and (4) pressure. Among them, surface tension-based systems can be autonomous and are excellent candidates for POC diagnostics (Safavieh and Juncker 2013; Safavieh et al. 2015). In an example, Pereira et al. designed an integrated magnetic immunosensor coupled with a gold electrode for the detection of *H. pylori*-specific IgG antibodies (Fig. 4a). Their results show the potential usefulness of their made-up microbiochip for the early assessment of IgG antibodies to *H. pylori* (Pereira et al. 2010). In another example, Gao et al. designed an electrokinetically driven microfluidic platform for detection of *H. pylori* (Gao et al. 2005; Safavieh and Juncker 2013). The assay took only 30 min and the chips stayed active for at least 10 days. Using a similar microfluidic immunoassay approach, Goa et al. also designed a simple electrokinetically controlled immunoassay chip for diagnosis of *H. pylori*. In this chip, an H-shaped microchannel network was fabricated using PDMS. Multibiomarker immobilization was done by adsorbing the biomarkers onto a PDMS-coated glass slide with the aid of a microfluidic network (Fig. 4b). The assay time was only 25 min (Gao et al. 2005; Kakaç et al. 2010; Lin et al. 2004). Lin et al. (2004) also employed a pressure-driven microfluidic platform for rapid detection of *H. pylori*. The system could provide accurate readings within 30 min, while the required solution volumes were 100-fold less than conventional ELISA systems (Fig. 4c). The same group also developed a heterogeneous PDMS-based microfluidic immunoassay with antibody-functionalized RLS nanoparticles described as labels for the detection of *H. pylori*. This approach has the same detection limit as a conventional ELISA system, while it allows long-term preservation (Fig. 4d) (Lin et al. 2005).

Miniaturized systems provide a strong tool for rapid diagnosis of *H. pylori* and possess superiority over conventional systems in terms of processing time, required facilities, and used reagents and samples. A key point in their widespread utilization is the feasibility of extracting the data by users. The systems that utilize calorimetric readouts are easy to use for negative/positive results. However, these systems are not easy to use for measuring the antigen concentration unless they are paired with an image processing tool. This can be achieved by utilization of smartphones which are widespread. Electrical-based sensing platforms on the hand are easier for reporting concentrations.

Commercial diagnostic kits

Two types of markers, namely DNA and protein markers, are used in commercial diagnostic kits for diagnosing *H. pylori* infection. The accuracy of these markers varies from test to test and depends greatly on the type of sample and the type of assay (Vaira and Vakil 2001). DNA markers are detected by means of a PCR test and will give very sensitive results. A few commercial DNA diagnostic kits are available for *H. pylori*, but most tests are performed in specialized laboratories with trained personnel (el-Zaatari et al. 1997).

Protein-based markers are popular for *H. pylori* detection, as they are manufactured into cheap user-friendly forms, such as immobilized strips. Besides that, protein-based diagnostic kits are also available in ELISA and latex agglutination test cards (Glassman et al. 1990). ELISA kits are the most sensitive and specific test for diagnosis of *H. pylori* infection; however, these tests are expensive and time consuming. Another lab-based serological method is immunoblotting. Immunoblotting is also performed as a lab-based serological method. These assays detect anti-*H. pylori* antibodies in either fecal, whole blood, serum, or urine (Glassman et al. 1990; Miwa et al. 2001; Sasidharan and Uyub 2009; Simor et al. 1996; Zuniga-Noriega et al. 2006). Most of the commercial antibody-based tests for detecting *H. pylori* infection use bacterial lysate or partially purified bacterial antigens. Only a few recombinant antigen-based tests have recently been introduced into the market. Due to higher level of sensitivity and specificity, most commercial diagnostic assays apply a mixture of antigenic extracts over a single antigen. However, the names of antigens used in commercial kits are usually undisclosed (Table 1) (Andersen and Espersen 1992; Glassman et al. 1990; Manes et al. 2005; Pelerito et al. 2006; Simor et al. 1996).

The cost of diagnostics is also one of the main factors for developing rapid kits. To decrease the cost, several factors should be monitored: (1) use the least expensive reagents, (2) production cost for mass production, (3) miniaturization, and (4) quality control. In addition, for clinical use of diagnostic devices, environmental conditions, such as high temperatures (35~45 °C), humidity, insufficient water, and unreliable electricity are of importance (Lee et al. 2010).

Lateral flow tests, also called immunochromatographic assays, are simple, cheap, efficient, and easy to apply. They do not require a reader device or expensive equipment, and they can be performed by an untrained person (Hujakka et al. 2003; Khalilpour et al. 2014b). Therefore, lateral flow tests are simple tools intended to detect the presence of *H. pylori* antigenic protein in sample without requiring for costly equipment.

Conclusions

Rapid diagnosis and treatment plays a major role in inhibiting the spread of *H. pylori* infection. Moreover, aberrant diagnosis and treatment may lead to drug misuse subject to unpredictable side effects. There are many immunoassays that have

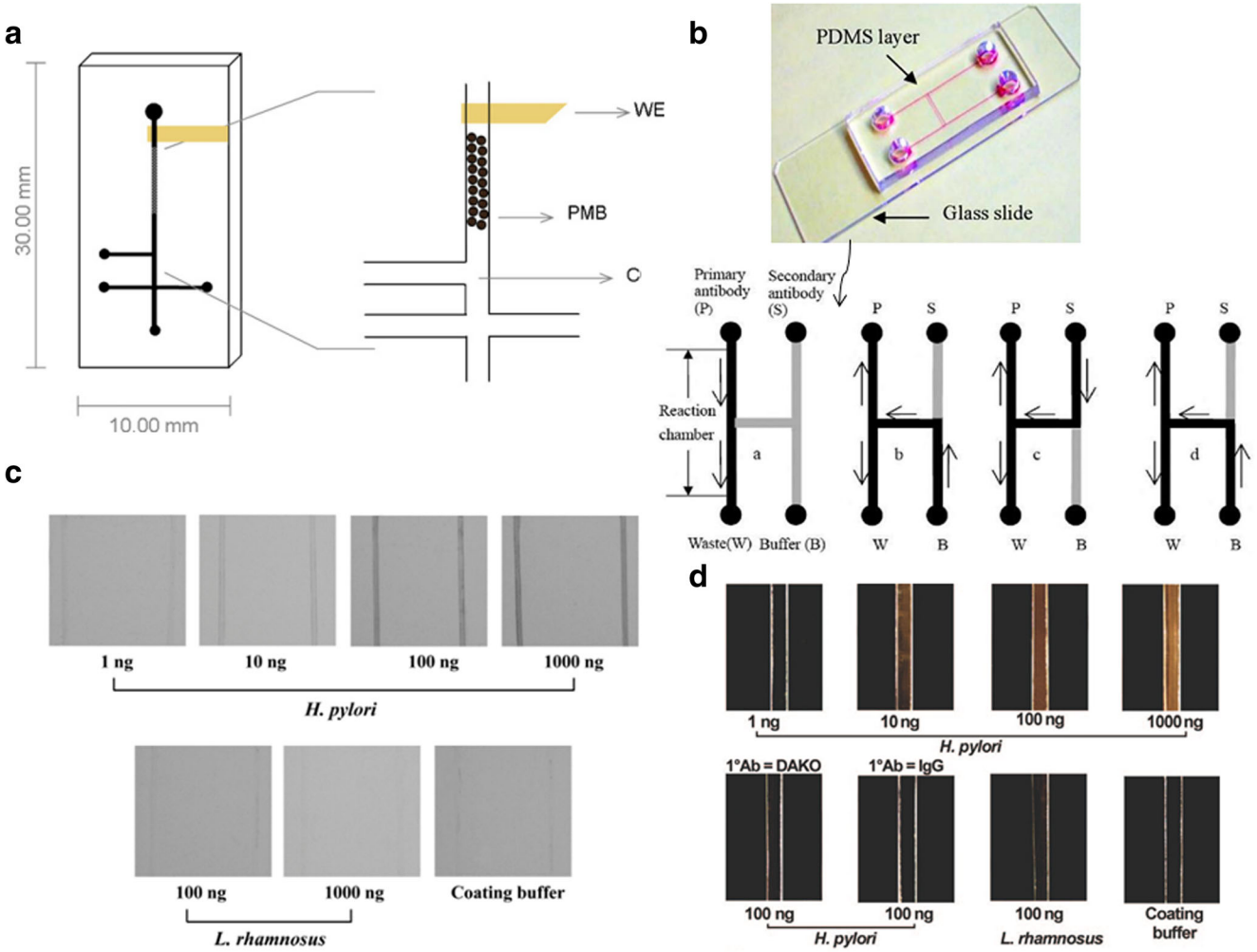


Fig. 4 Nano/microfluidic technologies for detection of *H. pylori* infection. **a** Schematic of an integrated magnetic immunosensor coupled with a gold electrode for the detection of *H. pylori*-specific IgG antibodies. **b** Schematic of an immunoassay chip with an H-shaped microchannel, a sequential steps of an automatic IA processes. **c** *H. pylori* antigen detection using the PDMS microchannel ELISA. **d** *H. pylori* antigen detection in PDMS microchannels with the RLS nanoparticles as the readout for the heterogeneous immunoassay. Reprinted with permission from Kakaç et al. (2010); Lin et al. (2005),; Lin et al. (2004), and Pereira et al. (2010)

been developed for the diagnosis of anti-*H. pylori* class serum antibodies (IgG/IgM/IgA) (Khalilpour et al. 2014a; Khalilpour et al. 2013). The diagnosis of *H. pylori* infection markers via serology is the easiest noninvasive method for diagnosing an infection as it requires only a few drops of blood from the finger and the results are available in less than 5 min. However, serological diagnosis is still not widely recognized because of the prevalence of *H. pylori* antibodies posttreatment (Rosenstock et al. 2000). A new range of markers have been made into commercial diagnostic kits and provide better accuracy and cost-effectiveness. Diagnostic kits that utilize stool antigens and carbon-labeled urease breath tests (Manes et al. 2001a; Manes et al. 2001b) have opened new options for diagnosing active *H. pylori* infections (Vaira and Vakil 2001). *H. pylori* strains are known to differ in their pathogenicity from country to country. This phenomenon is also supported by a few published studies stating that different strains from different countries might differ genetically and thus might differ in pathogenicity (Das and Paul 2007; Kusters et al. 2006; Malaty 2007b; Robinson et al. 2007). The accuracy of the diagnostic biomarkers varies from test to test, depending greatly on type of sample and type of diagnostic assay (Vaira and Vakil 2001). Many commercial ELISA kits for serological diagnosis of *H. pylori* antibodies have been considered to provide reliable results when used in the Western countries (Meijer et al. 1997a). However, when these serological kits were tested in Asian or middle east countries, the diagnostic accuracy of these tests were found to be lower, such as reports from Chinese (Leung et al. 1999) and Japanese patient populations (Miwa et al. 2001). These differences may be due to some reasons, such as cross-reactivity to other intestinal bacteria which vary in

Table 1 List of common commercial kits for diagnosis of *H. pylori* infection

Test format	Type of sample	Type of antigen	Sensitivity	Specificity
ELISA kit	Human stool	Recombinant CagA Recombinant VacA Recombinant UreB Recombinant 26KD	Not available	Not available
Rapid qualitative immunochromatographic	Human stool	CagA, VacA, CagII, CagC	95 %	Not available
IgA ELISA assay	Human serum or plasma	Recombinant antigens	Not available	Not available
ELISA kit	Human serum	Recombinant CagA	Not available	Not available
IgA ELISA kit	Human serum or plasma	Recombinant CagA Recombinant urease	90.0 %	95.0 %
IgG ELISA kit	Human serum or plasma	CagA (120KD) VacA (87KD) urease	93.7 %	93.3 %
Rapid *H. pylori* antigen test card	Human stool	Not available	Not available	Not available
Immunochromatographic rapid assay	Human stool	Not available	94.0 %	96.7 %
IgG chemiluminescence ELISA (CLIA)	Human serum or plasma	Not available	99 %	97 %
IgA ELISA	Human serum or plasma	Not available	Not available	Not available
IgM chemiluminescence ELISA	Human serum or plasma	Not available	Not available	Not available
IgA ELISA kit	Human serum and plasma	Recombinant CagA	Not available	Not available

different parts of the world, the presence of *H. pylori* strain heterogeneity in different geographic areas (Ito et al. 1997), and varying immunological responses to antigenic proteins of *H. pylori* in different patient populations (Khanna et al. 1998). A lot of money is being spent on purchasing diagnostic kits which may not be suitable for patients in other countries and may not be as sensitive as kits made from antigens using locally isolated *H. pylori* (Khalilpour et al. 2013; Meijer et al. 1997b).

Therefore, the development of lateral flow tests using recombinant forms of antigenic proteins could hopefully solve the limitation of *H. pylori* diagnostic kits (Khalilpour et al. 2014a). Combination of potential diagnostic markers could be used as an ideal clinical diagnostic reagent for diagnosis of *H. pylori* infection with effective applications, globally (Khalilpour et al. 2013). Nevertheless, identification of diagnostic biomarkers for local usage is essential. Microfluidic systems for clinical assays could also enable on-chip detection of blood-associated infectious diseases in simple, mass-producible, and thus, cheap setting (Chung et al. 2009). Diagnosis of *H. pylori* infection could be enhanced by modification of current detection methods related to nano/microfluidics interface using lateral flow and diffusion (Bhattacharyya and Klapperich 2007; Eteshola and Leckband 2001; Morozov et al. 2007).

Future directions

H. pylori has been an increasing source of gastrointestinal disorders in the world, and its proper diagnosis is of high priority. Part of the research is focused on identifying biomarkers that are specific and reliable for its detection. These biomarkers have been detected in different sources including serum and stool. Thus, it is expected that either new biomarkers will be identified or existing markers will be better characterized to facilitate the disease diagnosis. Another active area of research is on developing tools for detecting these markers in biomarkers. Current strategies for *H. pylori* detection are based on culture of biopsy samples or the use of ELISA-based immunoassays. However, these systems are not easy-to-use and require central facilities, which reduce their success as point of care and rapid diagnostic tools. Thus, there is an increasing need to create on-chip immunoassays. The existing technologies that have potential for further development include lateral assays with high sensitivity and low background noise. In addition, autonomous capillary-driven microfluidic systems hold a great promise for developing highly sensitive assays. It is expected that along with the future development of these technologies, novel diagnostic tools for detection of *H. pylori* will emerge. In addition, the rapid progress in the development of smartphones and flexible electronics and their integration with lateral assays and

microfluidic platforms will lead to the development of point of care that utilizes optical and electrochemical systems.

Compliance with ethical standards This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

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