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Review

Comparison of the lateral flow immunoassays (LFIA) for the diagnosis of *Helicobacter pylori* infection

Cebrail Karakus, Barik A. Salih *

Fatih University, Faculty of Science, Department of Biology, B.cekmece, 34500 Istanbul, Turkey

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ABSTRACT

Helicobacter pylori infection is the most common human infection where approximately 50% of the world populations are infected. The diagnosis of such infection is mainly done by endoscopy where gastric biopsies are examined for the presence of *H. pylori*. Such invasive approach is costly, time consuming and generally requires more than one test to confirm the infection. Serology on the other hand is a non-invasive approach that can detect *H. pylori* exposure. The lateral flow immunoassays (LFIA) support the serological approach and have the advantage of being fast, economic and require no additional equipment or experience. In this review the principles, components of the LFIA, sensitivities and specificities of the commercially available *H. pylori* test strips were compared and discussed.

Contents

1. Introduction

It is well known that half of the world populations are infected with *Helicobacter pylori* mainly those of the developing countries (Kimmel et al., 2000). The majority of subjects however are asymptomatic and only few percentages develop peptic ulceration that might progress to gastric cancer (Sipponen, 1998; Sipponen and Marshall, 2000). The diagnosis of *H. pylori* infection in such patients is an important approach for the selection of therapy and for the follow up of eradication success. *H. pylori* infection can be diagnosed by invasive and non-invasive techniques. The invasive technique (endoscopy) relies on the collection of gastric biopsy specimen to detect *H. pylori* by rapid urease test, culture, PCR and/or histopathology

Abbreviations: LFIA, lateral flow immunoassay; ICA, immunochromatographic assay; PCR, polymerase chain reaction; RUT, rapid urease test; UBT, urea breath test; ELISA, enzyme-linked immunosorbent assay; hCG, human chorionic gonadotropin.

* Corresponding author. Tel.: +90 2128663300; fax: +90 2128663402.
E-mail address: basalih@fatih.edu.tr (B.A. Salih).

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(Ricci et al., 2007). This is a time consuming and expensive approach unlike the non-invasive tests such as ^{13}C-urea breath test (UBT), stool antigen test and serology tests. The UBT and stool antigen tests detect the presence of *H. pylori* and are called active tests. Serology however (e.g. the ELISA test) detects anti-*H. pylori* antibodies which is an indicator for *H. pylori* exposure and is called passive test (Sciortino, 1993; Kimmel et al., 2000; Ricci et al., 2007). The lateral flow immunoassay (LFIA) is another alternative for the ELISA test used in serological and fecal diagnosis of *H. pylori* infection. It is intended particularly for a quick diagnosis and as a single use supporting test at a health care unit. Kato et al. (2004) compared the widely used stool antigen ELISA test with the lateral flow stool antigen test and reported that the later showed good specificity and sensitivity in the detection of *H. pylori* infection in children. The LFIA is an immunochromatographic assay (ICA) that is commercially available for a wide array of targets such as infectious agents (bacteria, viruses) (Nakasone et al., 2007; Cui et al., 2008; Kawatsu et al., 2008; Peng et al., 2008), hormones (Posthuma-Trumpie et al., 2008), drugs (Zhang et al., 2006; Zhu et al., 2008; Xie et al., 2009), pesticides (Zhou et al., 2004; Guo et al., 2009) and mycotoxins (Kolosova et al., 2007; Wang et al., 2007b). A visual qualitative (on/off) signal is enough for most applications. The best-known and first developed (LFIA) was the pregnancy test for the detection of human chorionic gonadotropin (hCG) hormone in urine sample (Leuvering et al., 1980; van Amerongen et al., 1993). Several investigators have also developed their own lateral flow test strips for the detection of various target molecules (Tanaka et al., 2006; Kolosova et al., 2007; Wang et al., 2007a, 2011; Chiao et al., 2008; Kawatsu et al., 2008; Peng et al., 2008; Zhao et al., 2008; Li et al., 2009; Xie et al., 2009; Xu et al., 2009; Omidfar et al., 2010; Yu et al., 2011). In general the lateral flow test strips have the advantages of being rapid (2–5 min), easy to use (self-performing), cost effective, portable, highly sensitive and specific. They do not require complicated equipment and technical expertise that are critical parameters for point-of-care, and have long shelf life at room temperature (12–24 months) (Posthuma-Trumpie et al., 2009; Ngom et al., 2010). The aim of this article is to review the components and the principles of lateral flow immunoassay (LFIA) devices and to compare the currently available rapid *H. pylori* commercial test devices.

2. Principles of the LFIA

The LFIA strips are designed to detect the presence of an analyte (antigen or antibody) by a specific labeled-antigen or labeled-antibody.

2.1. Test strip

The test strip consists of four sections; sample pad (cellulose), conjugate pad (glass fiber), membrane (nitrocellulose) and absorbent pad (cellulose) which are laminated onto a sheet of plastic backing orderly to allow cutting and handling. The pads overlap the membrane to allow a continuous flow path for the sample. The sample pad allows the diffusion of the sample into the conjugate pad that is impregnated with detector reagent (labeled-antigen or labeled-antibody) depending on the application. If the sample contains an analyte, it will bind to the detector reagent and the complex will continue to flow and then irreversibly binds capture reagent (antigen or antibody) at the test line on the membrane and forms a colored line. A continuous flow of the sample through the strip toward the control line will always form a colored line an indicator for a proper function of the test device. The absorbent pad at the end of the strip wicks the fluid through the membrane to ensure a continuous flow and thus maintains a clear background. Strips can be housed in a plastic holder (cassette), where only the sample application window and a reading window are exposed, for protection and easier handling (Millipore, 2008; Posthuma-Trumpie et al., 2009; Ngom et al., 2010).

2.2. Antibodies

The affinity of the specific antibody mostly influences the sensitivity of the test. Both monoclonal and polyclonal antibodies have been used in these tests; however the type of such antibodies used in commercial LFIA strips were not usually referred to in the manufacturer data sheets. Several investigators used either monoclonal antibodies or polyclonal antibodies both in the conjugate pad and on the test line (Shyu et al., 2002; Chiao et al., 2004, 2008; Kawatsu et al., 2006, 2008; Tanaka et al., 2006; Nakasone et al., 2007; Peng et al., 2008; Jiang et al., 2011; Yu et al., 2011). Others used both monoclonal and polyclonal antibodies on the same strip (Wang et al., 2011; Yang et al., 2011). The control line is coated with primary or secondary (anti-IgG) antibody depending on the application to capture excess detector reagents regardless of the presence or absence of target analyte (Posthuma-Trumpie et al., 2009; Ngom et al., 2010). The control line and the clear background that appear on the reading window are the indicators of an internal positive and internal negative procedural control.

2.3. Label particles

The majority of the available commercial LFIA strips (around 94%) use colloidal gold particles (red–pink color) for labeling, while the rest uses colored latex particles. According to the recent review articles many investigators also used colloidal gold particles for labeling (Posthuma-Trumpie et al., 2009; Ngom et al., 2010) while few others used colored latex particles (Gussenhoven et al., 1997; Greenwald et al., 2003). The size of the gold particles varies between 2 and 150 nm, but generally 15–25 nm particles were used (Zhou et al., 2004; Zhang et al., 2006; Nakasone et al., 2007; Zhao et al., 2008; Li et al., 2009; Xie et al., 2009; Omidfar et al., 2010). The advantages of gold particles are being stable, easy to use and have convenient surfaces to accelerate the antibody–antigen recognitions, which increases the immunoassay signals and allows a smooth flow through the membrane (DiScipio, 1996).

3. LFIA strips for the diagnosis of *H. pylori*

Several LFIA strips are currently commercially available for the diagnosis of *H. pylori* infection. This is a qualitative test that is used either to detect anti-*H. pylori* antibodies in blood samples (whole blood, serum and/or plasma) or to detect *H. pylori* antigens in stool samples. Both are intended to aid in the diagnosis of *H. pylori* infection in adult patients with

symptoms of gastrointestinal disorders and to monitor the success of eradication in treated patients. In this review a 22 commercially available test strips for the detection of serum anti-*H. pylori* antibodies (Table 1) and 14 other commercially available test strips for the detection of stool *H. pylori* antigen (Table 2) were compared.

3.1. LFIA for the detection of anti-H. pylori antibodies

The test strips are available in two formats:

1. Sandwich format: In this format *H. pylori* antigens coated on gold particles were placed into the conjugate pad, *H. pylori* unlabeled antigens were immobilized on the test line and similarly anti-*H. pylori* antibodies were immobilized on the control line (Fig. 1a). The addition of a sample drop onto the sample pad (Fig. 1b) leads to a lateral flow of the sample fluid containing anti-*H. pylori* antibodies toward the conjugate pad where it binds to the antigen coated on gold particles. The complex then flows to the test line where it binds to the immobilized unlabeled antigen and results in a red color line. The flow of the sample fluid will continue toward the control line where the remaining antigen coated gold particles will bind to the immobilized anti-*H. pylori* antibodies and give a red color line.
2. Indirect format: In this format either *H. pylori* antigens (Fig. 2) or antibodies (Fig. 3) were immobilized on the test line. In the antigen coated indirect format (Fig. 2), anti-human IgG antibodies coated on gold particles were placed into the conjugate pad, *H. pylori* unlabeled antigens were immobilized on the test line and similarly protein A was immobilized on the control line (Fig. 2a). The addition of a sample drop onto the sample pad (Fig. 2b) leads to a lateral flow of the sample fluid containing specific antibodies toward the conjugate pad where it binds to the antibody coated on gold particles. The complex then flows to the test line where it binds to the immobilized unlabeled antigen and results in a red color line. The flow of the sample fluid will continue toward the control line where the remaining immune-complexes will bind to the immobilized protein A and give a red color line. This line will turn positive whether the sample contains antibodies to *H. pylori* or not since the gold labeled anti-human IgG antibodies will bind to protein A. In the antibody coated indirect format (Fig. 3), *H. pylori* antigens coated on gold particles were placed into the conjugate pad, anti-human IgG antibodies were immobilized on the test line and similarly anti-*H. pylori* antibodies were immobilized on the control line (Fig. 3a). The addition of a sample drop (Fig. 3b) onto the sample pad leads to a lateral flow of the sample fluid containing specific antibodies toward the conjugate pad where it binds to the antigen coated on gold particles. The complex then flows to the test line where it binds to the immobilized antibody and results in a red color line. The flow of the sample fluid will continue toward the control line where the remaining antigen coated gold particles will bind to the immobilized anti-*H. pylori* antibodies and give a red color line.

Table 1
Principles and performances of commercially available lateral flow test strips for the detection of antibodies to *H. pylori* in serum samples.

	Commercial test[a]	Assay format	Label	Sensitivity (%)	Specificity (%)
1	Rapid Response™ *H. pylori*	NA	NA	95.1	94.1
2	CLIAwaived™ *H. pylori*	NA	NA	95.1	94.1
3	OneStep *H. pylori* RapiCard™	Sandwich	Gold	95.1	94.1
4	INSTANT-VIEW® *H. pylori*	Sandwich	Gold	95.1	94.1
5	Clarity *H. pylori*	Sandwich	Gold	95.9	89.1
6	BioStar® Acceava® *H. pylori*	Sandwich	Gold	95.9	89.1
7	ICON® HP-One-Step	Sandwich	Gold	95.9	89.1
8	BioSign® *H. pylori*	Sandwich	Gold	95.9	89.1
9	QuickView™ *H. Pylori*	Sandwich	Gold	100	97
10	Immunospec *H. Pylori*	Sandwich	Gold	100	97
11	Clearview® *H. pylori*	Indirect	NA	89	89
12	iScreen™ *H. pylori*	Indirect	NA	89	89
13	*H. pylori* Rapid Test	Indirect	NA	89	89
14	ACON® *H. pylori*	NA	NA	89	89
15	QuickVue® One-Step *H. pylori*	Indirect	NA	90	78
16	SureStep™ *H. pylori*	Sandwich	Gold	92.6	82.4
17	LINK2™ *H. pylori*	Sandwich	Latex	88.6	85.1
18	NOVAtest® One-Step *H. pylori*	Sandwich	Gold	95.9	89.6
19	Accu-Tell® *H. pylori*	Sandwich	Gold	92	96.6
20	RTA Labs *H. pylori* Test	Sandwich	Gold	92	96.6
21	RAPIRUN *H. pylori*	Indirect	Gold	84.7	89.3
22	ASSURE® *H. pylori*	Indirect	Gold	92	93

[a] The information was gathered from the manufacturers' data sheet. NA: not available.

Table 2
Principles and performances of commercially available lateral flow test strips for the detection of *H. pylori* antigens in stool samples.

	Commercial test[a]	Assay format	Label	Sensitivity (%)	Specificity (%)
1	ImmunoCard STAT!® HpSA	Sandwich	Latex	90.6	91.5
2	QuickView™ *H. pylori* Antigen	Sandwich	Gold	94	96.7
3	Immunospec *H. pylori* Antigen	Sandwich	Gold	94	96.7
4	OneStep *H. pylori* Antigen	Sandwich	Gold	94	96.7
5	HELISTOOL *H. pylori*	Sandwich	Gold	94	96.7
6	Rapid *H. pylori* Antigen	Sandwich	Gold	94	96.7
7	*H. pylori* Stool Antigen (HPSA)	Sandwich	Gold	94	96.7
8	StrongStep® *H. pylori* Antigen	Sandwich	NA	98.5	98.1
9	Accu-Tell® *H.pylori* Antigen	Sandwich	Gold	99.9	99.9
10	RAPID Hp StAR™	Sandwich	Gold	91.2	82.4
11	*H. pylori* Antigen Rapid Test	Sandwich	Latex	NA	95
12	SD BIOLINE *H. pylori* Ag	Sandwich	Gold	100	100
13	*H. pylori* Ag Card Test	Sandwich	Gold	94	98.6
14	Rapidan Tester® *H. pylori*	Sandwich	NA	99.9	99.9

[a] The information was gathered from the manufacturers' data sheet. NA: not available.

In regard to these test strips the following information were obtained from the manufacturer data sheets: the sensitivity was in the range of 85–100% (average 93%) while the specificity was in the range of 85–100% (average 90%); of these 50% showed a

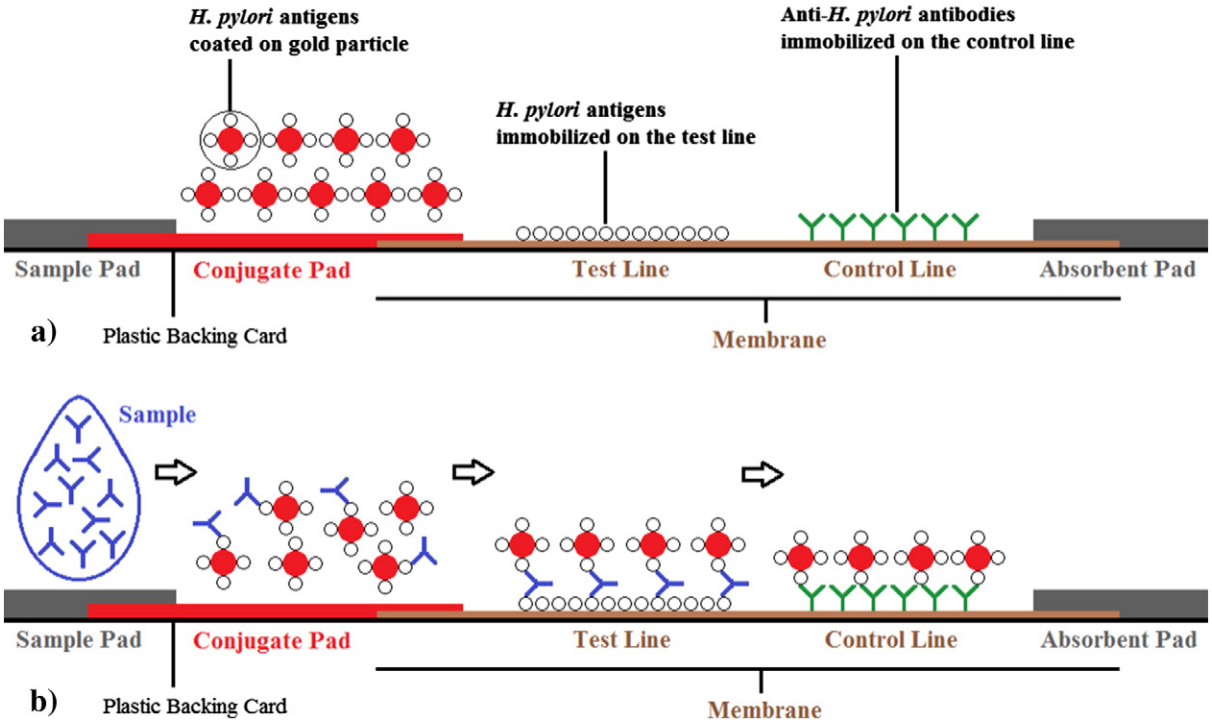

Fig. 1. Schematic representation of a lateral flow test strip (sandwich format for the detection of anti-*H. pylori* antibodies) showing the constituents of the strip before (a) and after (b) the addition of the sample.

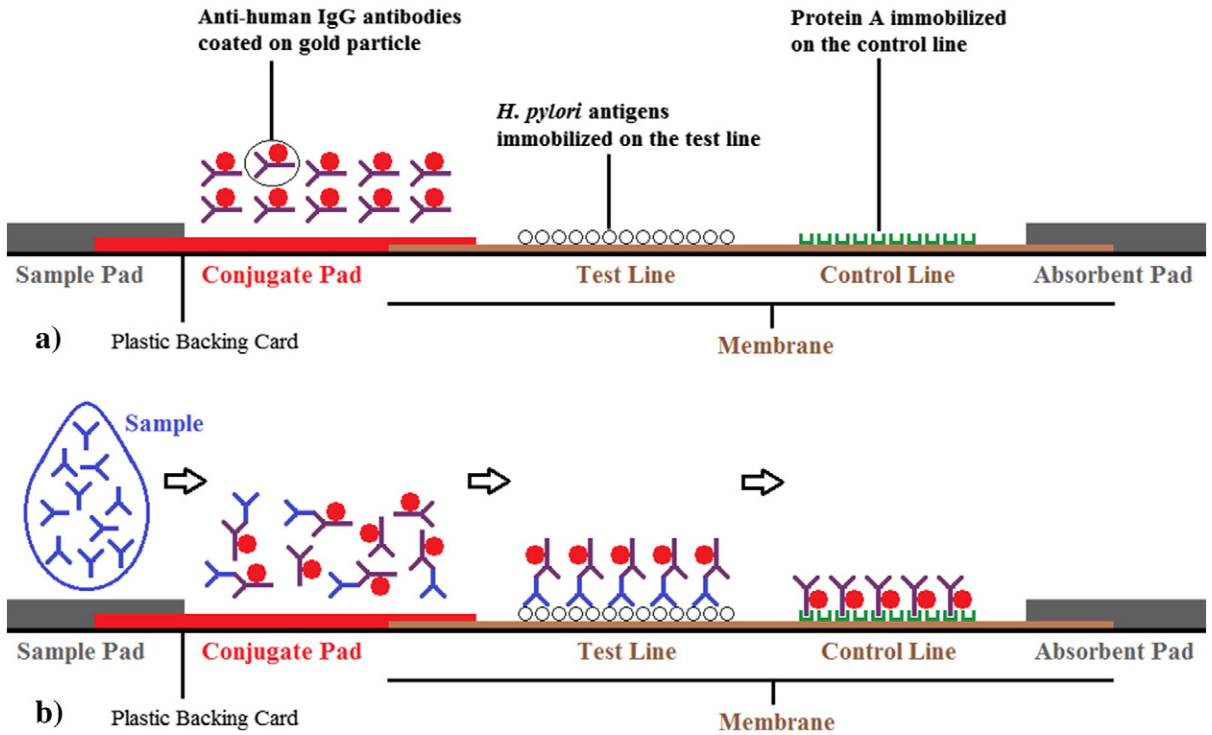

Fig. 2. Schematic representation of a lateral flow test strip (indirect format using antigen immobilized on the test line for the detection of anti-*H. pylori* antibodies) showing the constituents of the strip before (a) and after (b) the addition of the sample.

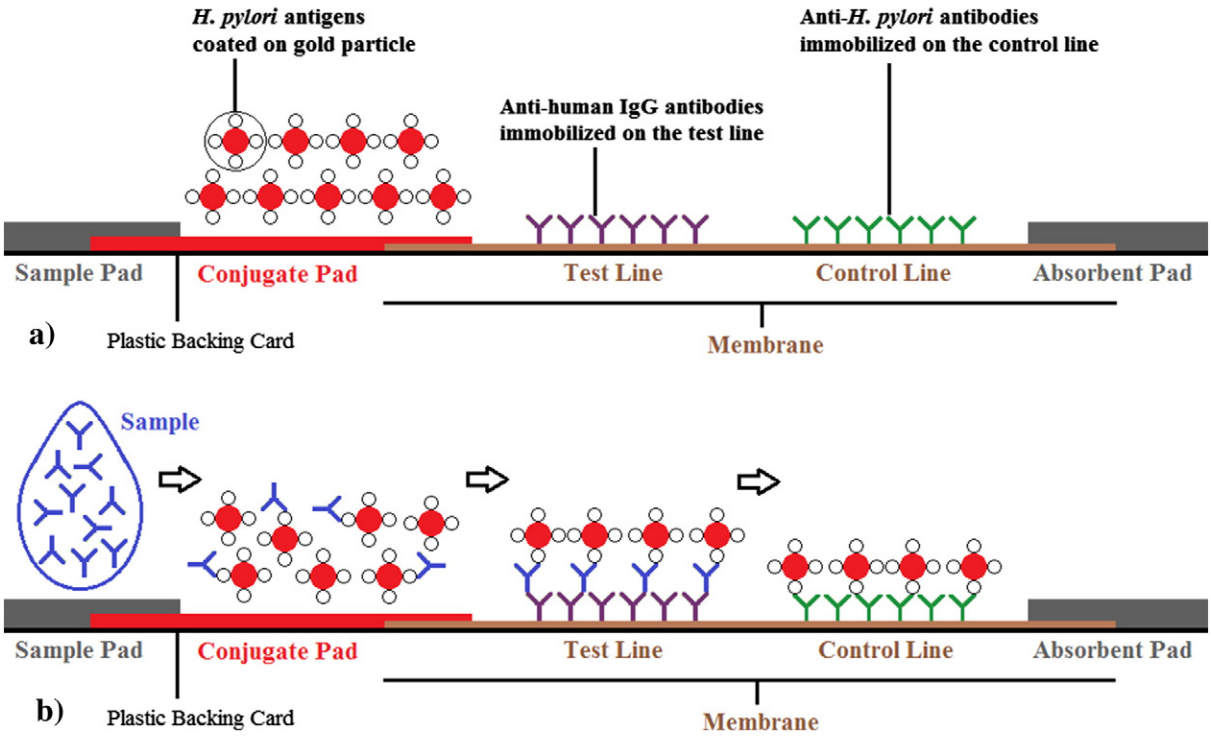

Fig. 3. Schematic representation of a lateral flow test strip (indirect format using antibody immobilized on the test line for the detection of anti-*H. pylori* antibodies) showing the constituents of the strip before (a) and after (b) the addition of the sample.

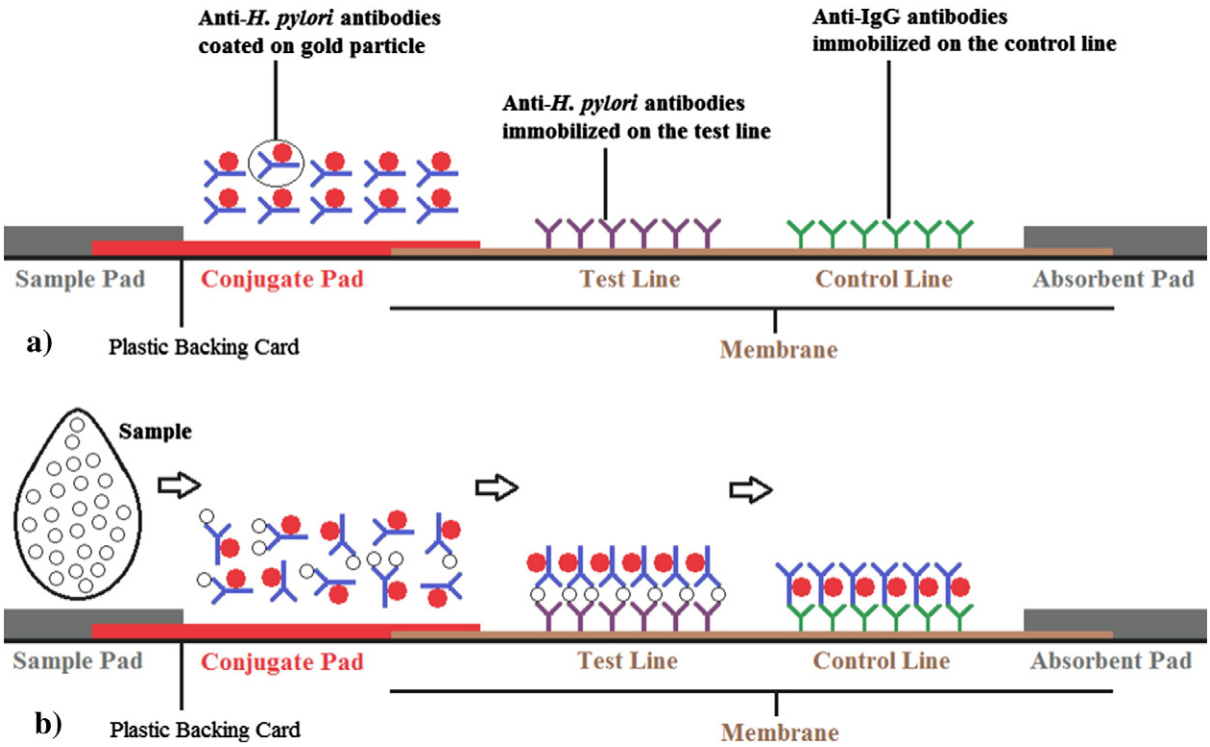

Fig. 4. Schematic representation of a lateral flow test strip (sandwich format for the detection of antigens) showing the constituents of the strip before (a) and after (b) the addition of the sample.

sensitivity of >95%, while only 20% had a specificity of >95%; the performance characteristics were evaluated by comparison with culture, histology, RUT, UBT and/or ELISA results and the anti-*H. pylori* IgG used in these test strips showed no cross reactivity with closely related organisms such as *C. jejuni*, *C. fetus*, *C. coli*, and *E. coli*. The detection time of these strips varied between 2 and 15 min and the stability was between 12 and 24 months. It appears that these test strips had the advantages of being fast, sensitive, specific and are reliable to be used in diagnosis even though they only detect *H. pylori* exposure.

3.2. LFIA for the detection of H. pylori antigens

The test strips used for the detection of *H. pylori* antigens were based on the sandwich format (Fig. 4). On the test strip anti-*H. pylori* antibodies coated on gold particles were placed into the conjugate pad, another anti-*H. pylori* antibodies were immobilized on the test line and similarly anti-IgG antibodies were immobilized on the control line (Fig. 4a). Both monoclonal and polyclonal antibodies were used in these strips. Some used gold conjugate monoclonal antibody in the conjugate pad and non-conjugate monoclonal antibody on the test line; few used gold conjugate monoclonal antibody in the conjugate pad and polyclonal antibody on the test line, while others did not mention the type of antibodies used in their manufacturer data sheets. Monoclonal antibodies are more specific than polyclonal antibodies since they are directed against a single epitope. The stool specimen is pretreated with sample diluents before application to the test strip. The addition of a sample drop onto the sample pad (Fig. 4b) leads to a lateral flow of the sample fluid containing antigens toward the conjugate pad where it binds to the antibody coated on gold particles. The complex then flows to the test line where it binds to the immobilized antibody and results in a red color line. The flow of the sample fluid will continue toward the control line where the remaining antibody coated gold particles will bind to the immobilized anti-IgG antibodies and give a red color line.

Similarly the following information were obtained from the manufacturer data sheets: the sensitivity was 90–100% (average 95%) and the specificity was 80–100% (average 96%); the performance characteristics of these test strips were evaluated as above and the test showed no cross reactivity when stools were spiked with a list of different microorganisms. The detection time of these strips varied between 2 and 20 min and the stability was between 12 and 24 months. These strips showed high specificity and sensitivity and were able to directly detect *H. pylori* infection.

In conclusion, we have found that around 44% of the LFIA strips showed sensitivity and specificity of over 95% as indicated in the manufacturer data sheets. The majority of these test strips (68%) that detect anti-*H. pylori* antibodies in serum samples were based on the sandwich format while 32% were based on the indirect format. Although the detection of these antibodies is only an indicator of *H. pylori* exposure, the LFIA strips do support the serological approach and have the advantage of being fast, economic and require no additional equipment or experience. On the other hand, the test strips that detect *H. pylori* antigens in stool samples were highly sensitive and specific and can directly detect the presence of *H. pylori*. The detailed schematic figures of the different test strip formats depicted in this review should help a great deal in understanding the basic principles of LFIA tests.

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